Type of Title: Research Article

Title: Targeting Regulatory Factors Associated with the *Drosophila Myc cis*-Elements by Reporter Expression, Gel Shift Assay, and Mass Spectrometric Protein Identification

Authors

Jasmine Kharazmi [1], Thomas Brody [2], Cameron Moshfegh [3]

Authors Affiliations

Jasmine Kharazmi [1]: University of Zurich, Department of Reproductive Endocrinology, Campus Schlieren, Zurich Switzerland. Electronic address: jasmine.kharazmi@gmail.com

Thomas Brody [2]: The Interactive Fly,4701 Willard Avenue, Suite 1636, Chevy, MD, 20815. Electronic address:interactivefly5@gmail.com; brodyt66@gmail.com

Cameron Moshfegh [3]: Department of Health Sciences and Technology, ETH Zurich, Zurich Switzerland. Electronic address: cameron.moshfegh@gmail.com

Correspondence: Jasmine Kharazmi, University of Zurich, Campus Schlieren, Wagistrasse 14, CH-8259, Schlieren, Zurich, Switzerland, Electronic address: jasmine.kharazmi@sekur.com

Abstract

Transcription factor MYC is highly responsive to mitogenic stimuli to modulate the expression of several targets involved in cell growth, protein biogenesis, proliferation, and differentiation. However, the regulation of MYC at the level of transcription is still incompletely understood. In previous work, we showed that most of the regulatory elements associated with *Drosophila Myc* are located within the 5' UTR and the intergenic regions of *Myc*. In this study, we developed a highly sensitive and selective Solid Surface Magnetic Enrichment Protocol (SSMEP) and used it to enrich DNA-Protein complexes compounded with the *Myc cis*-elements. Using smaller truncations, Electrophoretic-Mobility Shift Assay (EMSA) and protein identification by Mass Spec, we identified a cis-regulatory module (CRM) within the proximal 5' UTR that might be sufficient to activate lacZ reporter in the ovary and embryos. Furthermore, each of the CRMs from either the upstream region or the DPE within the intergenic region in conjunction with the proximal 5'-UTR regulatory unit recapitulates the missing expression in the larval tissues, the ovary, and in embryos. Finally, the Downstream Promoter Element (DPE) shows promoter activity and mimics the *Myc* wild type expression when fused in frame to the adjacent cluster of binding sites. Further *In vivo* functionality tests will be required to confirm the relevance of associated factors and identified signaling cascades as *Myc* regulators. In addition, the highly selective and sensitive protocol can be used for other model organisms and biomedical research projects and/or synthetic biological studies.

Key Words: Reporter Assay, Electrophoretic-Mobility Shift Assay, Solid Surface Magnetic Enrichment, Liquid Chromatography Mass Spectrometry, *Drosophila Myc*, Transcription

Abbreviations: **SSMEP**, Solid Surface Magnetic Enrichment Protocol; **EMSA**, Electrophoretic-Mobility Shift Assay; **CRM**, *Cis*-Regulatory Module; **DPE**, Downstream Promoter Element; **SCF**, Soluble Cytoplasmic Fraction; **SNF**, Soluble Nuclear Fraction; **Myc-CRM**, *Drosophila Myc Cis*-Regulatory Module; **W-CRM**, Wnt Cis-Regulatory Module; **pmol**, picomole; **DBCO-PEG23-amine**, DiBenzoCycloOctyne-Poly Ethyl Glycol-amine; **LC-MS/MS**, Liquid Chromatography Mass Spectrometry; **Scm**, Scrambled negative control; **ORF**, open reading frame.

Introduction

Developmental patterning of gene expression underlies complex interplays between transcription factors associated with enhancers, and promoters (BERMAN *et al.* 2004; SMITH *et al.* 2023), orchestrating cell growth, proliferation, apoptosis, and differentiation for a developing organism (EDGAR *et al.* 2001; PEARSON AND CREWS 2014; JIMENO-MARTIN *et al.* 2022). During early development, the stage of cell fate specification and organ shaping, gene activity must be tightly spatiotemporally controlled to confer the organism's normal body size and organ function (SMALL AND ARNOSTI 2020; RAY-JONES AND SPIVAKOV 2021).

MYC is an evolutionarily conserved powerful early super oncogene, and regulation of *MYC* gene requires tight transcriptional and post-transcriptional control during early development and later during adult life (CENCIONI *et al.* 2023), as well as during pathological disruptions and over the course of evolution (GALLANT *et al.* 1996; LEE *et al.* 2012; LI *et al.* 2013; HSIEH *et al.* 2019; THEVENON *et al.* 2020; REITER *et al.* 2023). Target gene activation upon binding of Myc/Max heterodimers to E-box sequences increases expression of S-phase cyclins, DNA replication (DANESHVAR *et al.* 2011), growth, and cell cycle progression (DE LA COVA AND JOHNSTON 2006; PROCHOWNIK 2022). In contrast, target genes involved in cell cycle arrest are expressed upon the binding of Myc in conjunction with other Myc-associated bHLHzip factors such as Miz1 (FIORESI *et al.* 2022) which counteracts the *Myc* proliferative signal. Similarly, Mad/Max or Mnt/Max heterodimers can compete with Myc for binding to the same E-box sequences thus repressing transcriptional activation by Myc/Max heterodimers (GRANDORI *et al.* 2000; OSTER *et al.* 2000; LUSCHER 2001; ZHOU AND HURLIN 2001; LOO *et al.* 2005). Like its human ortholog MYC, further important aspects of Myc biology include its role in regenerative growth/proliferation and stem cell biology. For example, Myc and Wingless play a crucial role in tissue regeneration, after surgical injury of imaginal discs or apoptosis-induced tissue damage (SMITH-BOLTON *et al.* 2009). Other studies have shown that somatic stem cells (SSCs) residing in each terminally differentiated organ have the potential to maintain and regenerate corresponding tissue after damage (QUINN *et al.* 2013). MYC family proteins have been shown to play an important role in replenishing organs after cancer treatment or injuries. Examples include midgut maintenance (REN *et al.* 2013), neuroblast maintenance, neural stem cell proliferation (RUST *et al.* 2018; SAMUELS *et al.* 2020), and epidermis regeneration (ZANET *et al.* 2005).

Since deregulated MYC expression has been implicated in a wide range of diseases ranging from neurodegenerative cases (MARINKOVIC AND MARINKOVIC 2021; ANATSKAYA AND VINOGRADOV 2022) to cancer (DHANASEKARAN *et al.* 2022; CENCIONI *et al.* 2023) and diabetes (LUO *et al.* 2021; ROSSELOT *et al.*

2021), tightly controlled *MYC* regulation plays a pivotal role in the physiology and homeostasis of an organism in early developmental stages and during adult life. Evolutionary conservation and functional similarities of *Drosophila Myc* and human *cMYC* (GALLANT *et al.* 1996) has made fruit fly, with its short life cycle and well-established genetics, a convenient model organism to study *Myc* biology. *Myc* patterning is highly responsive to signaling molecules and mitogenic stimulation during development and disease. Literature indicates that signaling pathways such as, Dpp, Wingless, Notch (JOHNSTON *et al.* 1999; HERRANZ *et al.* 2008), and Hippo (NETO-SILVA *et al.* 2010), and Ecdysone (DELANOUE *et al.* 2010) can positively and/or negatively regulate the expression of *Myc* maintaining balance between cell growth/proliferation and differentiation. Despite intensive studies of *Myc* regulation, many open questions remain to be addressed in order to understand the sophisticated and multi-level signaling cascades influencing *Myc* transcription.

In the previous work, we located a variety of *cis*-regulatory modules (CRMs) as Conserved Sequence Blocks (CSBs) throughout the *Myc* gene body and two main transcription start sites. The first one is a TATA-box containing promoter at the proximal 5'-end, and the second is a downstream promoter element (DPE) located in the 2nd intron (KHARAZMI *et al.* 2012). Here we further truncated the tested *Myc-lacZ* transgenes containing the promoter and other conserved regulatory elements. Our findings confirm that the dynamic expression of *Myc* during oogenesis and early embryogenesis is achieved through initiation of transcription at multiple sites, including a third newly identified start site at the 3'-end of intron 1 within the proximal 5'-UTR that contains the strong developmental enhancer P37/P38 (ChrX:3,374,908..3,374,960). We also found that high expression in larval discs and brain requires at least one additional late or differentiation enhancer in addition to the regulatory elements for the embryo and ovary. In our analysis of DNA/protein interaction complexes we have identified key regulatory factors associated with the *Myc cis*-elements, most of which are cell cycle regulators or belong to the known signaling pathways including noncanonical/canonical Wingless, Notch, DPP, Hedgehog, MAPK, JAK/STAT, insulin-like receptor (INR), and Hippo. The compounded factors and their link to developmental and metabolic signals relevant to *Myc* transcriptional and post-transcriptional regulation will be discussed.

Material and Methods

I. Generation of *lacZ* reporter fly strains

Constructs pJ2.1, pJ7, pJ8, pJ8.4, pJ8.5 J2.1, J7, J8, J8.4, J8.5 (Table S1.1) and intermediate vectors pCaSpeR4, pCaSpeR-NLSlacZ, SKII-dmyc5 and SKII-In2 were engineered and generated previously (KHARAZMI *et al.*

2012). Inserts for the transgenes J7.1-J7.3 are derived from the genomic sequences in SKII-dmyc5 and were subcloned into pCaSpeR-NLSlacZ.

J7 promoter (ChrX:3,373,200-3,375,005) contains 100 base pairs (bps) upstream of the main transcription start, noncoding exon1, intron1 and noncoding sequences of exon2 (Table S1.1). Transgene J7.1 is derived from J7 construct and lacks the sequences upstream of the main transcription start site containing TATA1-GC1-Inr1 sequences. To obtain the transgene J7.1 the intermediate plasmid SKII-dmyc5 and the vector Plasmid pCaSpeR-NLSlacZ were each separately digested with SacII and Acc65I. After isolation on 1.5% agarose gel the 5'-SacII/Acc65I-3' linearized/dephosphorylated vector and 1806 bp genomic 5'-SacII/Acc65I-3' insert (ChrX:3,373,200-3,375,005) were ligated overnight to create the transgene J7.1 (Table S1.1).

To obtain the promoter sequences for transgene J7.2, a fragment of 2164bp from the *Myc* 5' noncoding sequences was PCR amplified with the primers J7.2-F and J7.2-R (Table S1.2) and the forward primer introduced a SacII restriction site at the 5'-end of the PCR fragment, and the plasmid SKII-dmyc5 was used as template. PCR conditions in the thermal cycler were once cycle of initial denaturation at 98 °C for 30 seconds, 30 cycles of denaturation at 98 °C for 10 seconds, annealing at 58 °C for 30 seconds, and extension at 72 °C for 2 minutes, one cycle of final extension at 72 °C for 5 minutes, held at 4 °C. The PCR product was digested with 5' SacII-Acc65 3' to isolate 1295 bp genomic insert fragment containing 14bp from the 3' end of exon1, In1 and the noncoding region of exon2 sequences (ChrX:3,373,710-3,375,005). The insert was subcloned into 5' SacII-Acc65 3' linearized and dephosphorylated pCaSpeR-NLSlacZ in front of reporter lacZ to obtain transgene J7.2. The promoter of J7.2 contains 100 bp upstream of the main transcription start and the noncoding exon1.

To obtain the genomic sequences for the J7.3 promoter, a fragment of 641bp on the SKII-dmyc5 plasmid was amplified with the primers J7.3-F and J7.3-R (Table S1.2). The genomic fragment (ChrX:3,373,088..3,373,728) was blunt ligated (CIAP, Roche Diagnostics, Indianapolis, IN) into the Acc65I linearized, Klenow-treated, and dephosphorylated pCaSpeR-NLSlacZ vector in front of reporter lacZ to obtain transgene J7.3. The primer set J7.4-F and J7.4-R (Table S1.2) was used to amplify 1562bp from the *Myc* genomic sequences in the plasmid pJ2.1 enhancer region containing an E-box with the sequence 5'-CAGCTG-3' (Table S1.3, primers IR5-P1 and Az5-P2-IR3). The J7.4-F primer introduced a SacII restriction site at the 5' end of the PCR fragment, and the primer J7.4-R introduced Acc65I site at the 3' end of the fragment. After digestion the insert fragment of 1527bp genomic sequence (ChrX:3,369,648..3,371,174) 5' SacII-Acc65I 3' was ligated into the 5' SacII-Acc65I 3' linearized and dephosphorylated vector pCaSpeR-NLSlacZ to obtain the intermediate plasmid pJ7.4-temp.

The pJ7 promoter (ChrX:3,373,047..3,375,005) (Table S1.1) was amplified with the primer pair J7-F and J7-R (Table S1.2) and the Acc65I site was introduced at the 5'-end of the fragment. The reverse primer anneals 116bp downstream of the *Myc* fragment in the multiple cloning site and amplifies Acc65I site at the 3' end of the genomic fragment in the multiple cloning site (MCS). After digestion with Acc65I, the obtained 5' Acc65I-Acc65I 3' insert was ligated into Acc65I linearized and dephosphorylated pJ7.4-temp vector at the 3'-end of J2.1 enhancer region to obtain the transgene pJ7.4 with a composite promoter element (Table S1.1). For the creation of Transgene J7.5 a sequence of 932bp from the J8 enhancer region containing the conserved cluster of 12bp, 5'-CGC**GTGGGAA**AA-3', (*Hairless* (H) consensus binding site **GTGGGAA** is in the cluster) was amplified with the primers In2E-F-Not and In2E-R-Asp (Table S1.2) and (Table S1.3, primers IR5-P29 and IR5-P29-Az3), and at the 5'-end of the fragment a NotI site and at the 3'-end Acc65I restriction site was introduced. The 5' NotI-Acc65I 3' genomic insert fragment (ChrX:3,379,324..3,380,255) was swapped with the full-length In2 in J8 transgene to obtain the pJ7.5-temp intermediate product. Plasmid pJ7.4 was digested with the restriction enzyme Acc65I to obtain the pJ7 promoter sequences as insert and then was introduced into Acc65I linearized/dephosphorylated pJ7.5-temp vector at the end of J8 enhancer sequences and in front of lacZ reporter to obtain the transgene J7.5 (Table S1.1). For details on transfections, plasmid DNA preparations, phage ϕC31 integrase transgenesis system, and random P-element transgenesis system see *Kharazmi et al., 2012* section Materials and Methods.

The transgenes J2.1, J7, J7.1-J7.5, J8, J8.4, and J8.5 including *Myc* genomic sequences were sequenced at the distal site with the primers pcaF and Acc651_F (Table S1.2), and at the proximal site with the primers pCAR and pZR (Table S1.2), each time towards the *Myc* gene. All genomic, intermediate plasmids, engineered transforming vectors, and transgenes were sequenced by Microsynth AG, Balgach, Switzerland. Except for the standard primers (provided by Microsynth), all other oligonucleotides and sequencing primers were designed using the software tool DNASTAR Lasergene module PrimerSelect and synthesized at Microsynth AG.

II. *D. melanogaster* stocks and X-Gal staining assays

Transgenic lines J2.1, J8, J8.4, and J8.5 were generated by random P-element transgenesis and phage ϕC31 integrase transgenesis system (KHARAZMI *et al.* 2012), while J7.1-J7.5 strains were generated by random P-element insertion in the embryos from genotype *y[1] w[*1118*]* (performed by Rainbow Transgenic Flies Inc,

CA.). A *dpp-lacZ* fly stock (KHARAZMI *et al.* 2012) was used as a positive control, while *w[1118]* fly stock was used as negative control (Table S1.4). Fly stocks were grown on standard medium at 25 °C, and all the crosses took place at 25 °C. Larval and adult female tissues for lacZ staining were dissected from homozygous F2 generations. For the transgenic lines J2.1, J8, J8.4, and J8.5 X-Gal staining was performed according to standardized protocol (SONG *et al.* 2004; KHARAZMI AND MOSHFEGH 2013), and X-Gal staining of the tissues taken for the *Myc-lacZ* lines J7-J7.5 was performed according to the protocol adapted from Walter Gehrig lab.

III. Protein extraction from *Drosophila* embryos

Wild-type Canton-S flies (Bloomington *Drosophila* Stock Center; donor: Kyoto Stock Center, Kyoto Institute of Technology, Bloomington Stock Number #64349, originally Stock Number #1) (Table S1.4) were maintained at 25°C at 75 to 80% humidity in population cages and fed with yeast paste. Embryos were deposited onto apple-agar petri-dishes and collected from 0 to 12hr after egg laying and then stored at 4°C before use. Embryos from up to 3 successive days (72hr) were collected and stored at 4°C. Embryos were harvested by washing the petri-dishes with embryo collection solution (0.7% (w/v) NaCl 0.04% (v/v) Triton X-100) followed by extensive washes (5 times) with water to remove all traces of yeast. Embryos were dechorionated by immersion for 2 ½ minutes in 50% v/v bleach solution at room temperature followed by thorough rinses with running water at room temperature (three times for 5 minutes). After drying with paper towels the embryos were transferred into pre-chilled beaker and combined with chilled (4°C) 3ml/gram of embryos Buffer I (15 mM Hepes, K+, pH 7.6; 10 mM KCI; 5 mM MgCI; 0.1 mM EDTA; 0.5 mM EGTA; 350 mM sucrose; 1 mM dithiothreitol; 1 mM sodium metabisulfite; 0.2 mM phenylmethylsulfonyl fluoride; 1 mM benzamidine-HC1) and dispersed in the buffer with a glass rod.

Embryos were disrupted with a 40-ml Wheaton Dounce homogenizer in a 4° C room until the suspension became very milky. The homogenate was immediately filtered through a single layer of Miracloth (Calbiochem cat. #475855) into a Sorvall GSA centrifuge bottle and nuclei were pelleted in a Sorvall GSA rotor at 8000 rpm for 15 minutes at 4 °C. The supernatant, Soluble Cytoplasmic Fraction (SCF) was removed and stored at -80 °C. After wiping off lipids from the walls of the bottle with Kim wipes, tan-colored loose nuclei pellet was suspended in chilled buffer I (3 ml/g embryos) without suspending the yellow yolk pellet. The nuclei were dispersed by a 40-ml Wheaton Dounce homogenizer with a loose B pestle with a single stroke of the pestle and then transferred to clean centrifuge bottles, and re-pelleted in a Sorvall GSA rotor at 8000 rpm for 15 minutes at 4 °C. After decanting the supernatant and wiping off lipids, nuclei were swirled in buffer I (1 ml/g embryos).

Nuclei were homogenized with two or three strokes of the pestle, repelleted. at 8000 rpm for 15 minutes at 4 °C, supernatant was removed, and the nuclei were suspended in (0.5 ml /g nuclei) HEMG / 0.1M KCl buffer (25 mM Hepes, K+, pH 7.6; 0.1 M KCI;

12.5 mM MgCI; 0.1 mM EDTA; 20% (v/v) glycerol; 1.5 mM dithiothreitol; 1 mM sodium metabisulfite; 0.1 mM phenylmethylsulfonyl fluoride; 1 mM benzamidine-HCI). The nuclear suspension was incubated on ice for 20 minutes (max. 60 minutes) with occasional swirling and shaking, and then the mixture was subjected to ultracentrifugation in a Beckman SW28 rotor at 24,000 rpm (100,000 g) for 1 hour at 4 °C. The topmost thin gray-white lipid layer was removed with a spatula and discarded; the yellow liquid layer underneath comprising the Soluble Nuclear Fraction (SNF) was carefully aspirated, and immediately aliquoted in 0.1 to 1.0 ml aliquots, and after quick-freezing in liquid nitrogen stored at -80 °C. The third gray layer and the pellet at the bottom of the bottle composed of DNA and nuclear debris remained untouched. For protein quantification of SCF and SNF fractions, extracts were loaded across a 10% SDS-PAGE gel, separated by electrophoresis for 1 hour at a voltage of 80, Coomassie Blue stained (LUO *et al.* 2006; HARRIS *et al.* 2007) and imaged with the Odyssey Classic Infrared Imaging System in the 700nm. Additionally, the protein concentration was measured with the BCA Assay kit (Pierce™ BCA Protein Assay Kit cat# 23225, Thermo Scientific™).

IV. Designing of EMSA oligonucleotide pairs using Myc-CRMs as targets for protein binding

EMSA primers were designed from the *Myc* conserved *cis*-regulatory sequences within the proximal upstream and the large intron region. dTCF/Pan site, a Wnt-dependent *cis*-regulatory module (W-CRM) was used as positive control (Table S1.3). A sequence from the noncoding region of the *Mdm2* gene of the following mouse strain and the following Website were used to design a scrambled oligonucleotide as a negative control:

http://www.bioinformatics.org/sms2/shuffle_dna.html;(gi|372099100|ref|NC_000076.6|:117686685-117712961) Mus musculus strain C57BL/6J. For the visualization of double-stranded oligonucleotides, one strand was modified with the non-hazardous near-infrared fluorescence IRDye Dyomics 781 at one end and the complementary strand was modified with the same IRDye at the opposite end so that after annealing the two Dye molecules, one at the 5'-end and the other at the 3'-end were in each other's vicinity. To avoid steric effect and crowdedness for the subsequent protein binding after magnetic bead attachment, such oligo nucleotide pair was ordered twice, and in one pair the reactive Azide moiety (N-hydroxysuccinimide (NHS) ester) was attached to one strand at the free 5'-end and in the next pair the Azide group was attached to the 3'-end of the complementary strand. Since it was not clear whether proteins bind to one or both strands, this strategy helped

to preserve the bound proteins in the DNA protein complexes after multiple processes of washes. The oligonucleotides were synthesized at Microsynth AG. Considering the key determining factors, namely low salt concentration and the rate of temperature decrease that influence the efficiency of the hybridization of the complementary oligonucleotide pairs to duplexes (Table S1.5), final concentration of annealing reaction mix consisted of oligonucleotides mixture 1 pmol/µl (1:1 molar ratio) and NaCl concentration was 50mM. Thermal cycler was programmed as follows: Annealing of strand pairs for the removal of secondary structures was one cycle of initial denaturation at 95°C for 5 min; *40 cycles of hybridization at 95°C (**1°C/cycle) for 1 min; annealing at 55°C for 30 min; *20 cycles of hybridization at 55°C (1°C/cycle) for 1 min, and held at 4°C (*The number of cycles in hybridization steps was determined by the Tm of strand pairs to be annealed (the cycles above are an example for oligonucleotide pair with a Tm of 55°C in 50mM NaCl, pH 8.0); (**decrease of cycler's heating 1°C per min). Double-stranded oligonucleotides were subjected to electrophoresis in the Mini-PROTEAN electrophoresis system ((Bio-Rad Laboratories Inc.) in non-denaturing conditions on a 5% Precast TBE PAGE gel (Bio-Rad Laboratories Inc., cat. #4565014) for 1 hour at a voltage of 70 and visualized in wet PAGE gel with the Odyssey Classic Infrared Imager at the wavelength of 800nm.

V. Electrophoretic -Mobility Shift Assay (EMSA) using double-stranded azide and Dyomics 781 modified oligonucleotides

1.35 picomole of double-stranded targets (dsAzide-DNA-Dyo781) (Table S1.5) were added to the binding mixtures containing 10x binding buffer (100 mMTris, 500 mM KCl, 10 mM DTT; pH 7.5): **2 µL**; 25 mM DTT, 2.5% Tween®-20: **2 µL**; poly (dI.dc), 1 µg/ µL in 10 mMTris, 1 mM EDTA; pH 7.5: **1 µL**; 100 mM $MgCl_2$: **1 µL**; 1% NP (Nonylphenoxypolyethoxyethanol): **1 µ**L; protein (SCF in cytoplasmic samples and SNF in nuclear samples): **200 µg**; ultrapure water to a final volume of **20 µL**. Reactions were incubated in the dark for 30 minutes. After addition of 2 µL of 10x Orange Loading Dye (Odyssey® Infrared EMSA Kit) the mixtures and PageRuler protein marker (Thermo Fisher, cat. #26616) were subjected to electrophoresis in the Mini-PROTEAN electrophoresis system in non-denaturing conditions in 5% Precast TBE PAGE gel for 1 hour at a voltage of 80 and monitored in wet PAGE gel with the Odyssey Classic Infrared Imager at the wavelength of 800nm. The acquired EMSA images were processed in Adobe Photoshop CS6 by adjusting image brightness and contrast.

VI. Creation of chimeric carboxylic magnetic Bead-PEG-DNA construct

Based on existing technology we have developed a new protocol for protein enrichment from whole cell lysate and embryonic extracts for protein identification by MS analysis. We have built a chimeric construct consisting of magnetic bead attached covalently to a DBCO-PEG23-amine linker that is attached to a ds DNA oligonucleotide via copper-free Click Chemistry reaction. The PEG linker is hydrophilic, and its length allows sufficient steric movement on the Bead surface to allow sufficient spatial space for protein binding. The oligonucleotide is modified at one end with an IRDye for infrared detection and on the other end with an azide group for a covalent bonding with the PEG-linker via the Click reaction.

A). Activation and covalent coupling of ligand to the magnetic microspheres: Dynabeads® MyOne™ Carboxylic Acid (Invitrogen by Thermo Fisher cat. #65001) were resuspended by rolling the vial for >30 minutes and 1 mL (10 mg) was transferred to a new tube. The tube was placed in a magnet rack for 2 minutes and supernatant was aspirated. Magnetic beads were washed twice in 1 mL of 100 mM MES (2-[N-morpholino]ethane sulfonic acid, MW 213.25) buffer pH 6.00 and resuspend in 100 µL of the same buffer (final concentration of 10 µg/µL). In a separate 2mL tube, 140 µL magnetic beads (10 µg/µL), 84 µL of 1250mM EDC (N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride, MW 191.7) (Thermo Fisher, cat.# 77149) (75nmole EDC/1µg bead), and 42 µL of 100mM linker Dibenzocyclooctyne-Poly Ethyl Glycol-amine (DBCO-PEG23-amine, free-end amine salt) (BroadPharm San Diego, CA, cat. #BP-25103; formula C67H113N3O25) (3nmol PEG/1µg bead), and 1xPBS, pH 7.5/0.1% Triton X-100 were mixed to a final volume of 300 µL. The reaction mix was placed in a HulaMixer™ Sample Mixer (Thermo Fisher, cat.# 15920D) and incubated overnight at room temperature in the dark by gentle rotation (7rpm) for 12 to 16hrs. After activating carboxylic sites on the magnetic beads by EDC and amide-bonding between activated carboxylic group of coated beads and amine group of DBCO-PEG23-amine, Dynabeads® magnetic beads were washed 3 times for 10 minutes with storage buffer (PBS 100mM/NaCl 0.15M, pH 7.5 /0.1% Tween®-20) to remove the excess of EDC and PEG molecules.

B). Inactivation of the unbound carboxylic sites on Dynabeads® Magnetic Beads: The unbound carboxylic sites on Dynabeads® magnetic beads were quenched with 1 mL of 50mM Tris buffer pH 7.4 /0.01% Tween®-20

(each round incubated >30 minutes), then ligand-bound magnetic beads (DBCO-Beads) were gently washed 3 times with 1 mL of storage buffer and resuspended in the same buffer 10 µg/µL concentration and stored at 4° C for less than a week for later use.

C). Copper-free click chemistry (alkyne-azide) reaction: For the click chemistry reaction (covalent tethering of azide-modified DNA molecules to coated beads) 140 µL DBCO-Beads (10 µg/µL) were washed twice in 1 mL of 100 mM MES buffer pH 6.00 and resuspend in 140 µL of storage buffer (final concentration of 10 µg/µL). 7 µL of

double-stranded oligonucleotide (InfraRed Dyomics 781/Azide end-modified, 0.45pmole/µL) and storage buffer were added to the beads to a volume of 300 µL. The mixture was incubated for 4½ hours in HulaMixer™ at room temperature in the dark with 7rpm rotation. After the click chemistry reaction, the magnetic Bead-PEG-DNA construct was washed 4 times for 10 minutes with storage buffer to remove all traces of unbound DNA molecules. The construct was resuspended in 500 µL of storage buffer and stored at 4° C for less than 2 days for usage in protein binding experiments.

VII. Enrichment of DNA/protein complex using magnetic Bead-PEG-DNA pulldown construct

The magnetic bead-PEG-DNA construct in storage buffer was carefully resuspended and the supernatant was aspirated by replacement of the tube in magnetic rack and rinsed once with storage buffer, and then resuspended in 140 µL of the same buffer to a final concentration of 10 µg/ µL. Protein binding reactions for the soluble cytoplasmic fraction (SCF) and soluble nuclear fraction (SNF) samples were set up using Odyssey® Infrared EMSA Kit (Part #829-07910): **a)** Magnetic bead-PEG-DNA construct (10 µg/ µL): **1400 µg**; **b)** 10x binding buffer: **10 µL**; **c)** 25 mM DTT, 2.5% Tween®-20: **10 µL**; **c)** poly (dI.dc), 1 µg/ µL: **5 µL**; **d)** 1% NP: **5 µ**L; **e)** 100 mM $MgCl_2$: **5 µL**; **f)** Protein (SCF in cytoplasmic samples and SNF in nuclear samples): **200 µg**; **g)** ultrapure water was added to a final volume of **100 µL**. Sample tubes were incubated in the dark for 30 minutes by occasional manual flipping to keep the mixture in suspension. After incubation and aspiration of supernatants, the Beads-PEG DNA/protein complexes were gently washed 4 times for 5 minutes with storage buffer. Once more the residual liquid traces were removed, and the samples were stored at -80° C.

VIII. Sample processing and protein identification by mass spectrometry (LC-MS/MS)

a) Sample preparation: Proteins of each sample (Table S1.6) were denatured and reduced by suspending the beads (only beads and Beads-PEG DNA/protein complexes) in 8 M Urea, 100 mM Ammonium bi-carbonate with 5 mM DTT and incubated for 1 hr at room temperature. The reduced/denatured proteins were alkylated at cysteine residues by adding iodoacetamide to a final concentration of 15 mM and incubated for 1 hr. The reduced/alkylated samples were diluted 8 times with 100 mM ammonium bicarbonate and the supernatants were

collected after centrifugation at 20.000 x g for 5 min. Subsequently, samples were treated with 0.2 µg trypsin for 16 hrs at 37° C. The tryptic peptides were isolated and desalted by micropurification (reverse phase) prior to MS analysis. Myc-CRM samples, SCF and SNF extracts (starting materials), a Scrambled oligo (Scm) as negative control, from now on use only Scm), a configuration of W-CRM as Positive Controls (ARCHBOLD *et al.* 2014), along with the raw beads to track possible contaminations, were processed. **b) MS analysis**: Peptides were dissolved in 0.1% formic acid and loaded on a trap column (2 cm x 100 µm inner diameter). The peptides were eluted from the trap column and separated in 15-cm analytical column (75 µm inner diameter). The column material in both columns were ReproSil-Pur C18-AQ 3 µm resin (Dr. Maisch GmbH, Ammerbuch-Entringen, Germany). Peptides were eluted using a flow rate of 250 nL/min and a 30 min gradient from 5% to 35% phase B (0.1% formic acid and 90% acetonitrile). Samples were analyzed by high resolution LC-MS/MS setup using an EASY-nLC 1200 system (Thermo Scientific) connected to a Thermo Eclipse

Tribrid Mass Spectrometer (Thermo Scientific. **c) MS data processing**: The acquired MS raw data was processed in Proteome Discoverer 2.5 (Thermo Electronic) Software tool. The data was searched against *Drosophila. melanogaster* (Fruit fly) proteome (https://www.uniprot.org/proteomes/UP000000803). To filter non-*Drosophila* contaminants, the data was search against a contaminant database from the Max Planck Institute (MPI) (Germany) (https://lotus1.gwdg.de/mpg/mmbc/maxquant_input.nsf/7994124a4298328fc125748d0048fee2/$FILE/contaminants.fasta). Search parameters were trypsin as protease; minimum peptide length of 6 residues; Precursor Mass Tolerance of 10 ppm; Fragment Mass Tolerance of 0.02 Da and dynamic modifications with oxidation on Met and acetylation of the N-terminus. The search results were used for precursor ion quantification based on summed abundances of unique peptides without any normalization.

IX. Software processing of proteomics raw data

After filtering of MS data and removal of contaminants using the Thermo Fisher Scientific™ Proteome Discoverer™ 2.5 software, the protein candidates with a high Protein FDR Confidence, using a low False Discovery Rate (FDR) threshold of 1%, were processed with the software tool ComplexBrowser (MICHALAK *et al.* 2019), which can be accessed as web service at https://computproteomics.bmb.sdu.dk/app_direct/ComplexBrowser/. ComplexBrowser is a newly developed tool for the identification of protein complexes in experimental datasets using manually curated information from CORUM (Comprehensive Resource of Mammalian Protein Complexes) and complex portal databases

after quality control and interactive visualizations of obtained proteomics data and normalization of the data as recommended (MICHALAK *et al.* 2019). For *Drosophila* protein complexes, we curated protein complexes for our data manually and used ComplexBrowser only for the quality control of our protein candidates. For each sample pair including Negative Control, Myc-CRMs, and Positive Control, ComplexBrowser was used to generate the boxplot of log2 of the abundance intensities with median normalization, histograms of the coefficient of variation (CV) of abundance intensities to assess replicate variation, and the scatter plots comparing log of abundance intensities with Pearson correlation values. Statistical analysis through volcano plots served to represent changes in protein hits of protein complexes. Volcano plots were generated using the open source and online web tool VolcaNoseR (GOEDHART AND LUIJSTERBURG 2020), which can be accessed as web service at (https://huygens.science.uva.nl/VolcaNoseR2/). Volcano plots show log2 of abundance ratios for all protein candidates in each sample group over Scm on the x-axis and minus log10 of the q-value on the y-axis, with settings of a significance (q-value) threshold of 2 and a fold (abundance ratio) threshold of 1.5.

We used tools available on the Web and the community resources such as UniProt (https://www.uniprot.org/), The Interactive Fly (https://www.sdbonline.org/sites/fly/aimain/1aahome.htm), String (https://string-db.org/), and more importantly the comprehensive resource the FlyBase (https://flybase.org/reports/FBgn0013263.htm), and the available literature to manually find protein complexes of the identified protein hits.

Results

I. *Myc* P1/P2 distal enhancer patterns larval brain and discs while the intron 1 3'-end P37/P38 enhancer drives *Myc* expression in adult female tissues

Our previous work showed that the J2.1 transgene containing 7.2kb of the upstream noncoding region controls *Myc* expression in larval and adult female tissues (KHARAZMI *et al.* 2012). However, the smallest J7 transgene 1.928kb in size (ChrX: 3,373,088..3,375,005), consisting of 100 bp upstream of exon 1, noncoding exon1, intron 1, and noncoding 5'-region of exon2, is sufficient for expression in embryos and ovary but is inactive in larval brain and imaginal tissues (KHARAZMI *et al.* 2012). This result suggested that sequences within the J2.1 transgene might contain a late enhancer for tissue-specific patterning in larval imaginal discs and brain and further showed that the 1.928kb sequences within proximal 5'-UTR might contain regulatory elements important for oogenesis and embryogenesis (Figure1 A-B, Table S1.1).

In this work, we set out to examine both J2.1 and J7 transgenes in more detail and isolate enhancer elements responsible for *Myc* expression in ovary, larval discs, and brain. The role of each noncoding Myc-CRM in the J7 transgene (Figure 1B) (TATA-box containing P31/P32 consisting of 44 bps and P37/P38 53 bps sequence upstream of exon 1) was tested for its functionality in embryos and ovaries. We initially deleted the P1 promoter that harbors the Inr/TATA-box-containing Myc-CRM P31/P32, (J7.1 transgene) (Figure 1C). Additionally, as shown in J7.2 this region was further truncated by removing 1295 bps (Figure 1D), (J7.2 transgene). In these three transgenes *Myc-lacZ* expression in embryos and the ovary did not change. However, in the case of J7.3 transgene, when the intron 1 and noncoding exon 2 sequences, including the P37/P38 enhancer cluster, were deleted but the upstream sequences including the Myc-CRM P31/P32 within the P1 promoter and the entire exon1 were retained, no detectable expression was observed in adult female tissues (Figure 1E). These results mean that the endogenous *Myc* expression in adult female tissues is independent of P1 promoter activity. Further, they suggest that the 100 bps upstream of the exon 1 sequences are not required for *Myc-lacZ* activity in the embryos and ovary. Finally, we conclude that the P37/P38 enhancer, 53 base pairs in length (ChrX:3,374,908..3,374,960) (Table S1.3) is necessary and sufficient for the endogenous patterning of *Myc* expression in highly proliferating embryonic and ovarian cells in the early stages of development. Previous studies have shown that enhancers can undergo histone modifications, can bind to RNA polymerase II, and can initiate transcription in lineage-specific cell types during early development and differentiation (CATARINO *et al.* 2017; BARRAL AND DEJARDIN 2023; LINDHORST AND HALFON 2023). The transcriptional capacity of enhancers is particularly evident during early development, cellular differentiation, disease, and evolution (ANDERSSON *et al.* 2014; CATARINO *et al.* 2017; DAO *et al.* 2017; CATARINO AND STARK 2018; LINDHORST AND HALFON 2023). Based on previous findings and the current results, we refer to the Myc-CRM P37/P38 super enhancer (Figure 1, A-D and F; Table S1.3) as the *Myc* oocyte element.

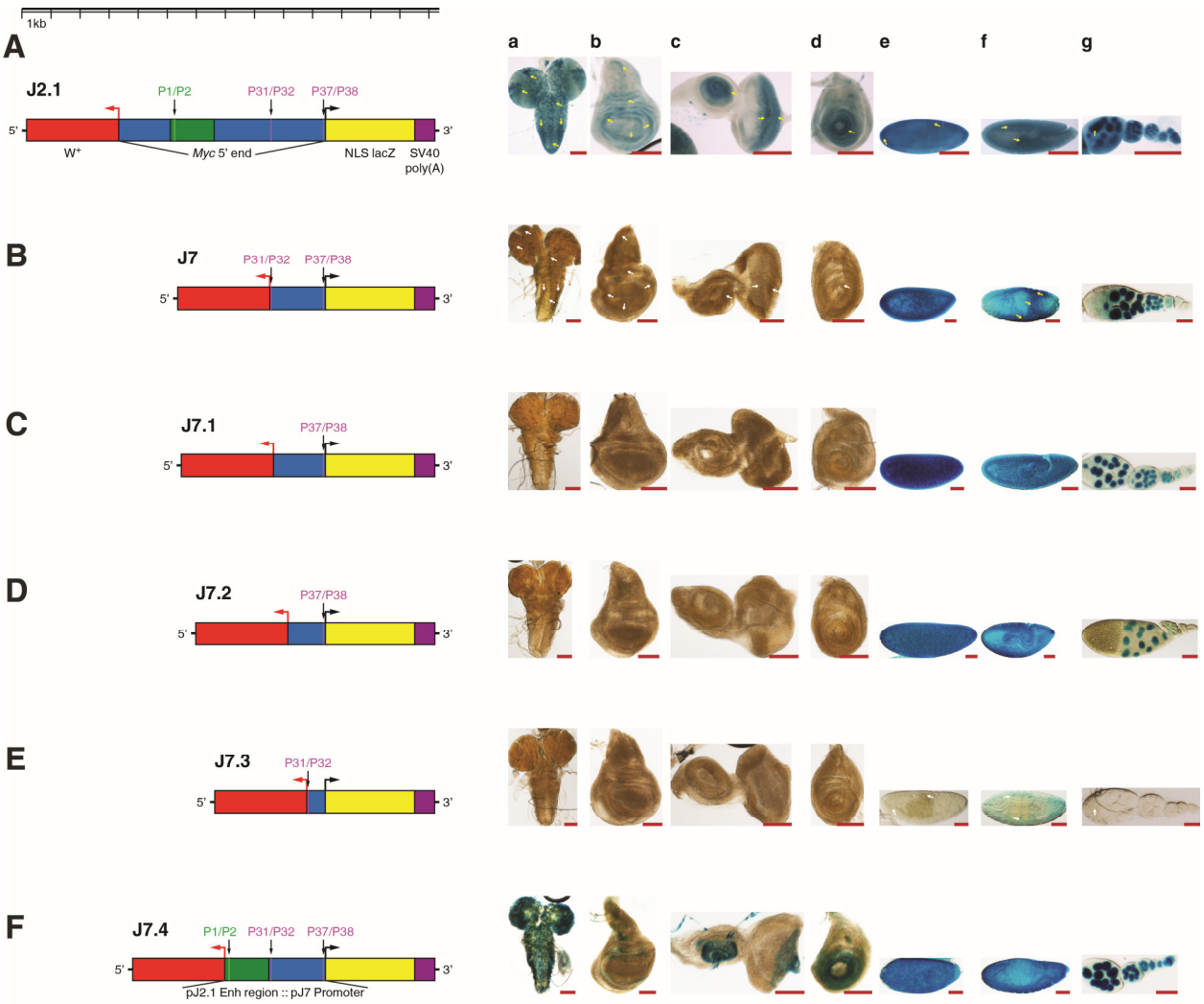


Figure 1. **Partitioning *Myc* 5' regulatory sequences detects a late developmental enhancer and an oocyte element**. (**A**) J2.1 contains a 7.159-kb regulatory region (*dmyc* 5' end, indicated by blue and green), lacking ORF sequences from the *Myc* 5'-end. These sequences contain a region with many conserved sequence blocks, the so-called J2.1 enhancer region (indicated by green) within which the upstream enhancer P1/P2 resides. The TATA-box-containing Myc-CRM P31/P32 and the proximal oocyte enhancer P37/P38 (indicated by blue) are located downstream of the enhancer region. (**B**) J7 is the smallest truncation derived from J2.1, 1.923-kb in size. Sequences upstream of the P31/P32 Myc-CRM and the P1/P2 enhancer are removed. (**C-E**) J7.1-J7.3 are truncations derived from J7. J7-1 and J7.2 contain the P37/P38 enhancer. However, J7.3 contains the P31/P32 promoter, but the P37/P38 enhancer is deleted. (**F**) The regulatory region in J7.4 is a composite element created by in frame fusion of sequences containing the P1/P2 enhancer (indicated by green in J2.1) to J7 regulatory sequences. Expression patterns of the *lacZ* reporter in the tested larval discs and brain panel (**a-d**), and in embryos and ovary panel (**e-g**) are shown. The **J2.1** transgene expresses *lacZ* in all the tested tissues, and the activity reflects the *Myc* endogenous mRNA distribution (yellow arrowheads) (KHARAZMI *et al.* 2012). In transgenes **J7**, **J7.1**, and **J7.2** *lacZ* patterning is abolished in the larval discs and brain (a-d) but retained in the embryos and ovary. Truncation **J7.3** fails to express *lacZ* in any of the tested tissues. In the composite **J7.4** transgene containing all the three Myc-CRMs P1/P2,

P31/P32, and P37/P38, but lacking the intervening sequences between the first two elements (compared to J2.1), reporter activity is restored in the larval brain and leg disc, embryos, and ovary; however, in the eye and wing imaginal discs the expression is weaker than the expression in the eye and wing discs of the full-length transgene J2.1. **Abbreviations**: **a**, brain; **b**, wing disc; **c**, eye-antennal disc; **d**, leg disc; **e** and **f**, embryos; **g**, ovary. **Note**: The sequences and coordinates of the Myc-CRMs P1/P2, P31/P32, and P37/P38 are indicated in Supplemental Table S1.3 and S1.5. β-Gal staining was done overnight at 25˚C on discs and brain taken from 3rd instar larvae and adult female embryos and ovary (**g**). Scale bar in (**a–g**) indicates 100 µm.

As a next step, we tested the P1/P2 enhancer within the conserved regulatory region of J2.1 transgene for its tissue specificity in larval brain and imaginal discs. The most convenient approach was to fuse the J2.1 enhancer region harboring the P1/P2 element to the J7 promoter in frame and test this J7.4 transgene for its functionality in larval tissues (Figure 1F). Interestingly, by staining for larval and adult female tissues endogenous *Myc* patterning was observed in all examined tissues. However, the expression in eye and wing discs was not as strong as seen for the J2.1 transgene. This result suggests that the upstream P1/P2 enhancer cluster is necessary for the *Myc* patterning in larval tissues and that the distance between the enhancer and promoter is required for a strong transcriptional activation, as is the case in the J2.1 transgene. Further, the result shows that the P1/P2 enhancer is a late developmental enhancer and is active during the time of the onset of differentiation in larval limb, eye, wing, and brain patterning and during pupal development.

II. Initiator-Downstream Promoter Element (Inr-DPE) requires the action of neighboring enhancer cluster for *Myc* pattering

Transcriptional interplays between enhancers and core promoters are vital and essential for gene expression in space and time during development (KUTACH AND KADONAGA 2000; BUTLER AND KADONAGA 2001; LEVINE 2010; SHIR-SHAPIRA *et al.* 2019; DREOS *et al.* 2021; YOKOSHI *et al.* 2022). Multiple DNA regulatory motifs have been identified, among which the TATA-box is the best investigated element (GOLDBERG *et al.* 1979; KADONAGA 2012). In addition, a conserved TATA-less regulatory element, namely Downstream Promoter Element (DPE) was first identified and best investigated in *Drosophila* (BURKE AND KADONAGA 1997; KADONAGA 2002; OHLER AND WASSARMAN 2010; KADONAGA 2012), and later in humans (DREOS *et al.* 2021). Previously, by scanning *Myc* 8kb intron 2 sequences with the phylogenetic footprinting tools, *cis*-Decoder-*EvoPrinter* (BRODY *et al.* 2007; YAVATKAR *et al.* 2008) we had identified multiple conserved sequence blocks (Myc-CRMs) in this region. A search using the Lasergene Bioinformatic tool GeneQuest module identified a

TATA-less initiator-promoter element, comparable to a DPE consensus sequence in most *Drosophila* genes: this element proved to be active unidirectionally (KHARAZMI *et al.* 2012). The transgene J8, containing the 8kb full-length intron 2 sequences within which the P29/P30 enhancer cluster and the TATA-less DPE promoter reside, drove *Myc-lacZ* expression in third instar larval imaginal discs, in the brain, and in adult female tissues (Figure 2A). The two truncations derived from the (J8) transgene, (J8.4), containing only the enhancer P29/P30 and the transgene (J8.5), retaining the DPE, failed to express *lacZ* in the tested tissues (Figure2, B-C). These results suggested to us that neither the enhancer nor the DPE was capable of initiating a transcriptional burst. We hypothesize that the core promoter element DPE functions as a regulatory unit to modify the action of the enhancer transcription dynamics throughout development. Indeed, a core promoter element serves as a modulator of a developmental enhancer in differential gene activation during highly proliferative stages of early development (YOKOSHI *et al.* 2022).

We next tested whether the transcriptional activity of the enhancer P29/P30 depends only on a DPE element, or alternatively a TATA-box promoter might also serve as a regulatory module for *Myc-lacZ* expression in all tissues as seen in full-length reporter construct J8 (Figure2A). To this end the enhancer P29/P30 was inserted in front of the TATA-box promoter in J7 to obtain the reporter J7.5 (Figure1B, Figure2D). J7.5 recapitulated almost all aspects of the endogenous *Myc* activity in larval brain and discs, and the early embryos and ovary (Figure2D). For β-gal staining of tissues taken from the positive and negative control flies see Figure S1, a-g in Supplemental Figures.

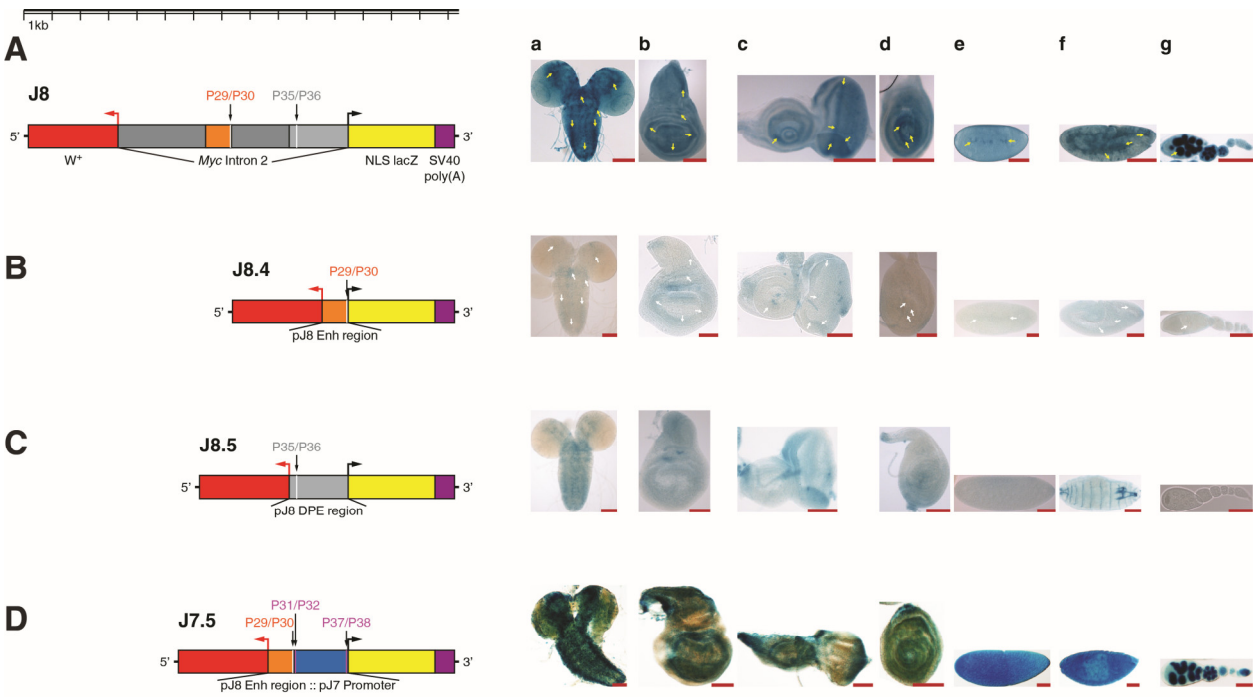


Figure 2. **The Downstream Promoter Element (DPE) within the large intron requires the adjacent enhancer cluster P29/P30 for transcription activation**. Reporters J8, J8.4, and J8.5 are adapted from the previous work (KHARAZMI *et al.* 2012). (**A**) J8 contains the 8 kb full-length intron 2 sequences (indicated by dark gray, orange, white, and light gray). The 932 bps enhancer region (indicated by orange) contains multiple conserved sequence blocks. The enhancer cluster P29/P30 (45 bps in length) is in the enhancer region; P29/P30 contains a conserved cluster of 12 bps , 5'-CGCGTGGGAAAA-3' (the Hairless binding site GTGGGAA is in the cluster). The 2.5 kb sequences at the 3'-end of intron 2 contains the DPE-containing Myc-CRM P35/P36 (47 bps in length). (**B**) J8.4 contains only the enhancer region (indicated by orange) including the enhancer cluster P29/P30 (indicated by white). (**C**) J8.5 contains only the downstream sequences of intron 2 (indicated by light gray) including the DPE-containing Myc-CRM P35/P36 (indicated by white). (**D**) The composite regulatory unit in the J7.5 transgene is under control of the J8 enhancer region containing the P29/P30 enhancer cluster fused to the J7 promoter (indicated by white and blue). Panel (**a-g**) shows expression patterns of the *lacZ* reporter in the tested larval discs and brain (**a-d**), and in embryos and ovary (**e-g**). In the **J8** transgene the larval imaginal discs and brain (a-d) and adult female embryos (e-f) and ovary (g) the expression reflects the *Myc* endogenous mRNA patterning (yellow arrowheads) (KHARAZMI *et al.* 2012). The **J8.4** truncation containing only the enhancer region and the **J8.5** under control of the DPE-containing downstream sequences are not capable of mimicking the endogenous *Myc* patterning (white arrowheads). The composite promoter in **J7.5**, consisting of the J8 enhancer region including the Myc-CRM P29/P30 subcloned in front of the J7 promoter, rescued *lacZ* expression in larval discs and brain and adult female tissues compared to J8.4 and J8.5 expression. **Abbreviations**: **a**, brain; **b**, wing disc; **c**, eye-antennal disc; **d**, leg disc; **e** and **f**, embryos; **g**, ovary. **Note**: The sequences and coordinates of the Myc-CRMs P29/P30, P31/P32, P35/P36, and P37/P38 are indicated in Supplemental Table S1.3 and S1.5. β-Gal staining was done overnight at 25˚C on discs and brain taken from 3rd instar larvae and adult female embryos and ovary. Scale bar in (**a–g**) indicates 100 µm.

III. Selection of *Myc cis*-elements as targets for the identification of *Myc* regulators and W-CRM as a positive control

Previously we identified *in silico* conserved *cis*-Regulatory Modules (CRMs) and promoter sequences within the noncoding sequences of the *Myc* locus that expressed the *lacZ* reporter positively in embryos, ovary, and larval brain and imaginal tissues (KHARAZMI *et al.* 2012; KHARAZMI AND MOSHFEGH 2013). Based on *lacZ* activity in larval and adult female fly tissues, we chose 5 oligonucleotides as 5 targets to be tested for the assembling of gene regulatory proteins to these sequences. As for the negative control, we generated a scrambled oligonucleotide from the noncoding region of the *Mdm2* gene of Mus musculus strain C57BL/6J (Materials and Methods, Section IV). As positive control a binding site for the Wingless/Wg regulator (W-CRM), dTCF/Pan was used (Figure 3; Table S1.3, primers IR5-Pos1 and Az5-Pos2-IR3). dTCF/Pan is a final effector of the

Wingless pathway for the expression of target genes. This dTCF/Pan site consists of an HMG domain with a basic tail (BT) of murine LEF-1 site for binding the HMG site (ARCHBOLD *et al.* 2014). *Drosophila* dTCF/Pan shares 92% homology with the HMG domain of LEF1. The HMG site is attached to a spacer followed by the C-clamp sequence (ARCHBOLD *et al.* 2014). In the normal state, the dTCF/Pangolin transcription factor is cytoplasmic. Upon activation of Wingless signaling, dTCF/Pangolin is translocated into the nucleus to drive the expression of Wingless/Wg target genes (SCHWEIZER *et al.* 2003; ARCHBOLD *et al.* 2014). DNA-protein complexes containing *Myc* trans-activators, and the complexes with the positive and negative controls were subjected to MS analysis for protein identification. The analysis procedure and the results obtained will be presented in the following sections.

Figure 3. **A variant of W-CRM containing HMG and Helper sites was used as positive control for protein identification studies**. HMG-Helper site pair orientation has been termed as Akimbo (AK) with six bps spacing (AK6) (ARCHBOLD *et al.* 2014). A right pointing arrow indicates the consensus sequence is read 5' to 3' on the ''top'' strand, a left pointing arrow indicates the consensus is read 5' to 3' on the ''bottom'' strand. The sequences shown for HMG (magenta) and Helper site (green) are identical to the ones used for DNA binding experiments and synthetic Wnt reporter constructs, and the sequence showed highest binding affinity binding to TCF/Pan *in vitro*. Figure taken from (ARCHBOLD *et al.* 2014).

IV. Annealed double-stranded oligonucleotides (targets) and protein extracts went through several control tests prior to developing a highly selective and sensitive methodology for protein identification

For *Myc* target sequences, the positive and the negative control oligonucleotides were annealed to double-stranded molecules (Figure 4a-c, Table S1.5). Annealed oligonucleotides were pegylated (SHCHEPINOV *et al.* 1997) for further control experiments prior to protein identification (Figure 4d). In order to test if the click chemistry reaction works and the azide moiety at the end of an oligonucleotide can be covalently bound to the DBCO group of DBCO-PEG23, we ran both pegylated and unpegylated oligonucleotides on a 5% PAGE gel and could show that pegylated DNA runs more slowly than the unpegylated oligonucleotide (Figure 4e). Furthermore, through Electro-Mobility-Shift-Assay (EMSA) and using positive control, we could show that

proteins can associate with a pegylated piece of DNA as efficiently as non-pegylated DNA (Figure 4f). For testing the range of limits for the usage of oligonucleotides with end modifications and valuable nuclear extracts, the next control experiments were followed by keeping the oligo concentration constant, and increasing the protein concentration incrementally, and scanning the DNA-protein super shifts for their intensity. For the positive control, increasing the concentration of the soluble cytoplasmic fraction (SCF) and soluble nuclear fraction (SNF) the intensity of super shifts containing DNA-protein complexes increased for both extracts, and the 2.25 picomole DNA was each time fully complexed by proteins (Figure 4g, lane 2-9). We obtained the same result for the *Myc* target P31/P32 with both protein fractions (Figure 4h, lane 2-7). However, although the amount of the DNA that we used for each lane, 1.35 picomole, was less than the one for the positive control, the portion of unbound oligonucleotides that migrated to the bottom of the gel was high and visible compared to the positive control. Based on the intensity of super shifts observed for the use of 100 and 150 µg of SCF (Figure 4h, lane 3-4) and SNF fractions (Figure 4h, lane 6-7), 200 µg of each fraction was used for each protein identification sample (see Material and Methods, section VII).

In the next experiment, as in the test with the positive control and the target P31/P32, we kept the oligo P37/P38 concentration constant, however we decreased the amount of SCF and SNF in increments and expected to see a decrease in the intensity of the shifted bands for both extracts. For the super shifts obtained from the SCF fraction, a decrease was seen as was expected, and all the oligo molecules were associated with proteins, and no free DNA molecules were detectable at the bottom of the gel (Figure 4i, lane 1-3). For the SNF fraction the super shifts became weaker with the decreased amount of protein, and most of the DNA was not bound and migrated to the bottom of the gel (Figure 4i, lane 4-6). This meant to us that 0.45 picomole of oligonucleotide is adequate for the isolation of DNA-protein complexes for protein identification. To confirm the specificity of the proteins assembled on the *Myc* target in the super shifts, we performed SCF and SNF protein binding assays with the *Myc* target P1/P2, in parallel to the scrambled oligo (Scm1/Scm2, short Scm) as a negative control. The SCF fraction showed a super shift for both, the negative control Scm (Figure 4j, lane 2 and 4) and the *Myc* target IR-P1/P2 (Figure 4j, lane 3 and 5). However, no binding was seen for the negative control Scm with the SNF fraction (Figure 4j, lane 6 and 8). For the *Myc* target IR-P1/P2 (Figure 4j, lane 7 and 9) the super shifts for both concentrations of 70 and 150 µg of SNF were evident and sharp, and the band became more intensive by increasing the protein concentration. We conclude that the binding of factors to Scm is unspecific. To further confirm the specificity of the association of the factors in SNF with the *Myc* sequences, we performed a competition EMSA with the *Myc* target IR5-P1/P2-IR3. Like other oligonucleotides used in the study, IR5-

P1/P2-IR3 was end-labelled with InfraRed Dye Dyomics 781, referred to as hot probe. Unlabeled primer pair P3/P4, referred to as cold probe (competitor) shares the same sequence with the IR5-P1/P2-IR3 (Table S1.3). With a 15-fold molar excess of the competitor, the intensity of the hot probe IR5-P1/P2-IR3 started decreasing (Figure 4k, lane 5), and with a molar excess of 30-fold of the cold probe the hot probe IR5-P1/P2-IR3 was fully outcompeted (Figure 4k, lane 6). This result is further proof for the specificity of the interaction between the DNA sequence and the factors in the SNF fraction assembled on the DNA.

After performing these control experiments and obtaining positive results, we used the Soluble Nuclear Fraction (SNF) that is enriched with transcription factors, as starting material for protein identification. The scrambled oligonucleotide (Scm) served as negative control and W-CRM dTCF/Pan as positive control. Since pegylated DNA worked for the recruitment of factors to the DNA sequence of interest, the created chimeric Magnetic Bead-PEG-DNA pulldown construct (See Section V of Results for the pulldown construction) and controls were placed on a glass slide with its chambers separated by silicon, and the IR-labeled DNA (in this experiment oligo P37/P38) could be visualized in a Licor InfraRed imager (Figure 4(l), panel 1a-1f and 2a-2f). For each target and the control oligos a corresponding pulldown construct was built and used to isolate factors complexed by the DNA (see Figure S2 A-D in Supplemental Figures).

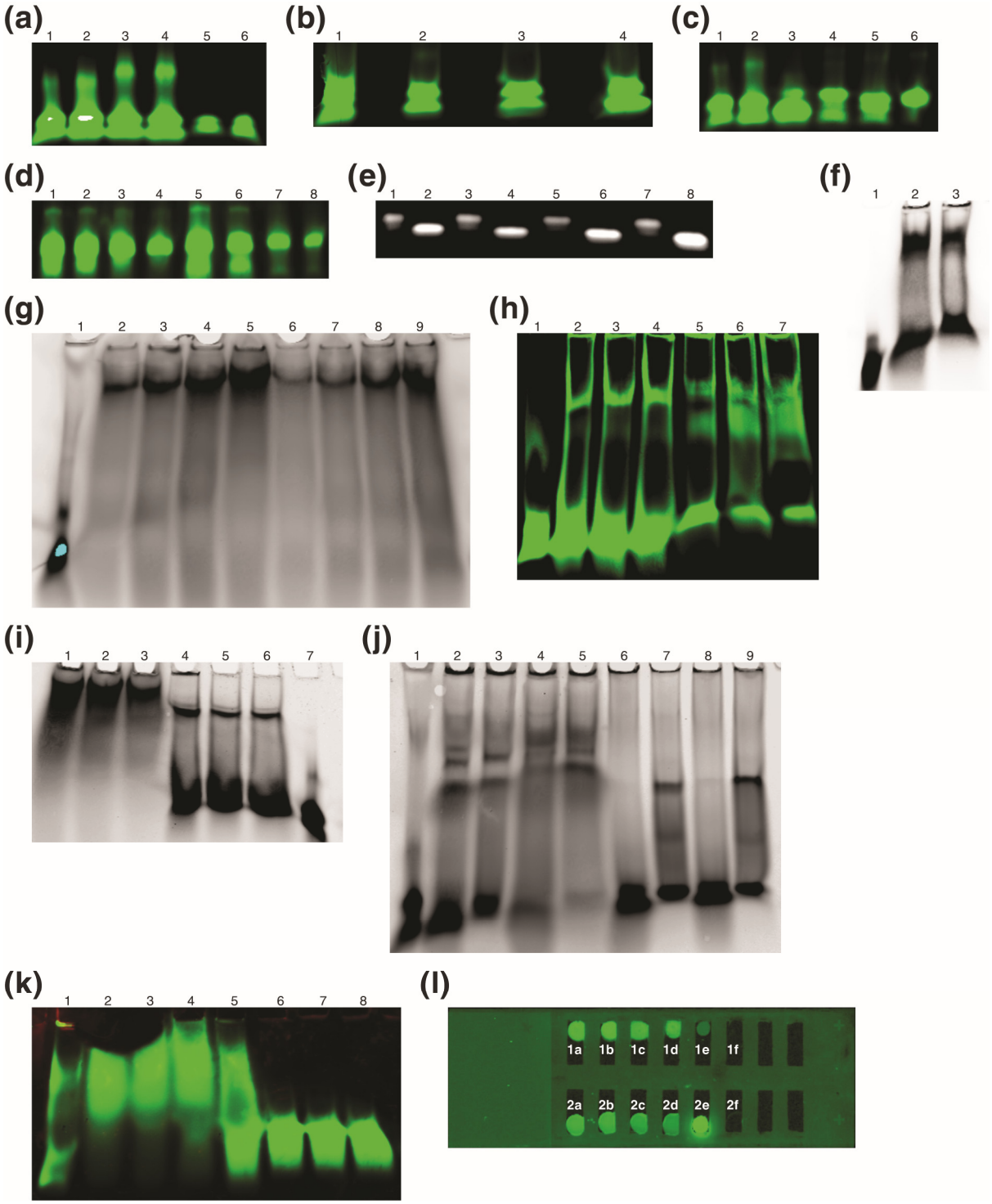


Figure 4. Annealing of the oligonucleotides to double-stranded molecules and EMSA assays after incubation with the *Drosophila* embryonic protein extracts. (**a**) **Annealing to IR-labeled positive control, 1-4**: azide-labeled, **5-6**: no azide labeling (lanes **1**, **3** & **5**: 0.9 and lanes **2**, **4** & **6**: 1.35 pmol oligo); (**b**) **Annealing to IR & azide-labeled oligos**, **1**: P31/P32, **2**: P32/P31, **3**: P37/P38, **4**: P38/P37:(each lane 1.35 pmol); (**c**) **Annealing to IR & azide-labeled oligos, 1**: Pos1/Pos2**, 2**: Pos2/Pos1, **3**: Scm1/Scm2, **4**: Scm2/Scm1, **5**: P1/P2, **6**: P2/P1 (each lane 1.35 pmol); (**d**) **Annealing & pegylation**: **1**: P31/P32, **2**: P31/P32, **3**: P32/P31, **4**: P32/P31, **5**: P35/P36, **6**: P35/P36, **7**: P36/P35, **8**: P36/P35 (lanes **1, 3, 5, &** **7**: 0.45 pmol; lanes **2, 4, 6, & 8**: 0.225 pmol); (**e**) **Unpegylated DNA migrates faster than pegylated**, **1**: Pos2/Pos1-Peg23: 0.2

pmol, **2**: Pos2/Pos1: 0.4 pmol, **3**: Pos2/Pos1-Peg-23: 0.3 pmol, **4**: Pos2/Pos1: 0.4 pmol, **5**: Pos1/Pos2-Peg-23: 0.2 pmol, **6**: Pos1/Pos2: 0.4 pmol, **7**: Pos1/Pos2-Peg23: 0.3 pmol, **8**: Pos1/Pos2: 0.6 pmol; (**f**) **SNF binding to non-pegylated IR & azide-labeled vs pegylated IR- & azide-labeled positive control 1**: Pos2/Pos1 only, **2**: Pos2/Pos1 + 100 µg SNF, **3**: Pos2/Pos1-PEG23 + 100 µg SNF (each lane 0.4 pmol); (**g**) **SNF & SCF binding to IR- & azide-labeled positive control by increasing protein concentration**, **1**: Pos2/Pos1 only, **2**: Pos1/Pos2 + 100 µg SCF, **3**: Pos1/Pos2 + 200 µg SCF, **4**: Pos1/Pos2 + 300 µg SCF, **5**: Pos1/Pos2 + 400 µg SCF, **6**: Pos1/Pos2 + 100 µg SNF, **7**: Pos1/Pos2 + 200 µg SNF, **8**: Pos1/Pos2 + 300 µg SNF, **9**: Pos1/Pos2 + 400 µg SNF; (each lane 2.25 pmol); (**h**) **SCF & SNF binding to IR- & azide-labeled P31/P32 by increasing protein concentration**, **1**: P31/P32-IR3 only, **2**: P31/P32 + SCF 70 µg, **3**: P31/P32 + SCF 100 µg, **4**: P31/P32 + SCF 150 µg, **5**: P31/P32 + SNF 70 µg, **6**: P31/P32 + SNF 100 µg, **7**: P31/P32 + SNF 150 µg (each lane 1.35 pmol); (**i**) **SCF & SNF binding to IR- & azide-labeled P37/P38 by decreasing protein concentration**, **1**: P37/P38 + 500 µg SCF, **2**: P37/P38 + 400 µg SCF, **3**: P37/P38 + 300 µg SCF, **4**: P37/P38 + 500 µg SNF, **5**: P37/P38 + 400 µg SNF, **6**: P37/P38 + 300 µg SNF, **7**: P37/P38 DNA only (each lane 0.45 pmol); (**j**) **SCF and SNF binding to IR- & azide-labeled P1/P2 & (Scm) negative control**: **1**: Scm1/Scm2 only, **2**: Scm1/Scm2 + 70 µg SCF, **3**: P1/P2 + 70 µg SCF, **4**: Scm1/Scm2 + 150 µg SCF, **5**: P1/P2 + 150 µg SCF, **6**: Scm1/Scm2 + 70 µg SNF, **7**: P1/P2 + 70 µg SNF, **8**: Scm1/Scm2 + 150 µg SNF, **9**: P1/P2 + 150 µg SNF (each lane 1.35 pmol); (**k**) **Competition EMSA between IR-labeled P1/P2 (hot, probe) & P3/P4 (cold, competitor)**: **1**: Probe only, **2**: Probe (1.35pmol) +130 µg SNF, **3**: Probe: Competitor = 1+1 plus 130 µg SNF, **4**: Probe: Competitor = 1+5 plus 130 µg SNF, **5**: Probe: Competitor = 1+15 plus 130 µg SNF, **6**: Probe: Competitor = 1+30 plus 130 µg SNF, **7**: Probe: Competitor = 1+45 plus 130 µg SNF, **8**: Probe: Competitor = 1+60 plus 130 µg SNF; (**l**) **Enrichment constructs Bead-Peg23-DNA (IR-labeled) on glass slide before protein binding**, **1a**/**1b**: Bead-Peg23-P37/P38 construct, **1c**/**1d**: Bead-Peg23-P38/P37 construct (**1a-1d**: each 3 µL of 0.3 pmol input/300 µL), **1e**: raw beads: 30 µg, **1f**: binding buffer only 30 µl, **2a**/**2b**: Supernatant of Bead-Peg23-P37/P38, **2c**/**2d**: Supernatant of Bead-Peg23-P38/P37 (**2a-2d**: each 30 µL of 0.3 pmol input/300 µL), **2e**: 0.3 pmol P37/P38 only, **2f**: beads buffer only: 30 µl; **Run conditions for the gel (e)**: run on 5% nondenaturing TBE PAGE gel (Biorad) for 2 hours at a voltage of 40. **Annealing and EMSA conditions**: run on 5% nondenaturing TBE PAGE gel (Biorad) for 55 minutes at a voltage of 100.

V. Magnetic Solid Surface Enrichment (MSSE) of *Myc* regulators and associated cofactors for identification by mass spectrometry

Affinity purification mass-spectrometry, footprinting, gel shift methodology and similar approaches have proven too often to be insufficient for reducing background noise and enhancing selectivity, sensitivity, and specificity of protein identification experiments in whole cell lysates by MS analysis. This obstacle is more evident for the identification of factors that are stoichiometrically present in low numbers, e.g., microproteins, hormone peptides, membrane bound receptors, and gene regulatory proteins. Other methods including immobilization of

nucleic acid-protein or protein-protein complexes on a solid surface followed by washing off unbound candidates and extraction of enriched proteins decreases background noise caused by chimeric spectra and hence false discovery rate (FDR); consequently the accuracy of identified proteins increases significantly with high confidence (LEITNER *et al.* 2010). In our study, we developed and used a chimeric Carboxylic Magnetic Bead-PEG-DNA construct (Figure 5, see also Materials and Methods section VI) to pull down only those proteins that were enriched on DNA sequences. The hydrophilic PEG-spacer increases the water solubility of the compound, reduces steric effect, confers enough spacing between the DNA molecule and the magnetic bead, and enhances the binding of the proteins to a target sequence (SHCHEPINOV *et al.* 1997). We tested this methodology for the identification of *Myc* regulators, and it proved to be significantly more convenient and convincing for optimal results with high selectivity and sensitivity in protein identification. Further, this basic formula can readily be manipulated for a variety of experimental goals in detecting other molecules such as antigens, antibodies, and other molecules. Finally, the construct can be used to study specific protein-miRNA interaction by replacing the DNA oligonucleotide with a miRNA hairpin or protein recognition sequence taken from a microRNA sequence.

As an example, it would be interesting to use this methodology to understand the role of oncogenes such as MYC in microRNA biology. It has been shown that MYC plays an important role in the regulation of oncogenic miRNAs such as the miR 17-92 complex (HE *et al.* 2005; TREIBER *et al.* 2017). The purpose of this study, as will be described in later sections, was to use the Bead-PEG23-Oligonucleotide construct to identify *Myc* transcriptional regulators.

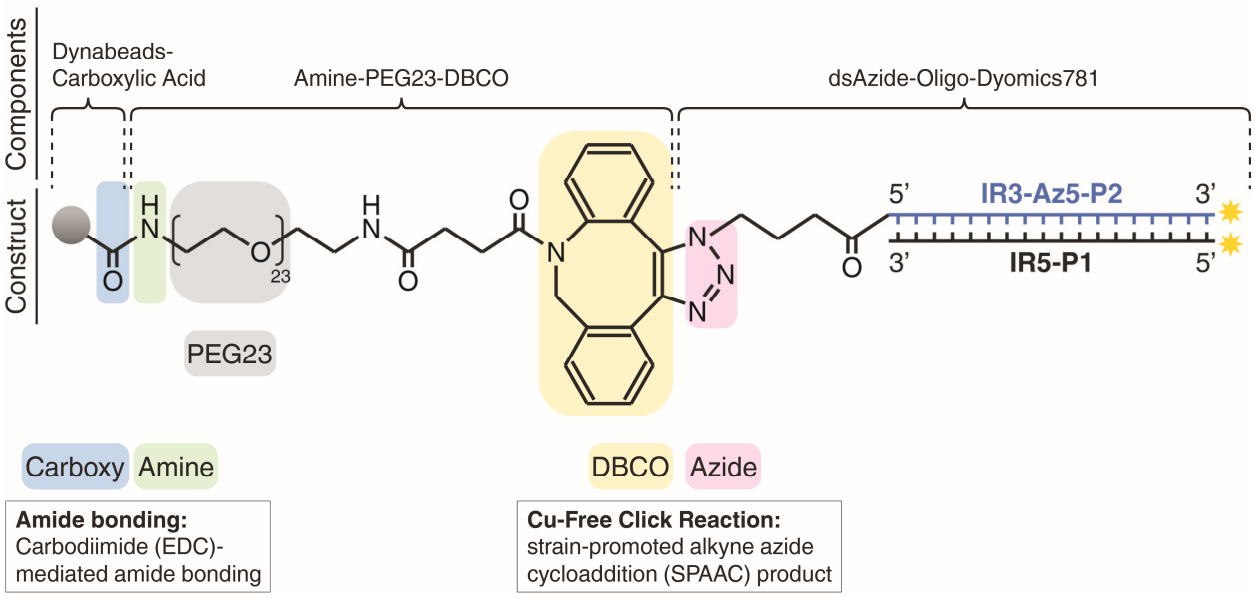


Figure 5. **Magnetic Solid Surface Construct (MSSC) designed and used for protein enrichment**. Amine-PEG23-DBCO is covalently bound to carbodiimide activated microspheric Dynabeads® MyOne™ Carboxylic acid beads via a covalent amide bonding. The chimeric structure underwent copper-free click chemistry reaction (JEWETT AND BERTOZZI 2010; EEFTENS *et al.* 2015) for covalent bonding between its reactive alkyne the DBCO moiety (Dibenzocyclooctyne) and the reactive Azide (N-hydorxysuccinimide (NHS) ester) end-modified oligonucleotide to obtain the Bead-Peg23-Oligonucleotide construct. The obtained excellent solid surface was used to biomagnetically separate, and affinity purify *Myc* regulators from *Drosophila* embryonic nuclear protein extracts (see Materials and Methods section VI for more detail).

VI. Soluble Nuclear Fraction (SNF) and Raw Beads as components of *in vitro* DNA-protein interaction

Generally, proteins interact with DNA either by sequence-specific binding, as is the case for transcription factors, or they may bind non-specifically as is the case for some replication proteins (NILKANTA AND BAGCHI 2018). Many factors favor DNA-protein interactions, among which adequate and proper hydrogen bonds, low salt concentration, pH value, and presence of divalent ions such as Zinc (Zn^{2+}) and Magnesium (Mg^{2+}) are important (PHIZICKY AND FIELDS 1995; DEY *et al.* 2012). For *in vitro* studies of DNA-protein interactions, *in vivo* conditions for biomolecules should be maintained as best as possible by performing experiments with non-denaturing agents. Before incubating different Myc-CRMs with SNF, we checked increasing concentrations of the nuclear fraction for its quality and quantity on a non-denaturing 5% Precast PAGE gel using low salt 0.5% TBE running buffer. After Coomassie Blue staining a smear of proteins with increasing intensity was observed in each lane (see Figure S2E, 1-9 in Supplemental Figures). Previous studies have shown that in-gel digestion for protein quantification and/or identification by MS analysis suffers from background noise causing low sensitivity and low selectivity in the obtained MS data and interpretation of dataset (GOODMAN *et al.* 2018). One main reason for the poor quality of the results may be the contamination of DNA-protein complexes with the unbound background proteins running alongside the DNA-protein complex on the gel. We troubleshot this problem by switching to magnetic beads solid surface support for protein identification. Mass spectrometric analysis of blank beads will be presented in the next section. This approach helped us to obtain highly selective datasets with low background noise and low false discovery rate (FDR) for all the samples. Proteins identified by Mass spectrometric analysis and processing of the protein categories will be presented in the next sections.

VII. MS data obtained for raw beads, Scm, embryonic extracts, and the Myc-CRMs

The raw beads, crude nuclear extract, and DNA-protein complexes were subjected to MS analysis for protein identification. The processing and analysis of the obtained dataset were performed using Thermo Fisher Scientific™ Proteome Discoverer™ 2.5 software and the software tool ComplexBrowser (Material and Methods, Section IX). We have performed the exact same data analysis of the blank sample (raw beads), both control samples the Scrambled (Scm) oligonucleotide (Negative Control), the positive control (Pos), and the Myc-CRM samples.

The blank beads result shows the identification of 26 proteins annotated to *D melanogaster* (see Table 2 and Image 2 in File S2). 12 of these proteins are low scoring with low confidence of the identification (ID). In addition, the identification of 10 of these proteins was based on a single unique peptide. We would normally exclude such ID prior to publication. Three of these proteins (Actin, Albumin and ATPase) were identified by peptides which are not unique for *Drosophila melanogaster* and could just as well be a contamination from a different source. The last protein (Collagen alpha I(III) chain, Accession P04258) was unique to Blank Beads and not found in SCF and SNF extracts and samples including Scm. The protein contains 1466 residues with a molecular weight of 138.4 kDa. Collagen contamination might originate from the surface of the coated magnetic beads that leeches off when boiling the beads. The beads were tested for protein contamination prior to their use for sample preparation. After the beads were treated with 8M urea, followed by boiling and running on a 1% SDS PAGE gel, few artifactual bands were visible upon intensive exposure of the gel to UV light (see Figure S2F in Supplemental Figures). Our overall conclusion is that raw beads can be used for biomagnetical separation and affinity purification of proteins and nucleic acids, considering the filtration of the collagen contaminant from the obtained MS data.

The original unbiased data file with the ungrouped replicates contained 3872 proteins (see Table 3 and Image 3 in File S3). Contaminants originating from the beads were removed from the original dataset (see Table 2 and Image 2 in File S2). The ComplexBrowser software requires ungrouped data input. Therefore, for the ComplexBrowser analysis the data file with ungrouped replicates was used. In the data file with ungrouped replicates, after application of the filter for the species *Drosophila*, and removal of the non-*Drosophila* contaminants (see Table 4 and Image 4 in File S4) 2224 proteins were obtained (see Table 5 and Image 5 in File S5). The unbiased data file with the grouped replicates resulted in 2264 factors (see Table 6 and Image 6 in File S6).

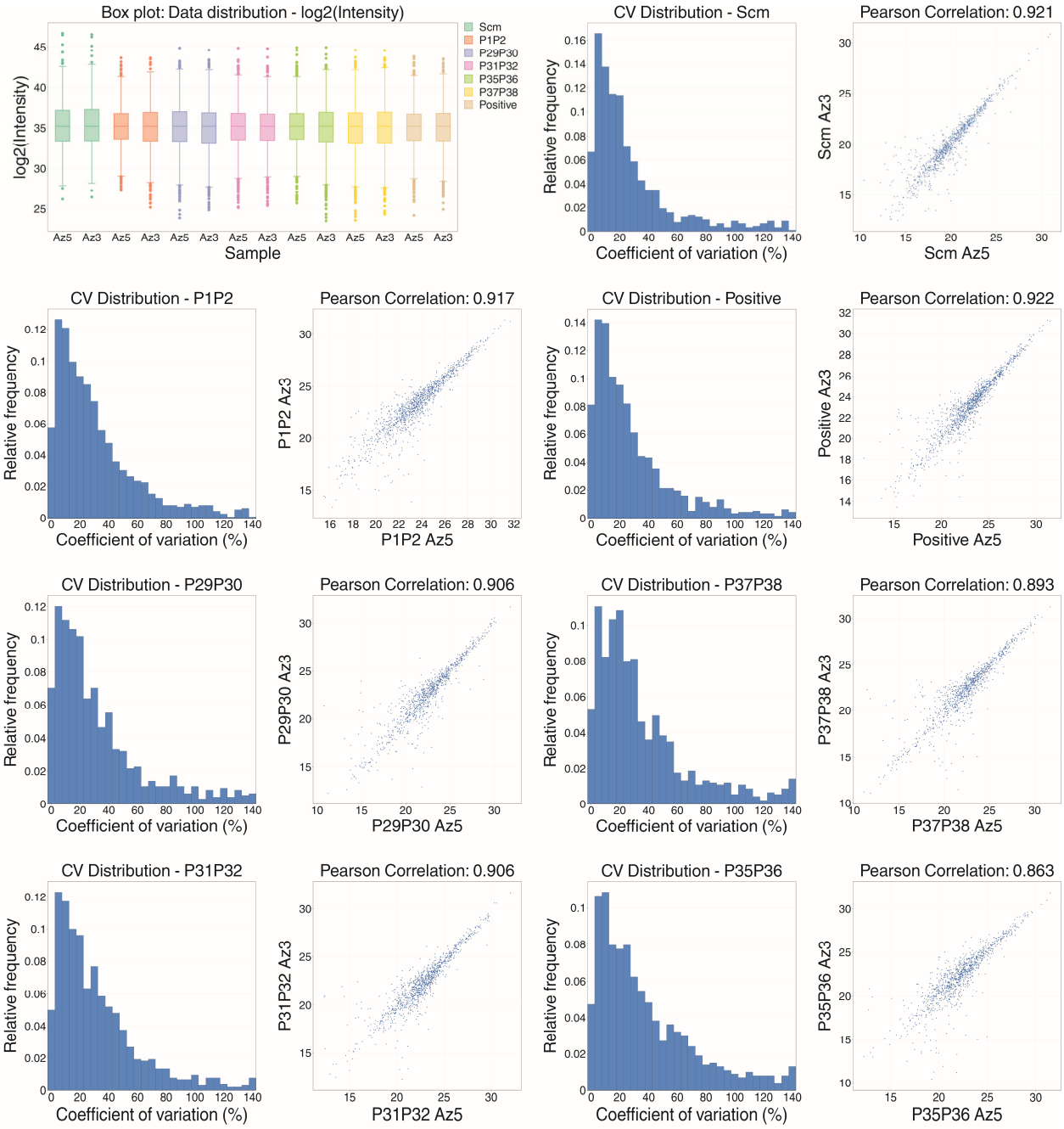
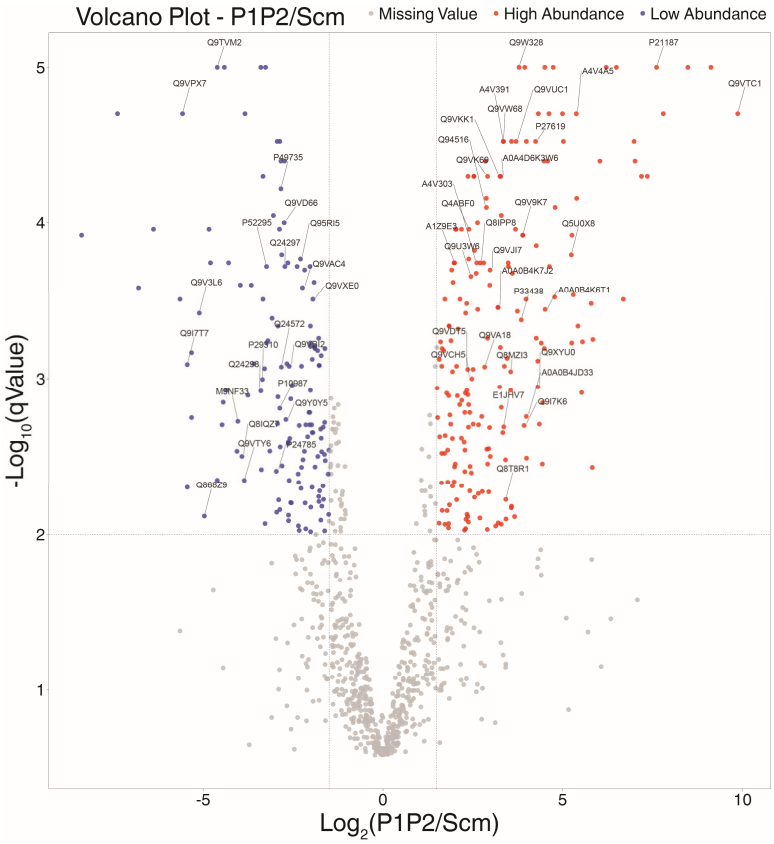


Figure 6. Bioinformatics label free dataset identification analysis visualization using the ComplexBrowser software tool showing P-Values for the Myc-CRMs and the control tests. Depiction of the box plot of log2-transformed intensities of label free identified factors in seven samples pairs including the negative control (Scm), Myc-CRMs, and positive control (Positive). Coefficient of Variation (CV) distribution of the label free intensities of all the proteins identified for two replicates within Scm, Positive, P1P2, P29P30, P31P32, P35P36, and P37P38 samples analyzed (total of seven samples) are indicated. Pearson correlations of the two replicates for each sample of total of seven samples are depicted above each correlation plot. **Note**. The Az5 and Az3 associated with the names of the samples represent the end-modification of each nucleotide at its 5'-end and the 3'-end, respectively (for further explanations see Materials and Methods, Section IX). The Azide (N-hydroxysuccinimide (NHS ester)) moieties were removed from the DNA-protein complexes during the protein

purification process prior to MS analysis. For more details on protein sample preparation see Material and Methods section IV, VI, and VIII.


Volcano Plot - P1P2/Scm
Missing Value
High Abundance
Low Abundance
-Log10(qValue)
Log2(P1P2/Scm)

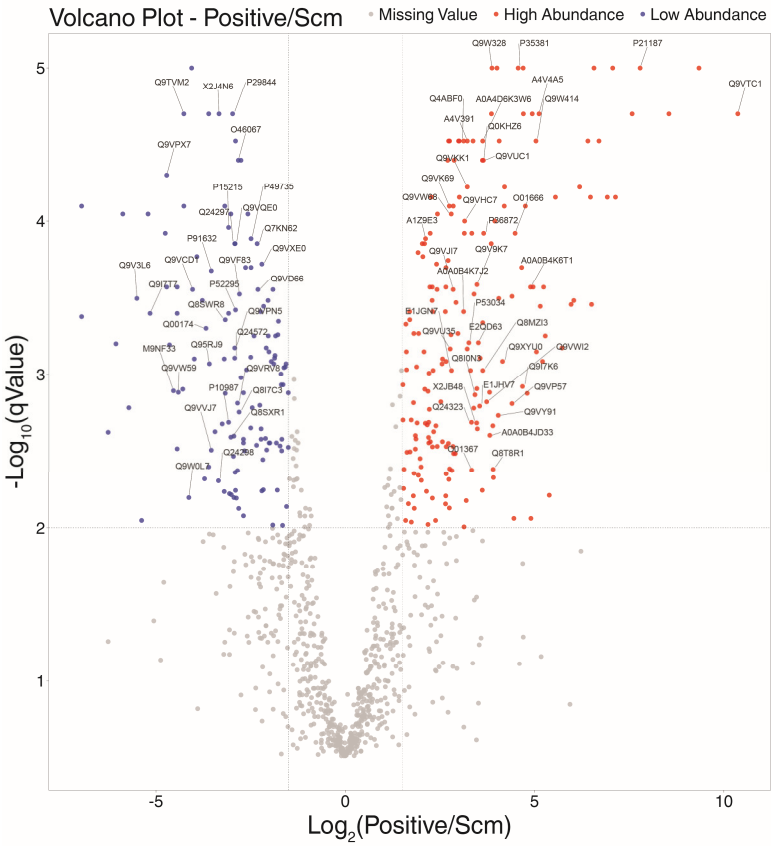
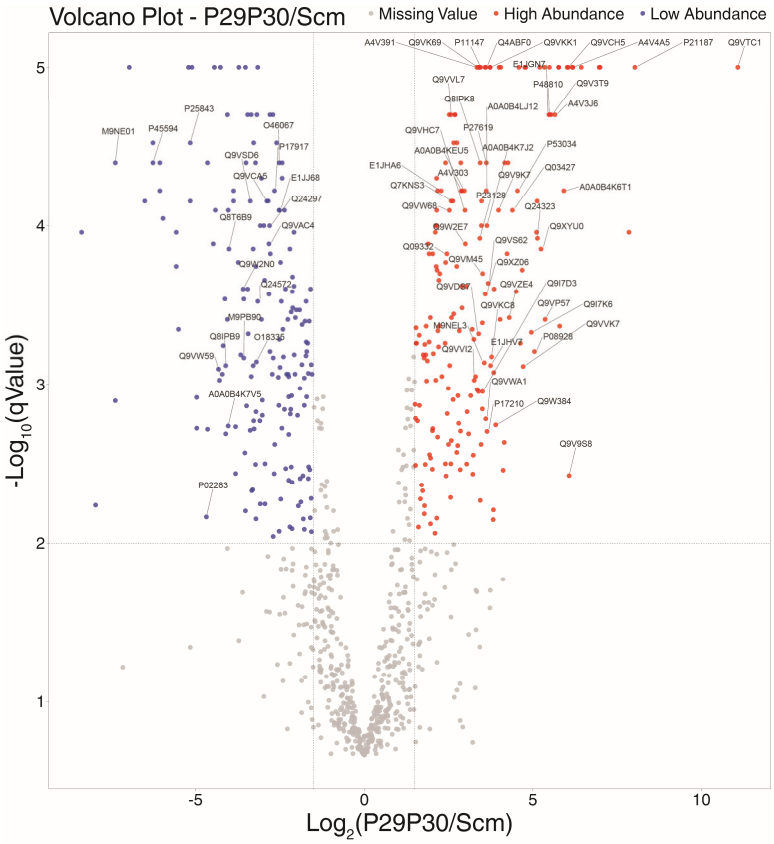

Volcano Plot - Positive/Scm
Missing Value
High Abundance
Low Abundance
-Log10(qValue)
Log2(Positive/Scm)

Figure 7. Visualization of Volcano Plots for the Myc-CRM P1/P2 and positive control using the ComplexBrowser software tool. Volcano plots show log2 of abundance ratios for the sample group P1/P2 and positive control (Positive) over the abundance ratio of negative control (Scm) on the x-axis and minus log10 of the q-value on the y-axis, with settings of a significance (q-value) threshold of 2 and a fold (abundance ratio) threshold 1.5.


Volcano Plot - P29P30/Scm
Missing Value
High Abundance
Low Abundance
-Log10(qValue)
Log2(P29P30/Scm)

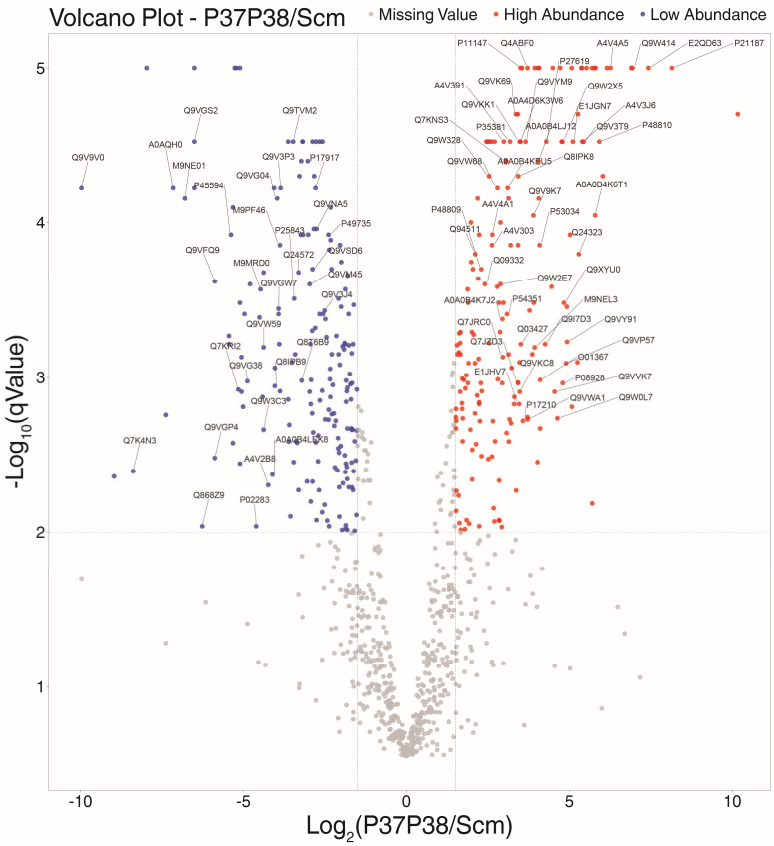
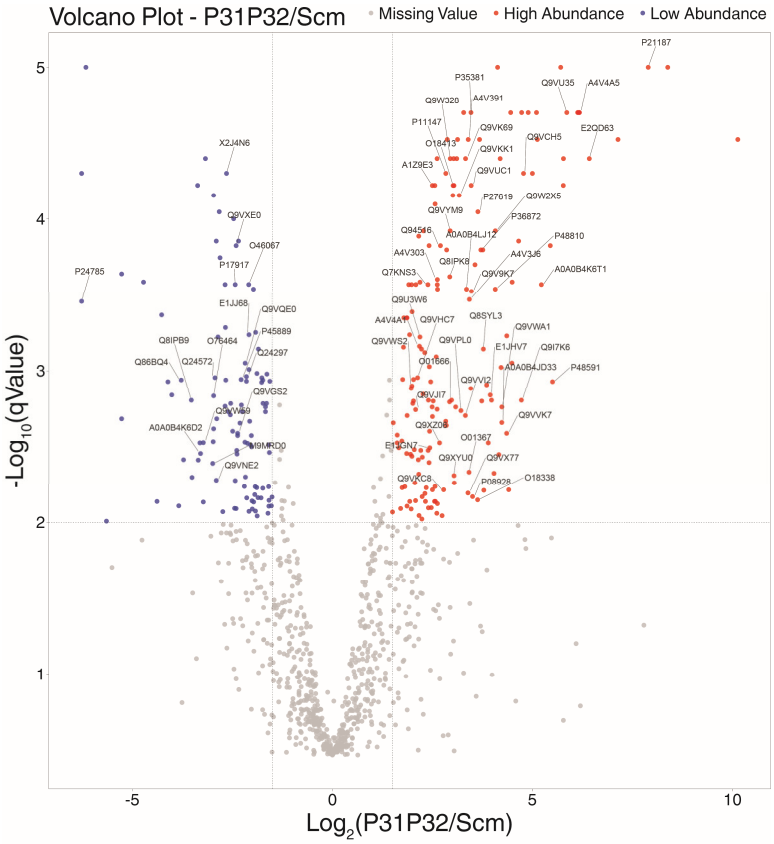

Volcano Plot - P37P38/Scm
Missing Value
High Abundance
Low Abundance
-Log10(qValue)
Log2(P37P38/Scm)

Figure 8. Visualization of Volcano plots for the Myc-CRMs P29/P30 and P37/P38 using the ComplexBrowser software tool. Volcano plots show log2 of abundance ratios for the sample group P29/P30 and P37/P38 over the abundance ratio of negative control (Scm) on the x-axis and minus log10 of the q-value on the y-axis, with settings of a significance (q-value) threshold of 2 and a fold (abundance ratio) threshold of 1.5.



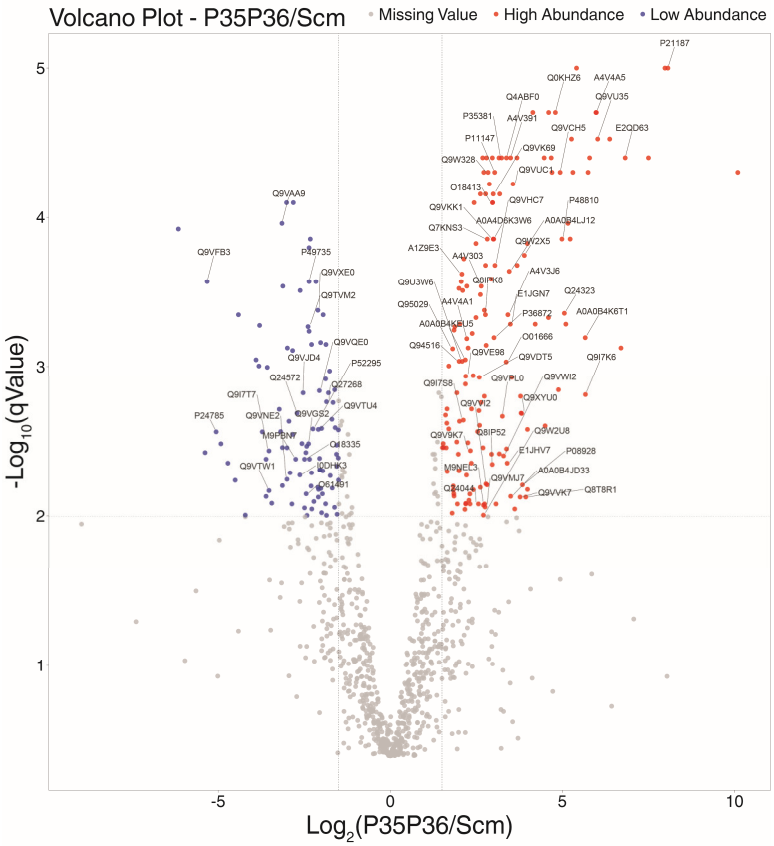


Figure 9. Visualization of Volcano plots for the Myc-CRMs P31/P32 and P35/P36 using the ComplexBrowser software tool. Volcano plots show log2 of abundance ratios for the sample group P31/P32 and P35/P36 over the abundance

ratio of negative control (Scm) on the x-axis and minus log10 of the q-value on the y-axis, with settings of a significance (q-value) threshold of 2 and a fold (abundance ratio) threshold of 1.5.

VIII. Generation of a Heat Map of the proteins compounded by each of the *Myc* targets and the control sequences for the detection of similar groups

The unbiased proteome result of 2264 proteins with the grouped replicates (see Table 6 and Image 6 in File S6) for the nuclear samples went through multiple general data quality filters, one of which was "the mass over charge ratio for sample is 10 times higher than for the scrambled control". Further filters included a threshold for false detection rate "protein FDR confidence combined has at least level high"; a confidence threshold against Scrambled negative control "found in sample groups has at most confidence Peak Found in sample groups Scm"; and a species proteome map for *Drosophila* "species map is true in species *Drosophila melanogaster* OX 7227". As an end-result, we came up with 1001 candidates to work with (see Table 7 and Image 7 in File S7). The obtained results were useful for the exclusion of contaminants as well as a good reference for the confirmation of the Myc-CRMs identified by phylogenetic footprinting (KHARAZMI *et al.* 2012) and the *Myc-lacZ* expression profiles (Figure 1, A-F and Figure 2, A-D). We processed the results for the target-associated proteins on pegylated DNA (Figure 4(f)) by generation of different heat maps (see Figure S3-S4 in Supplemental Figures) using Proteome Discoverer 2.5 (Thermo Scientific) software and looked for the similar and related groups in terms of functionally comparable to *Myc-lacZ* expression in the tested tissues (Figure 1 and Figure 2). Although *in vitro* association of the factors with the Myc-CRMs will require *in vivo* tests in the future, considering the expression analysis results, the pattern we obtain in *vitro* shows a specificity as *Myc* regulators for the tested targets.

IX. Assortment of proteins associated with each of the Myc-CRMs and positive control W-CRM into functional subclasses

After quality control and visualization of functionally similar sample pairs (Figure 6-9) the associated proteins with each *Myc* targets' pairs, most relevant to *Myc* expression, and the positive control pair were searched manually to find protein complexes to which a specific factor belonged (Table S8). Based on biological activity and molecular function, the identified proteins were categorized and subdivided into groups (Figure 10; see also supporting information, Table A-H in File S9). We differentiated between the unique factors associated with each target and those that associated with more than one tested target. We confirmed for those factors that are

already known from the literature for controlling *Myc* expression as relevant regulators of *Myc*. Such factors include but not limited to the components of Hippo, Wnt/Wg, Dpp, Hedgehog, and Notch signaling pathways. Those factors that are newly discovered or are not yet fully characterized, are potential candidates for future studies such as *in vivo* biochemical assays, and structural biological studies. In the heat map grouping (see Figure S3-S4 in Supplemental Figures) *Myc* distal enhancer P1/P2 and the positive control, dTCF/Pan site fall into the same group. In a previous study and the present work, we could show that the absence of upstream regulatory sequences containing the P1/P2 enhancer cluster leads to abrogation of expression in larval imaginal tissues and brain (KHARAZMI *et al.* 2012) (Figure 1B).

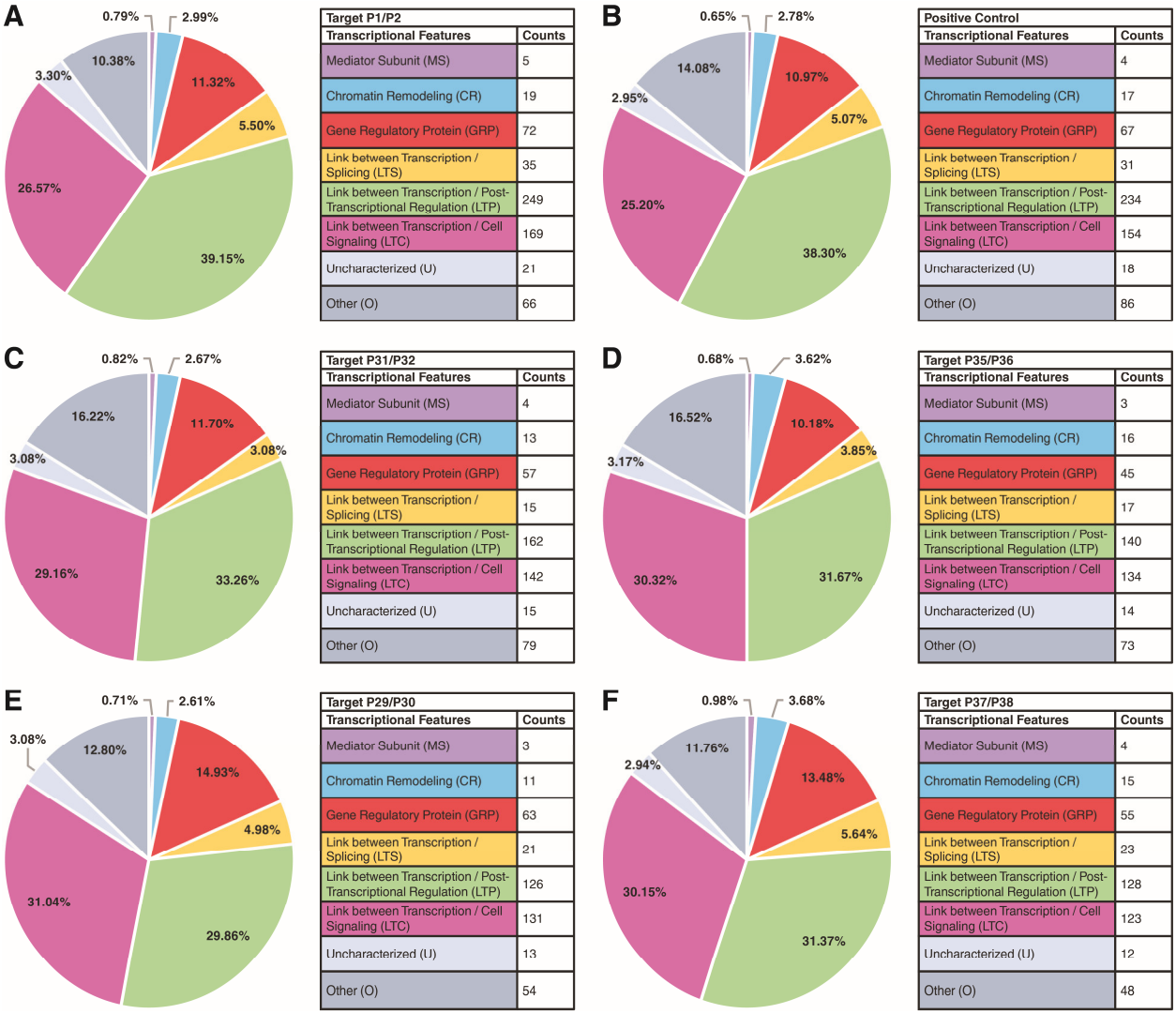


Figure 10. Classification of the subgroups of the factors associated with the Myc-CRMs and positive control based on their activity. Sample pairs with functional similarity obtained from Heat Map analysis (see Figure S3-S4 in Supplemental Figures) of the filtered proteins (see Table 7 and Image 7 in File S7) with high confidence are depicted next to each other. Subdivisions of the total 1001 analyzed regulatory factors revealed that 0.65-0.98% belong to the mediator complex, 2.61-

3.68% belong to chromatin remodeling, 10.18-14.93% are gene specific proteins, 3.08-5.64% belong to the splicing machinery, 29.86-39.15% are posttranscriptional regulators, 25.20-30.32% are cell signaling components, 2.94-3.30% comprise uncharacterized factors, and 10-38-16.52% are other regulatory factors than aforementioned. **A**: upstream 5'-end P1/P2 enhancer cluster; **B**: Positive Control (W-CRM); **C**: TATA-Box-containing Myc-CRM P31/P32; **D**: DPE-containing Myc-CRM P35/P36; **E**: intron 2 enhancer congregate P29/P30 near DPE; **F**: 5'-UTR containing the enhancer cluster P37/P38, the oocyte element. For the analysis of the pie charts data see Table A-H in File S9.

It has been shown that Wnt morphogens play a crucial role during early embryogenesis but also at later stages of development during differentiation, embryonic segmentation, organogenesis, and tissue homeostasis (HERRANZ *et al.* 2008; ARCHBOLD *et al.* 2014; STROEBELE AND ERIVES 2016; FRANZ *et al.* 2017). We hypothesize that the Myc-CRM P1/P2 is important for larval development and organ shape during morphogenesis, and hence we were looking for such factors. The two enhancer elements P1/P2 and dTCF/Pan show the same behavior of being active during early time of cell proliferation and later during pattern formation. Another important similar group is the two initiation sites, namely the TATA-Box-containing target P31/P32 and the DPE-containing *Myc* target P35/P36. Experiments with the *Myc-lacZ* reporter assays showed that P31/P32 and the DPE, are two separable and important transcription units within the *Myc* gene (KHARAZMI *et al.* 2012; KHARAZMI AND MOSHFEGH 2013), a result that the Heat Map representation also confirms as similar group in this study (see Figure S3-S4 in Supplemental Figures). The two developmental super enhancers the 5'-UTR P37/P38 and the downstream P29/P30 hold for a similar group. However, reporter studies showed that the activity of P29/P30 is dependent on the DPE-containing P35/P36 *cis*-element (Figure 2A) or the TATA containing promoter P31/P32 (Figure 2D), whereas P37/P38 can function autonomously and express *Myc* during the time of high rate of cell proliferation in the early embryos and ovary (Figure 1, C-D). The starting material embryonic extract SNF containing the full protein pool stands next to the neighboring group pair the P1/P2 and the positive control that are associated with a high amount of factors compared to the other groups (see Figure S3-S4 in Supplemental Figures). Scm stands next to the P31/P32 and the P35/P36 group that contain the less proteins compared to the other two groups (see Figure S3-S4 in Supplemental Figures).

X. Summary of the regulatory factors associated with positive control the W-CRM

A major group of canonical Wnt/β-catenin regulators belong to T-Cell Factor (TCF) family of proteins (CADIGAN 2012). The DNA recognition motif TCF/Pangolin containing HMG-Helper site was reported to

respond to Wingless signaling in a tissue-dependent manner (ARCHBOLD *et al.* 2014), thus a good candidate for us as a positive control.

The Wnt regulators associated with the W-CRM (Figure 11; see also supporting information, Table 3 in File S3 and Table S8) included casein kinase IIα (*CKIIα*), with roles in cell cycle (DUCAT *et al.* 2008), regulation of Hedgehog and Wnt signaling (GLOVER *et al.* 1983; DAHMUS *et al.* 1984; WILLERT *et al.* 1997; SIOMI *et al.* 2002; JIA *et al.* 2010) during proliferation, differentiation, and tissue patterning (KARANDIKAR *et al.* 2004; BOSE *et al.* 2006; WONG *et al.* 2011); Sorting nexin (*Snx6*), a component of retromer/tubulation complex (GENE ONTOLOGY CURATORS 2002) and a positive regulator of Wnt secretion (ZHANG *et al.* 2011); Galla-1 (*galla-1*), a component of the crb-galla-Xpd (CGX) complex required for proper mitotic chromosome segregation in early embryos, cell proliferation in developing wing discs (YEOM *et al.* 2015), and cell polarity (YEOM *et al.* 2015); adaptor molecule Cullin 1 (*Cul1*), part of Cullin-RING ubiquitin ligase complex and SCF ubiquitin ligase complex (SMELKINSON *et al.* 2007) with role in ubiquitination of cell cycle-specific proteins (SARIKAS *et al.* 2011) and a negative regulator of Wnt pathway (SWARUP AND VERHEYEN 2011; ROBERTS *et al.* 2012); Twins (*tws*), a component of TWS-Protein phosphatase Pp2A complex and regulator of Wnt signaling through stabilization of Armadillo/TCF during transcription (BAJPAI *et al.* 2004), cell fate specification and patterning in wing disc (UEMURA *et al.* 1993); Another transcription unit (*Atu*), involved in the formation of the nucleoplasmic transcriptional Wg/Wnt transactivation complex, pol II pre-initiation, transcription elongation (GO REFERENCE GENOME PROJECT 2011), and hemocyte proliferation during blood cell development (SINENKO *et al.* 2010); Rap1 GTPase (*Rap1*), a small GTPase from Ras superfamily with important role in planar cell polarity related morphogenetic events including imaginal disc patterning (O'KEEFE *et al.* 2009), border follicle cell migration (CHANG *et al.* 2018), and response to cAMP signaling (GO REFERENCE GENOME PROJECT 2011).

Some of the identified transcriptional regulatory factors have previously been identified as components of Wnt enhanceosome: the cofactor Chip (*chi*) (FIEDLER *et al.* 2015; VAN TIENEN *et al.* 2017), the E-box binding repressor Net (*Net, CG11450*) (BRENTRUP *et al.* 2000; PEYREFITTE *et al.* 2001), the global corepressor Groucho (*gro*) (CAVALLO *et al.* 1998; ARCE *et al.* 2009), and the corepressor Atrophin-Grunge (*Gug, Atro*) (HAECKER *et al.* 2007). Armadillo, segment polarity protein came up on the original list but was not enriched.

Transcription factor Mad, myeloid leukemia factor Mlf, Misshapen Msn, and Sumo were among the factors associated with the positive control: Mothers against dpp (Mad) is a conserved transcription factor and a component of BMP/Dpp signaling pathway that contributes to the induction of cell cycle arrest (FIRTH AND

BAKER 2005) during morphogenesis and organ development (LECUIT *et al.* 1996; WIERSDORFF *et al.* 1996; MARQUEZ *et al.* 2001; KIRILLY *et al.* 2005; DWORKIN AND GIBSON 2006). Mad is required by Wingless signaling during wing disc morphogenesis and embryonic segmentation (EIVERS *et al.* 2009). Mad functions as a transcription repressor and interacts with the components of the Wingless signaling such as Schnurri (*Shn*) during fate specification and patterning (YAO *et al.* 2006).

Myeloid leukemia factor (*Mlf*) interacts with the DNA bound transcription factors and protein-folding chaperones (MILLER *et al.* 2017) and is a modulator of transcription via interaction with the Hedgehog regulator Su(fu) during morphogenesis and cell proliferation (FOUIX *et al.* 2003), and blood cell development (BRAS *et al.* 2012; MILLER *et al.* 2017). Whether or not Mlf is a direct regulator of the Wnt/Wg during development and disease needs to be resolved.

The *Drosophila* Sumo and protein kinase Misshapen (*Msn*) might be potential regulator of Wnt/Wg signaling pathway. Small ubiquitin like modifier (*Sumo, smt3*), sole member of Sumo family proteins in *Drosophila*, is involved in epigenetic control of transcription via ubiquitin-like conjugation of proteins (CAPPADOCIA AND LIMA 2018; YAU *et al.* 2020). Sumo responds to TGF-β signaling (MILES *et al.* 2008) during embryonic patterning (Nie et al., 2009). Further, Sumo is a Hedgehog signaling modifier (ZHANG *et al.* 2017) and during wing disc morphogenesis Sumo interacts with the protein kinase Misshapen and certain other signaling pathways including Wingless to establish boundary formation between different cell populations in the developing wing disc (ROSALES-VEGA *et al.* 2023).

Chromatin modifying factors might act as potential regulators of Wnt/Wg: Besides the transcriptional regulated described above, there is strong evidence in this study that regulation of Wnt/Wg expression involves regulation of chromatin. Wnt/Wg facilitates diffuse chromatin architectural changes at the loci of target genes, and chromatin and histone modifying enzymes have been documented to interact with and regulate Wnt/Wg signaling pathway during development and disease (LIU *et al.* 2008; PARKER *et al.* 2008; CURTIS AND GRIFFIN 2012; KONIG AND SHCHERBATA 2015; TAKAHASHI AND TAKADA 2023). Here we identified a subset of chromatin and histone modifiers associated with the positive control W-CRM. These associated factors and corresponding protein complexes might be potential Wnt/Wg regulators.

High mobility group proteins HmgD and Hmg-2 and Wings apart-like protein interact with the tested W-CRM and induce chromatin changes: High mobility group (HMG) protein D (HmgD), a Wnt/Wg signaling

effector (ARCHBOLD *et al.* 2014) is involved in the induction of changes to chromatin-DNA architecture during gene expression (WAGNER *et al.* 1992; CHURCHILL *et al.* 1995; DRAGAN *et al.* 2003). Hmg-2 (*Hmg-2*), another High mobility group protein is involved in chromatin-DNA structural changes and gene expression (GO REFERENCE GENOME PROJECT 2011). HmgD and Hmg-2 both might be involved in the regulation of the *Drosophila wingless*/*wg* gene expression.

The X-linked wings apart-like (*wapl*) is involved in chromatin organization and preservation of healthy chromosome structure by facilitating adhesion of sister chromatids in certain heterochromatic areas, crucial for normal mitosis (VERNI *et al.* 2000). Wapl functions as Wnt/Wg modifier during wing size determination in the Mediterranean fruit fly (medfly) (CHO *et al.* 2013).

NURF, SWI/SNF chromatin-remodeling complex (Brahma), and Cdc73/PAF1 complex are involved in the regulation of Wnt/Wg target genes: Nucleosome remodeling factor NURF-38 (HAMICHE *et al.* 1999; MIZUGUCHI *et al.* 2001) interacts with the NURF complex and is required for the homeotic gene expression and larval hematopoiesis (SINENKO *et al.* 2010). Armadillo, cofactor of Wingless signaling has been implicated to interact with and recruit NURF complex to the promoters of Wingless signaling target genes and positively regulate transcription of these genes in cell cultures suggesting a potential role for NURF-38 in regulating *wingless/wg* transcription (SONG *et al.* 2009).

Snf5-related 1 (Snr1), a component of SWI/SNF chromatin-remodeling complex (Brahma) alongside the activation of Wnt/Wg signaling pathway induce higher order chromatin remodeling to establish gene-specific expression and control growth and morphogenesis (PARKER *et al.* 2008; RAMANUJAM *et al.* 2021).

Moira, BAP155 (*mor*, *CG18740*), Swi3 component of Brahma complex is involved in the regulation of trithorax homeotic gene transcription (CROSBY *et al.* 1999). Moira is involved in mitotic cell cycle regulation (BRUMBY *et al.* 2002) partly via negative regulation of Armadillo-dTCF target genes in *Drosophila* (COLLINS AND TREISMAN 2000) and TCF/β-catenin targets in mammals (NOLLET *et al.* 1999), and wing disc margin morphogenesis (TERRIENTE-FELIX AND DE CELIS 2009; CURTIS *et al.* 2011).

Antimeros (*atms*) and Another transcription unit (*Atu*) as parts of Cdc73/PAF1 complex, interact with the basal transcription machinery and bound transcription factors to regulation histone modifications and pol II pause release (ADELMAN *et al.* 2006). Atu participates in the assembly of Wnt/Wg transactivation complex and initiation of pol II transcription (GO REFERENCE GENOME PROJECT 2011) suggesting a potential role for Antimeros as a regulator of Wnt/Wg targets.

C-terminal binding protein (*CtBP*), isoform G (A0A0B4KHB2) and isoform F (A0A0B4KGG9) interact with repressive transcription factors and block developmental enhancers during transcription (ZHANG AND ARNOSTI 2011). CtBP is a regulator of Wnt/Wg target genes during embryonic segmentation and wing disc morphogenesis (FANG *et al.* 2006; BHAMBHANI *et al.* 2011) suggesting a potential role for this protein in the regulation of *wingless/wg* transcription.

SNF2L nucleosome remodeling factor SWI, a down regulator of Wingless target genes might regulate Wingless transcription: Imitation SWI (*Iswi*), part of NURF ACF, CHRAC, and RSF protein complexes (CORONA *et al.* 2000; EBERHARTER *et al.* 2001; BADENHORST *et al.* 2005) is involved in pol II transcription via chromatin remodeling (ITO *et al.* 1999; BURGIO *et al.* 2008; EMELYANOV *et al.* 2010), chromatin assembly (ITO *et al.* 1997; ITO *et al.* 1999), and nucleosome displacement (ITO *et al.* 1997; VARGA-WEISZ *et al.* 1997) during homeotic gene expression and development (BADENHORST *et al.* 2002; DOYEN *et al.* 2015). SWI represses Wg target genes by localizing to TCF binding sites within the target genes sequences (LIU *et al.* 2008). This suggests that Swi together with TCF might regulate Wingless transcription.

Components of ECM such as secreted heparan sulfates act as cofactors of Wnt/Wg signaling pathway: ECM protein Perlecan encoded by terribly reduced optic lobes (*trol*), a secreted heparan sulfate has crucial roles in cell fate specification and tissue pattering, maintenance of apico/basal polarity of epithelial cells (SCHNEIDER *et al.* 2006), and is involved in multiple signaling cascades including Hedgehog (PARK *et al.* 2003b), Wnt/Wg, and TGF-β (LINDNER *et al.* 2007). Trol might play a role in regulating *wingless/wg* expression.

Morphogens transporting molecules might be potential regulators of Wnt/Wg expression: Adaptor protein complex 2, α subunit (*AP-2α*) is involved in WNT5A-dependent internalization of FZD2, FZD4, and FZD5 in noncanonical Wnt signaling pathway (NARAYANAN AND RAMASWAMI 2001).

Vacuolar protein sorting 26 (*Vps26*) is a component of retromer complex and is required for tetrameric cycling and sustaining of Wingless and Dpp morphogens gradient during cell fate specification and tissue patterning (PORT *et al.* 2008).

Phosphomannomutase type 2 (Pmm2) enzyme is involved in synthesis of the GDP-mannose and dolichol-phosphate-mannose, is an effector molecule of Wingless pathway for the correct positioning of wings in adult

flies by maintaining N-linked glycosylation of the synaptic Mmp2 protein at the NMJ by enabling Dlp/Wg signaling during development (PARKINSON *et al.* 2016). AP-2α, Vps26, and Pmm2 might play a role in the regulation of *wingless/wg* gene.

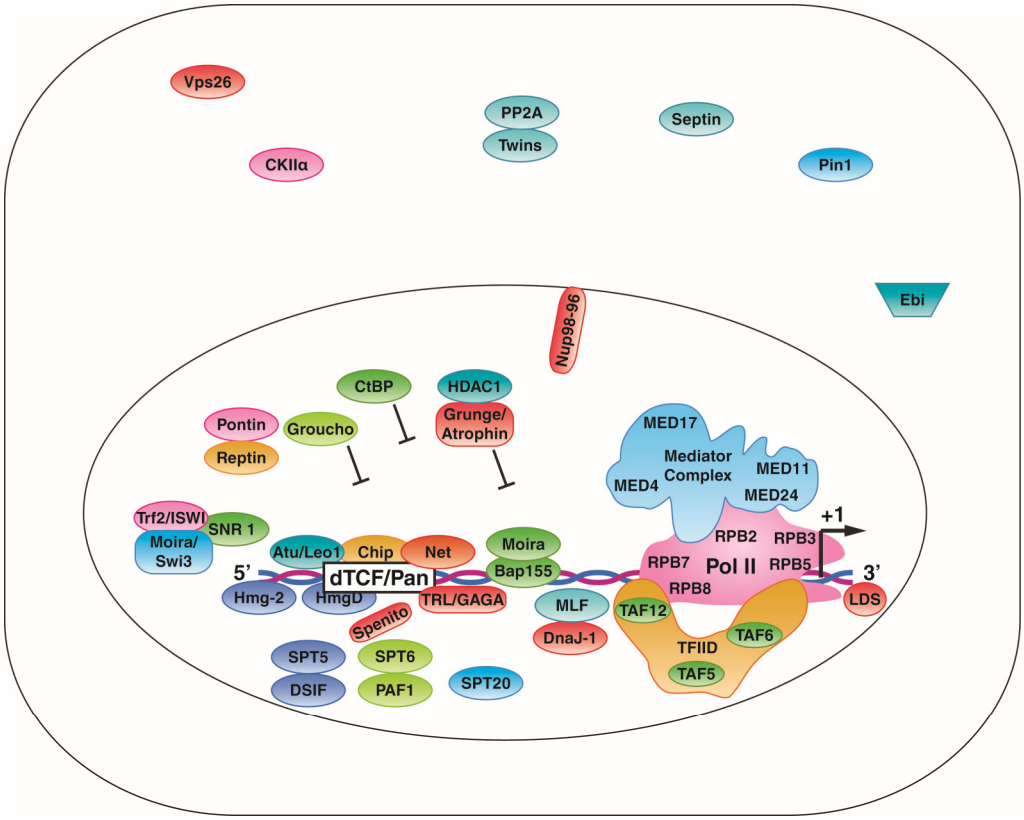
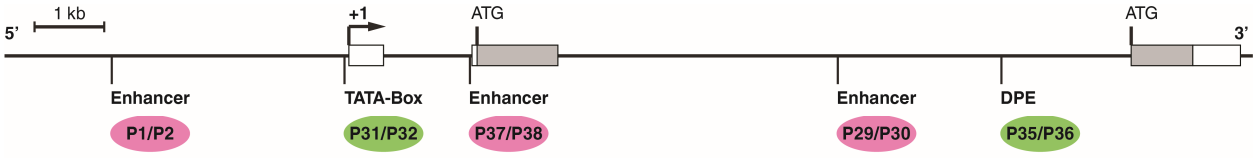


Figure 11. Schematic illustration of some of the identified factors associated with the positive control (W-CRM). These factors included some of the known Wnt/Wg regulators such as Homology Group proteins HmgD and Hmg-2, transcription factors Myeloid leukemia factor (Mlf), Chip (chi), Another transcription unit (Atu), Net (Net), Spenito (nito), and Trithorax-like (Trl), general repressors CtBP, Groucho, Atrophin-Grunge (Gug, Atro) as parts of the Wnt enhanceosome; components of the Mediator complex, RNA pol II subunits, and the TFIID complex (see Table 3 and Image 3 in File S3, Table S8). MED 24 was only associated with the Heat Map pair of the positive control and the Myc upstream enhancer cluster P1/P2 and is involved in larval salivary gland histolysis during metamorphosis, pupal development (WANG *et al.* 2010), and the response to ecdysone signaling (IHRY AND BASHIRULLAH 2014).

XI. Factors associated with the investigated Myc-CRMs based on their molecular activities and biological processes

MYC super-oncogene belongs to a family of regulatory genes that controls the expression of nearly 15% of the transcribed genes (ORIAN *et al.* 2005; CAFORIO *et al.* 2018; DHANASEKARAN *et al.* 2022). Among the identified

factors, we were interested in finding clues about *Myc* factors as potential *Myc* regulators. Based on the results obtained by the Heat Map (see Figure S3-S4 in Supplemental Figures) and by the Pie Chart analysis (Figure 10; see also supporting information, Table A-H in File S9), the putative *Myc* regulators were subdivided into different categories with regard to their role in different levels of *Myc* expression. With our novel protein purification strategy, we could capture if not all but most of the ubiquitous and tissue-specific factors, and components of RNA polymerase transcription regulator complex including general transcription factors (GTFs), mediators/cofactors, polymerases, gene-specific factors, Pol II-specific transcription factors, chromatin/histone modifiers, and posttranscriptional regulators such as RNA processing and gene silencing factors that play an essential role in controlling *Myc* mRNA levels and rate of protein biogenesis during development and disease (Figure 12; see also supporting information, Table A-G in File S10). Among the subdivisions, many kinases were identified, which are potential targets of transcription factor *Myc*, thus defining them as potential regulators of *Myc* (BOI *et al.* 2023). In each category of subdivisions of the factors, we will elucidate the relevance between their association with a certain Myc-CRM, the characteristics of their identity as potential regulators of *Myc* based on *Myc* biology, and the connections of the factors to developmental signals that converge on the *Myc* promoter and regulation of cell cycle. The ultimate goal is to characterize the early and/or late developmental enhancer elements and their trans-acting factors controlling *Myc* expression during cell growth, proliferation, and differentiation. On the other hand, we will screen for potential new transcription initiation sites in addition to the TATA-containing P1 promoter and the downstream DPE-containing P2 promoter that were identified previously (KHARAZMI *et al.* 2012). In the analysis we will also consider factors that control *Myc* posttranscriptionally to keep balance between *Myc* mRNA levels, translation and ribosome biogenesis, and protein turnover.



Transcriptional, Posttranscriptional, Translational *Myc* Regulators					
POLITR	11	8	6	5	6
POLITRAP	13	10	8	7	8
POLIITR	121	109	104	105	95
POLIITRAP	326	306	282	295	257
POLIIITR	9	7	6	5	5
POLIIITRAP	12	10	9	8	8
MTTR	6	3	1	3	4
MTTRAP	11	4	3	5	6
TN	23	21	19	22	21
TRSLAP	33	29	24	29	27
RNAPR	81	65	70	79	60
PPP	99	86	74	79	74
RRR	59	43	40	47	37
RRRAP	116	87	88	95	88
U	14	15	15	19	13

P1/P2: ChrX:3,369,776..3,369,815
P31/P32: ChrX:3,373,105..3,373,148
P37/P38: ChrX:3,374,908..3,374,960
P29/P30: ChrX: 3,380,185..3,380,229
P35/P36: ChrX:3,382,530..3,382,576

Figure 12. Depicted are subdivisions of the identified factors with each of the Myc-CRMs based on molecular functions and biological processes and the occurrence of the factors in each category. The three investigated enhancers, the upstream enhancer P1/P2, the 5'-proximal P37/P38, and the intron 2 enhancer P29P/30 are shown in magenta. The TATA-box-containing Myc-CRM P31P/32 and the DPE-containing Myc-CRM P35P/36 are shown in green. **Abbreviations**: **POLITR**, RNA Pol I Transcription ;**POLITRAP**, RNA Pol I Transcription-Associated Processes ;**POLIITR**, RNA Pol II Transcription; **POLIITRAP**, RNA Pol II Transcription-Associated Processes; **POLIIITR**, RNA Pol III Transcription; **POLIIITRAP**, RNA Pol III Transcription-Associated Processes; **MTTR**, Mitochondrial Transcription; **MTTRAP**, Mitochondrial Transcription-Associated Processes; **TN**, Translation; **TRSLAP**, Translation-Associated Processes; **RNAPR**, RNA Processing; **PPP**, Posttranscriptional/Posttranslational Processes; **RRR**, Replication-Repair-Recombination; **RRRAP**, Replication-Repair-Recombination-Associated Processes; **U**, Uncharacterized. **Note**: A complete list of *Myc* regulators at the level of transcription, post-transcription, post-translation, and other factors involved such as hormone receptors and protein modifications can be accessed via supplemental data (Table 3 and Image 3 in File S3). For the analysis of the transcriptional categories see (Table A-G in File S10).

Myc cell cycle cofactors associated with the Myc-CRMs might act as potential regulators of *Myc* expression

Most of the factors described here represent *Myc* cell cycle cofactors (Figure 13; see also supporting information, Table A-F in File S11) and are tightly linked to cell growth and patterning (EDGAR *et al.* 2001). It is well accepted that factors regulated by *Myc* feedback on Myc activity to tightly control *Myc* expression and establish wild-type body size during development. The results further show that many conserved developmental signaling pathways converge on conserved noncoding sequences at the *Drosophila Myc* locus to regulate *Myc*

expression depending on the developmental stage and the context within which the cell status is defined. Quite a large number of the factors explained in the Results Section show that signaling pathways such as Wnt/Wg (BARKER *et al.* 2000), Notch (ORIAN *et al.* 2007), TGF-β/Dpp (BARKER *et al.* 2000), EGFR (ORIAN *et al.* 2007), and Hedgehog (KENNEY *et al.* 2003; HALLIKAS *et al.* 2006) are at the fore front as regulators of *Myc* (Figure 13) and has been confirmed by the works of others as well.

Cdk1 (Cdc2) might control Myc levels during progression through cell cycle to establish cell size check point: Cdk1, cyclin-dependent kinase 1, regulated by Cdc25/String phosphatase (EDGAR *et al.* 2001) functions in association with Cyclin A, Cyclin B, and Cyclin B3. Phosphorylation of a wide range of cell cycle factors regulates entry into and progression through mitosis at the Gl/S (LEHNER AND O'FARRELL 1990) and G2/M transitions (LEHNER AND O'FARRELL 1990; STERN *et al.* 1993) in both the growing and proliferating cells and the differentiated larval cells lacking G2/M transition (EDGAR *et al.* 2001). The transcription factor Myc controls the activity of String to regulate Cdk1 and balance cell division and differentiation in embryos and discs (EDGAR *et al.* 2001). Previous work has shown that in metazoans a Cdk1-linked cell size checkpoint might exist under influence of the Myc/Max network by regulating ribosomal protein biogenesis and proteasomal degradation of cell cycle proteins (BONKE *et al.* 2013).

Wee1 tyrosine kinase regulates entry into the mitotic phase of cell cycle and might be involved in the regulation of Myc levels: Wee1, a non-specific protein kinase belongs to cell cycle check point factors (KIESSLING *et al.* 2009) and inhibits Cdc2 (Cdk1) to prevent premature entry into the mitotic phase of cell cycle (CAMPBELL *et al.* 1995). The entry into the G1/S transition (LARANJEIRO *et al.* 2013) and into the G2/M phase (MATSUO *et al.* 2003) are key determinants during normal cell growth and division, and aberrances of these check points lead to malignant transformation. The activity of Wee1 kinase (MASRI *et al.* 2013; KELLEHER *et al.* 2014) and MYC the growth regulator (BOUCHARD *et al.* 1999; PEREZ-ROGER *et al.* 1999) underly tight control during cell cycle transitional events by other proteins to synchronize cell cycle phases and prevent transmission of damaged DNA (FEILLET *et al.* 2014; KELLEHER *et al.* 2014). Like MYC the developmental protein Wee-1 is essential for embryonic syncytial blastoderm development (LEE AND ORR-WEAVER 2003). We identified Wee-1 associated with the upstream enhancer P1/P2 and the initiation sites the TATA and the DPE. This result suggests that Wee-1 kinase might play a crucial role in fine tuning Myc levels during cell cycle phases in proliferating and differentiating cells.

Cdk5, Cyclin-dependent kinase 5 might be involved in the control of Myc expression during neuronal differentiation: Cyclin5, a non-specific serine/threonine protein kinase favors degenerative diseases such as retinal degeneration via phosphorylation of MEKK1, activation of JNK cascade, and induction of apoptosis in response to endoplasmic stress signals (KANG *et al.* 2012). Cdk5 interacts with Rac GTPase and controls axonogenesis in *Drosophila* embryos, and together with the interacting partner P35 is essential for differentiation of post-mitotic neurons (CONNELL-CROWLEY *et al.* 2000). In mammals Cdk5, which is generally dysregulated in many cancer types, interacts physically with MYC through phosphorylation of MYC protein and inhibition of MYC inhibitors such as Bin1 to induce tumorigenesis (ITO *et al.* 2014; ZHANG *et al.* 2019).

Tousled-like kinase TLK is involved in morphogenesis in a cell cycle-dependent manner which might involve Myc control: TLK is a conserved non-specific serine/threonine protein kinase and the conserved anti-silencing protein 1 (ASF1) serves as substrate for TLK. TLK acts through cell cycle and shows high levels during S phase. The two proteins TLK and ACF are components of the chromatin assembly complex and phosphorylate each other during S phase in both the Mammals and the *Drosophila* to coordinate replication and chromatin organization (CARRERA *et al.* 2003), progression through cell cycle phases, and nuclear division (CARRERA *et al.* 2003) . Tousled-like kinase associates with the components of wingless signaling regulators such as Armadillo, DE-cadherin, and atypical protein kinase C (aPkc) as well as Notch signaling to induce protein degradation and apoptosis in adherence junctions to facilitate cell migration and modulation of morphogenesis (YEH *et al.* 2015; ZHANG *et al.* 2016).

'P-element somatic inhibitor' Psi plays a role in the regulation of Myc transcription: Psi, an RNA-binding splicing factor is phosphorylated by TLK (TALIAFERRO *et al.* 2013). Psi has dual roles: RNA processing of transposons in a tissue-specific manner (SIEBEL *et al.* 1994) and transcriptional regulation. Psi is required for the activation of *Myc* transcription by association with the basal transcription machinery and mediator complex at the *Myc* promoter (GUO *et al.* 2016). Psi and TLK were both associated with all the tested *Myc* targets and might both be required for the control of *Myc* expression, predominantly during S phase of the cell cycle.

SKP1-related A SkpA targets Myc for degradation during cell fate commitment and patterning: SkpA, a component of Skp, Cullin, and F-box (SCF)-containing ubiquitin ligase complexes (MURPHY 2003) plays an

essential role in centrosome duplication, chromosome condensation, endoreplication (MURPHY 2003), and microtubule assembly during mitosis (DUCAT *et al.* 2008). SKPA conveys information from signaling pathways to regulate cell fate specification and morphogenesis in developing tissues. The SCF/Slimb complex induces degradation of Kibra protein the negative regulator of the Hippo pathway to promote Hippo activity, independent of Yorkie-dependent transcription to control tissue patterning and growth (TOKAMOV *et al.* 2021). During neuronal remodeling and nervous system development SCF/E3 ligase complex targets components of insulin receptor, Pi3K, and Myc/TOR pathways for degradation to establish axon guidance and dendrite pruning (WONG *et al.* 2013). In conjunction with different signaling pathways the SCF/E3 ligase complex targets Myc and Cyclin E for degradation to downregulate cell cycle and initiate tissue patterning (SHCHERBATA *et al.* 2004; NICHOLSON *et al.* 2011).

Cell division cycle 6 protein CDC6, inhibitor of Myc transcriptional activity might control Myc expression during replication and repair process: is an essential component of the pre-replication complex with presence at literally all origins of replication in a cell (INTERPRO PROJECT MEMBERS 2004). Additionally, CDC6 contributes to enhancements between DNA replication and repair in early S phase and during mitosis (CREVEL *et al.* 2005; GO REFERENCE GENOME PROJECT 2011). CDC6 might have an influence on apoptosis and transcription (CREVEL *et al.* 2005). Both *in vitro* and *in vivo* studies have shown that CDC6 competes with the MYC binding partner Max to interact with MYC for binding to the E-boxes of target genes to inhibit transcriptional activity of MYC/Max heterodimers (TAKAYAMA *et al.* 2000).

Cell division cycle 6 CDC16 might target Myc for degradation and contribute to the control of Myc levels during cell fate specification: CDC16 is part of the anaphase promoting complex (APC/C) or Cyclosome and associates with the Shattered (*shtd*), the largest subunit of APC/E3 ubiquitin ligase to function (MARTINS *et al.* 2017). The APC/C is involved in degradation of Cyclins, String, and further cell cycle factors during G1 phase of cell cycle to induce cell fate specification in differentiating cells via modulation of Wingless signaling and control of post-mitotic processes (MARTINS *et al.* 2017). For example, G1 arrest is established in the morphogenetic furrow of the developing eye disc via degradation of Wingless pathway components to induce photoreceptor cell differentiation (MARTINS *et al.* 2017).

Rad50 and Mre11 might function as potential regulators of Myc during S phase of cell cycle: Rad50 and Mre11 are two conserved proteins and belong to the MRE11-RAD50-NBS1 (MRN) complex (CIAPPONI *et al.* 2006; GAO *et al.* 2009; BOSSO *et al.* 2019; ON *et al.* 2021) and have an essential role in DNA double-strand break repair (GORSKI *et al.* 2004), telomere maintenance, and chromosome organization (CIAPPONI *et al.* 2004). Disruption of Rad50 function leads to retarded development in third instar larval development and lethality during pupariation due to lack of double-strand break repair and induction of cell death (GORSKI *et al.* 2004). Rad50 contributes to G-quadruplex DNA binding for the unwinding and stabilization of G4 structures (GO REFERENCE GENOME PROJECT 2011). Rad50 is a predominant DNA damage complex component that senses double-strand break and replication stress defects during DNA synthesis and together with other factors induces apoptosis to eliminate damaged cells (GORSKI *et al.* 2004). In eukaryotes, conserved and tightly controlled mechanisms for cell cycle factors such as Myc confer each organ its integrity and precise shape during development (LIU *et al.* 2015). Whether or not Rad50 is directly involved in the regulation of *Myc* expression for a normal cell cycle remains to be resolved.

Replication factors such as PolD1, Pol32, Orc6, Prim1, and Prim2 might be involved in the regulation of Myc expression: DNA polymerase delta subunit 1 PolD1 is the catalytic subunit of PolD1 with a crucial role in high fidelity genome replication, including lagging strand synthesis and DNA recombination and repair (PECK *et al.* 1992; CHIANG *et al.* 1993; AOYAGI *et al.* 1994). PolD1 functions in highly proliferative embryonic cells to drive replication in earliest cell cycles (PECK *et al.* 1992; JI *et al.* 2019). PolD1 might be involved in controlling *Myc* expression during early development.

DNA polymerase delta subunit 2 PolD2, Pol31 is the accessory component of both the pol delta and pol zeta complexes and supports the function of polD3. PolD2 participates in both embryonic development and postembryonic in somatic cells (JI *et al.* 2019).

DNA polymerase delta subunit 3 PolD3, Pol32 is a part of multi-subunit DNA polymerases and is involved in DND replication and double-strand break repairs (TRITTO *et al.* 2015), and serves as processivity factor during genome replication (JI *et al.* 2019). Pol32 might associate with Myc during DNA replication and repair.

Origin recognition complex subunit 6, Orc6, serves as the smallest part of the origin recognition complex (ORC) (AKHMETOVA *et al.* 2015) and plays a crucial role in the initiation of replication in eukaryotic cells. Through interaction with Septin Orc6 is essential for replication and cytokinesis in *Drosophila* (HUIJBREGTS *et al.* 2009).

DNA primase subunit 1, Prim1 is a subunit of DNA polymerase alpha complex that together with the Prim2 subunit, Prim2 is responsible for the synthesis of short RNA primers required by DNA polymerase alpha to prime DNA synthesis at the origins of replication (VILLANI *et al.* 1980; KAGUNI *et al.* 1983; COTTERILL *et al.* 1987; KURODA *et al.* 1990; BAKKENIST AND COTTERILL 1994).

Chromosome fidelity factors such as Cutlet that regulate Myc transcriptional activity might serve as potential regulators of Myc during DNA synthesis: Chromosome cohesion factor Cutlet, CTF18-RFC (a component of one of the cell nuclear antigen loader complexes, CTF18-replication factor C) is important for the fidelity of chromosome transmission. Cutlet is involved in DNA clamp loading and positively regulates DNA polymerase activity during S phase of cell cycle (GENE ONTOLOGY CURATORS 2002). Cutlet might play a crucial role in the regulation of *Myc* expression during cell cycle.

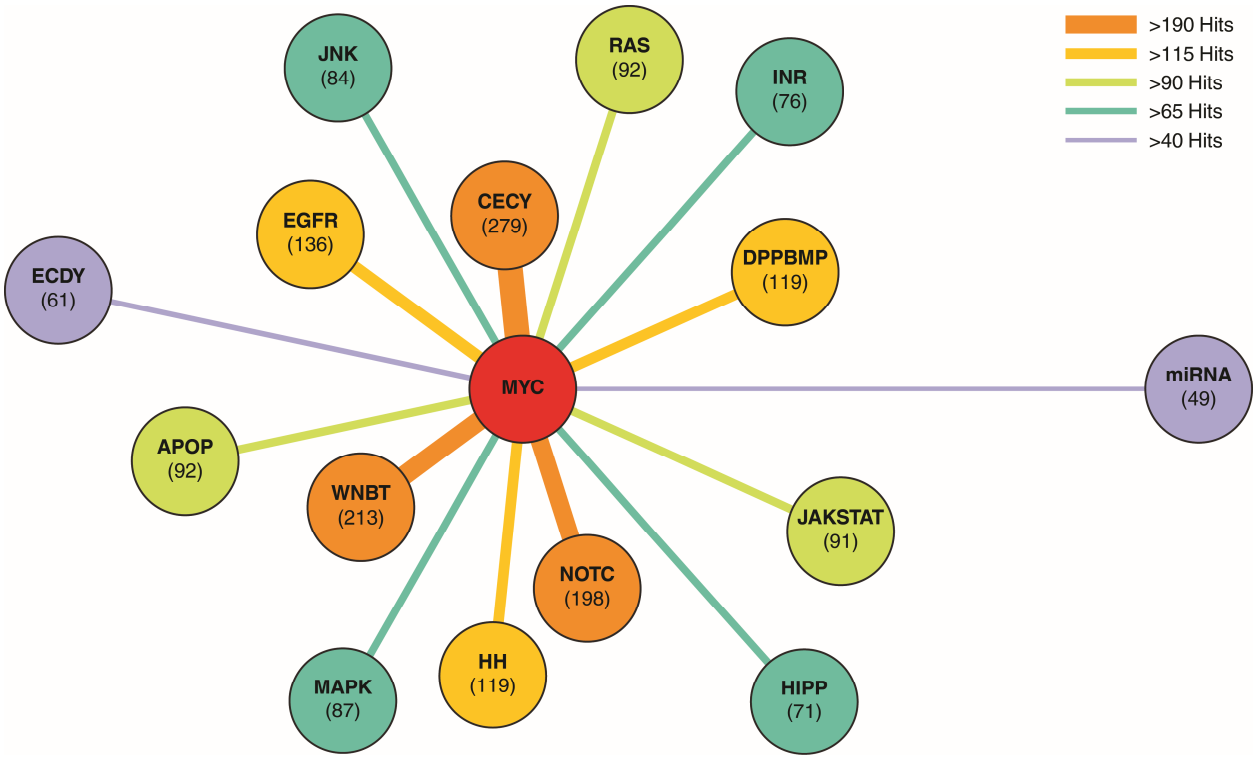


Figure 13. **Graphical illustration of cell cycle factors and components of signaling pathways as potential regulators of *Myc*-and *wingless/wg* expression**. The factors identified as potential regulators of *Myc* predominantly belong to cell cycle regulators and signaling pathways like Wingless (Wg), Notch (N), Hedgehog (HH), EGFR, BMP/DPP, MAPK, JAK/STAT, RAS signal transducers followed by several other signaling pathways. All the tested Myc-CRMs and the positive control (W-CRM) are dynamically associated with the identified cell cycle factors and the components of the listed pathways (see Table A-F in File S11). The numbers refer to the number of the protein counts/cell cycle or pathway. **Abbreviations**:

CECY, Cell Cycle; **WNBT**, canonical Wnt signaling (Wnt/β-Catenin/TCF); **NOTC**, Notch signaling pathway; **EGFR**, Epidermal Growth Factor Signaling Pathway; **DPPBMP**, DPP/BMP signaling pathway; **HH**, Hedgehog signaling pathway; **RAS**, RAS GTPase signaling pathway; **APOP**, Apoptosis pathway; **JAKSTAT**, JAK/STAT signaling pathway; **JNK**, Janus kinase signaling pathway; **INR**, Insulin-like receptor signaling pathway; **HIPP**, Hippo signaling pathway; **MAPK**, Mitogen-activated protein kinase signaling pathway; **ECDY**, Ecdysteroid signaling pathway; **miRNA**, miRNA signaling pathway.

Components of different signaling pathways as potential regulators of the *Myc* expression

Many of these factors belong to known signaling pathways playing an essential role in *Myc* expression (Figure 13). We decided to analyze these factors in order to be able to characterize the complexity of pathways converging on *Myc* promoter and enhancer elements.

Hedgehog signaling inhibitor Suppressor of Fused and 14-3-3 epsilon an interactor of Ras/MAPK and Hippo signaling might regulate Myc expression: Suppressor of Fused *Su(fu)* is a tumor suppressor protein kinase (HAN *et al.* 2015) and a conserved negative regulator of the Hedgehog signaling pathway by inhibiting transcription factors Ci/Gli ((METHOT AND BASLER 2000) via inhibition of recruitment of coactivator p300/CBP to the transcription regulator complex (WANG *et al.* 2000; SHI *et al.* 2014; HAN *et al.* 2015; OH *et al.* 2015; HAN *et al.* 2019). MYC has been shown to regulate the Hh component Gli (YOON *et al.* 2013; ABERGER AND RUIZ I ALTABA 2014) in certain types of tumors. Mechanistically, a regulatory circuit might exist between the regulation of *Drosophila* Myc and the Hedgehog signaling regulator Su(fu).

(*14-3-3ε*) encodes an acidic protein and is involved in cell proliferation and differentiation, tissue patterning, and organ size determination through interaction with multiple signaling pathways, predominantly Ras/MAPK (ASHTON-BEAUCAGE *et al.* 2014) and Hippo signaling pathways (REN *et al.* 2010; POJER *et al.* 2021). Transcriptional cofactor Yorkie (Yki/YAP) of the Hippo signaling pathway is a well-known coactivator of *Myc* transcription during cell growth and progression through cell cycle via translocation of (Yki/YAP) to the nucleus (NETO-SILVA *et al.* 2010). Phosphorylation of Yorkie promotes the binding of 14-3-3 epsilon to Yorkie and inhibition of Yorkie nuclear translocation (REN *et al.* 2010) suggesting an indirect role for14-3-3 epsilon in *Myc* downregulation during cell fate initiation and organ size determination.

Drosophila Sumo protein is involved in Myc protein Sumoylation and degradation: Small ubiquitin like modifier Sumo (*Sumo*, *smt3*) sole member of Sumo family proteins in *Drosophila*, responds to TGF-β (MILES *et*

al. 2008), Ras/MAPK (NIE *et al.* 2009), Smoothened (ZHANG *et al.* 2017), and Toll-NF-κB (KANOH *et al.* 2015) signaling pathways, and plays a crucial role during syncytial blastoderm mitotic cell cycle in embryonic patterning (NIE *et al.* 2009). Sumo contributes to wing morphogenesis through modulation of Hedgehog signaling during larval life cycle (ZHANG *et al.* 2017). Sumo has been reported to degrade Myc protein via Sumoylation to regulate Myc levels during development (GONZALEZ-PRIETO *et al.* 2015).

Notch regulator AP-2, host cell factor HCF and myeloid leukemia factor MLF are involved in cell growth and differentiation via modulation of Myc levels: Subunit of Adaptor protein complex AP-2 is involved in asymmetric cell division during nervous system development via modulation of Notch signaling (BARDIN *et al.* 2004). Further, AP-2α is required for intracellular vesicle trafficking (GONZALEZ-GAITAN AND JACKLE 1997; NARAYANAN AND RAMASWAMI 2001; WINDLER AND BILDER 2010), signaling receptor internalization and regulation of gene expression (MOORE *et al.* 2020), and the regulation of cell polarity (LIN *et al.* 2015). Notch signaling plays a pivotal role in regulating cell proliferation and differentiation by modulating transcription of Notch targets such as *Myc* (MAZUR *et al.* 2010). Numb protein together with AP-2, key determinants of cardiac progenitor differentiation downregulate *Myc* in a Pim1 background during mitosis to initiate cardiomyocyte fate (COTTAGE *et al.* 2010). Mammalian Ap-2 is involved in repression of *c-MYC* target genes (GAUBATZ *et al.* 1995) and inhibition of MYC-induced apoptosis (MOSER *et al.* 1997).

Coactivator Host cell factor HCF (*Hcf*) (MAHAJAN *et al.* 2003) is a component of the Ada2a complex (ATAC); Ash2-Sin3A-HCF interactions induce chromatin remodeling and histone modifications on the promoters of genes involved in cell growth and proliferation (GUELMAN *et al.* 2006a; SUGANUMA AND WORKMAN 2008; FURRER *et al.* 2010; SUGANUMA *et al.* 2012). DPE promoter requires Ada2 and DR1/NC2B to establish transcription initiation from this site (HSU *et al.* 2008). DR1-Drap1/NC2B is a bifunctional basal transcription factor that represses TATA while activating DPE to establish differential transcriptional bursting (WILLY *et al.* 2000). Ash2 and HCF were associated with all the examined Myc-CRMs indicating chromatin remodeling complexes containing Ash2-Sin3A-HCF components, might differentially control *Myc* transcription from multiple sites

DNA binding Myelodysplasia/myeloid leukemia factor MLF was associated with all the tested Myc-CRMs. MLF is required for cell growth, proliferation, and differentiation in developing eye via interaction with transcription factor DREF (JASPER *et al.* 2002), and MLF/DnaJ-1 is involved in lineage commitment of primary hematopoietic progenitors during blood cell development. Transcription factor MYC has been reported to play a

crucial role during hematopoiesis (LU *et al.* 2023), and in acute myeloid leukemia (AML) MYC and MLF are both overexpressed (BRAS *et al.* 2012). We identified both proteins MLF and DnaJ-1 in *Myc* samples indicating a putative role for MLF regulating Myc expression during cell proliferation, differentiation and patterning.

Transcription factor Mad downregulates Myc during G1/S transition to induce cell differentiation: Mothers against *dpp* Mad (*Mad*) is a pivotal pol II-specific Mad homology domain transcription factor with roles in a wide range of functions during development. As a core component of the Dpp/BMP signaling pathway Mad responds to Dpp/BMP ligands decapentaplegic (*dpp*), Screw (*scw*), and glass bottom boat (*gbb*) to regulate the expression of Dpp response target genes (*gbb*) (DAS *et al.* 1998b; INOUE *et al.* 1998; DAI *et al.* 2000; MULLER *et al.* 2003; YAO *et al.* 2006; WEISS *et al.* 2010; VUILLEUMIER *et al.* 2022). Mad induces proliferation arrest during G1/S transition (FIRTH AND BAKER 2005) and modulates differentiation and morphogenesis during limb development (MARQUEZ *et al.* 2001), wing patterning (LECUIT *et al.* 1996; DWORKIN AND GIBSON 2006), eye development (WIERSDORFF *et al.* 1996; LIU *et al.* 2023b), and follicle cell morphogenesis (KIRILLY *et al.* 2005). The Myc/Max/Mad network of transcription factors function as transcriptional activators or repressors in a tissue-specific manner during cell proliferation and differentiation (GRANDORI *et al.* 2000). Myc and Mad are two opposing transcription factors, and upon heterodimerization control the balance between cell fate determination and tissue size and shape (OKADA AND SHI 2018).

Pontin and Reptin control Myc expression during establishment of basolateral cell polarity: Pontin (*pont*) and Reptin (*rept*), components of the Scribble protein complex (Dlg1, LGL, Scribble) belong to RuvB-like DNA helicases (GO REFERENCE GENOME PROJECT 2011) and play key roles in multiple signaling pathways. They constitute components of chromatin remodeling complexes such as Tip60, SWRI (KUSCH *et al.* 2004), and Ino80 (KLYMENKO *et al.* 2006) that enhance DNA accessibility to transcription factors, replication machinery, and DNA damage/repair factors by modifying chromatin structure and nucleosome positioning (KUSCH *et al.* 2004). Pontin (Tip49/Pontin) and Reptin (Tip48/Reptin), Pontin with emphasized genetical interaction are essential for the normal function of Myc during growth and proliferation (BELLOSTA *et al.* 2005). The evolutionary conserved Pontin is at the nexus of a complex network of signaling cascades including June kinase, TNFα/Eiger, (WANG *et al.* 2018a; WANG *et al.* 2018b), Wingless/Wg (BAUER *et al.* 2000), and the Myc pathway (BELLOSTA *et al.* 2005) to control activation or repression of a set of developmental genes during patterning. We identified guanylate kinase Discs large 1 (*dlg1*), the scaffolding protein Scribble (*scrib*), and the

tumor suppressor protein Lethal giant larvae, LGL (*lgl*, *l(2)gl*, *CG2671*) associated with the examined Myc-CRMs (see Table 5 in File S5 and Table 6 in File S6). These proteins belong to the Scribble protein complex and play crucial roles in epithelial cell basolateral integrity and function (KHOURY AND BILDER 2020). The Scribble protein complex is involved in the modulation of signal transduction processes and control of cell proliferation (KHOURY AND BILDER 2020). Scribble function is essential for patterning of embryonic epithelium (GIBSON AND PERRIMON 2003), control of cell polarity and proliferation (ZEITLER *et al.* 2004), stem cell maintenance and division (ZENG *et al.* 2010), and cell competition. Cell polarity proteins such as aPKC, Scribble, and LGL (WAKEFIELD AND STUELTEN 2007; KIM *et al.* 2014), and growth regulator Myc the forefront indicator of cell fitness, interact to balance epithelial cell morphogenesis and polarity for a normal development (FAHEY-LOZANO *et al.* 2019).

Ecdysteroid receptor-related protein SMRTER regulates Myc expression during cell fate specification and metamorphosis: SMRTER (SMRT-related and ecdysteroid receptor interacting factor) (*Smr*), a Chromatin binding factor (TSAI *et al.* 2004; TSUDA *et al.* 2006; HECK *et al.* 2012) is part of a conserved transcription repressor complex that together with the Sin3A/HDAC complex negatively regulates ecdysone receptor signaling during fly development (KOZLOVA AND THUMMEL 2000). During follicle cell morphogenesis and wing disc patterning SMRTER interacts with Notch antagonist, Suppressor of Hairless Su(H) to inhibit transcription of Notch targets (HECK *et al.* 2012). Further, the corepressor complex consisting of Ebi/SMRTER/Su(H) is involved in modulation of EGFR and Notch signaling pathways to induce photoreceptor cell activity in the developing eye in *Drosophila* (TSUDA *et al.* 2006). Ecdysone signaling is an important regulator of *Myc* during cell growth and proliferation and induction of cell fate specification to control developmental timing with the production of a variety of cell types as progenitors of tissue patterners (DELANOUE *et al.* 2010; RAJAN AND PERRIMON 2010; GALLANT 2013).

The demethylase enzyme Little imaginal discs/Lysine demethylase 5 regulates Myc-dependent cell growth via direct binding to Myc: Little imaginal discs/Lysine demethylase 5 (*Kdm5*, *lid*, *CG9088*), a Trithorax Group protein, enables histone H3 demethylase activity (SECOMBE *et al.* 2007; LLORET-LLINARES *et al.* 2008; TARAYRAH *et al.* 2015). Lid/Kdm5 belongs to AT-rich interaction domain (ARID) transcriptional regulators (LIU AND SECOMBE 2015) and binds to DNA non-specifically to remodel chromatin (GO REFERENCE GENOME PROJECT 2011) and control general transcription (SECOMBE *et al.* 2007; LIU AND SECOMBE 2015). Lid/Kdm5 is

a transcriptional modulator of JAK/STAT signaling during male germline stem cell differentiation and maintenance (TARAYRAH *et al.* 2015). Lid is involved in the regulation of Myc-dependent cell growth via direct binding to Myc (SECOMBE *et al.* 2007), and the binding interaction between Lid homologs JARID1A, JARID1B, and MYC has also been observed in the mammalian system, indicating the evolutional conservation of the function of these proteins (SECOMBE *et al.* 2007).

LKRSDH might regulate Myc transcription during apoptosis and Modulo, a Myc downstream cofactor is a potential modulator of Myc levels: Lysine ketoglutarate reductase (*LKRSDH*) is a bifunctional enzyme that functions in the Lysine degradation pathway (CAKOUROS *et al.* 2008; ST CLAIR *et al.* 2017) and as a transcriptional corepressor regulates the expression of apoptotic genes through histone methylation in response to ecdysone signaling (CAKOUROS *et al.* 2008). Like Lid (*Kdm5*, *lid*), LKRSDH has roles in Myc-induced growth control (SECOMBE *et al.* 2007), and might have a feedforward function in regulating *Myc* transcription.
The nucleolar-localized and RNA recognition motif (RRM)-containing Modulo (*mod*) protein (PERRIN *et al.* 1999) is a downstream target of *Myc* that is required during cell growth and proliferation in imaginal tissues but not in endoreplicating larval tissues (PERRIN *et al.* 2003). Modulo is tightly controlled by Myc and plays an important role in biomass production and is required for nucleolus function (PERRIN *et al.* 1998). Mutations in Modulo show the minute phenotype, that is flies with small body size and female sterility (PERRIN *et al.* 1999). Here we identified Modulo associated with all the investigated Myc-CRMs, suggesting a possible role for Modulo in regulating *Myc*.

Wingless cofactor 'Short wing' is a potential regulator of Myc and the chromatin remodeler 'will die slowly' controls Myc expression during growth and morphogenesis: Short wing, Sw (*sw*), a dynactin binding (MORGAN *et al.* 2011) protein, and a subunit of the cytoplasmic dynein motor complex (NURMINSKY *et al.* 1998) contributes to wing and eye development (MYSTER AND PEIFER 2001), mitotic cell cycle (DZHINDZHEV *et al.* 2005; MORALES-MULIA AND SCHOLEY 2005), fate determination in 16-cell syncytium stage withing the egg chamber (MCGRAIL AND HAYS 1997), and neurogenesis (ZHENG *et al.* 2008). Since Sw was associated with all the investigated *Myc* targets, and both proteins, Myc and Sw, are essential during early embryonic development, cell fate specification, and oocyte maturation, there might be yet unknown direct or indirect regulatory circuits for Sw protein controlling *Myc* expression.

Protein 'will die slowly' (*wds*) is a WD40 domain containing chromatin remodeler, and in association with the Ada2a complex (ATAC) enables histone H3 acetylation and possibly H4 to regulate transcription (SUGANUMA AND WORKMAN 2008). As a part of TRX/MLL and NSL complexes (PASCUAL-GARCIA *et al.* 2014) Wds plays a crucial role in sustained transcriptional activation of neurogenic genes through interaction with the Myc-interacting protein Mad and Trx (YAO *et al.* 2018a). WD40 domain proteins have been implicated in *MYC* downregulation via ubiquitination and degradation of MYC protein during tissue patterning (GU *et al.* 2007).

Components of Ras/ERK, MAPK, and insulin signaling pathways are known regulators of Myc: Mitogen-activated protein kinase (*ERK*, MAPK, *rl*) is a core component of the RAS/MAPK pathway and binds to a wide range of proteins to phosphorylate downstream cytoplasmic and nuclear signal transducers and transcription factors. ERK plays a crucial role in varied cell fate decisions, and cell and tissue and body size control (BRUNNER *et al.* 1994; O'NEILL *et al.* 1994; SCHWEITZER *et al.* 1995; OELLERS AND HAFEN 1996; KARIM AND RUBIN 1999; BAKER *et al.* 2001; QIAO *et al.* 2006) by interacting with the components of multiple signaling pathways including epidermal growth factor receptor signaling pathway (DIAZ-BENJUMEA AND HAFEN 1994; SCHWEITZER *et al.* 1995; OELLERS AND HAFEN 1996; SCHNEPP *et al.* 1998; REICH AND SHILO 2002; MOLNAR AND DE CELIS 2013; YANG *et al.* 2016; OGURA *et al.* 2018), ERK1 and ERK2 cascade (OELLERS AND HAFEN 1996; CLEMENS *et al.* 2000; ROY *et al.* 2002; DOUZIECH *et al.* 2006; ASHTON-BEAUCAGE *et al.* 2014; GOYAL *et al.* 2017), fibroblast growth factor receptor (CLEMENS *et al.* 2000; OHSHIRO *et al.* 2002; SATO AND KORNBERG 2002; GRYZIK AND MULLER 2004; PETIT *et al.* 2004; CSISZAR *et al.* 2010; UKKEN *et al.* 2014; DU *et al.* 2018), insulin receptor (CLEMENS *et al.* 2000; XU *et al.* 2013; SLACK *et al.* 2015), Wingless (SINENKO *et al.* 2009; DRAGOJLOVIC-MUNTHER AND MARTINEZ-AGOSTO 2013), Sevenless (BIGGS *et al.* 1994; BRUNNER *et al.* 1994; KARIM *et al.* 1996; KUMAR *et al.* 1998; KITADATE *et al.* 2007), and Torso (Tor) signaling pathways (BRUNNER *et al.* 1994; GHIGLIONE *et al.* 1999; LIM *et al.* 1999; GOYAL *et al.* 2017). Ras/ERK cascade modulates spatiotemporal regulation of *Myc* in many different ways during development and malignant transformation (SEARS *et al.* 1999; PROBER AND EDGAR 2002; GREWAL 2015). Ras/ERK and Myc pathways are growth-promoting and pro-survival signaling pathways. *Myc* expression was shown to be elevated for compensatory proliferation in *Drosophila* damaged imaginal tissues expressing activator of apoptosis 'head involution defective' (hid), not solely in response to apoptotic pathway but rather in conjunction with the increased activity of Ras/ERK cascade (CRUCIANELLI *et al.* 2022).

MAPK-regulated corepressor-interacting protein (MCRIP) (*CG34159*) localizes to cytoplasmic stress granule and the nucleus, and consists of two variants, namely MCRIP1 and MCRIP2 (ICHIKAWA *et al.* 2015). At low concentration of ERK the isoform MCRIP1 is held in complex with CtBP in cytoplasm which allows the E-cadherin transcription to occur and sustain the integrity of epithelial cells. High concentration of ERK lead to phosphorylation of MCRIP1 by ERK, dissociation from CtBP, and translocation into the nucleus to interact with the DNA-bound corepressor partner ZEB1 on the E-cadherin promoter. The binding of the MCRIP1/ZEB1 heterodimer results in epigenetic silencing and transcriptional inhibition of E-cadherin (ICHIKAWA *et al.* 2015), promoting epithelial to mesenchymal transition (EMT) during development and diseases (ICHIKAWA *et al.* 2015). In adult malignancies the Zinc finger E-box binding ZEB1 and MYC are overexpressed in numerus cancer types (KATOH *et al.* 2013; LI *et al.* 2021; FRATINI *et al.* 2023). There is an E-box within the P1/P2 enhancer cluster (5'-CAGCTG-3') (Table S1.3) indicating MCRIP might be involved in direct or indirect regulation of *Myc* transcription and might also have an impact on *Myc* dysregulation in tumorigenesis.

Misshapen and Ajuba might control Myc activity during mitotic cell cycle and differentiation via interaction with the Hippo signaling pathway: The nuclear protein Ajuba (*jub*) functions as a transcriptional corepressor (GO REFERENCE GENOME PROJECT 2011) and is involved in follicle cell development (JIA *et al.* 2015) and mitotic cell cycle control (SABINO *et al.* 2011). Through its protein sequestering ability (RAUSKOLB *et al.* 2014), Ajuba inhibits activation of the Hippo pathway by Yorkie (*Yki*) during morphogenesis of epithelial layer and tissue size determination (DAS THAKUR *et al.* 2010; RAUSKOLB *et al.* 2014). Hippo signaling is an important regulator of *Myc* during cell proliferation, differentiation, and patterning (NETO-SILVA *et al.* 2010). Myc function is essential during embryonic epithelial morphogenesis and cell differentiation (NAIR *et al.* 2006), dosage compensation (MATHEWS *et al.* 2017), and oocyte maturation (MAINES *et al.* 2004). Since corepressor Ajuba associates with the pol II transcription regulator complex (GO REFERENCE GENOME PROJECT 2011), Ajuba might be involved in the regulation of *Myc* transcription.

Misshapen (*Msn*) a non-specific Ser/Thr protein kinase is a negative regulator of Notch signaling (MISHRA *et al.* 2015) and a positive regulator of Hippo signaling (LI *et al.* 2014; LI *et al.* 2015; MENG *et al.* 2015). Msn plays a crucial role during cell migration, epithelial morphogenesis, dorsal closure (SU *et al.* 1998; KADRMAS *et al.* 2004; KOPPEN *et al.* 2006), imaginal disc fusion, and thorax closure (KADRMAS *et al.* 2004). Misshapen, as a regulator of Hippo signaling is involved in Yorki nuclear localization and Hippo target gene activation (LI *et al.* 2015), which might infer the Misshapen-Warts-Yorkie circuit as potential regulator of *Myc* transcription.

Transcription factor Putzig is a potential transcriptional regulator of Myc during DNA synthesis, cell division, and patterning: Nuclear C2H2 Zinc finger transcription factor Putzig (*pzg*) is involved in proliferation and growth via expression of components of mitotic cell cycle and transcriptional stimulation of genes required for replication in a Trf2/DREF-dependent manner (KUGLER AND NAGEL 2007; KUGLER *et al.* 2011). Independent of DREF, Putzig can positively regulate Notch signaling in certain proliferating cell types via binding to the promoters of target genes in these cells and activation of transcription (KUGLER *et al.* 2011). In association with the nucleosome remodeling factor complex NURF Putzig positively regulates ecdysone receptor (EcR) signaling targets during growth and metamorphosis and negatively regulates JAK/STAT signaling pathway targets to modulate immune response (KUGLER *et al.* 2011). Trf2/DREF is an important transcriptional variant in regulating transcription of cell cycle components such as Myc and E2F, thus playing an important role in the control of cell division and tissue size (HYUN *et al.* 2005). Putzig and Trf2 were both associated with the Myc-CRMs, suggesting a role for Putzig and Trf2 in transcriptional regulation of *Myc*.

MYC cofactor Moesin plays a role in the regulation of Drosophila Myc during oogenesis and body plan specification: Moesin (*Moe*), the sole *Drosophila* Erzin, Radixin, Moesin protein is involved in a variety of processes including cortical cytoskeleton stability (CARRENO *et al.* 2008; JAYANANDANAN *et al.* 2014), epithelial integrity (YANG *et al.* 2012), cell shape including ovarian follicular morphogenesis (POLESELLO *et al.* 2002; SOLINET *et al.* 2013), and determination of left/right body symmetry (TANIGUCHI *et al.* 2007). Moesin belongs to c-MYC networks of cell cycle and apoptosis proteins and is a MYC cofactor (CIRIBILLI *et al.* 2015). We conclude that the Myc cofactor Moesin might be apart involved in the regulation of *Myc* expression during development.

Scribbler might be a potential regulator of Myc via interaction with multiple signaling pathways: Scribbler (*sbb*) (other names: Brakeless (*bks*); master of Thickveins (*mtv*)) is a transcriptional coregulator (HAECKER *et al.* 2007) and interacts with mediator complex (CRONA *et al.* 2015) and the nuclear corepressor Atrophin-Grunge (*Gug*, *Atro*) to repress transcription in a Tailless (*tll*)-mediated manner during early embryonic segmentation (HAECKER *et al.* 2007). In photoreceptor cells Scribbler represses the function of Runt (*Run*) (KAMINKER *et al.* 2002) to determine axon guidance and projection to medulla (HAECKER *et al.* 2007). Scribbler cooperates with Notch and Wingless signaling during wing disc morphogenesis and D/V boundary formation

(BEJARANO *et al.* 2008), and functions downstream of Hedgehog signaling to modulate the expression of TGF-β and Hedgehog target genes to determine many aspects of wing disc patterning (DWORKIN AND GIBSON 2006). Whether Scribbler is a regulator of *Myc* during development needs to be investigated.

Amun, a component of Notch signaling pathway might regulate Myc expression during many processes such as sensory organ development: Amun, isoform A (*Amun*) detectable in association with protein-containing complexes, is a nuclear glycosylase-containing domain transcriptional regulator and belongs to the components of the conserved Notch signaling pathway. It plays a crucial role during cell fate commitment, sensory organ development, chaeta development, compound eye morphogenesis, and wing disc patterning (SHALABY *et al.* 2009). Components of Notch signaling and Myc regulate the balance between proliferation and cell fate specification to propagate morphogenesis and tissue patterning (ORIAN *et al.* 2007; KOPECKY *et al.* 2011). Glycosylation is essential for Notch components to transduce the signal and express the target genes (HAINES AND IRVINE 2003).

Adhesion molecule RanBPM is a potential regulator of Myc during larval development: Ran-binding protein M (*RanBPM*) functions as a bridge between extracellular adhesion molecules and membrane receptors (DANSEREAU AND LASKO 2008) and contributes to germline stem cell maintenance, follicular cell of egg chamber cell adhesion, and female stem cell niche (DANSEREAU AND LASKO 2008). RanBPM is part of the E3 ubiquitin ligase complex named Glucose-Induced Degradation Deficient (GID), a complex in transition between sugar degradation and gluconeogenesis (ZAVORTINK *et al.* 2020). *Drosophila Myc* is essential during larval growth including endoreplication, body size, and behavior, and the function of RanBPM in the larval mushroom body is critical for proper development of behavior (DEMONTIS AND PERRIMON 2009; SCANTLEBURY *et al.* 2010) suggesting a putative functional and regulatory link between the two genes.

Nucleoporin Nup98-96 is a potential regulator of Myc during mitotic cell cycle: Member of nucleoporin proteins (*Nup98-96*), a precursor of two proteins Nup96 and Nup98 (PANDA *et al.* 2014) associates with nuclear membrane and chromatin (KRISTO *et al.* 2017) and directs mRNA export from the nucleus (KRISTO *et al.* 2017), translocation of SMAD, Mad, and other proteins into and out of the nucleus (CHEN AND XU 2010; KRISTO *et al.* 2017; WU *et al.* 2019), and is involved in the regulation of transcription (CAPELSON *et al.* 2010; PASCUAL-GARCIA *et al.* 2014), predominantly transcription upregulation of developmentally active genes and genes

regulating cell cycle such as *Myc* (KALVERDA *et al.* 2010). Nucleoporins (Nups) enhance the function of actively transcribing genes via enhancer-promoter loop anchoring and also provide a scaffold for poised genes enabling their immediate induction (PASCUAL-GARCIA *et al.* 2014) in processes such as maintenance of germline stem cells and preservation of their niche in collaboration with components of pathways such as TGF-β and EGFR signaling (PARROTT *et al.* 2011). In oncogenic diseases Nup98 collaborates with PHD finger and bromodomain transcription factors to upregulate the downstream target Pim1 to overexpress MYC (NOURA *et al.* 2024).

Wnt component transcription factor Net, and Notch antagonist* Hairless *are potential regulators of Myc transcription during development: The E-box binding (GO REFERENCE GENOME PROJECT 2011) Myc-type basic helix-loop-helix transcription repressor (*Net, CG11450*) is an essential developmental protein for cell proliferation, fate induction, and differentiation (PEYREFITTE *et al.* 2001). Net is a component of the Wnt enhanceosome and important for wing vein specification and morphogenesis by antagonizing EGFR activities in interveins territories (BRENTRUP *et al.* 2000; DE CELIS 2003). The Wingless signaling pathway is known as a major regulator of *Myc* during cell proliferation, differentiation and organ size determination (WU AND JOHNSTON 2010). Both proteins Net and Myc belong to basic helix-loop-helix family of transcription factors. There might exist a link between Net and downregulation of *Myc* during cell fate specification and differentiation via involvement of signaling cascades like Notch, Wingless, and EGFR.

Corepressor Hairless **(*H*)** (BROU *et al.* 1994; MAIER *et al.* 2011), a conserved silencer of Notch signaling forms transcriptional repressor complexes consisting of (H/Su(H)) and/or (H/Su(H)/Gro/CtBP) proteins (LYMAN AND YEDVOBNICK 1995; NAGEL *et al.* 2005; MAIER *et al.* 2011; SMYLLA *et al.* 2019; WOLF *et al.* 2019) to modulate Notch signaling during sensory organ development (NAGEL *et al.* 2000; CASTRO *et al.* 2005). (H)/(Su(H) controls the rate of cell proliferation and differentiation via Notch signaling during wing vein and wing margin morphogenesis (NAGEL *et al.* 2005) and plays an essential role in wing disc Dorso/Ventral compartment boundary formation by modulating the expression of Wingless (*wg*) and Vestigial (*vg*) (KOELZER AND KLEIN 2006). The work of others has shown that Hairless is involved in cell proliferation, cell death and initiation of differentiation and the regulation of *Myc* transcription (MULLER *et al.* 2005; ORIAN *et al.* 2007; HERRANZ *et al.* 2008).

Components of immune pathways such as Turandot Z were among the identified proteins associated with the Myc-CRMs: The secreted peptide Turandot Z (*Totz*) functions in stress situations and in conjunction with the Toll, JAK/STAT, Imd, and Mekk1/JNK pathways (BRUN *et al.* 2006) in response to UV damage, heat induction, paraquat-induced oxidative stress, and bacterial infection (EKENGREN AND HULTMARK 2001). In our proteomics approach for the identification of *Myc* regulators and the related pathways we came up with a wide range of components of conserved signaling pathways among which Toll, JAK/STAT, Imd, and Mekk1/JNK pathways were well enclosed (Figure 13; see also supporting information, Table A-F in File S11), and have been documented by the work of others as important regulators of *Myc* expression (CORDERO *et al.* 2012; ZORANOVIC *et al.* 2013; ALPAR *et al.* 2018; FAHEY-LOZANO *et al.* 2019; SCHAUB *et al.* 2019; WANG *et al.* 2019; CRUCIANELLI *et al.* 2022). Turandot Z was highly enriched in the DPE-containing *Myc* P35/P36 sample (see Table 3 in File S3), indicating a role for TotZ in *Myc* regulation.

XII. Proteins identified with the enhancer cluster P1/P2 are potential regulators of *Myc* during cell fate induction and differentiation

Next we examined those factors that were mostly uniquely associated with the upstream enhancer cluster P1/P2 as the absence of these conserved sequences abrogated the expression in larval brain and imaginal tissues (Figure 1B-E). These factors included DOC3, Wdr62, Chip, Mael, Piwi, Nclb/dPWP, Grip91, γSnap1, Pmm2, Pka-C1, RecQ4, and Top2. Potential functions of these factors are described below.

The T-box transcription factor Drosocross3 might define the P1/P2 enhancer as a tissue-specific cis-element: Drosocross3, only associated with the P1/P2 (Figure 14; see also supporting information, Table S8) (PFLUGFELDER *et al.* 2017) is a target gene of the TGF-β/Dpp signaling pathway that plays a role in organogenesis (HATTON-ELLIS *et al.* 2007) during third instar larval and early pupal development. DOC3 is involved in pupal thorax closure (LU *et al.* 2022), plays an essential role in proliferation arrest and completion of differentiation, cell fate specification, larval tissue patterning, notum specification, nephrocyte differentiation, limb development (PFLUGFELDER *et al.* 2017), and cardiogenesis by acting as an interplay between Dpp and Wingless inputs (REIM AND FRASCH 2005), and possibly by controlling *Myc* expression, the target of Dpp and Wingless signaling pathways.

Wdr62 might regulate Myc transcription in neuronal progenitor cells: WD repeat domain 62 (*Wdr62*) protein (Figure 14; see also supporting information, Table S8), is a cytoplasmic protein and is involved in the regulation of cytoskeleton and mitotic cell cycle (RAMDAS NAIR *et al.* 2016; ZEKE *et al.* 2016), and centriole duplication (GO REFERENCE GENOME PROJECT 2011). A study has shown that the JNK pathway is involved in the phosphorylation of JNK target Wdr62 in mitotically arrested progenitors of proneural cells and Myc protein is downregulated in these cells. However, in parallel, phosphorylation of Aurora kinase A (AURK) leads to association of the AURK with the phosphorylated Wdr62 and localization of Wdr62 to the microtubule poles during neuronal differentiation and brain development (ZEKE *et al.* 2016). This result indicates that the association of the Wdr62 protein with the P1/P2 enhancer might regulate Myc levels during differentiation of the neuroprogenitor cells into brain neurons.

Transcription factor Chip integrates Notch and Wingless signaling pathways to regulate the Myc during cell fate specification and tissue patterning: The LIM-homeodomain-binding transcription factor and enhancer facilitator Chip (*Chip*) (Figure 14; see also supporting information, Table S8) is essential for nervous system development by partnering with the Apterous protein (VAN MEYEL *et al.* 2000) and binding to different Chip-binding partners. Protein complexes containing Chip integrate multiple signaling pathways such as Notch and WNT/Wg to induce sensory organ precursor (SOP) cells fate, chaeta morphogenesis, eye development (BIRYUKOVA AND HEITZLER 2005; ROIGNANT *et al.* 2010), wing disc morphogenesis (RINCON-LIMAS *et al.* 2000), leg development, thoracic bristle formation (ZENVIRT *et al.* 2008), and cardiogenesis (ROIGNANT *et al.* 2010). A study has shown that the *Drosophila* Chip (*chi*) homolog in mouse 'LIM domain-binding protein 1' Ldb1 (*ldb1*) with essential roles during embryonic development contains a binding site for MYC, and that Ldb1 physically interacts with MYC (YAMASHITA *et al.* 1998). Perturbances in (*ldb1*) expression cause embryonic lethality and liver cancer in adult mice (TEUFEL *et al.* 2010). Microarray analysis showed expression of MYC in hepatocellular carcinoma cells, upregulation of the MYC downstream target Cyclin D1, and downregulation of p21 (TEUFEL *et al.* 2010).

Maelstrom and Piwi interact with Myc and are potential regulators of Myc expression: HMG box protein Maelstrom (*mael*) (Figure 14; see also supporting information, Table S8) is part of the meiotic complex and is involved in ncRNA-, piRNA-, and miRNA-mediated metabolic processes, gene silencing, heterochromatin formation (FINDLEY *et al.* 2003; LIM AND KAI 2007; PEK *et al.* 2009; SIENSKI *et al.* 2012) cell fate specification

and differentiation (CLEGG *et al.* 1997; PEK *et al.* 2012; HAYASHI *et al.* 2014), and germline maintenance (LIM AND KAI 2007; PEK *et al.* 2009). Further, Mael induces malignant transformation in collaboration with Myc and Ras pathways (KIM *et al.* 2017).

P-element induced wimpy testis Piwi (*Piwi*) exhibits the ability of silencing retrotransposons in part by physical interaction with piRNAs. Piwi and HMG protein Maelstrom colocalize with transcribing pol II to numerus genome instability hot spots embedded within transposons (SIENSKI *et al.* 2012). Repression of transposable elements by Piwi during transcription in ovarian somatic cells is dependent on the transcription factor Maelstrom, which in turn impacts chromatin organization and regulation (SIENSKI *et al.* 2012; ILYIN *et al.* 2017). Retrotransposon gypsy insulator is inserted into the first intron at the 5'-UTR of the *Myc* gene, 418 bps upstream of the translation initiation site (GALLANT *et al.* 1996). Piwi was associated with the upstream enhancer P1/P2 and the TATA-containing promoter P31/P32, and Mael was only found in association with the P31/P32 promoter. Both proteins Piwi and Mael might play an important role in repressing gypsy element during early embryonic development and during oogenesis from the *Myc* TATA promoter and the oocyte element initiation sites.

Drosophila chromatin associated-factor dPWP/Nclb regulates Myc levels during animal growth in response to nutrients: The chromatin associated factor (*nclb*, *dPWP*), 'no child left behand' functions in the Myc and TORC1 signaling pathway to regulate animal growth in response to nutrients by Pol I-mediated ribosomal gene expression, and possibly through pol III transcription (LIU *et al.* 2017). In *Drosophila* larvae dPWP regulates the localization of Cdk7 to the nucleolus, a component of pol I elongation factor TFIIH (LIU *et al.* 2017). Its function is crucial for germline stem cell maintenance in early germ cell cysts, female germ cell differentiation and egg chamber development (CASPER *et al.* 2011). Overexpression of dPWP has been reported in human squamous cell head and neck cancer with poor prognosis (LIU *et al.* 2017).

Gamma-tubulin ring protein 91 might be involved in Myc regulation during meiosis and mitosis: Gamma-tubulin ring protein 91 (*Grip91*) (Figure 14; see also supporting information, Table S8) is a component of γ-tubulin structural protein complex (OEGEMA *et al.* 1999) and plays a crucial role during male meiotic nuclear division and spindle organization (BARBOSA *et al.* 2003). During Mitosis Grip91 facilitates the formation of spindle assembly, aligning and segregation of duplicated chromosomes (TARIQ *et al.* 2020). Grip91

and Myc are two important proteins during cell division cycle, cell proliferation and maintenance of germline divisions.

γSnap1 is indirectly involved in Myc regulation during cell proliferation and differentiation: γSoluble NSF attachment protein 1 (γ*Snap1*) (Figure 14; see also supporting information, Table S8) is a N-ethylmaleimide-sensitive fusion protein that is involved in synaptic vesicle internalization and protein transport and activation of Notch signaling during wing Dorsal/Ventral (D/V) patterning (BEJARANO *et al.* 2008). Activated Notch activates Wingless in the D/V boundary cells and combined collaboration of Wingless and Notch signaling coordinates cell proliferation and differentiation in wing primordium by controlling the activity of target genes such as *Myc* (GIRALDEZ AND COHEN 2003).

Pmm2 and Claret enzymes might regulate Myc expression during proliferation and tissue patterning: Phosphomannomutase (*Pmm2*) (Figure 14; see also supporting information, Table S8) plays an essential role in GDP-mannose biosynthetic process and protein glycosylation at the neuromuscular junctions (NMJ). Synaptic Mmp2 glycosylation by Pmm2 occurs through modulation of Wnt/Wg signaling with the involvement of heparan sulfate proteoglycan coreceptor Dally-like protein (Dlp), thus acting via multiple cascades to control patterning and differentiation during developmental processes and adult life (PARKINSON *et al.* 2016). Dpp represses the protooncogene gene *Myc*, and in an autoregulatory loop antagonizes the positive effects of Wingless/Wnt signaling on cell proliferation to limit stem cell number and the activity of Myc (PENNETIER *et al.* 2012).

Claret (*ca*) (Figure 14; see also supporting information, Table S8) belongs to the Ras superfamily and the guanine nucleotide exchange factor family of proteins like Ran-GTPase. Claret is essential for eye color biosynthetic process and eye pigment precursor transport via vesicular trafficking pathway (REAUME *et al.* 1991; MA *et al.* 2004; HARRIS *et al.* 2011), regulation of lipid storage (WANG *et al.* 2012), cell cycle progression, and centrosome duplication (REN *et al.* 1994; SAZER AND DASSO 2000). In a study it was shown that v-Myc directly binds to the *Ran* promoter to drive the expression of the *Ran* gene (YUEN *et al.* 2013). Whether Claret, a member of the Ran-GTPase family might regulate *Drosophila Myc* expression, or alternatively Myc might be involved in *claret* expression remains to be resolved.

Pka-C1 was associated with the Myc upstream enhancer P1/P2 and the oocyte element P37/P38: cAMP-dependent protein kinase catalytic subunit 1 (Pka-C1) contributes to Anterior/Posterior (A/P) axis pattern specification in larval discs, wing morphogenesis (PAN AND RUBIN 1995), synaptic transmission (YAO AND WU 2001), and rhythmic behavior (LEVINE *et al.* 1994). Protein kinases such as Pka belong to *Myc* target genes, and Myc is a phosphorylation substrate for cAMP-dependent Pka species during transcriptional activity, meaning a putative feedback regulatory loop between Pka kinases and Myc during cell proliferation and differentiation (BOI *et al.* 2023).

DNA helicase RecQ4 with an essential role during replication in highly proliferative cells was associated only with the Myc enhancer P1/P2: ATP-dependent DNA helicase (*RecQ4*) (Figure 14; see also supporting information, Table S8) is important for genome stability, DNA metabolism including duplex unwinding and DNA double-strand break repair (CAPP *et al.* 2009), larval development (WU *et al.* 2008), mitotic cell cycle, and oogenesis (XU *et al.* 2009). It has been shown that in chorion genes of follicle cells RecQ4 plays an important role in viability and fertility and is actively involved in DNA replication during development (WU *et al.* 2008). RecQ4 binding to the *Myc* upstream enhancer cluster might have an impact on *Myc* regulation during oogenesis and larval development.

Myc enhancer cluster P1/P2 plays an essential role in larval and pupal development: If not all but most of the factors associated with the enhancer cluster P1/P2 contribute to cytoskeletal structure, tissue patterning, dorsal closure, segmentation, and larval and pupal development. We conclude that the *Myc* enhancer P1/P2, predominantly functions as a patterning tissue-specific enhancer and plays a crucial role by the onset of cell fate specification and differentiation during larval and pupal development. We reason that the factors associated with the P1/P2 enhancer and the basal transcription protein complex integrate developmental signals from pathways such as Wnt/Wg, Notch, Hedgehog, EGFR, and Dpp/BMP (Figure 14 and Figure 15) into transcription of target genes in certain cell types to pattern the tissues (GALLANT *et al.* 1996; PROBER AND EDGAR 2002; MAINES *et al.* 2004; ZANET *et al.* 2005; WU AND JOHNSTON 2010; TAVARES *et al.* 2017).

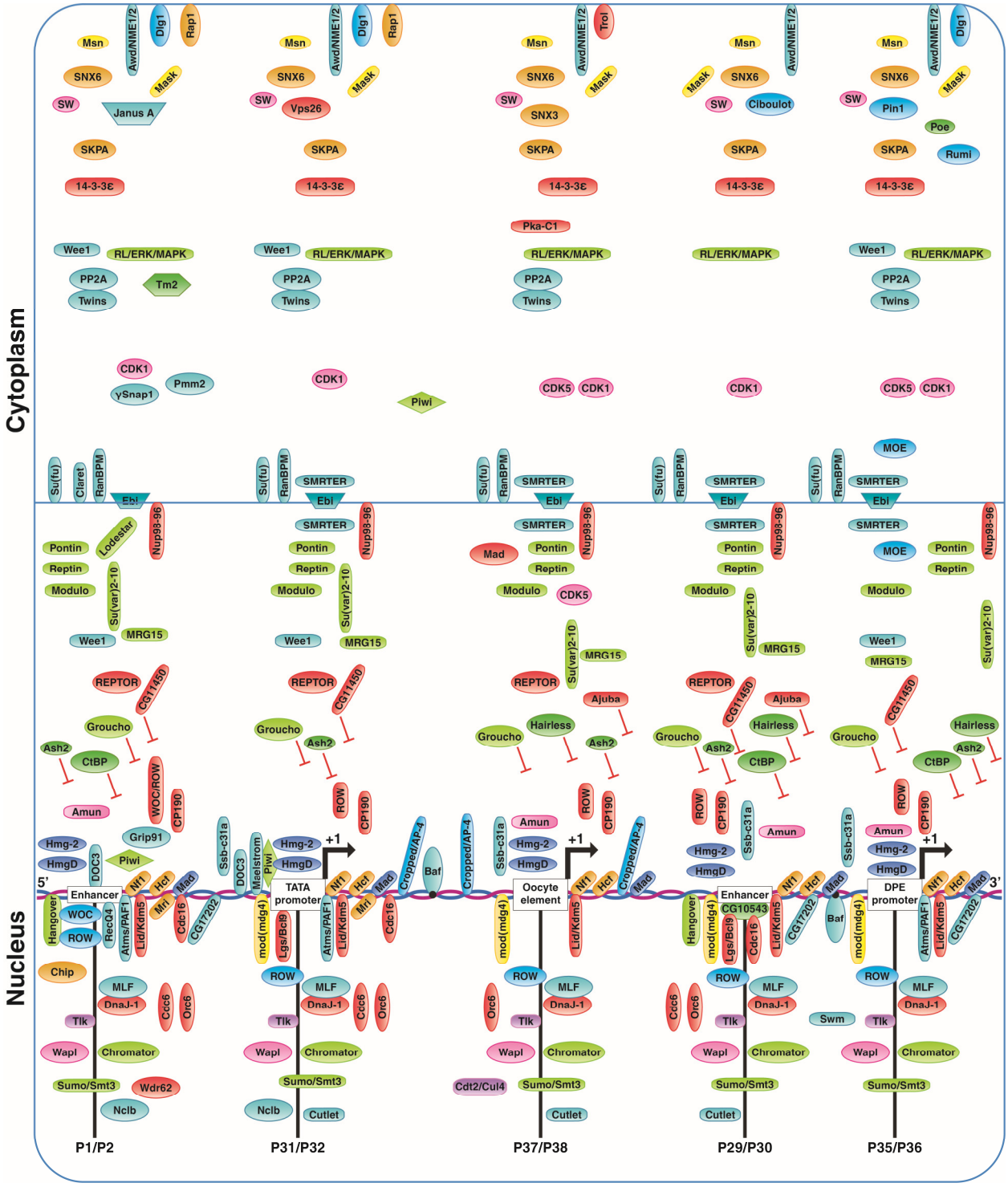


Figure 14. **Schematic illustration of the components of signaling pathways, cell cycle factors, boundary elements, and other gene regulatory proteins with potential role in *Myc* regulation**. Drosocross 3 (DOC3)– inhibitor of proliferation and pro-differentiation completion transcription factor was only associated with the upstream enhancer cluster P1/P2. Modulo, Myc partner for cell growth and proliferation, HMG group proteins HmgD and Hmg-2, Myeloid leukemia factor (Mlf), Host cell factor (Hcf), and transcription factor Mad, competitor of Myc for heterodimerization with another bHLH-Zip transcription factor like Max were associated with all the tested *Myc* targets. Barrier to autointegration factor (BAF) with roles in cell cycle and oocyte karyosome formation was only associated with the start sites P37/P38 and P35/P36. Piwi,

piRNA interacting factor was associated with the upstream enhancer and the TATA promoter, and Piwi interactor Maelstrom was only associated with the TATA-containing target. Hairless, Notch antagonist was compounded with the DPE promoter, the nearby enhancer P35/P36, and the super enhancer cluster P37/P38, the oocyte element. For more information about the association of the illustrated factors with the Myc-CRMs and the abundance ratio of the factors (see Table3 in File S3, Table 7 in File S7 and Table S8).

XIII. Here we describe core promoter factors, chromatin modulators, and gene-specific factors associated with the Myc-CRMs

RNA polymerase complex: RNA pol II subunit A RPII215 (*RPB1*) is the largest subunit of RNA pol II complex. RPB1 serves as a scaffold for the assembly of basal transcription factors and is involved in transcription initiation (ZEHRING *et al.* 1988; BRICKEY AND GREENLEAF 1995; INTERPRO PROJECT MEMBERS 2004) and elongation (BRICKEY AND GREENLEAF 1995).

RNA pol II subunit B Polr2B (*RpII140*, *RPB2*) is the second largest component of the pol II core complex and is essential for RNA polymerase transcriptional activity (FALKENBURG *et al.* 1987; AOYAGI AND WASSARMAN 2000) of nearly all start sites (GREENLEAF 1983; HAMILTON *et al.* 1993).

RNA pol II subunit C, (*Polr2C*, *Rpb3*) responds to heat shock and triggers transcription of the Hsp70 gene (YAO *et al.* 2007).

RNA pol II subunit E (*Polr2E*, *RPB5*) possesses polymerase activity for transcription from pol II, pol I and pol III promoters for tRNA synthesis by pol III and transcription elongation by pol I. (AOYAGI AND WASSARMAN 2000; GENE ONTOLOGY CURATORS 2002; GO REFERENCE GENOME PROJECT 2011).

RNA pol II subunit G (*Polr2G, RPB7*) binds to ssDNA and RNA and is involved in regulation of gene expression, translation initiation (GO REFERENCE GENOME PROJECT 2011), and pol II transcription (AOYAGI AND WASSARMAN 2000).

RNA pol II, I and III subunit H (*Polr2H, RPB8*) is capable of 5'-3' polymerase activity (INTERPRO PROJECT MEMBERS 2004) and Pol I, pol II, and pol III transcription (AOYAGI AND WASSARMAN 2000; GENE ONTOLOGY CURATORS 2002; GO REFERENCE GENOME PROJECT 2011). Polr2H is involved in tRNA transcription by RNA pol III (GENE ONTOLOGY CURATORS 2002).

A wide array of literature confirms the binding of mammalian MYC and *Drosophila* Myc to the proximal region of promoter and interaction with RNA Pol II complex (GREWAL *et al.* 2005; RAHA *et al.* 2010b; DE PRETIS *et al.* 2017; BALUAPURI *et al.* 2019).

TBP-TFIID subunits: We identified TBP-associated factors TAF5, TAF6, and TAF12 in complex with the tested Myc-CRM samples; these TAFs were present in the enhancer cluster samples P1/P2, P29/P30, P37/P38 and the TATA-containing P31/P32 sample. TAF6 was absent from the DPE-containing Myc-CRM P35/P36 sample (Figure 15). TAF5 and TAF6 associate with the TFIID complex (HANSEN AND TJIAN 1995; WRIGHT *et al.* 2006; GO REFERENCE GENOME PROJECT 2011), TAF12 recruits Taf4 and TAF6 recruits TAF9 to TBP-TFIID to endow stability to pre-initiation complex (YOKOMORI *et al.* 1993). A core TFIID complex consists of TAF4, TAF5, TAF6, TAF9, and TAF12 to form a stable pre-initiation complex (WRIGHT *et al.* 2006). We conclude that a robust bursting from *Myc* start sites contains all the essential parts of TFIID. TAF5 and TAF6 are important mediators of promoter responses to protein complexes associated with the enhancers (SCHEER *et al.* 2012) to regulate developmental transcription (AOYAGI AND WASSARMAN 2000). In addition, TAF6 and T AF12 are components of the SAGA complex with histone acetyltransferase (HAT) and ubiquitin protease capability (GUELMAN *et al.* 2006b). The HAT activity of SAGA and the interaction with TBP and the bound transcription factors is essential for promoter opening and pol II promoter escape (HANSEN AND TJIAN 1995; WRIGHT *et al.* 2006). The SAGA protease activity degrades unwanted transcription factors and enhances tissue-specific transcription in certain cell types to induce initiation of differentiation. SAGA is enriched at the 5'-end of the genes indicating paused SAGA can immediately facilitate reinitiation for further rounds of transcription (WEAKE *et al.* 2011). We conclude that *Myc* transcription as an immediate early gene seems to be guaranteed with the assembly of TFIID core components at the initiation sites and the poised complex at the 5'-end. Pol II pre-initiation complex and SAGA, together with the factors bound to the upstream and downstream Myc enhancers integrates the information from developmental signals into transcriptional regulatory programs controlling *Myc* transcription.

Trf2/DREF and Trl/GAF as potential regulators of Myc: TBP-related factor 2 (Trf2, TLF), a general transcription factor associates with the DNA replication element binding factor DREF (BASHIRULLAH *et al.* 2007; SHIMA *et al.* 2007) (TRF2/DREF), recognizes and binds specifically to core promoter sequences (OHLER *et al.* 2002; KEDMI *et al.* 2014; WANG *et al.* 2014), and interacts with the basal transcription machinery and transcription factors-occupied enhancers to modulate gene expression. For example, via interaction with the components of ecdysteroid pathway TRF2/DREF controls regulation of developmental gene expression during spiracle morphogenesis (SHIMA *et al.* 2007) and pupal development (HOCHHEIMER *et al.* 2002; BASHIRULLAH *et*

al. 2007). TRF2/DREF is an important transcriptional regulator of cell cycle components such as Myc and E2F, thus playing an important role in controlling cell division and tissue size (HYUN *et al.* 2005). *Drosophila Myc* is a regulator of DREF (BELLOSTA AND GALLANT 2010) in S2 cells, ovaries, and imaginal discs (THAO *et al.* 2008).

Trithorax-like TRL, the GAGA transcription factor GAF (Trl/GAF) is a TrxG group C2H2 zinc finger transcription factor and binds specifically to core promoter (AGIANIAN *et al.* 1999). Trl/GAF recruits Polycomb group (PcG) complex for the induction of chromatin remodeling (FARKAS *et al.* 1994; ESPINAS *et al.* 1999; BONCHUK *et al.* 2011; OGIYAMA *et al.* 2018). TrxG and Polycomb groups both are essential for chromatin mediated transcription of homeotic and developmental genes (MATHARU *et al.* 2010). In the ovaries TRL/GAF is essential for the follicular cell morphogenesis and germline cell differentiation, mutations of which cause female sterility (OGIRENKO *et al.* 2008). Trl/GAF was enriched in the distal enhancer cluster P1/P2 and the initiation sites TATA-containing P31/P32 and the oocyte element P37/P38. We argue that TRL/GAF might regulate *Myc* transcription at least from TATA promoter and the oocyte element with the involvement of PcG during development.

Cabeza, a member of the TFIID complex holds as a potential regulator of Myc: Cabeza (*caz*, *dFUS*), also known as sarcoma-associated RNA binding fly homolog (SARFH) and P19 (IMMANUEL *et al.* 1995), a member of TFIID group (hTAFII68/dCabeza) (AOYAGI AND WASSARMAN 2000) is the only *Drosophila* homolog of human TLS/FUS (BERTOLOTTI *et al.* 1996; MALLIK *et al.* 2018) and binds to ssDNA/RNA. Cabeza can integrate transcription initiation via association with the pre-initiation complex (PIC) (BERTOLOTTI *et al.* 1996; AOYAGI AND WASSARMAN 2000), and gene expression through mRNA processing (HEROLD *et al.* 2009) during development and adult life (WANG *et al.* 2011; XIA *et al.* 2012; AZUMA *et al.* 2014; SHIMAMURA *et al.* 2014; AZUMA *et al.* 2018). Abnormalities in protein function results in severe progressive neurodegenerative amyotrophic lateral sclerosis (ALS) disease (XIA *et al.* 2012; TAYLOR *et al.* 2016; MALLIK *et al.* 2018). In addition to TFIID complex, like FUS and TAF15, Cabeza belongs to the TET (TAF15-EWS-TLS) or FET (FUS-EWS-TLS) protein family (CHAU *et al.* 2016). 'N-Myc downstream regulated gene-1' (*NDRG1*) is the binding partner of TAF15, colocalizes with TAF15 to the nucleus and promotes cell proliferation (LI *et al.* 2023). Xrp1, an AT-hook binding transcription factor in *Drosophila* (BLANCO *et al.* 2020) interacts genetically with *caz*, and majority of Xrp1-interacting factors are involved in gene expression (MALLIK *et al.* 2018). Cabeza

might establish specific subcomplexes of TFIID to mediate differential activation of *Myc* expression by various classes of activators and TAFs during development and disease.

Transcription factors HmgD, Hmg-2 might regulate Myc: High mobility group transcription factors HmgD, Hmg-2 (DRAGAN *et al.* 2003), corepressor Groucho (GIAGTZOGLOU *et al.* 2003; HELMAN *et al.* 2011), and CtBP are downstream effectors of signaling pathways such as Wnt/Wg (LEVANON *et al.* 1998) and TGF-β/Dpp (DORFMAN *et al.* 2002) that converge signaling inputs on the transcription regulator complex at the promoter for the context-dependent activation or repression of target genes like *Myc* and accommodate a controlled transcription during development (BARKER *et al.* 2000; DUMAN-SCHEEL *et al.* 2004; HERRANZ *et al.* 2008).

RNA polymerase II transcription coactivator Ssb-c31a is involved in Myc transcription: The single-stranded DNA binding protein (*Ssb-c31a*) binds sequence-specific to NssBF element, a sequence of 26 nucleotide in the long terminal repeat (LTR) of the *D. melanogaster* 1731 retrotransposon and represses the promoter activity of this retrotransposon (LACOSTE *et al.* 1995). As a general transcriptional coactivator Ssb-c31a interacts with TAFs to connect upstream transcriptional activators to the general transcription machinery (INTERPRO PROJECT MEMBERS 2004), which might indicate involvement of Ssb-c31a in *Myc* regulation.

Insulator Isha was identified in association with the samples P29/P30, P37/P38, P31/P32, and P35/P36: 'Suppressor of hairy wing' Su(Hw) mRNA adaptor protein Isha (*Isha*, *CG4266*) associates physically with the *Su(Hw)* mRNA in vitro and in vivo. Isha binds to and recruits *Su(Hw)* mRNA to chromatin to the insulator protein complex and negatively regulates transcription by interacting with the RNA pol II full complex and with the pol II elongating complex (BAG *et al.* 2022). No evidence exists whether Isha is directly involved in the regulation of *Myc* transcription.

SAYP might play a crucial role in the formation of initiation complex on Myc promoter: The nuclear protein enhancer of yellow 3 (*e(y)3*, *SAYP*), a component of the SWI/SNF (Brahma) chromatin remodeling complex (CHALKLEY *et al.* 2008; BARISH *et al.* 2020) and TFIID complex activates Yolk Protein and functions as a transcriptional regulator (SHIDLOVSKII *et al.* 2005). SAYP can activate euchromatic genes on polytene chromosomes during embryogenesis and oogenesis and repress activity from heterochromatic regions

(SHIDLOVSKII *et al.* 2005). TBP has been reported to interact with the c-MYC to propagate transcription through assembly of the initiation complex at the start site (WEI *et al.* 2019). SAYP can combine Brahma with TFIID to form a coactivator supercomplex for an efficient transcription initiation (VOROBYEVA *et al.* 2009). SAYP might play a crucial role in transcription initiation from both *Myc* promoters the TATA and the DPE.

PAF1 and PAF1C associated with the P29/P30 enhancer cluster: Another transcription unit (*Atu*), ortholog of human LEO1 is part of the Cdc73/Paf1 complex and is an RNA polymerase-associated factor 1 complex (PAF1C) (CHEN *et al.* 2015; GOODMAN *et al.* 2019). PAF1C is recruited to the phosphorylated RNA pol II through the PAF1 complex and is involved in the release of proximal-promoter pause and transcription elongation (GO REFERENCE GENOME PROJECT 2011; QIU *et al.* 2012). PAF1C might interact with the DPE bound Antimeros/PAF1 to facilitate histone modifications and pol II pause release (ADELMAN *et al.* 2006).
14-3-3zeta protein Leonardo, Leo (*14-3-3zeta, leonardo, leo*) isoforms, resulting from alternative mRNA splicing are effectors of multiple signaling pathways, mainly Ras/MAPK cascade and cell cycle control (LI *et al.* 1997; YANO *et al.* 2006; ASHTON-BEAUCAGE *et al.* 2014). Leo is a component of RNA polymerase-associated factor 1 complex (PAF1C) and was associated with both the enhancer cluster P29/P30 and the DPE containing target P35/P36. Leo is involved in recruitment of Myc to open promoters of target genes for the transcriptional activity of *Myc* during cell growth and proliferation (GERLACH *et al.* 2017). Taken together, Antimeros/PAF1, Atu/Cdc73/PAF1, and Leo/PAF1C might contribute to proximal pause release and regulation of transcription elongation from the *Myc* DPE promoter.

Insulator proteins Mod(mdg4) and CP190 might modulate Myc transcription: Modifier of mdg4 *mod(mdg4)* functions in various processes including male meiosis, chromosome segregation (SOLTANI-BEJNOOD *et al.* 2007; MATSUI *et al.* 2011), programmed cell death (HARVEY *et al.* 1997), chromatin organization (GERASIMOVA *et al.* 1995), regulation of transcription (GO REFERENCE GENOME PROJECT 2011), and enhancer blocking via interaction with the Su (Hw) and CP190 (GERASIMOVA *et al.* 1995; SAVITSKY *et al.* 2016).
Centrosomal protein 190kD (*CP190*), a BTB/POZ domain transcription factor (BONCHUK *et al.* 2011) functions as an insulator protein (CHODAGAM *et al.* 2005). CP190 is involved in the establishment of heterochromatic genomic boundaries with enhancer-blocking effects (CUARTERO *et al.* 2014) through physical interaction with the DNA-binding proteins such as CTCF, Su(Hw) (MOHAN *et al.* 2007), and Mod (mdg4) (PAI *et al.* 2004;

CAPELSON *et al.* 2010). The insulator activity of CP190/CTCF/Su(Hw) links homeotic gene regulation to body patterning during development (MOHAN *et al.* 2007). The function of gypsy insulator complex might imply the regulation of *Myc* transcription during development.

Protein ROW might be involved in the regulation of Myc transcription: The putative zinc finger transcription factor 'Relative Of Woc' ROW (*row*) plays an essential role in expression of chromatin protein HP1c, a component of chromatin with an essential role in gene expression. For ROW binding to methylated H3 and chromatin and regulation of gene expression, HP1c requires both the Relative of Woc' Row and the 'Without Children' WOC proteins. Through an autoregulatory transcriptional feedback loop (FONT-BURGADA *et al.* 2008; ABEL *et al.* 2009) HP1c can remodel chromatin and fine tune pol II transcription. Myc has a global impact on chromatin structural changes within multiple genomic loci via recruitment of chromatin modifying complexes during transcription (KNOEPFLER *et al.* 2006), which would indicate a putative role for ROW and WOC in regulating *Myc* transcription.

Histone deacetylase Bin1/SAP18 recruits the Sin3A/HDAC1 repressor complex to the promoters for silencing of certain genes: Bicoid interacting protein 1 Bin1/SAP18 (*Bin1, SAP18*), the histone deacetylase complex subunit SAP18 facilitates formation of the Sin3A-HDAC1 corepressor complex (MATYASH *et al.* 2009) to silence transcription of Bicoid target gene at the tip of the embryo during germband retraction (ZHU *et al.* 2001). SAP18 interacts with GAGA factor (GAF), and SAP18/GAF associates with the Polycomb group (PcG) response elements (PREs) at the loci of silenced homeotic genes of bithorax complex to activate transcription (ESPINAS *et al.* 2000). SAP18-mSin3-Su(fu) repressor complex inhibits the Hedgehog signaling by repression of Gli-mediated transcription and induction of cell fate commitment and morphogenesis (CHENG AND BISHOP 2002). MYC can recruit Sin3A-HDAC1 protein complex to the promoters of certain target genes such as cell cycle inhibitor p21 to silence these genes (LIU *et al.* 2023a). SAP18 function might be required for the regulation of *Myc* transcription during cell proliferation and differentiation during development.

Chromatin assembly factor 1, p55 (Caf1-55, p55, Caf1, NURF, NURF55, NURF-55) with roles in replication and transcription: Caf1-55 (Nurf55/p55) was compounded by all the tested Myc-CRMs. *Drosophila* Caf-1 (*dCaf-1*), containing p55 as main component and part of multiprotein complex is involved in DNA replication, chromatin assembly and histone modification (TYLER *et al.* 1996). Caf-1/p55 complex is

involved in assembly of nucleosomes onto newly synthesized DNA, and in collaboration with the histone deacetylase complex Sin3 induces H3K27me3 histone marks on chromatin during replication and transcription and contributes to regulation of transcriptional patterning during development (TYLER *et al.* 1996; SPAIN *et al.* 2010). Caf-1 binds to transcription *cis*-regulatory regions and is involved in cell cycle regulation via replication-coupled histone modification and gene regulation (YAO *et al.* 2018b). Through association with H3 and H4 and Polycomb Repressive Complex 2 (PRC2) contributes to cell survival, segment identity and tumor suppression (ANDERSON *et al.* 2011; NOWAK *et al.* 2011). Caf1-55 was associated with all the tested Myc-CRMs suggesting a role for Caf1-55 in epigenetic regulation of *Myc* transcription during development and disease.

SWI and MRG15 are potential regulators of Myc expression in different biological settings: SWI (*Iswi*), an ATPase component of the NURF complex (BADENHORST *et al.* 2005) and part of several other chromatin remodeling complexes (CORONA *et al.* 2000; EBERHARTER *et al.* 2001) plays a crucial role during pol II transcription via induction of changes to chromatin structure, histone octamer sliding, and replication-dependent chromatin assembly (ITO *et al.* 1997; VARGA-WEISZ *et al.* 1997; HAMICHE *et al.* 1999; ITO *et al.* 1999; EBERHARTER *et al.* 2001; LANGST AND BECKER 2001; BURGIO *et al.* 2008; EMELYANOV *et al.* 2010). NURF complex interacts with the *c-MYC* promoter to modulate *c-MYC* transcription during hematopoietic stem cell fate commitment and differentiation (XIA *et al.* 2014), and misexpression of *c-MYC* in many cancer types (ZHAN *et al.* 2019). In *Drosophila* components of NURF complex, NURF-38 and NURF-55 interact with Myc/Max heterodimers to regulate growth through insulin receptor and mTOR signaling (LIU *et al.* 2020).
The MORF-related gene 15 (*MRG15*) belongs to the Tip60/NuA4-HAT chromatin remodeling complex and is involved in acetylation of phosphorylated H2AV and exchange with the unmodified form of H2AV at the DNA lesions (KUSCH *et al.* 2004). MRG15 assists repair-dependent chromatin remodeling (KUSCH *et al.* 2004), positive regulation of gene expression, and transcription (INTERPRO PROJECT MEMBERS 2004; HUANG *et al.* 2017). The Myc and c-MYC network are substrates for Tip60/NuA4 within different biological settings such as neuroblast differentiation and establishment of cell polarity, regulation of cell cycle genes and replication, and stem cell self-renewal (PATEL *et al.* 2004; RAVENS *et al.* 2015; RUST *et al.* 2018; ZHAO *et al.* 2018) suggesting that MRG15 might regulate *Myc* expression in complex with Tip60/NuA4.

Snr1 might be involved in in the regulation of Myc expression: (Snr1, BAP45) functions as a tumor suppressor gene during S phase of cell cycle by suppressing Myc target genes such as CycE (BRUMBY *et al.*

2002). By binding on SET domain of proteins and methylation of lysine residue on histone tails Snr1 maintains expression of a subset of genes important for cell differentiation and tissue patterning while suppressing other factors such as Myc (MARENDA *et al.* 2003; MARMORSTEIN 2003; TERRIENTE-FELIX AND DE CELIS 2009; CURTIS *et al.* 2011; KOE *et al.* 2014)

Bap111 is a potential regulator of Myc transcription from multiple initiation sites: The DNA binding Brahma associated protein 111kD (*Bap111, BAF57*) is a component of the Brahma Associated proteins complex (KAL *et al.* 2000; MOHRMANN *et al.* 2004; CHALKLEY *et al.* 2008; BARISH *et al.* 2020; MASHTALIR *et al.* 2020), Polybromo-containing proteins complex, and High Mobility Group (HMG) box transcription factors (PAPOULAS *et al.* 2001). Bap111 is involved in chromatin remodeling and induction of transcriptional bursting at the promoters of developmental genes (PAPOULAS *et al.* 2001) such as *Myc* via integration of information from different signaling pathways (COLLINS AND TREISMAN 2000; MARENDA *et al.* 2003; LUO *et al.* 2011; VINAYAGAM *et al.* 2013; SONG *et al.* 2017).

BAP155/Moira and SPT20 regulate Myc TRanscription from multiple sites: Brahma-associated protein 155 kDa BAP155/Moira, a component of the SWI/SNF (Brahma) chromatin remodeling complex and trithorax group (TrxG) (CROSBY *et al.* 1999; MOLLER *et al.* 2005). BAP155 plays a crucial role in transcriptional regulation of *Drosophila* and human (*Myc*, *MYC*) as the key targets during embryonic development and disease (SHI *et al.* 2013; GUO *et al.* 2022; CHAMBERS *et al.* 2023). In *Drosophila* BAP155 is involved in wing disc patterning (TERRIENTE-FELIX AND DE CELIS 2009; CURTIS *et al.* 2011), negative regulation of neuroblast proliferation (JANSSENS *et al.* 2014), and negative regulation of G1/S cell cycle transition via downregulation of S phase Cyclin E (BRUMBY *et al.* 2002).

SPT20 (*Spt20*) is a bifunctional component of the SAGA complex with involvement in histone acetylation and chromatin remodeling and activation of pol II transcription (GENE ONTOLOGY CURATORS 2002; INTERPRO PROJECT MEMBERS 2004; GO REFERENCE GENOME PROJECT 2011). SPT20/SAGA contributes to tissue-specific protein degradation during patterning via ubiquitin protease activity (WEAKE *et al.* 2011) and differential transcription regulation in interaction with TBP and tissue-specific transcription factors (WEAKE *et al.* 2009). SPT20 was associated with the upstream enhancer cluster P1/P2, the downstream intronic enhancer P29/P30, and the TATA containing P31/P32 Myc-CRMs suggesting a putative role for SPT20 regulating *Myc* transcription.

Histone methyltransferase Set1/Ash2 is directly involved in the regulation of Myc transcription: The DNA binding and transcriptional coregulator Ash2 enzyme (PEREZ-LLUCH *et al.* 2011) (absent, small, or homeotic discs 2) (*ash2*) is a member of the histone methyltransferase (HMT) complex and trithorax-group (TrxG) gene family. Ash2 plays an essential role in H3K4 trimethylation (H3K4me3) via interaction with the Sin3A/HCF complex to remodel chromatin and regulate gene expression, partly in response to ecdysone and EGFR signaling (LAJEUNESSE AND SHEARN 1995; ANGULO *et al.* 2004; BELTRAN *et al.* 2007; CARBONELL *et al.* 2013). Ash2 has been reported to contribute to regulation of developmental genes such as *MYC* and has been found in protein complexes containing MYC (LUSCHER-FIRZLAFF *et al.* 2008; WAN *et al.* 2013). In a protein interaction experiment Ash2 coimmunoprecipitated with (FUSE binding protein) (FBP1, FUBP1) both of which are the part of SET1 (COMPASS) multiprotein complex and FUBP1 has been shown to interact with the far upstream element of *MYC* and modulate *MYC* regulation (DUNCAN *et al.* 1994; ZAMAN *et al.* 2014).

Here, we identified an array of chromatin remodeling factors that converge on *Myc cis*-elements to control *Myc* regulation. Myc and its cofactors are important modifiers of chromatin architecture during cell growth (MARINHO *et al.* 2011). Chromatin remodeling processes, genomic architectural changes, and *Myc* expression are tightly intertwined during development and disease.

XIV. Factors regulating *Myc* from TATA promoter

Factors associated with the TATA promoter belong to a wide array of groups of protein complexes with vital functions governing different aspects of chromatin dynamics, pol II function, trans-actors and RNA processing candidates. Proteins RPB1, RPB2, RPB3, RPB5, RPB7, and RPB8 constituted RNA pol II complex. Subunits of the TFIID/MED complexes comprised TAF5, TAF6, TAF12, MED4, MED11, MED13/Skuld (TRAP), MED17, and MED25. Associates of the transcription factor TFIIH consisted of Cyclin H (*CycH*) and dPWP. Further factors important for transcription included Cabeza/dFUS, *SAYP*, Trf2, Bin1/SAP18, HDAC1, Snr 1, mod(mdg4), CP190, Isha, SWI, MRG15, Set1/Ash2, Bap111, TRL, Snr1, SPT20, and BAP155/Moira.

Basal transcription machinery regulating Myc from TATA promoter: The largest subunit of RNA pol II RPB1, important for initiation (ZEHRING *et al.* 1988; BRICKEY AND GREENLEAF 1995; INTERPRO PROJECT MEMBERS 2004) and elongation (BRICKEY AND GREENLEAF 1995), together with the second largest component

Polr2B essential for transcriptional activity (FALKENBURG *et al.* 1987; AOYAGI AND WASSARMAN 2000) of nearly all start sites (GREENLEAF 1983; HAMILTON *et al.* 1993) confer stability to pol II complex.

Rpb3 is required for the expression of heat-induced *Hsp70* gene (YAO *et al.* 2007). Bag1, a downstream target of *Myc* together with the cofactor Hsp70 builds the Bag1/Hsp70 chaperone complex to counteract Myc apoptotic function and drive cell cycle (GENNARO *et al.* 2019) suggesting an evidence for the composition of RNA pol II components linking *Myc* control to a multitude of Myc cofactors to regulate cellular processes.

RPB7 interacts with open chromatin and by binding to ssDNA/RNA connects transcription termination to translation initiation (GO REFERENCE GENOME PROJECT 2011) and RNA processing.

The composition of the identified subunits of RNA pol II complex show that RNA pol II enzyme can be formed on the *Myc* TATA promoter in a context dependent manner.

MED 24, compounded with the upstream enhancer cluster P1/P2 might be recruited to MED4; MED11, MED13/Skuld, MED17, and MED25 on TATA promoter for a proper response to *Myc* regulation during development. MED13/Skuld (TRAP) is one of the four soluble subunits of mediator complex (Kto/Med12, Skd/Med13, Cdk8, CycC), Cdk8-CycC and MED12-MED13 work as pairs during cell growth and tissue patterning (LONCLE *et al.* 2007).

TAF5, TAF6, and TAF12 can potentially recruit further components to the TFIID complex. Depending on the composition of the TFIID, mediator, and RNA pol II complexes different pre-imitation (PIC) complexes take form for the spatial-temporal patterning of *Myc* from the TATA promoter.

We argue that a firm and robust RNA pol II complex can potentially be shaped on the *Myc* TATA promoter endowing pre-initiation complex stability (Figure 15).

TAF-TBP and TAF-Trf2 regulating Myc from the TATA promoter: Transcription factor MYC functions as a differential regulator of transcription of most target genes to control growth and proliferation and activate developmental programs (KRESS *et al.* 2015; ZAYTSEVA AND QUINN 2017). Similarly, *Myc* patterning might be under control of differential regulatory switches during cell division and differentiation (KRESS *et al.* 2015). During neuronal stem cell renewal and differentiation TAF-TBP and TAF-Trf2 both function in *Drosophila* and the target genes express different transcription factors and RNA binding proteins (NEVES AND EISENMAN 2019). During spermatogenesis TAF7L paralogous to TAF7 interacts with Trf2 to regulate expression of postmeiotic genes in a tissue-specific manner (ZHOU *et al.* 2013; HERRERA *et al.* 2014). Since TAFs are a priori parts of TFIID and SAGA complexes and our proteome discovery showed signatures for TAFs and Trf2 on TATA

promoter, we conclude that the selective regulatory programs TAF-TBP and TAF-Trf2 might direct *Myc* transcription from the TATA promoter.

Promoter melting, initiation, and elongation on the Myc TATA promoter: Cyclin H (*CycH*), enriched on the TATA-containing P31/P32 Myc-CRM, comprises part of the Cdk-activating kinase (CAK) subunit of the TFIIH core subcomplex. The heterotrimeric TFIIH subcomplex Cdk7-CycH-Mat1 plays a fundamental role during transcription and cell cycle control (PEISSERT *et al.* 2020). TFIIH phosphorylation of RNA pol II-CTD enhances mediator dissociation from pre-initiation complex (PIC) and pol II promoter escape (LAROCHELLE *et al.* 2001; WONG *et al.* 2014). The cell cycle control, DNA excision repair, and transcriptional activities of TFIIH might assign a role for TFIIH in regulating *Myc* transcription from TATA promoter.

Suppressor of stem-loop mutation Ssl1 (p44/Ssl1) is part of TFIIH core subcomplex and p44/Ssl1 associates with the XPD subunit of TFIIH to increase the helicase activity of XPD and facilitate promoter clearance (WANG *et al.* 1995). The P1/P2 enhancer associated with the p44/Ssl1 might fold onto TATA promoter and contribute to promoter clearance at the pre-initiation complex during *Myc* transcription.

Enhancers of yellow 3 (*e(y)3), SAYP*), a component of SWI/SNF (Brahma) complex and a multidomain-containing coactivator can combine Brahma chromatin remodeling function with the TAF9-containing TFIID complex to form a coactivator supercomplex for an efficient transcription initiation during early development (SHIDLOVSKII *et al.* 2005).

SPT6, pol II elongation factor and histone H3 chaperone (ADELMAN *et al.* 2006) recruits SPT5 component of DSIF elongation factor to transcription regulatory apparatus and facilitates Cdk7-dependent loading onto RNA pol II complex (BALUAPURI *et al.* 2019). MYC function is required during pol II pause and pause release and processivity of elongation of literally all transcribed genes (BALUAPURI *et al.* 2019). MYC binds directly to SPT5 and engage with promoters to load SPT5 onto RNA pol II in a Cdk7-dependent manner to facilitate processivity of transcription elongation (BALUAPURI *et al.* 2019). Conversely, in MYC overexpressing tumor cells MYC sequesters SPT5 into inactive protein complexes to block transcription of anti-proliferative genes such as P21 (BALUAPURI *et al.* 2019). SPT6 was enriched in all the tested *Myc* samples, however, SPT5 came up on the original list but was not enriched (see Table 3 in File S3). We conclude that SPT6 might interact with the elongation complex on the *Myc* promoter and recruit SPT5 for a fast and processive elongation of *Myc* transcripts.

Topoisomerase type II (Top2) is an important enzyme for the removal of supercoils that emerge during meiotic and mitotic crossovers and transcription (LEE *et al.* 1989; STRICK *et al.* 2000). Top2 accommodates the passage of DNA through transient double-strand break repair by unwinding and relaxing DNA to detangle chromosomes (STRICK *et al.* 2000). Collaboration between MYC and Top2 has well been documented during development and disease (DANG 2012; MATIAS-BARRIOS AND DONG 2023). Top2 and chromatin condensation factor Barren interact *in vivo* with Polycomb group (PcG) and bind to PcG response elements and bithorax binding sites to control epigenetic expression of homeotic genes (LUPO *et al.* 2001). Top2 and Barren were enriched only in the upstream enhancer P1/P2 sample (see Table 3 in File S3, Table S8) which might indicate involvement of Top2 in epigenetic control of *Myc* expression from TATA promoter via interaction with Barren and components of PcG.

Chromatin repressive complex SAP18/HDAC1 associated with the *Myc cis*-elements, has context-dependent vital roles during embryonic body patterning. SAP18 interacts with Kruppel and recruits enhancer-specific Sin3A/HDAC1 repressor complex (MATYASH *et al.* 2009) to repress transcription of certain genes such as Bicoid during embryonic germband extension (ZHU *et al.* 2001). SAP18/GAF associates with Polycomb response elements (PREs) to activate transcription of silenced homeotic genes (ESPINAS *et al.* 2000). SAP18-mSin3-Su(fu) repressor complex inhibits Gli-mediated transcription during morphogenesis (CHENG AND BISHOP 2002). MYC can recruit Sin3A-HDAC1 complex to the promoters of certain targets to inhibit transcription (LIU *et al.* 2023a), suggesting a potential role for SAP18-Sin3A-HDAC1 in the regulation of *Myc* transcription during development.

Set1/Ash2, part of COMPASS complex is involved in H3K4 mono- and dimethylation providing chromatin accessibility to transcription factors, adjustment of transcription fidelity, and enhancement of splicing events (COLLINS *et al.* 2019; ZHU *et al.* 2024). Mammalian far upstream protein FBP1 interacts with Set1/Ash2 to activate transcription and DNA repair (ZAMAN *et al.* 2014). Psi, *Drosophila* sole homolog of mammalian FBP1-3 associates with the pol II complex and *Myc* intergenic sites to activate transcription during cell growth and morphogenesis, accordingly (GUO *et al.* 2016). FBP1 and Psi both bind to ssDNA suggesting a potential role for Set1/Ash2 in *Myc* regulation through activators such as Psi.

Half pint (*hfp*), ssDNA/RNA binding protein and *Drosophila* homolog of Fuse Interacting Repressor (FIR) associates with the transcription machinery and Hfp/FIR counteracts Psi function to inactivate *Myc* (GUO *et al.* 2016) and enhance (alternative) transcripts processing. Ash2, Psi, and Hfp were complexed by all the investigated Myc-CRMs.

Termination transcription initiated from the Myc TATA promoter: Cpsf160, AAUAAA recognition factor was associated with the three upstream enhancer clusters P1/P2 and plays a key role in RNA 3'-end formation (GLOVER-CUTTER *et al.* 2008) and pre-mRNA polyadenylation via interaction with the poly(A) polymerase complex (GLOVER-CUTTER *et al.* 2008). Cpsf160 and partners such as CPSF6, CPSF73, and CPSF100 colocalize with and bind to the *c-MYC* mRNA during 3'-formation and mRNA degradation by the FBW7 proteasomal pathway (GLOVER-CUTTER *et al.* 2008; TRAN *et al.* 2014; SIM *et al.* 2024). Cpsf160 might be involved in transcription termination from the TATA promoter and might polyadenylate *Myc* nascent transcripts.

Translation factor Poly(A) binding protein PABP, complexed by all the *Myc cis*-elements couples transcription termination and pre-mRNA processing to translation initiation, predominantly during oogenesis and maternal to zygotic (MZT) transition (MOUNT AND SALZ 2000; CLOUSE *et al.* 2008; HEROLD *et al.* 2009; WANG *et al.* 2017). PABP interacts with the quadruplex G4 DNA binding factors during transcription and alternative polyadenylation (LI *et al.* 2020), and the MYC promoter contains G4 structures with roles during replication, transcription, and polyadenylation (SIDDIQUI-JAIN *et al.* 2002). PABP might be important for transcription termination and alternative polyadenylation of *Myc* transcripts.

XV. Factors regulating *Myc* transcription from the DPE promoter

We were interested in isolating factors binding to the downstream promoter element DPE, contained in the Myc-CRM P35/P36. Reporter studies showed that the enhancer P29/P30 proximal to DPE promoter is necessary for the DPE promoter to drive *Myc-lacZ* patterning (Figure 2A). We identified a subset of factors associated with both *cis*-elements that might initiate *Myc* transcription from the DPE promoter.

Pre-initiation complex (PIC) and initiation at the Myc DPE promoter: RNA pol II subunits identified with the TATA promoter were also present in DPE-containing Myc-CRM P35/P36 sample, providing evidence for the assembly of a stable RNA pol II complex on the DPE promoter (Figure 15).

TFIID subunits compromised Taf5 and Taf12, and mediator subunits were composed of MED4, MED11, MED17, and MED25. Taf6 and MED13/Skuld (TRAP), compounded by the TATA-containing P31/P32 promoter were absent from the DPE sample. Other components of the mediator accessory subcomplex (MED12/Kto, Cdk8, CycC) were also absent from the DPE. The mediator tail module

Med12.Med13.Cdk8.CycC functions as a repressor; MED12/Kto and MED13/Skuld repress Hox gene *Ubx* during developmental switches, a phenotype similar to PcG silencing of certain Hox genes (GAYTAN DE AYALA ALONSO *et al.* 2007). This result suggests that repression of *Myc* transcription from the DPE promoter might not occur via mediator inhibitory subcomplex.

Myc downstream enhancer cluster P29/P30 might round off bursting from the DPE promoter: We identified Taf6 and TFIIEα associated with the enhancer cluster P29/P30 proximal to DPE. In humans and yeast, TAF6 has been reported to recruit TAF9 to PIC, and in flies to TFIID complex (WEAKE *et al.* 2009).

Transcription factor TFIIEα contacts TAF6 (OHKUMA *et al.* 1991), and TFIIEα/TFIIEβ interacts with RNA pol II and GTFs *inv vitro* (WANG *et al.* 1997). TFIIE is essential for PIC formation, activation of PIC via promoter melting, and initiation of elongation (OKAMOTO *et al.* 1998). TFIIEα can either associate with TFIIH and enhance TFIIH function, or function independent from TFIIH (OKAMOTO *et al.* 1998). TFIIE was shown to be among the class II transcription factors associating with pol II and other GTFs on the *c-MYC* promoter to trigger initiation *in vitro* (SZENTIRMAY AND SAWADOGO 1991).

From the TFIIH complex we identified Cyclin H, a component of the kinase subcomplex that is required for pol II-CTD phosphorylation, PIC destabilization and pol II promoter escape (LAROCHELLE *et al.* 2001; WONG *et al.* 2014). Another identified TFIIH subunit, p44/Ssl1 together with the XPD subunit elevate TFIIH helicase activity and assist pol II promoter clearance (WANG *et al.* 1995).

We propose that TAF6 associated with the enhancer cluster P29/P30 might complement the GTFs and pol II complex bound to the DPE to form a stable PIC. The factors TFIIEα, Cdk7-CycH/TFIIH, and Ssl1/TFIIH compounded by the enhancer P29/P30 could potentially contribute to promoter melting, pol II CTD phosphorylation, dissociation of MED/TFIID complex, activation of PIC, pol II promoter scape and transcription initiation.

Finally, we pulled down the general transcription factor TRF2 by the enhancer P29/P30. TRF2 is a core promoter recognition factor and part of TFIID complex (AOYAGI AND WASSARMAN 2000; ANDERSEN *et al.* 2017). Potentially, a TAF-TRF2 association with the DPE promoter is imaginable for an initiation-competent RNA pol II complex.

Transcription elongation and termination from the DPE promoter: In addition to TFIIEα and the subunits of TFIIH, we identified a few factors that might contribute to coordination of transcription steps from the DPE

promoter. Cabeza/dFUS (hTAFII68/FUS), a member of TFIID group (AOYAGI AND WASSARMAN 2000) and a ssDNA/RNA binding protein can facilitate transcription initiation via interaction with the pre-initiation complex (PIC) (BERTOLOTTI *et al.* 1996; AOYAGI AND WASSARMAN 2000). Cabeza was compounded by all the Myc-CRMs.

Enhancers of yellow 3 (*e(y)3), SAYP*), a component of SWI/SNF (Brahma) complex and a multidomain-containing coactivator can couple chromatin remodeling with the TAF9-containing TFIID complex to form a coactivator supercomplex for an efficient transcription initiation during early development (SHIDLOVSKII *et al.* 2005). SAYP was compounded by the TATA and DPE promoter.

PAF1 (Antimeros) interacts with basal transcription machinery and sequence-specific transcription factors, recruits other chromatin factors like SPT6 and FACT and is involved in H3K4 trimethylation and control of pol II pause release during elongation (ADELMAN *et al.* 2006; GO REFERENCE GENOME PROJECT 2011). PAF1 was associated with the DPE promoter and might regulate *Myc* transcription from the DPE promoter.

Spt6, the central factor of activated RNA pol II elongation complex interacts with all four histones and can recruit SPT5 a member of DSIF, and together with PAF1 contribute to successful processivity of pol II elongation (MILLER *et al.* 2023). Spt6 was associated with all the tested Myc-CRMs suggesting a crucial role for Spt6 in regulating *Myc* transcription from multiple sites. PAF1 was compounded by the TATA and DPE promoter.

Suppressor of sable *Su(sable)*, CPSF6, and Poly(A)-binding protein PABP (*pAbp*) were associated with the DPE and the enhancer P29/P30, and CPSF160 was compounded by the enhancer P29/P30. Wdr82 and CPSF100 came up on the original list but were not enriched (see Table 3 in File S3). RNA during protein Su(sable) promotes transcription termination of aberrant RNAs (VOELKER *et al.* 1991; TURNAGE *et al.* 2000; KUAN *et al.* 2004; KUAN *et al.* 2009; BREWER-JENSEN *et al.* 2016). Su(sable) interacts with Wdr82 to promote transcription termination of heat shock inducible genes containing repetitive sequences and degradation of RNAs via exosomes (BREWER-JENSEN *et al.* 2016). Wdr82 is one of the unique components of Compass complex (Wdr82-Set1/Compass) in *Drosophila* and enables methylation of H3K4 to activate transcription (SHILATIFARD 2012). Transcription regulation by MYC is dependent on MYC interaction with transcription regulator complexes and epigenetic factors (GOUW *et al.* 2019). MYC and Wdr82 interact physically, and the complex has been found in certain transcriptional complexes at the promoter of MYC targets (MORELLI *et al.* 2023), and references therein. Su(sable) and Wdr82 might be involved in regulating *Myc* transcription termination and transition to RNA processing by CPSFs and PABP complexes.

Transcription factors and other coregulators complexed by the enhancer cluster P29/P30: CG10543, compounded only by P29/P30 and a pol II-specific C2H2 Zinc finger transcription factor binds to a sequence containing a GGGA core in the DNA major groove (WOLFE *et al.* 2000). CG10543 can activate or repress transcription (GENE ONTOLOGY CURATORS 2002; INTERPRO PROJECT MEMBERS 2004) and is essential for gravitaxis in *Drosophila* (ARMSTRONG *et al.* 2006). *Myc* target P29/P30 contains a Hairless consensus binding site within which the core sequence GGGA resides (Table S1.3). Hairless itself was compounded by both the enhancer P29/P30 and the DPE-containing sample P35/P36. CG10543 might be a *Myc* regulator possibly via modulation of Notch signaling.

Putzig (*pzg*), a sequence-specific transcription factor (YAO *et al.* 2018b) is involved in chromatin activation and transcription of targets of signaling pathways including Notch, Ecdysone and JAK/STAT in certain proliferating cells (EGGERT *et al.* 2004; KUGLER *et al.* 2011). For the activation of replication-related genes Putzig/Trf2/DREF binds to the promoters of these genes (KUGLER AND NAGEL 2007). Trf2/DREF is an important transcriptional variant in regulating transcription of cell cycle components such as Myc and E2F, thus playing an important role in controlling cell division and tissue size (HYUN *et al.* 2005). Putzig/Trf2/DREF might regulate *Myc*, possibly during G1/S phase. A repressor complex consisting of CG10543/Hairless, explained above and Groucho/CtBP also compounded by P29/P30 enhancer and the DPE might counteract Notch activation to control *Myc* transcription during differentiation.

Histone H2A variant is present in about 25% of nucleosomes (INTERPRO PROJECT MEMBERS 2004). At the DNA lesion sites phosphorylated His2Av is acetylated by the dTip60 chromatin remodeling complex and exchanged with the unmodified form during DNA repair (KUSCH *et al.* 2004). His2Av is required for *Drosophila* adult stem cell differentiation and self-renewal (MORILLO PRADO *et al.* 2013), and histone variants have been implicated to occupy transcribing regions of the genome in both plants and animals (CONERLY *et al.* 2010). In humans, the histone H2A variant H2AZ has been shown to occupy transcription start sites (TSSs); here we identified His2Av compounded by the *Myc* P1/P2 and P29/P30 enhancer clusters. In the case of B-cell lymphomagenesis H2AZ facilitates Myc-induced transcriptional transformation towards tumor cell development (CONERLY *et al.* 2010). In *Drosophila* His2Av was shown to positively regulate IMD signaling to modulate immune response (TANG *et al.* 2021).

Turandot Z (*Totz*), a secreted peptide that is involved in fly stress response (EKENGREN AND HULTMARK 2001) was compounded by the DPE promoter. We show the evidence that His2Av, compounded by the enhancer

P29/P30 might collaborate with the TotZ via interaction with the transcription complex on the DPE promoter to regulate *Myc* expression in response to different stress-induced stimuli.

The Activator Protein-4/Cropped is a sequence-specific pol II transcription factor (KING-JONES *et al.* 1999) and is involved in growth regulation, cell proliferation, and organ size determination, and is modulated by the Sin3A chromatin modifier during larval somatic muscle development (DOBI *et al.* 2014). *AP-4* is a downstream direct target of MYC, and the MYC binding to the E-box CACGTG in the first intron of the *AP-4* gene leads to induction of mitotic cell cycle reentry and maintenance of cell proliferation in a progenitor-like manner (JUNG *et al.* 2008). AP-4 overexpression has been reported in many cancer types such as MYCN-expressing neuroblastoma cells and concomitant expression with MYC in colorectal cancer (JACKSTADT *et al.* 2013; XUE *et al.* 2016). AP-4 might play a role in the regulation of *Myc* transcription.

XVI. Enhancer cluster P37/P38 might transcribe eRNAs in early embryos and ovary

The minimal sequences from the proximal 5'-UTR, retaining the P37/P38 enhancer cluster recapitulated the *Myc* endogenic expression in embryos and ovary in all the truncations (Figure 1A-D and F). We decided to isolate all the proteins essential for early development during oogenesis and embryogenesis and the factors that confer the oocyte element the supremacy as key developmental switch to express *Myc* transcripts during early development. Factors enriched on the P37/P38 target play predominant role in early embryos, oocyte maturation, and cell fate induction. Transcription initiation from the *Myc* super enhancer oocyte element adds another layer to differential bursting from multiple sites.

Formation of pre-initiation/initiation complex on the enhancer cluster P37/P38: RPB2 is the second largest subunit with 5'-3' polymerase activity and is present in all pol II complexes (FALKENBURG *et al.* 1987; AOYAGI AND WASSARMAN 2000). RPB3 localizes to the cytoplasm, nucleus and polytene chromosomes (YAO *et al.* 2007; PANKOTAI *et al.* 2010) and is involved in transcription by RNA pol II (INTERPRO PROJECT MEMBERS 2004; YAO *et al.* 2007). RPB5 and RPB8 both required by the RNA pol I, pol II, and pol III contribute to synthesis of mRNAs, rRNAs, and tRNAs (AOYAGI AND WASSARMAN 2000; GENE ONTOLOGY CURATORS 2002; GO REFERENCE GENOME PROJECT 2011) RPB7 not only enables pol II transcription (AOYAGI AND WASSARMAN 2000) but also binds to mRNA and can initiate translation (GO REFERENCE GENOME PROJECT 2011).

Like the TATA and DPE initiation sites, we identified Taf5, Taf6, and Taf12 compounded by the enhancer cluster P3//P38.

Mediator subunits MED11, MED13/Skuld, MED17, and MED25 were associated with P37/P38. Mediator complex serves as a hub for linking pol II preinitiation complex at the start site to transcription factors to modulate gene regulation (GTFs) (GU *et al.* 2002; JANODY *et al.* 2003; PARK *et al.* 2003a). MED13/Skuld and Sin3A as global transcription regulators are required for cell proliferation, differentiation and tissue patterning during development (DOBI *et al.* 2014). Pygopus, a nuclear key component of the Wnt/Wg pathway interacts with the MED12-MED13 to associate with the pre-initiation complex and convey the signaling stimuli to RNA pol II complex and trigger expression of Wnt/Wg targets genes (CARRERA *et al.* 2008). In other developmental settings function of MED13 and MED17 are important for the induction of cell fate commitment, segment identity (BOUBE *et al.* 2000), and tissue patterning (LONCLE *et al.* 2007; BEJARANO *et al.* 2008; CARRERA *et al.* 2008). MED25 plays a role in nervous system development (KOIZUMI *et al.* 2007) and immune response through NF-kappa-β pathways (VALANNE *et al.* 2010).

The mRNA binding protein dFUS/Cabeza (*dFUS, caz*) (LASKO 2000), a member of TFIID class of proteins is involved in transcription initiation (AOYAGI AND WASSARMAN 2000), transcription termination and mRNA processing (HEROLD *et al.* 2009).

Chromatin remodelers and other transcriptional regulators associated with P37/P38: Nuclear Zinc Finger protein Simjang (*simj*), a highly conserved repressor constitutes the regulatory component p66 of the Mi-2/NURD complex (KON *et al.* 2005). Simjang is involved in induction of chromatin remodeling and histone deacetylation to repress transcription (KON *et al.* 2005). Simjang function is required for the induction of heart fate map (KIM *et al.* 2004) and brain synaptic development (WILLEMSEN *et al.* 2013). Both NURD and Sin3 complexes are involved in *Myc* regulation during development and disease (HARPER *et al.* 1996; ALLAND *et al.* 1997; SATOU *et al.* 2001; KADAMB *et al.* 2013; HATA *et al.* 2019) suggesting a role for Simjang/p66/NURD complex a direct role in regulating *Myc* expression during development.

Highly conserved DNA binding protein 'Barrier-to-autointegration factor' Baf is involved in chromosome condensation and organization (GO REFERENCE GENOME PROJECT 2011; NIKALAYEVICH AND OHKURA 2015). In the oocyte nucleus BAF induces karyosome formation during cell cycle (LANCASTER *et al.* 2007). Functions of NURD and phosphorylated BAF are required for inhibiting attachment of chromosomes to nuclear matrix regions and hence helping karyosome development (LANCASTER *et al.* 2007; NIKALAYEVICH AND OHKURA 2015). Baf was associated with the oocyte element and might modulate *Myc* expression during karyosome formation.

The uncharacterized protein (CG6026) functions as a transcriptional coregulator and is involved in chromatin remodeling and pol II transcription during pseudohyphal growth (GO REFERENCE GENOME PROJECT 2011). The structural features and protein motifs are unknown and the connection of CG6026 to *Myc* has not been investigated. Here we identified CG6026 in association with the oocyte element P37/P38 (see Table 3 in File S3).

Calmodulin (*Cam*), a calcium sensor is a component of the Ca^{2+}/Calmodulin signaling pathway (MARTIN AND BAYLEY 2004) with an expression peak during embryonic nervous system development (ANDRUSS *et al.* 1997). Calmodulin is involved in ovarian germline maintenance (ANDRUSS *et al.* 1997), cell movement (TOTH *et al.* 2005), and mitosis (GOSHIMA *et al.* 2007). MYC binds to Calmodulin and high levels of Calmodulin increase transcription and oncogenic activity of MYC in cell cultures (RAFFEINER *et al.* 2017). Calmodulin might be a potential regulator of Myc transcription.

Terribly reduced optic lobes (*trol*) is an extracellular matrix heparan sulfate Perlecan protein with a crucial role during fate commitment and tissue pattering by integration of multiple signaling cascades including Hedgehog (PARK *et al.* 2003b), Wingless/WNT, TGF-β (LINDNER *et al.* 2007), FGFR (PARK *et al.* 2003b; DRAGOJLOVIC-MUNTHER AND MARTINEZ-AGOSTO 2013), and EGFR (TAKEMURA AND NAKATO 2017). Trol plays a role in the maintenance of epithelial-cell polarity in ovarian follicle cells (SCHNEIDER *et al.* 2006) and epithelial morphogenesis in the egg chamber (ISABELLA AND HORNE-BADOVINAC 2016; CREST *et al.* 2017). Dally-like, a heparan sulfate proteoglycan activates Dpp pathway to antagonize Wingless signaling and control *Myc* expression during establishment of larval hematopoietic niche size and progenitor stem cell (PSC) differentiation (PENNETIER *et al.* 2012). Trol might function in a similar way to control *Myc* expression during cell differentiation.

Spaghetti Squash (*sqh*), the regulatory light chain of nonmuscle type 2 myosin (WANG *et al.* 2023) plays a role during cell cycle, cell proliferation (SOMMA *et al.* 2002; ROYOU *et al.* 2004; DEAN AND SPUDICH 2006), morphogenesis (SIMOES *et al.* 2006; SHARMA *et al.* 2018), embryonic gastrulation, syncytial cellularization (VASQUEZ *et al.* 2014; HE *et al.* 2016), and oogenesis (FULGA AND RORTH 2002; NICOLAS *et al.* 2009; SHARMA *et al.* 2018; DOERFLINGER *et al.* 2022). Based on these functions, Spaghetti Squash may play an important role as a *Myc* regulator during cell growth, proliferation, and differentiation.

Lethal-(2)-denticleless (*l(2)dtl/Cdt2*) codes for Cdt2 protein and serves as an adapter for Cul4-based E3 ubiquitin ligase modification of proteins during S phase and DNA damage repair during mitotic G2 checkpoint (GO REFERENCE GENOME PROJECT 2011). Cdt2 is required during *Drosophila* embryogenesis, male fertility and

larval development (SLOAN *et al.* 2012). Cdt2 was compounded only by the oocyte element suggesting that Cdt2 might regulate *Myc* from the oocyte element during development.

Abnormal wing disc AWD (*awd*) is a nucleoside-diphosphate kinase (LASCU *et al.* 1992) with roles in vesicle trafficking, receptor internalization (IGNESTI *et al.* 2014), and GDP to GTP conversion (MORERA *et al.* 1995). Human homolog of (*awd*) metastasis suppressor gene (*NmE1/2*) is essential for tissue patterning (DAMMAI *et al.* 2003; HSOUNA *et al.* 2010; SERAFINI *et al.* 2020). Awd shows high levels during oogenesis and plays a role in follicular epithelial cell integrity and morphogenesis by modulating the levels and spatial endocytic distribution of adherens junction components to establish and maintain egg chamber integrity (WOOLWORTH *et al.* 2009). Known *Myc*-regulating signaling pathways such as Notch and EGFR require the activity of Awd/NmE1/2 (DAMMAI *et al.* 2003; IGNESTI *et al.* 2014) suggesting that Awd might be an indirect regulator of *Myc* during oocyte development and cell differentiation.

Survival motor neuron (*Smn*) is a member of the SMN (BORG *et al.* 2015) and SMN-Gemmin2 complex (KROISS *et al.* 2008) and participates in a multi-protein interaction network (CAUCHI *et al.* 2008). SMN is an RNA binding protein with Tudor domain and is essential for oocyte maturation and viability, and higher levels of the protein are required for neuromuscular development (CHAN *et al.* 2003). A study done on culture cells (Rat-1 cells) showed that SMN induced apoptosis through activation of the *c-MYC* gene in a manner similar to Bcl-2 and IAP-2 action (VYAS *et al.* 2002). Coilin, an interactor of SMN and a member in the large SMN protein complex localizes to the nucleoplasm (HEBERT AND MATERA 2000). SMN might regulate *Myc* during oogenesis.

Post-transcriptional/-translational factors associated with the oocyte element: Cleavage and polyadenylation factor 5 (Cpsf5) and 160 (Cpsf160) enable transcription termination and mRNA processing. Both factors were associated with the oocyte element.

Upstream of N-ras *(Unr*), lncRNA binding RNA chaperone (PATALANO *et al.* 2009; MILITTI *et al.* 2014) is involved in posttranscriptional regulation of gene expression (KAKUMANI *et al.* 2020) with roles in translation (ABAZA *et al.* 2006; DUNCAN *et al.* 2006) and dosage compensation (PATALANO *et al.* 2009; MILITTI *et al.* 2014). Unr binds to the IRES sequence at the 5'-end of *c-MYC* mRNA and is involved in cap-independent translation of *c-MYC* mRNA (MITCHELL *et al.* 2001). MYC and Max suppress the expression of Unr, which is dependent on the cell's developmental stage (ANDERSON AND CATNAIGH 2015). These findings suggest that Unr might play role in translational regulation of *Drosophila Myc*.

SBDS ribosome maturation factor (*Sbds*), a nucleolar protein enables cytosolic ribosome assembly, translation, protein biogenesis, and cell growth (TAKAI *et al.* 2020). MYC proteins play an essential role in the regulation of genes important for ribosome assembly and biogenesis and translation during cell growth and proliferation (GOMEZ-ROMAN *et al.* 2003; SCHMIDT 2004; VAN RIGGELEN *et al.* 2010; PIAZZI *et al.* 2019). There might exist a role for the SBDS in regulating *Myc* expression.

The eukaryotic translation initiation factor 3 subunit j (*eIF3j*) is a conserved components of the translation initiation complex (ANDERSEN AND LEEVERS 2007). The translation initiation factor 3, eIF3 is the largest subunit and is present in all the mammalian translation initiation complexes and exhibit essential role in the formation and initiation of translation in highly proliferative cells (ASANO *et al.* 1997).

Nucleostemin 3 (*Ns3*), evolutionary conserved is a body size regulator with GTPase activity (HARTL *et al.* 2013), functions in the export of the 60S ribosomal subunit from nucleus and is a global growth controller (HARTL *et al.* 2013). Literature shows that regulation of *Myc* mRNA translation rate and mRNA and protein stability are key factors controlling *Myc* activity during development and adult life (SAMUELS *et al.* 2020; ZACARIAS-FLUCK *et al.* 2024).

Effete (*eff, UbcD1*) is an E2 ubiquitin-conjugating enzyme (RYOO *et al.* 2002; HERMAN-BACHINSKY *et al.* 2007; ASAOKA *et al.* 2016) with involvement in multiple processes. Effete is involved in ubiquitination/degradation of mitotic cyclins to regulate female germline stem cell maintenance (CHEN *et al.* 2009) and ubiquitination/proteolysis of a telomeric polypeptide during mitosis and male meiosis to maintain chromosome structure (CENCI *et al.* 1997). Effete might be involved in Myc degradation during early cell division cycles.

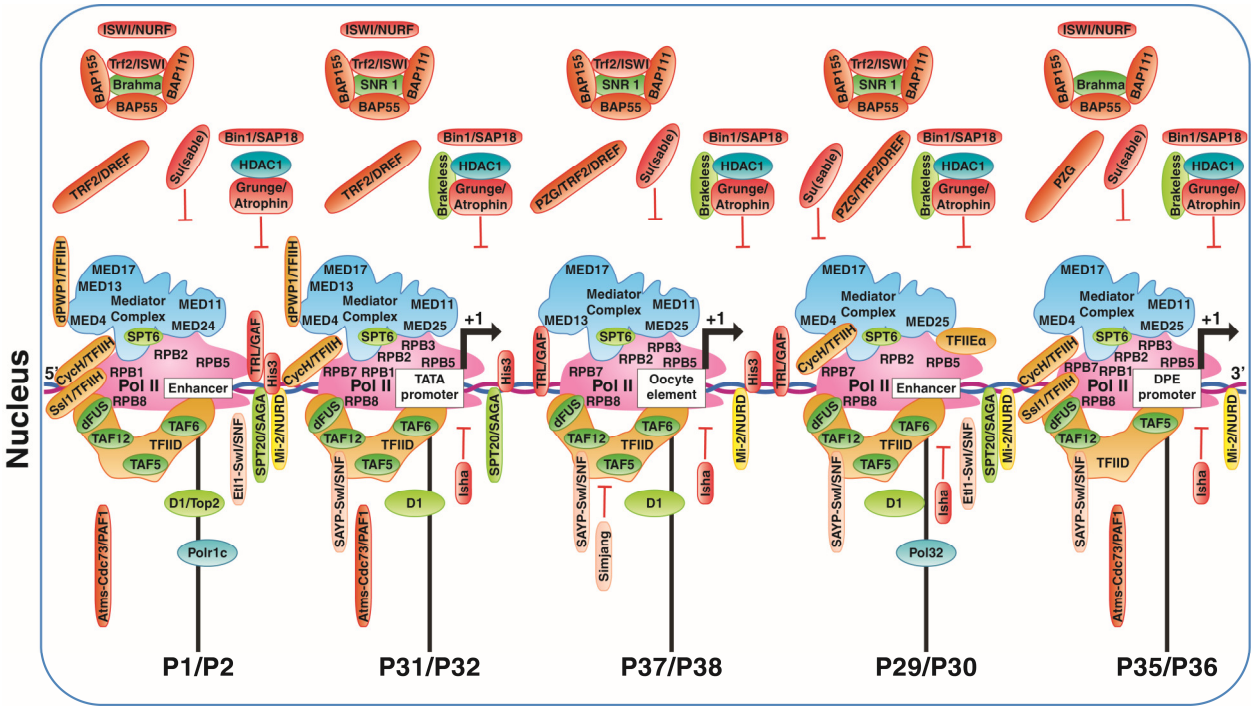


Figure 15. Core promoter and basal transcription factors, chromatin remodelers, and histone modifiers associated with the investigated Myc-CRMs. Subunits of the Mediator complex, transcription factor TFIID complex, and RNA pol II complex were at most detected on all the three P31/P32, P37/P38 ,and P35/P36 initiation sites. MED13/Skuld was found in complex with the P1/P2 enhancer cluster in addition to the start sites P31/P32 and P37/P38, and MED25 was complexed with the enhancer cluster P29/P30 in addition to the three initiation sites P31/P32, P37/P38 and P35/P36. Some of the components of the histone and chromatin modifiers, factors associated with boundary elements, and other transcription factors and cofactors are illustrated as well. Most of the factors have been explained in the Results and Discussion sections. For more information about the association of the illustrated factors with the Myc-CRMs and abundance ratio of each factor see (see Table3 in File S3, Table 7 in File S7 and Table S8).

XVII. Posttranscriptional regulation of *Myc*

A considerable number of the identified proteins belong to the factors that participate in RNA transport, ribosome biogenesis, translation, mRNA processing, posttranscriptional silencing, and protein metabolism. Examples of these factors as potential regulators of *Myc* expression will be described here (Figure 16; see also supporting information, Table 3 in File S3).

The enzymes described here might regulate different aspects of Myc posttranscriptionally: O-GlcNAc transferase 'Super sex combs' Ogt/Sxc (*Ogt/Sxc*) enables the addition of acetylglucosamine moieties to the cytoplasmic and nuclear proteins (GAMBETTA *et al.* 2009) such as members of Polycomb group, hence plays a

key role in the regulation of epigenetic gene silencing and developmental gene expression (AKAN *et al.* 2016). MYC is a direct target of Ogt, and O-GlcNAcylation stabilizes MYC protein in humans during development and disease (ITKONEN *et al.* 2013; ITKONEN *et al.* 2019; LUANPITPONG *et al.* 2021).

N-myristoyl transferase (*Nmt*) is an important protein that contributes to meristoylation of the developmental proteins (NTWASA *et al.* 1997) during cell shape changes and completion of dorsal closure (HARDEN 2002). MYC binding partner MAX is myristoylated posttranslationally prior to caspase cleavage during apoptosis, differentiation, and development (MARTIN *et al.* 2011), resulting in downregulation of MYC.

Peptidylprolyl isomerase Pin1 (*Pin1*), mammalian homolog of *Drosophila* Dodo (*dod*) facilitates rhomboid gene expression via degradation of chorion factor 2 (CF2) and induction of dorsoventral patterning of the follicular epithelial cell layer within the egg chamber (HSU *et al.* 2001). MYC is a known target of Pin1 for ubiquitination and proteasomal degradation for tight control of MYC protein stability during cell cycle transitions. Disruption of limits on MYC accumulation contributes to oncogenesis in humans (YEH *et al.* 2004; YEH *et al.* 2006; HELANDER *et al.* 2015). Further, Pin1 directly associates with MYC and through recruitment of p300 and indirect involvement of ABL protein can acetylate MYC protein which might facilitate transcriptional activity of MYC (SANCHEZ-AREVALO LOBO *et al.* 2013).

Spaghetti/RNA Pol II-associated protein 3 (*Spag/RABP3*) is a cochaperone containing TPR domain and can associate with HSP70 and HSP90 (GO REFERENCE GENOME PROJECT 2011) to mediate protein-protein interactions (DAS *et al.* 1998a), leading to the formation of multi protein complexes (D'ANDREA AND REGAN 2003) such as the assembly of the macromolecule complex with RNA pol II providing a scaffold connecting pol II to the regulatory protein complexes essential for the pol II protein complex stabilization and transcriptional regulation (RODRIGUEZ AND LLORCA 2020). In complex with ribonucleoproteins Spag/RABP3 can mediate protein folding immediately at the end of translation completion (BOULON *et al.* 2008; ECKERT *et al.* 2010). Spag/RABP3 might acts as a potential regulator of *Myc*.

Large proline-rich protein CG7546 (*CG7546*, *clone 2.45*) is part of the BAT3 complex and homolog to human BCL2-associated athanogene 6 (BAG6) (BRODERICK *et al.* 2010) (BAG cochaperone 6). BAG6 functions in the ubiquitin-dependent ERAD pathway for the degradation of cytoplasmic proteasome (GO REFERENCE GENOME PROJECT 2011) and regulation of DNA damage response and apoptosis (KRENCIUTE *et al.* 2013). MYC plays an important role in the induction of cell death (THOMPSON 1998). BAG6 might be involved in the regulation of *Myc* expression via protein degradation processes.

EGF-domain serine glucosyl-/xylol transferase Rumi (*rumi*), a Notch modulator and an ER enzyme contributes to O-linked protein modifications (ACAR *et al.* 2008; TAKEUCHI *et al.* 2011; SETHI *et al.* 2012; YU *et al.* 2016). Myc and Notch signaling interact during induction of cell fate and lineage commitment to produce progenitors of differentiation for a variety of tissue types most probably in G1 phase of cell cycle (SINGH AND DALTON 2009). Glucosylation of EGF repeats by Rumi are essential for correct folding and cleavage of Notch to activate the target genes (YU *et al.* 2016).

Purity of essence Poe (*poe*) exhibits calmodulin-binding and E3 ubiquitin ligase activity (XU *et al.* 1998; GENE ONTOLOGY CURATORS 2002) with roles in glial cell and cell growth (YAGER *et al.* 2001), and sperm individualization (FABRIZIO *et al.* 1998; ARAMA *et al.* 2003). Myc plays a key role during *Drosophila* sperm maturation process and glial cell growth and differentiation (HEMPEL *et al.* 2006; REDDY AND IRVINE 2011; OTA AND KOBAYASHI 2020; SHOHAYEB *et al.* 2020). Poe might be involved in regulating *Myc* expression via degradation of Myc protein during glial cell differentiation and sperm maturation.

These factors might have an impact on Myc mRNA processing and translation: Bicaudal (*bic/Btf3*) is the β subunit of the nascent polypeptide-associated complex (NAC). The subunits α and β enable the activity of the ribosomal translational machinery (MARKESICH *et al.* 2000; BRAAT *et al.* 2004; KOGAN *et al.* 2017; KOGAN *et al.* 2022). In addition to chaperone activity NAC complex might function as a coactivator during developmental transcription and cell differentiation (ROSPERT *et al.* 2002; LIU *et al.* 2010). Quantitative proteomics approaches have colocalized the coexpression of BTF3, MYCN, *N-myc* downstream regulated gene 1 (NDRG1), and the *MYC* downstream target Ornithine Decarboxylase 1 (ODC1) in epithelial cancer cells (BELLO-FERNANDEZ *et al.* 1993; TU *et al.* 2007; HOGARTY *et al.* 2008; SYMES *et al.* 2013) suggesting complex interactions between the MYC/Max network and other players including BTF3.

The conserved small CCHC-type zinc finger protein CNBP (*CNBP*) binds to ssDNA/RNA and plays a key role during development and disease (ANTONUCCI *et al.* 2014). CNBP binds to G-rich regions in mRNAs and stabilizes their structure by inhibition of G-quadruplex formation (INTERPRO PROJECT MEMBERS 2004; GO REFERENCE GENOME PROJECT 2011). CNBP synthesizes *Myc* protein via IRES-dependent translation of *Myc* mRNAs, and absence of CNBP cause embryonic lethality (ANTONUCCI *et al.* 2014). Further, CNBP is involved in *Drosophila* wing disc morphogenesis and control of wing size by modulating Myc protein levels (ANTONUCCI *et al.* 2014). CNBP is a *myc* regulator and was compounded by the upstream enhancer P1/P2, the proximal enhancer P37/P38 the oocyte element, and the DPE promoter.

(F-box-like/WD40 repeat-containing protein Ebi (*ebi*), *Drosophila* homolog of mammalian (*TBL1*) is a nuclear conserved repressor and has been identified in association with the transcription repressor complex (TSUDA *et al.* 2006) and histone deacetylase complex (GO REFERENCE GENOME PROJECT 2011). Ebi integrates multiple developmental signaling pathways such as JNK (LIM *et al.* 2012), EGFR (DONG *et al.* 1999), Notch (TSUDA *et al.* 2002; MARYGOLD *et al.* 2011; NGUYEN *et al.* 2016), and Wingless (MATSUZAWA AND REED 2001) to regulate cell cycle switches, gene regulation, and induction of differentiation and patterning. Ebi might have a direct and/or indirect role in controlling *Myc* expression during development.

Pre-mRNA processing factor 31 (*Prp31*) (Figure 16; see also supporting information, Table 3 in File S3) is a splicing factor and is involved in pre-mRNA processing/splicing (MOUNT AND SALZ 2000; HEROLD *et al.* 2009) and photoreceptor development (RAY *et al.* 2010). Low levels of the protein or loss of the (*Prp31*) gene leads to embryonic lethality in mice (BUJAKOWSKA *et al.* 2009). MYC plays a crucial role in controlling expression of splicing machinery factors to maintain proper splicing of pre-mRNAs during development and disease (KOH *et al.* 2015; ABOU FAYCAL *et al.* 2016; PHILLIPS *et al.* 2020; JABLONOWSKI *et al.* 2023) suggesting a potential role for Prp31 in regulatory *Myc*.

Spenito (*nito*) (Figure 16), an RNA-binding protein (LASKO 2000) acts downstream of the Wnt signaling pathway and is required during larval development (CHANG *et al.* 2008). In association with the WMM complex, Spenito mediates (m6A) methylation of mRNAs to increase the efficacy of alternative mRNA splicing of species such as Sex-lethal (*Sxl*) during sex determination and dosage compensation (YAN AND PERRIMON 2015). *Myc*, *Sxl* and transformer (*tra*) genes control male and female body size dimorphism (MATHEWS *et al.* 2017).Spenito might have a role in regulating *Myc* expression during sex determination among other processes.

Suppressor of forked Su(f) was enriched in P1/P2 and P37/P38 enhancer clusters. Su(f) enables mRNA processing (INTERPRO PROJECT MEMBERS 2004; GO REFERENCE GENOME PROJECT 2011) and high concentrations of Su(f) are found during mitosis in proliferating cells (MITCHELSON *et al.* 1993). Su(f) negatively regulates the gypsy transposon and inhibits gypsy mRNA accumulation (PARKHURST AND CORCES 1986). The retrotransposon gypsy is inserted at the proximal 5'-end region in *Myc* into the first intron (GALLANT *et al.* 1996) near the P37/P38 enhancer cluster giving Su(f) more significance for being compounded by the enhancer P37/P38.

La-related protein 4B (*Larp4B*) is an RNA binding protein and is involved in downregulation of cell growth by posttranscriptional inhibition of *Myc* mRNA translation. Overexpression of Larp4B leads to reduced cell size and small organ size (FUNAKOSHI *et al.* 2018).

Pumilio (*pum*), an RNA binding protein is involved in the modulation of RNA stability, translation repression (DEAN *et al.* 2002; KIM *et al.* 2012), and RNA degradation. Target mRNAs deadenylated by Pop2 are utilized by repression domains (RDs) of Pumilio for destruction during stem cell differentiation, neurogenesis, and embryogenesis (ARVOLA *et al.* 2020). Pumilio plays role in the regulation of Hunchback mRNA in early embryos, germline stem cell proliferation and migration (ASAOKA-TAGUCHI *et al.* 1999; WICKENS *et al.* 2002), and fate specification in the sensory organ precursor (SOP) cells via EGFR signaling (KIM *et al.* 2012). Pumilio and Pop2 might be involved in negative regulation of *Myc* mRNA levels during bristle and wing vein development by integrating inputs from multiple signaling pathways.

Dead-box-1, an RNA helicase plays roles in RNA trafficking, nucleic acid duplex unwinding (GENE ONTOLOGY CURATORS 2002), translation and protein synthesis (RAFTI *et al.* 1996). In the early embryo Ddx1 positively regulates cell proliferation and controls body size and germ cell development (RAFTI *et al.* 1996; GERMAIN *et al.* 2015). The human homolog of *Ddx1* has been shown to be amplified in a series of MYCN-amplified tumors such as retinoblastoma (GODBOUT AND SQUIRE 1993; MANOHAR *et al.* 1995; SQUIRE *et al.* 1995; GODBOUT *et al.* 1998). In pediatric neuroblastoma, the MYC-binding partner Max showed strong interaction with the Ddx1 protein (JIN *et al.* 2021) suggesting that Ddx1 might be indirectly involved in *Myc* regulation through interaction with the Myc-binding partner Max.

Abstrakt (*abs*), a DEAD/H-Box RNA helicase is involved in RNA splicing (HEROLD *et al.* 2009; GO REFERENCE GENOME PROJECT 2011) and is a Notch regulator in certain sibling progenitor cells to control mRNA levels of Inscuteable (*insc*) during neurogenesis (IRION *et al.* 2004). Abstrakt function is required for the regulation of epithelial cell polarity in the oocyte and during embryogenesis (IRION AND LEPTIN 1999). Dead Box helicases have been shown to be direct targets of MYC/Max heterodimers (GRANDORI *et al.* 1996).

Drosophila Barentsz (*btz*), vertebrate homolog (*CASC3*, *MLN51*) is a component of the Exon Junction Complex (EJC) pathway and marks intron removal sites on spliced mRNAs, a process essential for mRNA translation, localization, and decay of nonsense-mediated mRNAs. EJC components MLN15/Barentsz, eIF4A3, Ddx1, and Mago/Y14 (LE HIR AND ANDERSEN 2008; CHAZAL *et al.* 2013) are required during transcription termination and RNA processing (ISHIGAKI *et al.* 2014). High levels of EJC are present in the nuclei of the cells during late oogenesis and EJC is associated with pol II elongation and termination complexes in cycling and differentiating cells (KISELEV *et al.* 2017). Y14 and Myc were shown to colocalize to nucleus in pre-/vitellogenic stage of oocytes (KISELEV *et al.* 2017). Mago came up on the original list but was not enriched (see Table 3 in File S3); however, Barentsz, Ddx1, and another subunit of the EJC complex, partner of Y14 and Mago (Pym) (GHOSH *et*

al. 2014) were enriched on the Myc-CRMs suggesting that EJC might play an important role in terminating *Myc* transcription and processing of mRNAs.

Smaug (*smg*), is the founding member of the SMAUG family of sequence-specific RNA-binding proteins. Smaug functions as translation repressor (DEAN *et al.* 2002; KIM AND BOWIE 2003) during maternal-to-zygotic transition in early embryos and induces degradation of hundreds of maternal mRNAs by recruitment of the CCR4-NOT deadenylase complex (JESKE *et al.* 2006; ZAESSINGER *et al.* 2006; CHARTIER *et al.* 2015), thereby controlling embryonic patterning (SMIBERT *et al.* 1999), activation of zygotic transcription, and early embryonic cell cycles (LIANG *et al.* 2008).These activities might indicate a role for Smaug in the regulation of *Myc* as an immediate early gene.

Obelus (*obe*) belongs to the Ski2 family of DNA helicases (VICHAS *et al.* 2015) and is required for adherence junction arrangement and alternative splicing of Crumbs (*crb*) mRNA, a transmembrane protein responsible for apico-basal cell polarity during embryogenesis and oogenesis (VICHAS *et al.* 2015). Myc, a master regulator of growth and proliferation must be tightly under control to keep balance between cell polarity and proliferation rate and avoid malignant transformation (GRIFONI *et al.* 2013). Here, we found Obelus protein, a regulator of apico-basal cell polarity in association with the DPE containing Myc-CRM P35/P36. Obelus might be a potential regulator of *Myc* mRNA and protein levels during early development.

How/Struthio (*how*, *struthio*), 'held out wings' is a KH domain conserved RNA binding protein (BAEHRECKE 1997) with role in alternative-/splicing of mRNAs (VOLOHONSKY *et al.* 2007; GO REFERENCE GENOME PROJECT 2011). Isoform how(L) destabilizes RNA, whereas How(S) induces stabilization of the RNA (VOLOHONSKY *et al.* 2007) during embryonic cell differentiation and morphogenesis (WALSH AND BROWN 1998). How functions in various signaling cascades including EGFR (MARTIN-BERMUDO 2000; EDENFELD *et al.* 2006), MAPK (TOLEDANO-KATCHALSKI *et al.* 2007), ERK (NIR *et al.* 2012), DPP/BMP (ISRAELI *et al.* 2007), Hedgehog (LOBBARDI *et al.* 2011), Wnt-TCF (MARSDEN AND DESIMONE 2001) (RIESE *et al.* 1997), Insulin-like receptor, Ras, and Akt (LASKO 2003) to control cell cycle progression and induce differentiation. The mouse homolog of How/Struthio, Quaking a tumor suppressor RNA binding protein contributes to control of cell cycle progression and promotes differentiation pathways (BIEDERMANN *et al.* 2010). How/Struthio might control *Myc* expression during cell cycle progression and induction of fate specification.

Scaffold attachment factor B (Saf-B), an RNA and DNA binding protein regulates alternative mRNA splicing (PARK *et al.* 2004) and pol II transcription (GO REFERENCE GENOME PROJECT 2011). *Drosophila* SAF-B can establish a link between the nuclear matrix, chromosomes, and transcription regulator complex to maintain

nucleosome-chromosome structure and nucleus organization (ALFONSO-PARRA AND MAGGERT 2010). Experiments with cultured human embryonic carcinoma cells showed that MYC contains a nuclear matrix attachment region (MAR) within the 3'-UTR sequences to which proteins such as transcription factor Sox2 binds (LEI *et al.* 2005). MARs function as boundary elements that are located between genes and serve as protecting shield during replication and transcription of the genes (BODE *et al.* 1996; KOHWI-SHIGEMATSU *et al.* 1997; BODE *et al.* 2003). Saf-B might provide a hub for the integrity of nuclear structure during *Myc* transcriptional regulation.

Ribosomal proteins (Rps) associated with the Myc-CRMs included the constituents of Rp large subunit (60S) RpL5, RpL8, RpL9, RpL13, RpL13A, RpL14, RpL19, RpL27A, RpL31, RpL32, RpL36, RpL38, parts of the small subunit (40S) RpS3, RpS3A, RpS6, RpS5a6, RpS10b, RpS13, RpS24, RpS26, RpS28b, RpS29, and the mitochondrial ribosomal proteins mRpL16. Myc-CRMs associated mRNA translation factors noteworthy to mention included eukaryotic translation initiation factors eIF2α, eIF3B, eIF3C, eIF3J, eIF3I, eIF4A, eIF4B, eIF4E1, eIF4H1, translation elongation factor eEF1α1, translation release and termination factor eRF1, mitochondrial initiation factor mIF2 and mitochondrial elongation factor mEFTu1.

The aforementioned ribosomal proteins and the translation initiation factor eIF2α as part of the eIF-2 complex have been classified as 'Minute' as heterozygous animals grow slowly and exhibit small body size and short bristles (MARYGOLD *et al.* 2007). Initiation factor eIF3I, a component of the eIF-3 complex stimulates binding of mRNA to the 40S ribosome (LASKO 2000; GO REFERENCE GENOME PROJECT 2011) to specifically target and initiate translation of a subset of mRNAs involved in cell proliferation (ASANO *et al.* 1997). Translation initiation factors eIF4B plays a role in cap-dependent translation, cell survival, and proliferation (HERNANDEZ *et al.* 2004). Translation initiation factor eIF4E1 is a component of the eIF4F cap-binding complex and plays a crucial role in cap-dependent translation of mRNA (LAVOIE *et al.* 1996).

Ribosomal genes belong to Myc target genes (ORIAN *et al.* 2003; ORIAN *et al.* 2005). Notably, a high percentage of RPs and translation factors identified here were compounded by the tissue-specific enhancer cluster P1/P2 and are mostly involved in cell growth and tissue patterning. For instance, the X- linked mRpL16 (*mRpL16*) (MARYGOLD *et al.* 2007), enriched only on P1/P2 enhancer is expressed in adult head, embryonic/larval midgut, larval muscle system, larval visceral muscle cell, and spermatozoon (HAMADA *et al.* 2005; DENG AND MELLER 2006; WORRINGER AND PANNING 2007). Translation release factor eRF1 complexed only by P1/P2 induces release of nascent peptide chain in response to termination codons UAA, UAG and UGA. Mutations in eRF1 gene end in readthrough of stop codons with maternal-effect nonsense suppression and lethality in larval stages

(CHAO *et al.* 2003). We conclude that components of ribosome machinery and translation protein complexes might regulate *Myc* mRNA and protein levels during cell and tissue growth mainly with the involvement of enhancer cluster P1/P2.

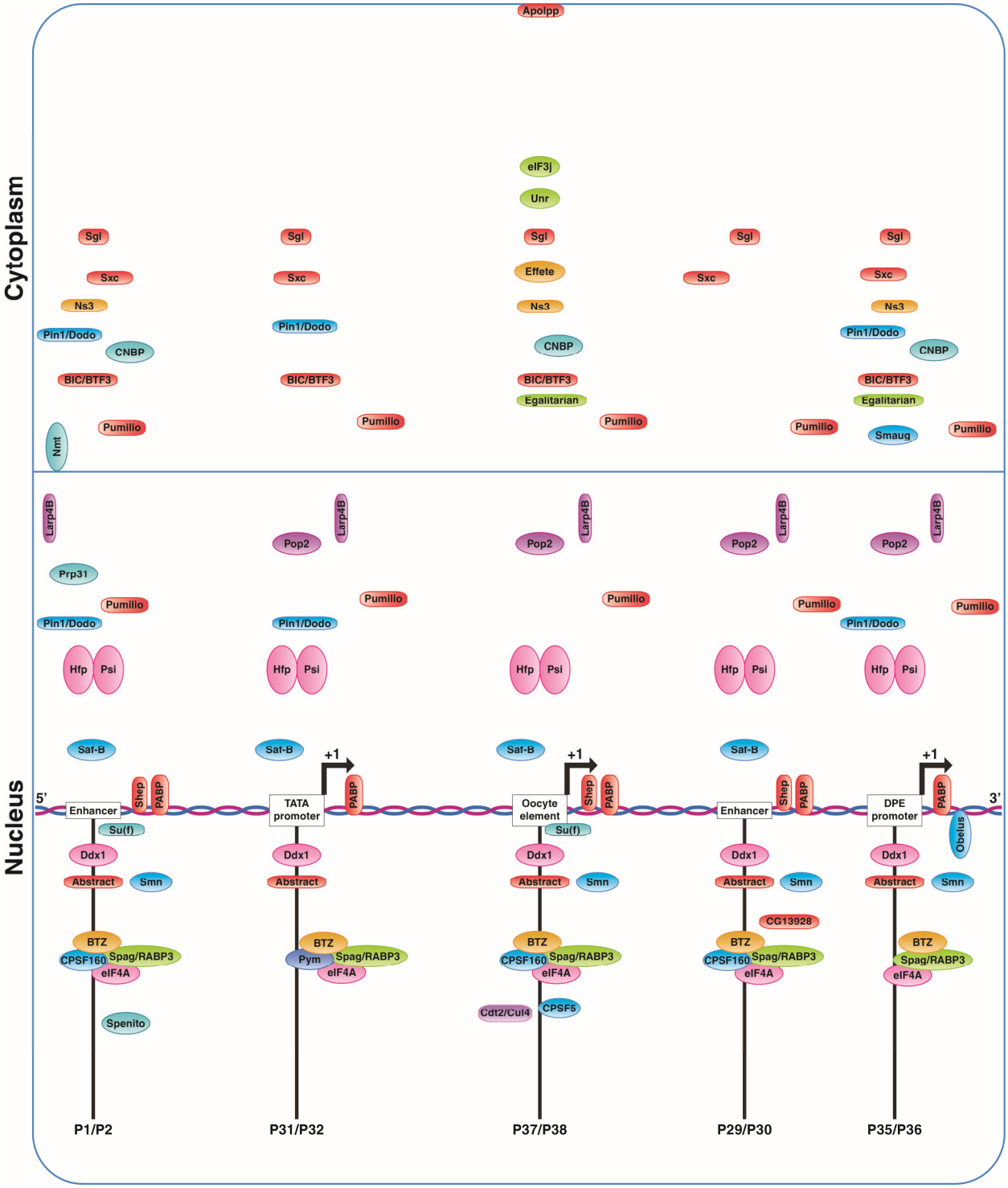


Figure 16. Examples of the factors that might act as posttranscriptional regulators of the *Drosophila Myc* gene. We identified Upstream of N-ras (**Unr**), an RNA chaperon with a role in *Myc* mRNA translation only in association with the

oocyte element P37/P38. Conserved small CCHC-type ZnF nucleic acid binding protein **CNBP**, regulator of Myc protein synthesis was associated with the Myc-CRMs P1/P2, P37/P38, and P35/P36. Core components of Exon Junction Complex (EJC) included Barentsz (**BTZ**), eukaryotic initiation factor 4A (**eIF4A**) (essential RNA helicase and Cap binding translation initiation factor), Dead-box-1 (**Ddx1**), Partner of Y14 and Mago (**Pym**), and Spaghetti (**Spag, RABP3**) (identified in association with the pol II transcription regulator complex and RNP protein folding complex). From the protein group cleavage and polyadenylation factors, involved in nascent mRNA 3'-formation **CPSF160** was identified in association with the P1/P2, P29/P30, and P37/P38 targets, and **CPSF5** was compounded only with the oocyte element (P37/P38) initiation site. Poly(A) binding protein **PABP** was associated with all the targets. Both proteins Half pint **Hfp** and P-element somatic inhibitor **Psi** with roles in transcription and RNA processing, and **Larp4B**, RNA binding protein and translation inhibitor of *Myc* mRNA were complexed on all the targets. For more information about the association of the illustrated factors with the Myc-CRMs and abundance ratio of the factors see (see Table3 in File S3, Table 7 in File S7 and Table S8).

Discussion

This study used a newly developed highly sensitive and selective Solid Surface Magnetic Enrichment Protocol (SSMEP) to purify proteins from *Drosophila* embryonic nuclear extracts and subsequently identified *Myc* regulators by Mass Spec analysis. The goal of this project was to discover potential trans-regulators responsible for *Myc* regulation at the level of transcription, post-transcriptional control, and post-translational protein stability and decay. We justify the chosen target sequences, i.e. P1/P2, P29/P30, P31/P32, P35/P36, and P37/P38 by the identification of conserved Myc-CRMs by *in silico* phylogenetic alignment of the sequenced genomic loci of *Myc* in *Drosophila* species by *cis*-Decoder (KHARAZMI *et al.* 2012) and reporter expression under the control of *Myc cis*-elements (Figures 1 and Figure 2; see also supporting information, Table S1.3). The datasets obtained show that mitogenic stimuli trigger transcription through a cascade of multiprotein complexes down to nucleus and provoke assembly of pre-initiation complexes on *Myc* promoters accompanied by binding of trans-acting factors to different sets of enhancers in the gene body.

To confirm our results with the identified factors by Myc-CRMs, we used a Wnt *cis*-Regulatory Module (W-CRM) as a positive control that has been designed and tested in *Drosophila* and *in vitro* with expressed and purified proteins (ARCHBOLD *et al.* 2014). In our experiment the W-CRM sequence (Figure 3) was tested with crude nuclear embryonic extracts and potential *wingless/wg* regulators were identified (Figure 11; see also supporting information, Table 3 in File S3 and Table S8). In addition to the components of the Wnt/Wg signaling pathway, among others, components of Notch, Hedgehog, Dpp, EGFR, and MAPK were associated with the W-CRM. Developmental collaboration between Wnt/Wg and other pathways are known; for example,

Wingless and Notch cooperate in wing pattern formation (COUSO *et al.* 1995). We believe the tested W-CRM containing TCF/LEF site can be used as a positive control in similar studies.

Developmental signals and cell cycle factors control Myc expression: Frontiers of *Myc* regulators are *Myc* cofactors controlling cell cycle phases (Figure 13) meaning that cell growth and proliferation correlates with *Myc* expression levels (BRETONES *et al.* 2015). Following the cell cycle factors many of the identified proteins are products of the target genes of Wingless, Notch, EGFR signaling pathways succeeded by the components of Dpp, Hedgehog, ecdysone and miRNA pathways. The known factors among our identified proteins corroborate the findings of others for the control of *Myc* expression.

Advances in *Myc* regulation indicate that a variety of cofactors act as *Myc* regulators, both transcriptionally and posttranscriptionally via positive and/or negative feedback loops. For example, the transcriptional coactivator Yorkie (*yki*), the *Drosophila* homolog of Yap in Hippo pathway, stimulates *Myc* transcription, and high levels of Myc protein represses *yki* (NETO-SILVA *et al.* 2010).

The complexity of *MYC* regulation is multilayered by inclusion of multiple initiation sites and differential regulation to orchestrate early ubiquitous and at later stages patterned expression of *MYC* during development (LIU *et al.* 2006). Analyses of the most relevant identified trans-actors reveals that *Myc* gene is at the nexus of diverse signal transducers for delivering a message and triggering appropriate promoter response to developmental stage and physiological needs of cell types. Cdk5, Scribbler/Brakeless/Thickveins, and SAP18/HDAC1 compounded by Myc-CRMs, exemplify tight regulation of *Myc* in conjunction with cell cycle factors and cell signaling components. Cyclin5 controls axonogenesis in *Drosophila* embryos in interaction with Rac GTPase and by partnering with P35 induces differentiation of post-mitotic neurons (CONNELL-CROWLEY *et al.* 2000). Given induction of MYC by Cdk5 in tumor cells via inhibition of MYC suppressor Bin1 (ITO *et al.* 2014; ZHANG *et al.* 2019) and regulation of CDKs by MYC (BRETONES *et al.* 2015) we postulate that Cdk5 might regulate *Myc* expression during onset of tissue patterning in *Drosophila*.

Transcriptional coregulator Scribbler, in association with the corepressor Atrophin/Grunge, an interactor of histone deacetylase complex represses transcription during early embryonic segmentation and later during axon projection from photoreceptor cells to medulla (HAECKER *et al.* 2007). Through modulation of Notch, Wingless, and Hedgehog signaling target gene expression, Scribbler determines many aspects of wing disc patterning (DWORKIN AND GIBSON 2006). Scribbler requires binding to *cis*-elements of Kruppel to repress transcription (HAECKER *et al.* 2007).

Chromatin repressive complex SAP18/HDAC1 might reflect the complexity of the signaling network relevant to *Myc* regulation. SAP18/HDAC1 functions in various settings during body patterning and requires tethering to Kruppel CRMs, as does Scribbler to recruit enhancer-specific Sin3A/HDAC1 repressor complex (MATYASH *et al.* 2009) to repress transcription of certain genes such as Bicoid during embryonic germband extension (ZHU *et al.* 2001). It is fair to assume that Scribbler, Grunge, SAP18/HDAC1 and the signaling pathways linked to these proteins might form a subset of activating and repressing complexes to pattern *Myc* during cell and tissue growth.

To sum up, transcription factor MYC, master regulator of transcription is under tight control of cell signaling and cell cycle control programs, and deregulation drives the cells toward malignant transformation and metabolic adversities.

The distal enhancer cluster P1/P2 controls Myc transcription during tissue patterning and DNA replication: A large subset of the P1/P2-associated proteins constitute components of the conserved signaling pathways with activities in the induction of cell differentiation and morphology changes, and patterning during larval and pupal development. P1/P2 enhancer cluster 5'-GAAGCTTT**TTGTTG**T**TTTTG**CAGCTGCC**GTTT**TCGCCCTT-3' (ChrX:3,369,776..3,369,815) contains an E-box (indicated by gray) within a sequence of conserved bases (bold letters). Net (*Net, CG11450*), the E-box binding (GO REFERENCE GENOME PROJECT 2011) bHLH transcription repressor was pulled down by the P1/P2 sequence. Net is antagonist of EGFR and part of Wnt enhanceosome with roles in proliferation, cell fate induction and wing vein formation (BRENTRUP *et al.* 2000; PEYREFITTE *et al.* 2001).

The uniquely associated factors encompassed tissue-specific T-box transcription factor DOC3 (TGF-beta, Dpp and Wg cofactor) (REIM AND FRASCH 2005; HATTON-ELLIS *et al.* 2007; LU *et al.* 2022), Spenito (Wg cofactor) (CHANG *et al.* 2008), and N-myristoyl transferase Nmt (TGF-β/Dpp and Wg cofactor) (HARDEN 2002). Interestingly, MED24 a sensor for developmental signals and essential for pupal growth (WANG *et al.* 2010) was only compounded by the P1/P2. One scenario would be that Wingless signaling might form a transcription inhibition complex with the transcription repressor Net and recruitment of further repressive factors on the E-box and mediate the information to pol II complex via MED24 to downregulate *Myc* during wing vein specification. A parallel activating complex might be formed with the involvement of the Wingless/Wg pathway by Phosphorylation of 'Enhancer of Rudimentary' ERH (GELSTHORPE *et al.* 2006) (enriched only on P1/P2 and W-CRM) by CKII at any of both target sites to counteract the repressive behavior of Net complex and

upregulate *Myc*. Similar regulatory switches are conceivable with the binding of trans-actor DOC3 to the *cis*-element P1/P2 for *Myc* control during cardiogenesis, neuronal development or the like. We hypothesize that complex regulatory programs initiating from distal enhancer P1/P2 govern differential expression of *Myc* spatio/temporally during larval and pupal development.

Association of Topoisomerase II (Top2) and RecQ4 helicase with the P1/P2 infer the role of this *cis*-element to the removal of torsional stress generated by DNA replication and transcription, and maintenance of genome integrity. DNA 3'-5' RecQ4 helicase has pivotal role during S phase in maintaining genome stability during DNA strand separation and exchange. This raise the possibility that RecQ4 might control *Myc* levels mainly during S phase and G1/S checkpoint to ensure healthy cell cycle progression.

Taken together, P1/P2 functions as an enhancer with dual roles, transmission of patterning signals to *Myc* promoters to establish differential expression of *Myc* during tissue growth, and relaxation of DNA topology by removal of supercoils during transcription and replication.

Myc *TATA-box containing P1 promoter might represent major bursting site:* Myc-CRM P31/P32, 5'-GA**GCGCGGC**AGTCTGGTACGATAG**AAATTTTATTTAA**GCCACAG-3' (ChrX:3,373,105..3,373,148) derived from the sequences within the P1 promoter retains the GC-box and the TATA-box (indicated by gray) (KHARAZMI *et al.* 2012). Most of the proteins associated with this target belong to RNA pol II complex, different steps of transcription, chromatin remodeling, cell cycle factors, and DNA replication/repair (Figure 14 and Figure 15; see also supporting information, Table 3 in File S3).

Basal transcription machinery at the Myc TATA promoter: We identified general transcription factors and components of RNA pol II indicative of the assembly of a functional pre-initiation complex (PIC) on TATA promoter. Literature has shown that purified TFIID incubated with the adenovirus major late promoter can form a stable PIC (VAN DYKE *et al.* 1989). The core promoter requires TFIID, TFIIA, TFIIB, TFIIE, TFIIF, TFIIH, and RNA pol II for the formation of PIC *in vivo* (THOMAS AND CHIANG 2006). The binding of TBP/TFIID to promoter nucleates the assembly of PIC on promoter (SMALE AND KADONAGA 2003) followed by recruitment of TFIIA for the stabilization of TBP/TFIID, and promoter contact with TFIIB to recruit RNA pol II to the complex (THOMAS AND CHIANG 2006).

TAF5, TAF6, TAF12, and dFUS/Cabeza subunits of the TFIID complex were compounded by TATA promoter; TAF12 recruits TAF4 and TAF12 can recruit TAF9 to TBP-TFIID complex *in vivo* (YOKOMORI *et al.* 1993). A stable core TFIID complex consists of TAF4, TAF5, TAF6, TAF9, and TAF12 (WRIGHT *et al.* 2006). TFIIA-S

(*Drosophila* isoform of TFIIA) (AOYAGI AND WASSARMAN 2000) came up on the original list but was not enriched. Most of the RNA pol II subunits required for transcription were associated with the TATA promoter. RPB1 the largest subunit of RNA pol II (GREENLEAF 1983; BRICKEY AND GREENLEAF 1995; INTERPRO PROJECT MEMBERS 2004), RPB2 the second largest component of the pol II core complex (FALKENBURG *et al.* 1987; AOYAGI AND WASSARMAN 2000), and the evolutionarily conserved RPB7 were among the identified pol II subunits. RPB7 is essential in *Drosophila* and can recruit and heterodimerize with the RPB4, both of which are vital for transcriptional and posttranscriptional events (PANKOTAI *et al.* 2010).

The activity of TATA-bound PIC might be sufficient for a basal level of *Myc* transcription. However, basal promoter requires the activity of additional GTFs and mediator complex, working independently or in collaboration for transmission of regulatory cues between upstream activators and/or repressors and initiation complex to fine tune *Myc* patterning in a cell-type or tissue-specific manner. Further GTFs compounded by the Myc-CRMs included TFIIEα, CycH-TFIIH, Ssl1-TFIIH, and topoisomerase II (Top2). TFIIE (α and β subunits) constitutes an important component of initiation complex that interacts with the bound pol II and contributes to promoter clearance. TFIIE can function independently or in collaboration with GTFs and TFIIH. TFIIH could potentially contribute to many aspects of initiation and elongation via helicase activity (p44/Ssl1 and XPD subunit) for promoter opening, transcription-coupled nucleotide excision repair, phosphorylation of pol II CTD, and E3 ubiquitin ligase activity. Top2 can instantly neutralize torsional stress upstream and downstream of transcribing machinery. dFUS/Cabeza subunit of TFIID can accommodate pol II complex alongside initiation, termination, and mRNA processing.

The compounded mediator subunits consisted of MED4, MED11, MED13/Skuld, MED17, and MED25, which might be suggestive of the presence of mediator complex with the large and accessory subcomplexes for bridging enhancers to GTFs. Mediator complex might transmit signaling stimuli from activators and repressors to TATA promoter and initiation complex for precisely patterned expression of *Myc*.

Finally, Trf2, associated with the TATA promoter might add a sophisticated option to *Myc* transcriptional regulation in addition to a TBP-dependent transcription. We hypothesize that both regulatory plans, the TAF-TBP and TAF-Trf2 initiation might be possible from the TATA promoter for expressing *Myc* throughout the development.

Myc DPE promoter and downstream enhancer cluster P29/P30 participate in the formation of basal transcription machinery on DPE promoter: Analysis of *Drosophila* promoter database indicates that 40% of

Drosophila promoters contain a DPE element (KUTACH AND KADONAGA 2000; THOMAS AND CHIANG 2006). In the previous work we located an initiator (Inr) and a DPE element, the P2 promoter within the *Myc* downstream intronic sequences (KHARAZMI *et al.* 2012). (Figure 2A). Here, the factors complexed by the DPE-containing target P35/P36 and the adjacent enhancer cluster P29/P30 show that these two elements constitute a functional initiation site within the downstream intronic sequences to cooperatively initiate a full size bursting from this site (Figure 14 and Figure 15). TAF6 and TFIIEα associated with the enhancer P29/P30 might complement the factors on DPE for the formation of a stable PIC (Figure 15). The strength and choice of transcription factors and the composition of a core promoter plays a crucial role in the robustness and size of the transcription initiation (SLOUTSKIN *et al.* 2024). *Myc* DPE promoter responds to mitogenic stimuli transduced by the conserved developmental signaling pathways and to abiotic stress. The outcome of each context-dependent response to an inducer contributes to changes in expression levels of *Myc* affecting cell cycle progression, cell proliferation rate, and differentiation. The DPE promoter has been reported to play a pivotal role during different developmental stages via translating input signals of evolutionarily conserved pathways into transcriptional regulatory programs to contribute to animal growth (SLOUTSKIN *et al.* 2024). Precisely designed genetic and functional studies will be needed to elucidate the molecular mechanism of the regulatory network at the *Myc* DPE promoter.

We have identified Myc enhancer cluster P37/P38 as a transcribing developmental super enhancer synthesizing eRNAs: Work of others has shown that microinjection of a plasmid containing *MYC* promoter into Xenopus oocytes and embryos leads to high levels of *MYC* expression during oogenesis, no expression in early fertilized embryos, expression reactivation at the midblastula transition (MBT) (PRIOLEAU *et al.* 1994). Although early embryos contain all the necessary GTFs for transcription complex assembly, high nuclear levels of histones hamper DNA binding of transcription factors (PRIOLEAU *et al.* 1994). To start transcription during the early stages of embryonic development, TBP-DNA must be established at the promoter complex in order to overcome histone inhibition (PRIOLEAU *et al.* 1994).

In addition to the three previously reported P0, P1, and P2 promoters (KHARAZMI *et al.* 2012), the proteomics discovery data indicate that the enhancer cluster P37/P38 within the proximal 5'-UTR regions may serve as a potential fourth initiation site at the *Myc* locus with the main activity during oogenesis and embryogenesis. P37/P38-compounding factors primarily indicate the commencement of TAF-TFIID and/or TAF-TRF2 transcription (Figure 15; see also supporting information, Table 3 in File S3). In order to supply cells with

sufficient biomass during oocyte maturation and embryogenesis, we expect that bursting from the oocyte element at the MBT transition and the start of zygotic gene expression will be a crucial source.

MYC is thought to be transcribed into mRNAs with varying lengths including enhancer RNAs (eRNAs). The mouse *MYC* gene generates transcripts of varying lengths throughout development (SPENCER AND GROUDINE 1991; BENASSAYAG *et al.* 2005; KUBICKOVA *et al.* 2023). In tumor cells with replication stress, the number of transcripts can increase up to five shorter variants to the cost of the loss of full-length MYC mRNAs (IBRAHIM *et al.* 2022).

Developmental enhancers can produce eRNAs independently of promoters (CATARINO AND STARK 2018; HENRIQUES *et al.* 2018). eRNAs are initially short-lived molecules and can be translated with or without polyadenylation procedure (KOCH *et al.* 2011). According to the association of key regulatory factors such as subunits of Pol II complex, subunits of TFIID and mediator complexes, GTFs, chromatin remodeling and histone-modifying enzymes, TRF2/GAF, and TRL (Figure 14 and Figure 15) it is reasonable to believe that the oocyte element P37/P38 serves as a transcribing super enhancer to empower *Myc* in its function as a master regulator of cell growth and proliferation and determinant of organ size. The P37/P38 also includes other factors, such as Effete/UbcD1, eIF3j, ribosome maturation factor SBDS, survival motor neuron Smn, and Cdt2 protein (encoded by the Lethal-(2)-denticleless gene) that point to their roles in oocyte maturation, female germline cell maintenance, sex determination, early phases of mitotic cell cycle, and establishment of germ layers.

Myc control prompts dynamic chromatin landscape and regulatory switches with gene-specific factors: Competition between chromatin changes and transcription complex assembly is a key determinant in the regulation of MYC transcription during development (KING *et al.* 1986; VRIZ *et al.* 1989; PRIOLEAU *et al.* 1994). We identified components of major chromatin assembly and histone modification complexes compounded by MYC-CRMs that is indicative of the vital roles of major remodeling complexes such as SAGA, SWI/SNF (Brahma), ATAC, NURF; NURD, ACF, and CHRAC throughout fly development.

Certain components of GTFs like TAF5 and TAF6 show interrelation to chromatin remodeling complexes for the modulation of cell-type-specific transcription. TAF5 and TAF6 (compounded by Myc-CRMs), subunits of TFIID are parts of the coactivator SAGA complex (WANG *et al.* 2020). SAGA exhibits histone acetyltransferase (HAT) and ubiquitin protease activity (GUELMAN *et al.* 2006b). SAGA links TBP to transcription factors, ensures promoter opening, degrades unwanted transcription factors to initiate differentiation in certain cell

types, and is enriched at the proximal 5'-ends of the genes (HANSEN AND TJIAN 1995; WRIGHT *et al.* 2006; WEAKE *et al.* 2011). 5'-end paused SAGA contributes to immediate reinitiation of transcription of genes like *Myc* via TAF-TRF2/pol II and/or TAF-TFIID/pol II pause release in response to active enhancers (WEAKE *et al.* 2011).

In addition, SAGA complex plays essential role during pol II elongation events. Transcription elongation factor SPT6 is a component of SAGA/TFTC complex and associates with both nucleosomes and actively transcribing pol II (ARDEHALI *et al.* 2009). SPT6 recruits other elongation factors such as SPT5 and PAF1 (SPT6 and PAF1/Antimeros both compounded by Myc-CRMs) and contributes to chromatin remodeling and pol II elongation (ANDRULIS *et al.* 2002; PETRUK *et al.* 2006; HSU *et al.* 2008; ARDEHALI *et al.* 2009). Taken together, our data show that SAGA complex might be actively involved in different steps of *Myc* transcriptional control.

SWI/SNF (Brahma), a known chromatin remodeling complex in *Drosophila* (MARENDA *et al.* 2004) might have essential role in regulating *Myc*. For instance, Snr1/BAP45 compounded by Myc-CRMs induces ATP-dependent chromatin remodeling over endosome-mediated signaling cascade to negatively regulate pol II transcription during cell proliferation and induce differentiation (DINGWALL *et al.* 1995; KAL *et al.* 2000; MARENDA *et al.* 2003; MOHRMANN *et al.* 2004; CHALKLEY *et al.* 2008; BONNAY *et al.* 2014; BARISH *et al.* 2020; MASHTALIR *et al.* 2020). In addition, Snr1/BAP45 inhibits CycE during S phase and induces histones tails methylation during differentiation and patterning (BRUMBY *et al.* 2002; KOE *et al.* 2014). This raises the possibility that Snr1/BAP45 might directly inhibit *Myc* transcription during S phase to promote cell differentiation.

A further function of chromatin assembly complexes might include association with the gene-specific factors to reach out to *Myc* promoter, or transcription factors might be parts of remodeling complexes and act as *Myc* regulators. For instance, Host cell factor HCF, compounded by Myc-CRMs and a cell cycle regulator (MAHAJAN *et al.* 2003) is a component of Ada2a complex (ATAC), and Ash2-Sin3A-HCF protein complex activates promoters of genes involved in growth and proliferation via histone modifications (GUELMAN *et al.* 2006a; SUGANUMA AND WORKMAN 2008; FURRER *et al.* 2010; SUGANUMA *et al.* 2012). In a similar manner heterodimers of HCF/MLF or MLF/DnaJ-1 might downregulate *Myc* transcription during tissue growth and organogenesis (JASPER AND BOHMANN 2002) and during hematopoiesis (BRAS *et al.* 2012; MILLER *et al.* 2017) in response to signaling cascades such as Hedgehog (FOUIX *et al.* 2003).

Myc mRNA and protein levels are under tight control during development: The vital role of Myc in controlling Pol I, II and III transcription to modulate various aspects of cell physiology including ribosome biogenesis and DNA replication has been evidenced by the works of others (RAHA *et al.* 2010a; MITCHELL *et al.* 2015). Two of the RNA polymerase subunits, RPB5 and RPB8 (complexed by the Myc-CRMs) exhibit polymerase activity from the promoters of pol I, pol II and pol III. Polr2E/RPB5, enabling pol II transcription, is involved in transcription elongation by pol I during rRNA synthesis and tRNA synthesis by pol III (AOYAGI AND WASSARMAN 2000; GENE ONTOLOGY CURATORS 2002; GO REFERENCE GENOME PROJECT 2011). RNA pol I, II and III subunit H, Polr2H/RPB8 enables pol I, pol II, and pol III transcription (AOYAGI AND WASSARMAN 2000; GENE ONTOLOGY CURATORS 2002; GO REFERENCE GENOME PROJECT 2011).

Myc function in controlling target gene expression during cell growth and proliferation (ORIAN *et al.* 2007) might be linked to control of *Myc* expression by these cofactors, which include components of RNA pol I, II, III, and factors involved in ribosome biogenesis and mRNA translation. The identification of ribosomal proteins and translation factors compounded by the Myc-CRMs might point to the potential roles of these proteins in controlling *Myc* mRNA and protein levels during cell growth, proliferation and differentiation. Myc-CRMs-compounded posttranscriptional/-translational factors are parts of the cytoplasmic 40S and 60S subunits of the eukaryotic 80S functional ribosomal apparatus, mitochondrial ribosomal proteins, and eukaryotic translation factors.

Point mutations in certain genes encoding ribosomal proteins (RPs) and mRNA translation factors including RpL15, RpS5a6 and eIF2α cause 'Minute' condition due to allelic loss-of-function leading to genetic haploinsufficiency (LAMBERTSSON 1998; MARYGOLD *et al.* 2007). Animals with 'Minute' syndrome show small body size, short and thin bristles, and reduced viability and fertility (QIAN *et al.* 1987; MARYGOLD *et al.* 2007). Ribosome biogenesis and protein synthesis are key factors sensed by cells for DNA replication and G1/S transition (PARDEE 1989; NORBURY AND NURSE 1992). Myc is a fine-tuning regulator of DNA synthesis and ribosome biogenesis to control cell and animal size (PARDEE 1989; NORBURY AND NURSE 1992; DESTEFANIS *et al.* 2020). 'Minute' syndrome might link haploinsufficiency of ribosomal genes to adversities in *Myc* mRNA and protein turnover. We propose that genes at 'Minute' loci might be main regulators of *Myc* in highly proliferative cells during early development.

DEAD-box RNA helicases constitute a large family of proteins and induce structural changes in RNA molecules required for the physiological function of RNAs. Many of the DEAD-box RNA helicase genes are targets of MYC/Max heterodimers (GRANDORI *et al.* 1996). Similarly, DEAD-box RNA helicases associate with the target

genes of the MYC/Max heterodimers, exhibit nucleic acid duplex unwinding ability and are essential for rRNA processing and ribosome biogenesis during cell growth and germline development (HIRLING *et al.* 1989; GRANDORI *et al.* 1996; LASKO 2013; KARA *et al.* 2023).

Members of the Dead-box RNA helicases including eIF4A (*eIF-4a*), Dead-box-1 (*Ddx1*), and Abstrakt (*abs*) were pulled down by the Myc-CRMs. eIF4A is dependent on eIF4B (also compounded by Myc-CRMs) for its helicase activity and together with the eIF4B and ribosomal protein S6 kinase (*S6k*), a component of insulin receptor signaling pathway (RADIMERSKI *et al.* 2002), selectively regulates translation of pro-survival mRNAs including MYC and Bcl-2 to promote cell proliferation (WOLFE *et al.* 2014; ROUX AND TOPISIROVIC 2018). eIF4A has been demonstrated to behave oncogenic and overexpress MYC and Notch1 through mRNA translation in acute lymphatic leukemia (ALL) (WOLFE *et al.* 2014).

Dead-box-1 (Ddx1), with roles in nucleic acid duplex unwinding (GENE ONTOLOGY CURATORS 2002) and protein synthesis (RAFTI *et al.* 1996) is a positive regulator of cell proliferation and germ cell development in the early embryos (RAFTI *et al.* 1996; GERMAIN *et al.* 2015). The human DDX1, with high similarity to the *Drosophila* Ddx1, has been found to be overexpressed and amplified with MYCN in a subset of retinoblastoma and neuroblastoma tumors, as well as some other cancer types like breast cancer (SQUIRE *et al.* 1995; GODBOUT *et al.* 1998; BALKO AND ARTEAGA 2011; GERMAIN *et al.* 2011), suggesting that Ddx1 plays a role in cell proliferation with potential connections to the regulation of growth regulators like *Myc*.

Abstrakt, a member of DEAD/DEAH-box RNA helicases is required for cell survival throughout the *Drosophila* life cycle with essential impact on cell morphology, mRNA processing and localization, and apoptosis (IRION AND LEPTIN 1999). Abstrakt is a Notch regulator via modulation of Inscuteable (*insc*) mRNA levels in certain sibling progenitor cells during neurogenesis (IRION *et al.* 2004). Abstrakt was highly enriched in all the Myc-CRM samples which might indicate a cell signature-dependent role for Abstrakt in regulating *Myc* expression. In conclusion, according to our proteomics data, Dead-box RNA helicases have a significant influence on the direct or indirect control of *Myc* expression across different developmental stages and in disease.

Concluding remarks

The tested Myc-CRMs in this study may contain overlapping binding sites for the binding of multiple transcription factors to these sequences, simultaneously or sequentially. Alternatively, the factors might compete for binding to the same site(s) depending on the transcriptional context. The exact sequences of binding sites,

the binding mechanism, and the allosteric conditions for the binding of factor(s) with or without involvement of putative adaptor molecules and mediators, remain to be investigated.

Considering the stringent filtering of the obtained data and implementation of different quality control approaches we could deliver credible datasets without background noise. The dataset represents a wide range of factors regulating *Myc* expression at different levels of gene control mechanisms. Extensive *in vivo* follow-up analyses are necessary to provide enough proof for the functionality of identified proteins and validate these results further. We believe our findings give a solid foundation for the scientific community to plan and carry out numerous exploratory studies to clarify the molecular and cellular mechanisms of the genes of interest, as well as their linkages to the powerful network of the super-oncogene MYC.

Acknowledgements: The authors thank C. Kerscher, Licor Biosciences for the analytical support with the Gel Shift Images, Prof. V. Schwämmle and Prof A. Rogowska-Wrzesinska, University of Southern Denmark for the analytical accommodation of the use of the Software Tool ComplexBrowser, C. Scavenius Sonne-Schmidt, Danish Technological Institute (DTI) for analytical support with the Mass Spec data, and D. Ackermann and C. Winiger from the Oligonucleotide services Microsynth AG, Switzerland for the synthesis of the novel oligonucleotides. The authors are thankful to Prof. H-P Lipp for the critical reading of the final version of the pre-submission manuscript and the members of BroadPharm company (Sandiego, Ca) for the synthesis of the free-end PEG-linker 'BP-25103 DBCO-PEG23-amine.

Author Contributions:

Jasmine Kharazmi (corresponding author), University of Zurich, Switzerland; Contributions: Conceptualization, Resources, Funding, Supervision, Methodology, Investigation, Formal analysis, Validation, Data curation and visualization, Project administration, Writing - review and editing

Cameron Moshfegh, ETH Zurich, Switzerland; Contributions: Funding, Methodology, Formal analysis, Data curation and visualization, Software, Review and editing

Thomas Brody, Compiler of The Interactive Fly, a cyberspace guide to *Drosophila* development

Contributions: Conserved DNA sequence analysis, Review and editing

All authors critically revised the manuscript and agreed to the published version.

Funding: This project did not receive external support.

Disclosure: The authors declare that they have no conflict of interest.

LITERATURE CITED

Abaza, I., O. Coll, S. Patalano and F. Gebauer, 2006 Drosophila UNR is required for translational repression of male-specific lethal 2 mRNA during regulation of X-chromosome dosage compensation. Genes Dev 20**:** 380-389.

Abel, J., R. Eskeland, G. D. Raffa, E. Kremmer and A. Imhof, 2009 Drosophila HP1c is regulated by an auto-regulatory feedback loop through its binding partner Woc. PLoS One 4**:** e5089.

Aberger, F., and A. Ruiz i Altaba, 2014 Context-dependent signal integration by the GLI code: the oncogenic load, pathways, modifiers and implications for cancer therapy. Semin Cell Dev Biol 33**:** 93-104.

Abou Faycal, C., S. Gazzeri and B. Eymin, 2016 RNA splicing, cell signaling, and response to therapies. Curr Opin Oncol 28**:** 58-64.

Acar, M., H. Jafar-Nejad, H. Takeuchi, A. Rajan, D. Ibrani *et al.*, 2008 Rumi is a CAP10 domain glycosyltransferase that modifies Notch and is required for Notch signaling. Cell 132**:** 247-258.

Adelman, K., W. Wei, M. B. Ardehali, J. Werner, B. Zhu *et al.*, 2006 Drosophila Paf1 modulates chromatin structure at actively transcribed genes. Mol Cell Biol 26**:** 250-260.

Agianian, B., K. Leonard, E. Bonte, H. Van der Zandt, P. B. Becker *et al.*, 1999 The glutamine-rich domain of the Drosophila GAGA factor is necessary for amyloid fibre formation in vitro, but not for chromatin remodelling. J Mol Biol 285**:** 527-544.

Akan, I., D. C. Love, K. R. Harwood, M. R. Bond and J. A. Hanover, 2016 Drosophila O-GlcNAcase Deletion Globally Perturbs Chromatin O-GlcNAcylation. J Biol Chem 291**:** 9906-9919.

Akhmetova, K., M. Balasov, R. P. Huijbregts and I. Chesnokov, 2015 Functional insight into the role of Orc6 in septin complex filament formation in Drosophila. Mol Biol Cell 26**:** 15-28.

Alfonso-Parra, C., and K. A. Maggert, 2010 Drosophila SAF-B links the nuclear matrix, chromosomes, and transcriptional activity. PLoS One 5**:** e10248.

Alland, L., R. Muhle, H. Hou, Jr., J. Potes, L. Chin *et al.*, 1997 Role for N-CoR and histone deacetylase in Sin3-mediated transcriptional repression. Nature 387**:** 49-55.

Alpar, L., C. Bergantinos and L. A. Johnston, 2018 Spatially Restricted Regulation of Spatzle/Toll Signaling during Cell Competition. Dev Cell 46**:** 706-719 e705.

Anatskaya, O. V., and A. E. Vinogradov, 2022 Polyploidy and Myc Proto-Oncogenes Promote Stress Adaptation via Epigenetic Plasticity and Gene Regulatory Network Rewiring. Int J Mol Sci 23.

Andersen, D. S., and S. J. Leevers, 2007 The essential Drosophila ATP-binding cassette domain protein, pixie, binds the 40 S ribosome in an ATP-dependent manner and is required for translation initiation. J Biol Chem 282**:** 14752-14760.

Andersen, P. R., L. Tirian, M. Vunjak and J. Brennecke, 2017 A heterochromatin-dependent transcription machinery drives piRNA expression. Nature 549**:** 54-59.

Anderson, A. E., U. C. Karandikar, K. L. Pepple, Z. Chen, A. Bergmann *et al.*, 2011 The enhancer of trithorax and polycomb gene Caf1/p55 is essential for cell survival and patterning in Drosophila development. Development 138**:** 1957-1966.

Anderson, E. C., and P. O. Catnaigh, 2015 Regulation of the expression and activity of Unr in mammalian cells. Biochem Soc Trans 43**:** 1241-1246.

Andersson, R., C. Gebhard, I. Miguel-Escalada, I. Hoof, J. Bornholdt *et al.*, 2014 An atlas of active enhancers across human cell types and tissues. Nature 507**:** 455-461.

Andrulis, E. D., J. Werner, A. Nazarian, H. Erdjument-Bromage, P. Tempst *et al.*, 2002 The RNA processing exosome is linked to elongating RNA polymerase II in Drosophila. Nature 420**:** 837-841.

Andruss, B. F., A. Q. Lu and K. Beckingham, 1997 Expression of calmodulin in Drosophila is highly regulated in a stage- and tissue-specific manner. Dev Genes Evol 206**:** 541-545.

Angulo, M., M. Corominas and F. Serras, 2004 Activation and repression activities of ash2 in Drosophila wing imaginal discs. Development 131**:** 4943-4953.

Antonucci, L., D. D'Amico, L. Di Magno, S. Coni, L. Di Marcotullio *et al.*, 2014 CNBP regulates wing development in Drosophila melanogaster by promoting IRES-dependent translation of dMyc. Cell Cycle 13**:** 434-439.

Aoyagi, N., S. Matsuoka, A. Furunobu, A. Matsukage and K. Sakaguchi, 1994 Drosophila DNA polymerase delta. Purification and characterization. J Biol Chem 269**:** 6045-6050.

Aoyagi, N., and D. A. Wassarman, 2000 Genes encoding Drosophila melanogaster RNA polymerase II general transcription factors: diversity in TFIIA and TFIID components contributes to gene-specific transcriptional regulation. J Cell Biol 150**:** F45-50.

Arama, E., J. Agapite and H. Steller, 2003 Caspase activity and a specific cytochrome C are required for sperm differentiation in Drosophila. Dev Cell 4**:** 687-697.

Arce, L., K. T. Pate and M. L. Waterman, 2009 Groucho binds two conserved regions of LEF-1 for HDAC-dependent repression. BMC Cancer 9**:** 159.

Archbold, H. C., C. Broussard, M. V. Chang and K. M. Cadigan, 2014 Bipartite recognition of DNA by TCF/Pangolin is remarkably flexible and contributes to transcriptional responsiveness and tissue specificity of wingless signaling. PLoS Genet 10**:** e1004591.

Ardehali, M. B., J. Yao, K. Adelman, N. J. Fuda, S. J. Petesch *et al.*, 2009 Spt6 enhances the elongation rate of RNA polymerase II in vivo. EMBO J 28**:** 1067-1077.

Armstrong, J. D., M. J. Texada, R. Munjaal, D. A. Baker and K. M. Beckingham, 2006 Gravitaxis in Drosophila melanogaster: a forward genetic screen. Genes Brain Behav 5**:** 222-239.

Arvola, R. M., C. T. Chang, J. P. Buytendorp, Y. Levdansky, E. Valkov *et al.*, 2020 Unique repression domains of Pumilio utilize deadenylation and decapping factors to accelerate destruction of target mRNAs. Nucleic Acids Res 48**:** 1843-1871.

Asano, K., T. G. Kinzy, W. C. Merrick and J. W. Hershey, 1997 Conservation and diversity of eukaryotic translation initiation factor eIF3. J Biol Chem 272**:** 1101-1109.

Asaoka-Taguchi, M., M. Yamada, A. Nakamura, K. Hanyu and S. Kobayashi, 1999 Maternal Pumilio acts together with Nanos in germline development in Drosophila embryos. Nat Cell Biol 1**:** 431-437.

Asaoka, T., J. Almagro, C. Ehrhardt, I. Tsai, A. Schleiffer *et al.*, 2016 Linear ubiquitination by LUBEL has a role in Drosophila heat stress response. EMBO Rep 17**:** 1624-1640.

Ashton-Beaucage, D., C. M. Udell, P. Gendron, M. Sahmi, M. Lefrancois *et al.*, 2014 A functional screen reveals an extensive layer of transcriptional and splicing control underlying RAS/MAPK signaling in Drosophila. PLoS Biol 12**:** e1001809.

Azuma, Y., T. Tokuda, Y. Kushimura, I. Yamamoto, I. Mizuta *et al.*, 2018 Hippo, Drosophila MST, is a novel modifier of motor neuron degeneration induced by knockdown of Caz, Drosophila FUS. Exp Cell Res 371**:** 311-321.

Azuma, Y., T. Tokuda, M. Shimamura, A. Kyotani, H. Sasayama *et al.*, 2014 Identification of ter94, Drosophila VCP, as a strong modulator of motor neuron degeneration induced by knockdown of Caz, Drosophila FUS. Hum Mol Genet 23**:** 3467-3480.

Badenhorst, P., M. Voas, I. Rebay and C. Wu, 2002 Biological functions of the ISWI chromatin remodeling complex NURF. Genes Dev 16**:** 3186-3198.

Badenhorst, P., H. Xiao, L. Cherbas, S. Y. Kwon, M. Voas *et al.*, 2005 The Drosophila nucleosome remodeling factor NURF is required for Ecdysteroid signaling and metamorphosis. Genes Dev 19**:** 2540-2545.

Baehrecke, E. H., 1997 who encodes a KH RNA binding protein that functions in muscle development. Development 124**:** 1323-1332.

Bag, I., Y. Chen, K. D'Orazio, P. Lopez, S. Wenzel *et al.*, 2022 Isha is a su(Hw) mRNA-binding protein required for gypsy insulator function. G3 (Bethesda) 12.

Bajpai, R., K. Makhijani, P. R. Rao and L. S. Shashidhara, 2004 Drosophila Twins regulates Armadillo levels in response to Wg/Wnt signal. Development 131**:** 1007-1016.

Baker, D. A., B. Mille-Baker, S. M. Wainwright, D. Ish-Horowicz and N. J. Dibb, 2001 Mae mediates MAP kinase phosphorylation of Ets transcription factors in Drosophila. Nature 411**:** 330-334.

Bakkenist, C. J., and S. Cotterill, 1994 The 50-kDa primase subunit of Drosophila melanogaster DNA polymerase alpha. Molecular characterization of the gene and functional analysis of the overexpressed protein. J Biol Chem 269**:** 26759-26766.

Balko, J. M., and C. L. Arteaga, 2011 Dead-box or black-box: is DDX1 a potential biomarker in breast cancer? Breast Cancer Res Treat 127**:** 65-67.

Baluapuri, A., J. Hofstetter, N. Dudvarski Stankovic, T. Endres, P. Bhandare *et al.*, 2019 MYC Recruits SPT5 to RNA Polymerase II to Promote Processive Transcription Elongation. Mol Cell 74**:** 674-687 e611.

Barbosa, V., M. Gatt, E. Rebollo, C. Gonzalez and D. M. Glover, 2003 Drosophila dd4 mutants reveal that gammaTuRC is required to maintain juxtaposed half spindles in spermatocytes. J Cell Sci 116**:** 929-941.

Bardin, A. J., R. Le Borgne and F. Schweisguth, 2004 Asymmetric localization and function of cell-fate determinants: a fly's view. Curr Opin Neurobiol 14**:** 6-14.

Barish, S., T. S. Barakat, B. C. Michel, N. Mashtalir, J. B. Phillips *et al.*, 2020 BICRA, a SWI/SNF Complex Member, Is Associated with BAF-Disorder Related Phenotypes in Humans and Model Organisms. Am J Hum Genet 107**:** 1096-1112.

Barker, N., P. J. Morin and H. Clevers, 2000 The Yin-Yang of TCF/beta-catenin signaling. Adv Cancer Res 77**:** 1-24.

Barral, A., and J. Dejardin, 2023 The chromatin signatures of enhancers and their dynamic regulation. Nucleus 14**:** 2160551.

Bashirullah, A., G. Lam, V. P. Yin and C. S. Thummel, 2007 dTrf2 is required for transcriptional and developmental responses to ecdysone during Drosophila metamorphosis. Dev Dyn 236**:** 3173-3179.

Bauer, A., S. Chauvet, O. Huber, F. Usseglio, U. Rothbacher *et al.*, 2000 Pontin52 and reptin52 function as antagonistic regulators of beta-catenin signalling activity. EMBO J 19**:** 6121-6130.

Bejarano, F., C. M. Luque, H. Herranz, G. Sorrosal, N. Rafel *et al.*, 2008 A gain-of-function suppressor screen for genes involved in dorsal-ventral boundary formation in the Drosophila wing. Genetics 178**:** 307-323.

Bello-Fernandez, C., G. Packham and J. L. Cleveland, 1993 The ornithine decarboxylase gene is a transcriptional target of c-Myc. Proc Natl Acad Sci U S A 90**:** 7804-7808.

Bellosta, P., and P. Gallant, 2010 Myc Function in Drosophila. Genes Cancer 1**:** 542-546.

Bellosta, P., T. Hulf, S. Balla Diop, F. Usseglio, J. Pradel *et al.*, 2005 Myc interacts genetically with Tip48/Reptin and Tip49/Pontin to control growth and proliferation during Drosophila development. Proc Natl Acad Sci U S A 102**:** 11799-11804.

Beltran, S., M. Angulo, M. Pignatelli, F. Serras and M. Corominas, 2007 Functional dissection of the ash2 and ash1 transcriptomes provides insights into the transcriptional basis of wing phenotypes and reveals conserved protein interactions. Genome Biol 8**:** R67.

Benassayag, C., L. Montero, N. Colombie, P. Gallant, D. Cribbs *et al.*, 2005 Human c-Myc isoforms differentially regulate cell growth and apoptosis in Drosophila melanogaster. Mol Cell Biol 25**:** 9897-9909.

Berman, B. P., B. D. Pfeiffer, T. R. Laverty, S. L. Salzberg, G. M. Rubin *et al.*, 2004 Computational identification of developmental enhancers: conservation and function of transcription factor binding-site clusters in Drosophila melanogaster and Drosophila pseudoobscura. Genome Biol 5**:** R61.

Bertolotti, A., Y. Lutz, D. J. Heard, P. Chambon and L. Tora, 1996 hTAF(II)68, a novel RNA/ssDNA-binding protein with homology to the pro-oncoproteins TLS/FUS and EWS is associated with both TFIID and RNA polymerase II. EMBO J 15**:** 5022-5031.

Bhambhani, C., J. L. Chang, D. L. Akey and K. M. Cadigan, 2011 The oligomeric state of CtBP determines its role as a transcriptional co-activator and co-repressor of Wingless targets. EMBO J 30**:** 2031-2043.

Biedermann, B., H. R. Hotz and R. Ciosk, 2010 The Quaking family of RNA-binding proteins: coordinators of the cell cycle and differentiation. Cell Cycle 9**:** 1929-1933.

Biggs, W. H., 3rd, K. H. Zavitz, B. Dickson, A. van der Straten, D. Brunner *et al.*, 1994 The Drosophila rolled locus encodes a MAP kinase required in the sevenless signal transduction pathway. EMBO J 13**:** 1628-1635.

Biryukova, I., and P. Heitzler, 2005 The Drosophila LIM-homeo domain protein Islet antagonizes pro-neural cell specification in the peripheral nervous system. Dev Biol 288**:** 559-570.

Blanco, J., J. C. Cooper and N. E. Baker, 2020 Roles of C/EBP class bZip proteins in the growth and cell competition of Rp ('Minute') mutants in Drosophila. Elife 9.

Bode, J., S. Goetze, H. Heng, S. A. Krawetz and C. Benham, 2003 From DNA structure to gene expression: mediators of nuclear compartmentalization and dynamics. Chromosome Res 11**:** 435-445.

Bode, J., M. Stengert-Iber, V. Kay, T. Schlake and A. Dietz-Pfeilstetter, 1996 Scaffold/matrix-attached regions: topological switches with multiple regulatory functions. Crit Rev Eukaryot Gene Expr 6**:** 115-138.

Boi, D., E. Rubini, S. Breccia, G. Guarguaglini and A. Paiardini, 2023 When Just One Phosphate Is One Too Many: The Multifaceted Interplay between Myc and Kinases. Int J Mol Sci 24.

Bonchuk, A., S. Denisov, P. Georgiev and O. Maksimenko, 2011 Drosophila BTB/POZ domains of "ttk group" can form multimers and selectively interact with each other. J Mol Biol 412**:** 423-436.

Bonke, M., M. Turunen, M. Sokolova, A. Vaharautio, T. Kivioja *et al.*, 2013 Transcriptional networks controlling the cell cycle. G3 (Bethesda) 3**:** 75-90.

Bonnay, F., X. H. Nguyen, E. Cohen-Berros, L. Troxler, E. Batsche *et al.*, 2014 Akirin specifies NF-kappaB selectivity of Drosophila innate immune response via chromatin remodeling. EMBO J 33**:** 2349-2362.

Borg, R. M., R. Bordonne, N. Vassallo and R. J. Cauchi, 2015 Genetic Interactions between the Members of the SMN-Gemins Complex in Drosophila. PLoS One 10**:** e0130974.

Bose, A., B. Kahali, S. Zhang, J. M. Lin, R. Allada *et al.*, 2006 Drosophila CK2 regulates lateral-inhibition during eye and bristle development. Mech Dev 123**:** 649-664.

Bosso, G., F. Cipressa, M. L. Moroni, R. Pennisi, J. Albanesi *et al.*, 2019 NBS1 interacts with HP1 to ensure genome integrity. Cell Death Dis 10**:** 951.

Boube, M., C. Faucher, L. Joulia, D. L. Cribbs and H. M. Bourbon, 2000 Drosophila homologs of transcriptional mediator complex subunits are required for adult cell and segment identity specification. Genes Dev 14**:** 2906-2917.

Bouchard, C., K. Thieke, A. Maier, R. Saffrich, J. Hanley-Hyde *et al.*, 1999 Direct induction of cyclin D2 by Myc contributes to cell cycle progression and sequestration of p27. EMBO J 18**:** 5321-5333.

Boulon, S., N. Marmier-Gourrier, B. Pradet-Balade, L. Wurth, C. Verheggen *et al.*, 2008 The Hsp90 chaperone controls the biogenesis of L7Ae RNPs through conserved machinery. J Cell Biol 180**:** 579-595.

Braat, A. K., N. Yan, E. Arn, D. Harrison and P. M. Macdonald, 2004 Localization-dependent oskar protein accumulation; control after the initiation of translation. Dev Cell 7**:** 125-131.

Bras, S., S. Martin-Lanneree, V. Gobert, B. Auge, O. Breig *et al.*, 2012 Myeloid leukemia factor is a conserved regulator of RUNX transcription factor activity involved in hematopoiesis. Proc Natl Acad Sci U S A 109**:** 4986-4991.

Brentrup, D., H. Lerch, H. Jackle and M. Noll, 2000 Regulation of Drosophila wing vein patterning: net encodes a bHLH protein repressing rhomboid and is repressed by rhomboid-dependent Egfr signalling. Development 127**:** 4729-4741.

Bretones, G., M. D. Delgado and J. Leon, 2015 Myc and cell cycle control. Biochim Biophys Acta 1849**:** 506-516.

Brewer-Jensen, P., C. B. Wilson, J. Abernethy, L. Mollison, S. Card *et al.*, 2016 Suppressor of sable [Su(s)] and Wdr82 down-regulate RNA from heat-shock-inducible repetitive elements by a mechanism that involves transcription termination. RNA 22**:** 139-154.

Brickey, W. J., and A. L. Greenleaf, 1995 Functional studies of the carboxy-terminal repeat domain of Drosophila RNA polymerase II in vivo. Genetics 140**:** 599-613.

Broderick, P., D. Cunningham, J. Vijayakrishnan, R. Cooke, A. Ashworth *et al.*, 2010 IRF4 polymorphism rs872071 and risk of Hodgkin lymphoma. Br J Haematol 148**:** 413-415.

Brody, T., W. Rasband, K. Baler, A. Kuzin, M. Kundu *et al.*, 2007 cis-Decoder discovers constellations of conserved DNA sequences shared among tissue-specific enhancers. Genome Biol 8**:** R75.

Brou, C., F. Logeat, M. Lecourtois, J. Vandekerckhove, P. Kourilsky *et al.*, 1994 Inhibition of the DNA-binding activity of Drosophila suppressor of hairless and of its human homolog, KBF2/RBP-J kappa, by direct protein-protein interaction with Drosophila hairless. Genes Dev 8**:** 2491-2503.

Brumby, A. M., C. B. Zraly, J. A. Horsfield, J. Secombe, R. Saint *et al.*, 2002 Drosophila cyclin E interacts with components of the Brahma complex. EMBO J 21**:** 3377-3389.

Brun, S., S. Vidal, P. Spellman, K. Takahashi, H. Tricoire *et al.*, 2006 The MAPKKK Mekk1 regulates the expression of Turandot stress genes in response to septic injury in Drosophila. Genes Cells 11**:** 397-407.

Brunner, D., K. Ducker, N. Oellers, E. Hafen, H. Scholz *et al.*, 1994 The ETS domain protein pointed-P2 is a target of MAP kinase in the sevenless signal transduction pathway. Nature 370**:** 386-389.

Bujakowska, K., C. Maubaret, C. F. Chakarova, N. Tanimoto, S. C. Beck *et al.*, 2009 Study of gene-targeted mouse models of splicing factor gene Prpf31 implicated in human autosomal dominant retinitis pigmentosa (RP). Invest Ophthalmol Vis Sci 50**:** 5927-5933.

Burgio, G., G. La Rocca, A. Sala, W. Arancio, D. Di Gesu *et al.*, 2008 Genetic identification of a network of factors that functionally interact with the nucleosome remodeling ATPase ISWI. PLoS Genet 4**:** e1000089.

Burke, T. W., and J. T. Kadonaga, 1997 The downstream core promoter element, DPE, is conserved from Drosophila to humans and is recognized by TAFII60 of Drosophila. Genes Dev 11**:** 3020-3031.

Butler, J. E., and J. T. Kadonaga, 2001 Enhancer-promoter specificity mediated by DPE or TATA core promoter motifs. Genes Dev 15**:** 2515-2519.

Cadigan, K. M., 2012 TCFs and Wnt/beta-catenin signaling: more than one way to throw the switch. Curr Top Dev Biol 98**:** 1-34.

Caforio, M., C. Sorino, S. Iacovelli, M. Fanciulli, F. Locatelli *et al.*, 2018 Recent advances in searching c-Myc transcriptional cofactors during tumorigenesis. J Exp Clin Cancer Res 37**:** 239.

Cakouros, D., K. Mills, D. Denton, A. Paterson, T. Daish *et al.*, 2008 dLKR/SDH regulates hormone-mediated histone arginine methylation and transcription of cell death genes. J Cell Biol 182**:** 481-495.

Campbell, S. D., F. Sprenger, B. A. Edgar and P. H. O'Farrell, 1995 Drosophila Wee1 kinase rescues fission yeast from mitotic catastrophe and phosphorylates Drosophila Cdc2 in vitro. Mol Biol Cell 6**:** 1333-1347.

Capelson, M., Y. Liang, R. Schulte, W. Mair, U. Wagner *et al.*, 2010 Chromatin-bound nuclear pore components regulate gene expression in higher eukaryotes. Cell 140**:** 372-383.

Capp, C., J. Wu and T. S. Hsieh, 2009 Drosophila RecQ4 has a 3'-5' DNA helicase activity that is essential for viability. J Biol Chem 284**:** 30845-30852.

Cappadocia, L., and C. D. Lima, 2018 Ubiquitin-like Protein Conjugation: Structures, Chemistry, and Mechanism. Chem Rev 118**:** 889-918.

Carbonell, A., A. Mazo, F. Serras and M. Corominas, 2013 Ash2 acts as an ecdysone receptor coactivator by stabilizing the histone methyltransferase Trr. Mol Biol Cell 24**:** 361-372.

Carreno, S., I. Kouranti, E. S. Glusman, M. T. Fuller, A. Echard *et al.*, 2008 Moesin and its activating kinase Slik are required for cortical stability and microtubule organization in mitotic cells. J Cell Biol 180**:** 739-746.

Carrera, I., F. Janody, N. Leeds, F. Duveau and J. E. Treisman, 2008 Pygopus activates Wingless target gene transcription through the mediator complex subunits Med12 and Med13. Proc Natl Acad Sci U S A 105**:** 6644-6649.

Carrera, P., Y. M. Moshkin, S. Gronke, H. H. Sillje, E. A. Nigg *et al.*, 2003 Tousled-like kinase functions with the chromatin assembly pathway regulating nuclear divisions. Genes Dev 17**:** 2578-2590.

Casper, A. L., K. Baxter and M. Van Doren, 2011 no child left behind encodes a novel chromatin factor required for germline stem cell maintenance in males but not females. Development 138**:** 3357-3366.

Castro, B., S. Barolo, A. M. Bailey and J. W. Posakony, 2005 Lateral inhibition in proneural clusters: cis-regulatory logic and default repression by Suppressor of Hairless. Development 132**:** 3333-3344.

Catarino, R. R., C. Neumayr and A. Stark, 2017 Promoting transcription over long distances. Nat Genet 49**:** 972-973.

Catarino, R. R., and A. Stark, 2018 Assessing sufficiency and necessity of enhancer activities for gene expression and the mechanisms of transcription activation. Genes Dev 32**:** 202-223.

Cauchi, R. J., K. E. Davies and J. L. Liu, 2008 A motor function for the DEAD-box RNA helicase, Gemin3, in Drosophila. PLoS Genet 4**:** e1000265.

Cavallo, R. A., R. T. Cox, M. M. Moline, J. Roose, G. A. Polevoy *et al.*, 1998 Drosophila Tcf and Groucho interact to repress Wingless signalling activity. Nature 395**:** 604-608.

Cenci, G., R. B. Rawson, G. Belloni, D. H. Castrillon, M. Tudor *et al.*, 1997 UbcD1, a Drosophila ubiquitin-conjugating enzyme required for proper telomere behavior. Genes Dev 11**:** 863-875.

Cencioni, C., F. Scagnoli, F. Spallotta, S. Nasi and B. Illi, 2023 The "Superoncogene" Myc at the Crossroad between Metabolism and Gene Expression in Glioblastoma Multiforme. Int J Mol Sci 24.

Chalkley, G. E., Y. M. Moshkin, K. Langenberg, K. Bezstarosti, A. Blastyak *et al.*, 2008 The transcriptional coactivator SAYP is a trithorax group signature subunit of the PBAP chromatin remodeling complex. Mol Cell Biol 28**:** 2920-2929.

Chambers, C., K. Cermakova, Y. S. Chan, K. Kurtz, K. Wohlan *et al.*, 2023 SWI/SNF Blockade Disrupts PU.1-Directed Enhancer Programs in Normal Hematopoietic Cells and Acute Myeloid Leukemia. Cancer Res 83**:** 983-996.

Chan, Y. B., I. Miguel-Aliaga, C. Franks, N. Thomas, B. Trulzsch *et al.*, 2003 Neuromuscular defects in a Drosophila survival motor neuron gene mutant. Hum Mol Genet 12**:** 1367-1376.

Chang, J. L., H. V. Lin, T. A. Blauwkamp and K. M. Cadigan, 2008 Spenito and Split ends act redundantly to promote Wingless signaling. Dev Biol 314**:** 100-111.

Chang, Y. C., J. W. Wu, Y. C. Hsieh, T. H. Huang, Z. M. Liao *et al.*, 2018 Rap1 Negatively Regulates the Hippo Pathway to Polarize Directional Protrusions in Collective Cell Migration. Cell Rep 22**:** 2160-2175.

Chao, A. T., H. A. Dierick, T. M. Addy and A. Bejsovec, 2003 Mutations in eukaryotic release factors 1 and 3 act as general nonsense suppressors in Drosophila. Genetics 165**:** 601-612.

Chartier, A., P. Klein, S. Pierson, N. Barbezier, T. Gidaro *et al.*, 2015 Mitochondrial dysfunction reveals the role of mRNA poly(A) tail regulation in oculopharyngeal muscular dystrophy pathogenesis. PLoS Genet 11**:** e1005092.

Chau, B. L., K. P. Ng, K. K. Li and K. A. Lee, 2016 RGG boxes within the TET/FET family of RNA-binding proteins are functionally distinct. Transcription 7**:** 141-151.

Chazal, P. E., E. Daguenet, C. Wendling, N. Ulryck, C. Tomasetto *et al.*, 2013 EJC core component MLN51 interacts with eIF3 and activates translation. Proc Natl Acad Sci U S A 110**:** 5903-5908.

Chen, D., Q. Wang, H. Huang, L. Xia, X. Jiang *et al.*, 2009 Effete-mediated degradation of Cyclin A is essential for the maintenance of germline stem cells in Drosophila. Development 136**:** 4133-4142.

Chen, F. X., A. R. Woodfin, A. Gardini, R. A. Rickels, S. A. Marshall *et al.*, 2015 PAF1, a Molecular Regulator of Promoter-Proximal Pausing by RNA Polymerase II. Cell 162**:** 1003-1015.

Chen, X., and L. Xu, 2010 Specific nucleoporin requirement for Smad nuclear translocation. Mol Cell Biol 30**:** 4022-4034.

Cheng, S. Y., and J. M. Bishop, 2002 Suppressor of Fused represses Gli-mediated transcription by recruiting the SAP18-mSin3 corepressor complex. Proc Natl Acad Sci U S A 99**:** 5442-5447.

Chiang, C. S., P. G. Mitsis and I. R. Lehman, 1993 DNA polymerase delta from embryos of Drosophila melanogaster. Proc Natl Acad Sci U S A 90**:** 9105-9109.

Cho, I. K., C. L. Chang and Q. X. Li, 2013 Diet-induced over-expression of flightless-I protein and its relation to flightlessness in Mediterranean fruit fly, Ceratitis capitata. PLoS One 8**:** e81099.

Chodagam, S., A. Royou, W. Whitfield, R. Karess and J. W. Raff, 2005 The centrosomal protein CP190 regulates myosin function during early Drosophila development. Curr Biol 15**:** 1308-1313.

Churchill, M. E., D. N. Jones, T. Glaser, H. Hefner, M. A. Searles *et al.*, 1995 HMG-D is an architecture-specific protein that preferentially binds to DNA containing the dinucleotide TG. EMBO J 14**:** 1264-1275.

Ciapponi, L., G. Cenci, J. Ducau, C. Flores, D. Johnson-Schlitz *et al.*, 2004 The Drosophila Mre11/Rad50 complex is required to prevent both telomeric fusion and chromosome breakage. Curr Biol 14**:** 1360-1366.

Ciapponi, L., G. Cenci and M. Gatti, 2006 The Drosophila Nbs protein functions in multiple pathways for the maintenance of genome stability. Genetics 173**:** 1447-1454.

Ciribilli, Y., P. Singh, R. Spanel, A. Inga and J. Borlak, 2015 Decoding c-Myc networks of cell cycle and apoptosis regulated genes in a transgenic mouse model of papillary lung adenocarcinomas. Oncotarget 6**:** 31569-31592.

Clegg, N. J., D. M. Frost, M. K. Larkin, L. Subrahmanyan, Z. Bryant *et al.*, 1997 maelstrom is required for an early step in the establishment of Drosophila oocyte polarity: posterior localization of grk mRNA. Development 124**:** 4661-4671.

Clemens, J. C., C. A. Worby, N. Simonson-Leff, M. Muda, T. Maehama *et al.*, 2000 Use of double-stranded RNA interference in Drosophila cell lines to dissect signal transduction pathways. Proc Natl Acad Sci U S A 97**:** 6499-6503.

Clouse, K. N., S. B. Ferguson and T. Schupbach, 2008 Squid, Cup, and PABP55B function together to regulate gurken translation in Drosophila. Dev Biol 313**:** 713-724.

Collins, B. E., C. B. Greer, B. C. Coleman and J. D. Sweatt, 2019 Histone H3 lysine K4 methylation and its role in learning and memory. Epigenetics Chromatin 12**:** 7.

Collins, R. T., and J. E. Treisman, 2000 Osa-containing Brahma chromatin remodeling complexes are required for the repression of wingless target genes. Genes Dev 14**:** 3140-3152.

Conerly, M. L., S. S. Teves, D. Diolaiti, M. Ulrich, R. N. Eisenman *et al.*, 2010 Changes in H2A.Z occupancy and DNA methylation during B-cell lymphomagenesis. Genome Res 20**:** 1383-1390.

Connell-Crowley, L., M. Le Gall, D. J. Vo and E. Giniger, 2000 The cyclin-dependent kinase Cdk5 controls multiple aspects of axon patterning in vivo. Curr Biol 10**:** 599-602.

Cordero, J. B., R. K. Stefanatos, K. Myant, M. Vidal and O. J. Sansom, 2012 Non-autonomous crosstalk between the Jak/Stat and Egfr pathways mediates Apc1-driven intestinal stem cell hyperplasia in the Drosophila adult midgut. Development 139**:** 4524-4535.

Corona, D. F., A. Eberharter, A. Budde, R. Deuring, S. Ferrari *et al.*, 2000 Two histone fold proteins, CHRAC-14 and CHRAC-16, are developmentally regulated subunits of chromatin accessibility complex (CHRAC). EMBO J 19**:** 3049-3059.

Cottage, C. T., B. Bailey, K. M. Fischer, D. Avitabile, B. Collins *et al.*, 2010 Cardiac progenitor cell cycling stimulated by pim-1 kinase. Circ Res 106**:** 891-901.

Cotterill, S., G. Chui and I. R. Lehman, 1987 DNA polymerase-primase from embryos of Drosophila melanogaster. DNA primase subunits. J Biol Chem 262**:** 16105-16108.

Couso, J. P., E. Knust and A. Martinez Arias, 1995 Serrate and wingless cooperate to induce vestigial gene expression and wing formation in Drosophila. Curr Biol 5**:** 1437-1448.

Crest, J., A. Diz-Munoz, D. Y. Chen, D. A. Fletcher and D. Bilder, 2017 Organ sculpting by patterned extracellular matrix stiffness. Elife 6.

Crevel, G., E. Mathe and S. Cotterill, 2005 The Drosophila Cdc6/18 protein has functions in both early and late S phase in S2 cells. J Cell Sci 118**:** 2451-2459.

Crona, F., B. Singla and M. Mannervik, 2015 Gene expression profiling of brakeless mutant Drosophila embryos. Data Brief 5**:** 134-137.

Crosby, M. A., C. Miller, T. Alon, K. L. Watson, C. P. Verrijzer *et al.*, 1999 The trithorax group gene moira encodes a brahma-associated putative chromatin-remodeling factor in Drosophila melanogaster. Mol Cell Biol 19**:** 1159-1170.

Crucianelli, C., J. Jaiswal, A. Vijayakumar Maya, L. Nogay, A. Cosolo *et al.*, 2022 Distinct signaling signatures drive compensatory proliferation via S-phase acceleration. PLoS Genet 18**:** e1010516.

Csiszar, A., E. Vogelsang, H. Beug and M. Leptin, 2010 A novel conserved phosphotyrosine motif in the Drosophila fibroblast growth factor signaling adaptor Dof with a redundant role in signal transmission. Mol Cell Biol 30**:** 2017-2027.

Cuartero, S., U. Fresan, O. Reina, E. Planet and M. L. Espinas, 2014 Ibf1 and Ibf2 are novel CP190-interacting proteins required for insulator function. EMBO J 33**:** 637-647.

Curtis, B. J., C. B. Zraly, D. R. Marenda and A. K. Dingwall, 2011 Histone lysine demethylases function as co-repressors of SWI/SNF remodeling activities during Drosophila wing development. Dev Biol 350**:** 534-547.

Curtis, C. D., and C. T. Griffin, 2012 The chromatin-remodeling enzymes BRG1 and CHD4 antagonistically regulate vascular Wnt signaling. Mol Cell Biol 32**:** 1312-1320.

D'Andrea, L. D., and L. Regan, 2003 TPR proteins: the versatile helix. Trends Biochem Sci 28**:** 655-662.

Dahmus, G. K., C. V. Glover, D. L. Brutlag and M. E. Dahmus, 1984 Similarities in structure and function of calf thymus and Drosophila casein kinase II. J Biol Chem 259**:** 9001-9006.

Dai, H., C. Hogan, B. Gopalakrishnan, J. Torres-Vazquez, M. Nguyen *et al.*, 2000 The zinc finger protein schnurri acts as a Smad partner in mediating the transcriptional response to decapentaplegic. Dev Biol 227**:** 373-387.

Dammai, V., B. Adryan, K. R. Lavenburg and T. Hsu, 2003 Drosophila awd, the homolog of human nm23, regulates FGF receptor levels and functions synergistically with shi/dynamin during tracheal development. Genes Dev 17**:** 2812-2824.

Daneshvar, K., A. Khan and J. M. Goodliffe, 2011 Myc localizes to histone locus bodies during replication in Drosophila. PLoS One 6**:** e23928.

Dang, C. V., 2012 MYC on the path to cancer. Cell 149**:** 22-35.

Dansereau, D. A., and P. Lasko, 2008 RanBPM regulates cell shape, arrangement, and capacity of the female germline stem cell niche in Drosophila melanogaster. J Cell Biol 182**:** 963-977.

Dao, L. T. M., A. O. Galindo-Albarran, J. A. Castro-Mondragon, C. Andrieu-Soler, A. Medina-Rivera *et al.*, 2017 Genome-wide characterization of mammalian promoters with distal enhancer functions. Nat Genet 49**:** 1073-1081.

Das, A. K., P. W. Cohen and D. Barford, 1998a The structure of the tetratricopeptide repeats of protein phosphatase 5: implications for TPR-mediated protein-protein interactions. EMBO J 17**:** 1192-1199.

Das, P., L. L. Maduzia, H. Wang, A. L. Finelli, S. H. Cho *et al.*, 1998b The Drosophila gene Medea demonstrates the requirement for different classes of Smads in dpp signaling. Development 125**:** 1519-1528.

Das Thakur, M., Y. Feng, R. Jagannathan, M. J. Seppa, J. B. Skeath *et al.*, 2010 Ajuba LIM proteins are negative regulators of the Hippo signaling pathway. Curr Biol 20**:** 657-662.

De Celis, J. F., 2003 Pattern formation in the Drosophila wing: The development of the veins. Bioessays 25**:** 443-451.

de la Cova, C., and L. A. Johnston, 2006 Myc in model organisms: a view from the flyroom. Semin Cancer Biol 16**:** 303-312.

de Pretis, S., T. R. Kress, M. J. Morelli, A. Sabo, C. Locarno *et al.*, 2017 Integrative analysis of RNA polymerase II and transcriptional dynamics upon MYC activation. Genome Res 27**:** 1658-1664.

Dean, K. A., A. K. Aggarwal and R. P. Wharton, 2002 Translational repressors in Drosophila. Trends Genet 18**:** 572-577.

Dean, S. O., and J. A. Spudich, 2006 Rho kinase's role in myosin recruitment to the equatorial cortex of mitotic Drosophila S2 cells is for myosin regulatory light chain phosphorylation. PLoS One 1**:** e131.

Delanoue, R., M. Slaidina and P. Leopold, 2010 The steroid hormone ecdysone controls systemic growth by repressing dMyc function in Drosophila fat cells. Dev Cell 18**:** 1012-1021.

Demontis, F., and N. Perrimon, 2009 Integration of Insulin receptor/Foxo signaling and dMyc activity during muscle growth regulates body size in Drosophila. Development 136**:** 983-993.

Deng, X., and V. H. Meller, 2006 roX RNAs are required for increased expression of X-linked genes in Drosophila melanogaster males. Genetics 174**:** 1859-1866.

Destefanis, F., V. Manara and P. Bellosta, 2020 Myc as a Regulator of Ribosome Biogenesis and Cell Competition: A Link to Cancer. Int J Mol Sci 21.

Dey, B., S. Thukral, S. Krishnan, M. Chakrobarty, S. Gupta *et al.*, 2012 DNA-protein interactions: methods for detection and analysis. Mol Cell Biochem 365**:** 279-299.

Dhanasekaran, R., A. Deutzmann, W. D. Mahauad-Fernandez, A. S. Hansen, A. M. Gouw *et al.*, 2022 The MYC oncogene - the grand orchestrator of cancer growth and immune evasion. Nat Rev Clin Oncol 19**:** 23-36.

Diaz-Benjumea, F. J., and E. Hafen, 1994 The sevenless signalling cassette mediates Drosophila EGF receptor function during epidermal development. Development 120**:** 569-578.

Dingwall, A. K., S. J. Beek, C. M. McCallum, J. W. Tamkun, G. V. Kalpana *et al.*, 1995 The Drosophila snr1 and brm proteins are related to yeast SWI/SNF proteins and are components of a large protein complex. Mol Biol Cell 6**:** 777-791.

Dobi, K. C., M. S. Halfon and M. K. Baylies, 2014 Whole-genome analysis of muscle founder cells implicates the chromatin regulator Sin3A in muscle identity. Cell Rep 8**:** 858-870.

Doerflinger, H., V. Zimyanin and D. St Johnston, 2022 The Drosophila anterior-posterior axis is polarized by asymmetric myosin activation. Curr Biol 32**:** 374-385 e374.

Dong, X., L. Tsuda, K. H. Zavitz, M. Lin, S. Li *et al.*, 1999 ebi regulates epidermal growth factor receptor signaling pathways in Drosophila. Genes Dev 13**:** 954-965.

Dorfman, R., L. Glazer, U. Weihe, M. F. Wernet and B. Z. Shilo, 2002 Elbow and Noc define a family of zinc finger proteins controlling morphogenesis of specific tracheal branches. Development 129**:** 3585-3596.

Douziech, M., M. Sahmi, G. Laberge and M. Therrien, 2006 A KSR/CNK complex mediated by HYP, a novel SAM domain-containing protein, regulates RAS-dependent RAF activation in Drosophila. Genes Dev 20**:** 807-819.

Doyen, C. M., G. E. Chalkley, O. Voets, K. Bezstarosti, J. A. Demmers *et al.*, 2015 A Testis-Specific Chaperone and the Chromatin Remodeler ISWI Mediate Repackaging of the Paternal Genome. Cell Rep 13**:** 1310-1318.

Dragan, A. I., J. Klass, C. Read, M. E. Churchill, C. Crane-Robinson *et al.*, 2003 DNA binding of a non-sequence-specific HMG-D protein is entropy driven with a substantial non-electrostatic contribution. J Mol Biol 331**:** 795-813.

Dragojlovic-Munther, M., and J. A. Martinez-Agosto, 2013 Extracellular matrix-modulated Heartless signaling in Drosophila blood progenitors regulates their differentiation via a Ras/ETS/FOG pathway and target of rapamycin function. Dev Biol 384**:** 313-330.

Dreos, R., A. Sloutskin, N. Malachi, D. Ideses, P. Bucher *et al.*, 2021 Computational identification and experimental characterization of preferred downstream positions in human core promoters. PLoS Comput Biol 17**:** e1009256.

Du, L., A. Sohr, G. Yan and S. Roy, 2018 Feedback regulation of cytoneme-mediated transport shapes a tissue-specific FGF morphogen gradient. Elife 7.

Ducat, D., S. Kawaguchi, H. Liu, J. R. Yates, 3rd and Y. Zheng, 2008 Regulation of microtubule assembly and organization in mitosis by the AAA+ ATPase Pontin. Mol Biol Cell 19**:** 3097-3110.

Duman-Scheel, M., L. A. Johnston and W. Du, 2004 Repression of dMyc expression by Wingless promotes Rbf-induced G1 arrest in the presumptive Drosophila wing margin. Proc Natl Acad Sci U S A 101**:** 3857-3862.

Duncan, K., M. Grskovic, C. Strein, K. Beckmann, R. Niggeweg *et al.*, 2006 Sex-lethal imparts a sex-specific function to UNR by recruiting it to the msl-2 mRNA 3' UTR: translational repression for dosage compensation. Genes Dev 20**:** 368-379.

Duncan, R., L. Bazar, G. Michelotti, T. Tomonaga, H. Krutzsch *et al.*, 1994 A sequence-specific, single-strand binding protein activates the far upstream element of c-myc and defines a new DNA-binding motif. Genes Dev 8**:** 465-480.

Dworkin, I., and G. Gibson, 2006 Epidermal growth factor receptor and transforming growth factor-beta signaling contributes to variation for wing shape in Drosophila melanogaster. Genetics 173**:** 1417-1431.

Dzhindzhev, N. S., S. L. Rogers, R. D. Vale and H. Ohkura, 2005 Distinct mechanisms govern the localisation of Drosophila CLIP-190 to unattached kinetochores and microtubule plus-ends. J Cell Sci 118**:** 3781-3790.

Eberharter, A., S. Ferrari, G. Langst, T. Straub, A. Imhof *et al.*, 2001 Acf1, the largest subunit of CHRAC, regulates ISWI-induced nucleosome remodelling. EMBO J 20**:** 3781-3788.

Eckert, K., J. M. Saliou, L. Monlezun, A. Vigouroux, N. Atmane *et al.*, 2010 The Pih1-Tah1 cochaperone complex inhibits Hsp90 molecular chaperone ATPase activity. J Biol Chem 285**:** 31304-31312.

Edenfeld, G., G. Volohonsky, K. Krukkert, E. Naffin, U. Lammel *et al.*, 2006 The splicing factor crooked neck associates with the RNA-binding protein HOW to control glial cell maturation in Drosophila. Neuron 52**:** 969-980.

Edgar, B. A., J. Britton, A. F. de la Cruz, L. A. Johnston, D. Lehman *et al.*, 2001 Pattern- and growth-linked cell cycles in Drosophila development. Novartis Found Symp 237**:** 3-12; discussion 12-18, 36-42.

Eeftens, J. M., J. van der Torre, D. R. Burnham and C. Dekker, 2015 Copper-free click chemistry for attachment of biomolecules in magnetic tweezers. BMC Biophys 8**:** 9.

Eggert, H., A. Gortchakov and H. Saumweber, 2004 Identification of the Drosophila interband-specific protein Z4 as a DNA-binding zinc-finger protein determining chromosomal structure. J Cell Sci 117**:** 4253-4264.

Eivers, E., L. C. Fuentealba, V. Sander, J. C. Clemens, L. Hartnett *et al.*, 2009 Mad is required for wingless signaling in wing development and segment patterning in Drosophila. PLoS One 4**:** e6543.

Ekengren, S., and D. Hultmark, 2001 A family of Turandot-related genes in the humoral stress response of Drosophila. Biochem Biophys Res Commun 284**:** 998-1003.

Emelyanov, A. V., A. Y. Konev, E. Vershilova and D. V. Fyodorov, 2010 Protein complex of Drosophila ATRX/XNP and HP1a is required for the formation of pericentric beta-heterochromatin in vivo. J Biol Chem 285**:** 15027-15037.

Espinas, M. L., S. Canudas, L. Fanti, S. Pimpinelli, J. Casanova *et al.*, 2000 The GAGA factor of Drosophila interacts with SAP18, a Sin3-associated polypeptide. EMBO Rep 1**:** 253-259.

Espinas, M. L., E. Jimenez-Garcia, A. Vaquero, S. Canudas, J. Bernues *et al.*, 1999 The N-terminal POZ domain of GAGA mediates the formation of oligomers that bind DNA with high affinity and specificity. J Biol Chem 274**:** 16461-16469.

Fabrizio, J. J., G. Hime, S. K. Lemmon and C. Bazinet, 1998 Genetic dissection of sperm individualization in Drosophila melanogaster. Development 125**:** 1833-1843.

Fahey-Lozano, N., J. E. La Marca, M. Portela and H. E. Richardson, 2019 Drosophila Models of Cell Polarity and Cell Competition in Tumourigenesis. Adv Exp Med Biol 1167**:** 37-64.

Falkenburg, D., B. Dworniczak, D. M. Faust and E. K. Bautz, 1987 RNA polymerase II of Drosophila. Relation of its 140,000 Mr subunit to the beta subunit of Escherichia coli RNA polymerase. J Mol Biol 195**:** 929-937.

Fang, M., J. Li, T. Blauwkamp, C. Bhambhani, N. Campbell *et al.*, 2006 C-terminal-binding protein directly activates and represses Wnt transcriptional targets in Drosophila. EMBO J 25**:** 2735-2745.

Farkas, G., J. Gausz, M. Galloni, G. Reuter, H. Gyurkovics *et al.*, 1994 The Trithorax-like gene encodes the Drosophila GAGA factor. Nature 371**:** 806-808.

Feillet, C., P. Krusche, F. Tamanini, R. C. Janssens, M. J. Downey *et al.*, 2014 Phase locking and multiple oscillating attractors for the coupled mammalian clock and cell cycle. Proc Natl Acad Sci U S A 111**:** 9828-9833.

Fiedler, M., M. Graeb, J. Mieszczanek, T. J. Rutherford, C. M. Johnson *et al.*, 2015 An ancient Pygo-dependent Wnt enhanceosome integrated by Chip/LDB-SSDP. Elife 4.

Findley, S. D., M. Tamanaha, N. J. Clegg and H. Ruohola-Baker, 2003 Maelstrom, a Drosophila spindle-class gene, encodes a protein that colocalizes with Vasa and RDE1/AGO1 homolog, Aubergine, in nuage. Development 130**:** 859-871.

Fioresi, R., P. Demurtas and G. Perini, 2022 Deep learning for MYC binding site recognition. Front Bioinform 2**:** 1015993.

Firth, L. C., and N. E. Baker, 2005 Extracellular signals responsible for spatially regulated proliferation in the differentiating Drosophila eye. Dev Cell 8**:** 541-551.

Font-Burgada, J., D. Rossell, H. Auer and F. Azorin, 2008 Drosophila HP1c isoform interacts with the zinc-finger proteins WOC and Relative-of-WOC to regulate gene expression. Genes Dev 22**:** 3007-3023.

Fouix, S., S. Martin-Lanneree, M. Sanial, L. Morla, C. Lamour-Isnard *et al.*, 2003 Over-expression of a novel nuclear interactor of Suppressor of fused, the Drosophila myelodysplasia/myeloid leukaemia factor, induces abnormal morphogenesis associated with increased apoptosis and DNA synthesis. Genes Cells 8**:** 897-911.

Franz, A., D. Shlyueva, E. Brunner, A. Stark and K. Basler, 2017 Probing the canonicity of the Wnt/Wingless signaling pathway. PLoS Genet 13**:** e1006700.

Fratini, L., M. G. S. Dalmolin, M. Sinigaglia, A. da Silveira Perla, C. B. de Farias *et al.*, 2023 ZEB1 is a Subgroup-Specific Marker of Prognosis and Potential Drug Target in Medulloblastoma. Neuromolecular Med 25**:** 64-74.

Fulga, T. A., and P. Rorth, 2002 Invasive cell migration is initiated by guided growth of long cellular extensions. Nat Cell Biol 4**:** 715-719.

Funakoshi, M., M. Tsuda, K. Muramatsu, H. Hatsuda, S. Morishita *et al.*, 2018 Overexpression of Larp4B downregulates dMyc and reduces cell and organ sizes in Drosophila. Biochem Biophys Res Commun 497**:** 762-768.

Furrer, M., M. Balbi, M. Albarca-Aguilera, M. Gallant, W. Herr *et al.*, 2010 Drosophila Myc interacts with host cell factor (dHCF) to activate transcription and control growth. J Biol Chem 285**:** 39623-39636.

Gallant, P., 2013 Myc function in Drosophila. Cold Spring Harb Perspect Med 3**:** a014324.

Gallant, P., Y. Shiio, P. F. Cheng, S. M. Parkhurst and R. N. Eisenman, 1996 Myc and Max homologs in Drosophila. Science 274**:** 1523-1527.

Gambetta, M. C., K. Oktaba and J. Muller, 2009 Essential role of the glycosyltransferase sxc/Ogt in polycomb repression. Science 325**:** 93-96.

Gao, G., X. Bi, J. Chen, D. Srikanta and Y. S. Rong, 2009 Mre11-Rad50-Nbs complex is required to cap telomeres during Drosophila embryogenesis. Proc Natl Acad Sci U S A 106**:** 10728-10733.

Gaubatz, S., A. Imhof, R. Dosch, O. Werner, P. Mitchell *et al.*, 1995 Transcriptional activation by Myc is under negative control by the transcription factor AP-2. EMBO J 14**:** 1508-1519.

Gaytan de Ayala Alonso, A., L. Gutierrez, C. Fritsch, B. Papp, D. Beuchle *et al.*, 2007 A genetic screen identifies novel polycomb group genes in Drosophila. Genetics 176**:** 2099-2108.

Gelsthorpe, M. E., Z. Tan, A. Phillips, J. C. Eissenberg, A. Miller *et al.*, 2006 Regulation of the Drosophila melanogaster protein, enhancer of rudimentary, by casein kinase II. Genetics 174**:** 265-270.

Gene Ontology Curators, -. 2002 Manual transfer of experimentally-verified manual GO annotation data to orthologs by curator judgment of sequence similarity. .

Gennaro, V. J., H. Wedegaertner and S. B. McMahon, 2019 Interaction between the BAG1S isoform and HSP70 mediates the stability of anti-apoptotic proteins and the survival of osteosarcoma cells expressing oncogenic MYC. BMC Cancer 19**:** 258.

Gerasimova, T. I., D. A. Gdula, D. V. Gerasimov, O. Simonova and V. G. Corces, 1995 A Drosophila protein that imparts directionality on a chromatin insulator is an enhancer of position-effect variegation. Cell 82**:** 587-597.

Gerlach, J. M., M. Furrer, M. Gallant, D. Birkel, A. Baluapuri *et al.*, 2017 PAF1 complex component Leo1 helps recruit Drosophila Myc to promoters. Proc Natl Acad Sci U S A 114**:** E9224-E9232.

Germain, D. R., K. Graham, D. D. Glubrecht, J. C. Hugh, J. R. Mackey *et al.*, 2011 DEAD box 1: a novel and independent prognostic marker for early recurrence in breast cancer. Breast Cancer Res Treat 127**:** 53-63.

Germain, D. R., L. Li, M. R. Hildebrandt, A. J. Simmonds, S. C. Hughes *et al.*, 2015 Loss of the Drosophila melanogaster DEAD box protein Ddx1 leads to reduced size and aberrant gametogenesis. Dev Biol 407**:** 232-245.

Ghiglione, C., N. Perrimon and L. A. Perkins, 1999 Quantitative variations in the level of MAPK activity control patterning of the embryonic termini in Drosophila. Dev Biol 205**:** 181-193.

Ghosh, S., A. Obrdlik, V. Marchand and A. Ephrussi, 2014 The EJC binding and dissociating activity of PYM is regulated in Drosophila. PLoS Genet 10**:** e1004455.

Giagtzoglou, N., P. Alifragis, K. A. Koumbanakis and C. Delidakis, 2003 Two modes of recruitment of E(spl) repressors onto target genes. Development 130**:** 259-270.

Gibson, M. C., and N. Perrimon, 2003 Apicobasal polarization: epithelial form and function. Curr Opin Cell Biol 15**:** 747-752.

Giraldez, A. J., and S. M. Cohen, 2003 Wingless and Notch signaling provide cell survival cues and control cell proliferation during wing development. Development 130**:** 6533-6543.

Glover-Cutter, K., S. Kim, J. Espinosa and D. L. Bentley, 2008 RNA polymerase II pauses and associates with pre-mRNA processing factors at both ends of genes. Nat Struct Mol Biol 15**:** 71-78.

Glover, C. V., E. R. Shelton and D. L. Brutlag, 1983 Purification and characterization of a type II casein kinase from Drosophila melanogaster. J Biol Chem 258**:** 3258-3265.

GO Reference Genome Project, -. 2011 Phylogenetic annotation using the Gene Ontology.

Godbout, R., M. Packer and W. Bie, 1998 Overexpression of a DEAD box protein (DDX1) in neuroblastoma and retinoblastoma cell lines. J Biol Chem 273**:** 21161-21168.

Godbout, R., and J. Squire, 1993 Amplification of a DEAD box protein gene in retinoblastoma cell lines. Proc Natl Acad Sci U S A 90**:** 7578-7582.

Goedhart, J., and M. S. Luijsterburg, 2020 VolcaNoseR is a web app for creating, exploring, labeling and sharing volcano plots. Sci Rep 10**:** 20560.

Goldberg, M. L., R. P. Lifton, G. R. Stark and J. G. Williams, 1979 Isolation of specific RNA's using DNA covalently linked to diazobenzyloxymethyl cellulose or paper. Methods Enzymol 68**:** 206-220.

Gomez-Roman, N., C. Grandori, R. N. Eisenman and R. J. White, 2003 Direct activation of RNA polymerase III transcription by c-Myc. Nature 421**:** 290-294.

Gonzalez-Gaitan, M., and H. Jackle, 1997 Role of Drosophila alpha-adaptin in presynaptic vesicle recycling. Cell 88**:** 767-776.

Gonzalez-Prieto, R., S. A. Cuijpers, R. Kumar, I. A. Hendriks and A. C. Vertegaal, 2015 c-Myc is targeted to the proteasome for degradation in a SUMOylation-dependent manner, regulated by PIAS1, SENP7 and RNF4. Cell Cycle 14**:** 1859-1872.

Goodman, J. K., C. G. Zampronio, A. M. E. Jones and J. R. Hernandez-Fernaud, 2018 Updates of the In-Gel Digestion Method for Protein Analysis by Mass Spectrometry. Proteomics 18**:** e1800236.

Goodman, L. D., M. Prudencio, N. J. Kramer, L. F. Martinez-Ramirez, A. R. Srinivasan *et al.*, 2019 Toxic expanded GGGGCC repeat transcription is mediated by the PAF1 complex in C9orf72-associated FTD. Nat Neurosci 22**:** 863-874.

Gorski, M. M., R. J. Romeijn, J. C. Eeken, A. W. de Jong, B. L. van Veen *et al.*, 2004 Disruption of Drosophila Rad50 causes pupal lethality, the accumulation of DNA double-strand breaks and the induction of apoptosis in third instar larvae. DNA Repair (Amst) 3**:** 603-615.

Goshima, G., R. Wollman, S. S. Goodwin, N. Zhang, J. M. Scholey *et al.*, 2007 Genes required for mitotic spindle assembly in Drosophila S2 cells. Science 316**:** 417-421.

Gouw, A. M., K. Margulis, N. S. Liu, S. J. Raman, A. Mancuso *et al.*, 2019 The MYC Oncogene Cooperates with Sterol-Regulated Element-Binding Protein to Regulate Lipogenesis Essential for Neoplastic Growth. Cell Metab 30**:** 556-572 e555.

Goyal, Y., G. A. Jindal, J. L. Pelliccia, K. Yamaya, E. Yeung *et al.*, 2017 Divergent effects of intrinsically active MEK variants on developmental Ras signaling. Nat Genet 49**:** 465-469.

Grandori, C., S. M. Cowley, L. P. James and R. N. Eisenman, 2000 The Myc/Max/Mad network and the transcriptional control of cell behavior. Annu Rev Cell Dev Biol 16**:** 653-699.

Grandori, C., J. Mac, F. Siebelt, D. E. Ayer and R. N. Eisenman, 1996 Myc-Max heterodimers activate a DEAD box gene and interact with multiple E box-related sites in vivo. EMBO J 15**:** 4344-4357.

Greenleaf, A. L., 1983 Amanitin-resistant RNA polymerase II mutations are in the enzyme's largest subunit. J Biol Chem 258**:** 13403-13406.

Grewal, S. S., 2015 Why should cancer biologists care about tRNAs? tRNA synthesis, mRNA translation and the control of growth. Biochim Biophys Acta 1849**:** 898-907.

Grewal, S. S., L. Li, A. Orian, R. N. Eisenman and B. A. Edgar, 2005 Myc-dependent regulation of ribosomal RNA synthesis during Drosophila development. Nat Cell Biol 7**:** 295-302.

Grifoni, D., F. Froldi and A. Pession, 2013 Connecting epithelial polarity, proliferation and cancer in Drosophila: the many faces of lgl loss of function. Int J Dev Biol 57**:** 677-687.

Gryzik, T., and H. A. Muller, 2004 FGF8-like1 and FGF8-like2 encode putative ligands of the FGF receptor Htl and are required for mesoderm migration in the Drosophila gastrula. Curr Biol 14**:** 659-667.

Gu, J. Y., J. M. Park, E. J. Song, G. Mizuguchi, J. H. Yoon *et al.*, 2002 Novel Mediator proteins of the small Mediator complex in Drosophila SL2 cells. J Biol Chem 277**:** 27154-27161.

Gu, Z., K. Inomata, K. Ishizawa and A. Horii, 2007 The FBXW7 beta-form is suppressed in human glioma cells. Biochem Biophys Res Commun 354**:** 992-998.

Guelman, S., T. Suganuma, L. Florens, S. K. Swanson, C. L. Kiesecker *et al.*, 2006a Host cell factor and an uncharacterized SANT domain protein are stable components of ATAC, a novel

dAda2A/dGcn5-containing histone acetyltransferase complex in Drosophila. Mol Cell Biol 26**:** 871-882.

Guelman, S., T. Suganuma, L. Florens, V. Weake, S. K. Swanson *et al.*, 2006b The essential gene wda encodes a WD40 repeat subunit of Drosophila SAGA required for histone H3 acetylation. Mol Cell Biol 26**:** 7178-7189.

Guo, A., H. Huang, Z. Zhu, M. J. Chen, H. Shi *et al.*, 2022 cBAF complex components and MYC cooperate early in CD8(+) T cell fate. Nature 607**:** 135-141.

Guo, L., O. Zaysteva, Z. Nie, N. C. Mitchell, J. E. Amanda Lee *et al.*, 2016 Defining the essential function of FBP/KSRP proteins: Drosophila Psi interacts with the mediator complex to modulate MYC transcription and tissue growth. Nucleic Acids Res 44**:** 7646-7658.

Haecker, A., D. Qi, T. Lilja, B. Moussian, L. P. Andrioli *et al.*, 2007 Drosophila brakeless interacts with atrophin and is required for tailless-mediated transcriptional repression in early embryos. PLoS Biol 5**:** e145.

Haines, N., and K. D. Irvine, 2003 Glycosylation regulates Notch signalling. Nat Rev Mol Cell Biol 4**:** 786-797.

Hallikas, O., K. Palin, N. Sinjushina, R. Rautiainen, J. Partanen *et al.*, 2006 Genome-wide prediction of mammalian enhancers based on analysis of transcription-factor binding affinity. Cell 124**:** 47-59.

Hamada, F. N., P. J. Park, P. R. Gordadze and M. I. Kuroda, 2005 Global regulation of X chromosomal genes by the MSL complex in Drosophila melanogaster. Genes Dev 19**:** 2289-2294.

Hamiche, A., R. Sandaltzopoulos, D. A. Gdula and C. Wu, 1999 ATP-dependent histone octamer sliding mediated by the chromatin remodeling complex NURF. Cell 97**:** 833-842.

Hamilton, B. J., M. A. Mortin and A. L. Greenleaf, 1993 Reverse genetics of Drosophila RNA polymerase II: identification and characterization of RpII140, the genomic locus for the second-largest subunit. Genetics 134**:** 517-529.

Han, Y., Q. Shi and J. Jiang, 2015 Multisite interaction with Sufu regulates Ci/Gli activity through distinct mechanisms in Hh signal transduction. Proc Natl Acad Sci U S A 112**:** 6383-6388.

Han, Y., B. Wang, Y. S. Cho, J. Zhu, J. Wu *et al.*, 2019 Phosphorylation of Ci/Gli by Fused Family Kinases Promotes Hedgehog Signaling. Dev Cell 50**:** 610-626 e614.

Hansen, S. K., and R. Tjian, 1995 TAFs and TFIIA mediate differential utilization of the tandem Adh promoters. Cell 82**:** 565-575.

Harden, N., 2002 Signaling pathways directing the movement and fusion of epithelial sheets: lessons from dorsal closure in Drosophila. Differentiation 70**:** 181-203.

Harper, S. E., Y. Qiu and P. A. Sharp, 1996 Sin3 corepressor function in Myc-induced transcription and transformation. Proc Natl Acad Sci U S A 93**:** 8536-8540.

Harris, D. A., K. Kim, K. Nakahara, C. Vasquez-Doorman and R. W. Carthew, 2011 Cargo sorting to lysosome-related organelles regulates siRNA-mediated gene silencing. J Cell Biol 194**:** 77-87.

Harris, L. R., M. A. Churchward, R. H. Butt and J. R. Coorssen, 2007 Assessing detection methods for gel-based proteomic analyses. J Proteome Res 6**:** 1418-1425.

Hartl, T. A., J. Ni, J. Cao, K. L. Suyama, S. Patchett *et al.*, 2013 Regulation of ribosome biogenesis by nucleostemin 3 promotes local and systemic growth in Drosophila. Genetics 194**:** 101-115.

Harvey, A. J., A. P. Bidwai and L. K. Miller, 1997 Doom, a product of the Drosophila mod(mdg4) gene, induces apoptosis and binds to baculovirus inhibitor-of-apoptosis proteins. Mol Cell Biol 17**:** 2835-2843.

Hata, T., H. Rajabi, H. Takahashi, Y. Yasumizu, W. Li *et al.*, 2019 MUC1-C Activates the NuRD Complex to Drive Dedifferentiation of Triple-Negative Breast Cancer Cells. Cancer Res 79**:** 5711-5722.

Hatton-Ellis, E., C. Ainsworth, Y. Sushama, S. Wan, K. VijayRaghavan *et al.*, 2007 Genetic regulation of patterned tubular branching in Drosophila. Proc Natl Acad Sci U S A 104**:** 169-174.

Hayashi, R., S. M. Wainwright, S. J. Liddell, S. M. Pinchin, S. Horswell *et al.*, 2014 A genetic screen based on in vivo RNA imaging reveals centrosome-independent mechanisms for localizing gurken transcripts in Drosophila. G3 (Bethesda) 4**:** 749-760.

He, B., A. Martin and E. Wieschaus, 2016 Flow-dependent myosin recruitment during Drosophila cellularization requires zygotic dunk activity. Development 143**:** 2417-2430.

He, L., J. M. Thomson, M. T. Hemann, E. Hernando-Monge, D. Mu *et al.*, 2005 A microRNA polycistron as a potential human oncogene. Nature 435**:** 828-833.

Hebert, M. D., and A. G. Matera, 2000 Self-association of coilin reveals a common theme in nuclear body localization. Mol Biol Cell 11**:** 4159-4171.

Heck, B. W., B. Zhang, X. Tong, Z. Pan, W. M. Deng *et al.*, 2012 The transcriptional corepressor SMRTER influences both Notch and ecdysone signaling during Drosophila development. Biol Open 1**:** 182-196.

Helander, S., M. Montecchio, R. Pilstal, Y. Su, J. Kuruvilla *et al.*, 2015 Pre-Anchoring of Pin1 to Unphosphorylated c-Myc in a Fuzzy Complex Regulates c-Myc Activity. Structure 23**:** 2267-2279.

Helman, A., E. Cinnamon, S. Mezuman, Z. Hayouka, T. Von Ohlen *et al.*, 2011 Phosphorylation of Groucho mediates RTK feedback inhibition and prolonged pathway target gene expression. Curr Biol 21**:** 1102-1110.

Hempel, L. U., C. Rathke, S. J. Raja and R. Renkawitz-Pohl, 2006 In Drosophila, don juan and don juan like encode proteins of the spermatid nucleus and the flagellum and both are regulated at the transcriptional level by the TAF II80 cannonball while translational repression is achieved by distinct elements. Dev Dyn 235**:** 1053-1064.

Henriques, T., B. S. Scruggs, M. O. Inouye, G. W. Muse, L. H. Williams *et al.*, 2018 Widespread transcriptional pausing and elongation control at enhancers. Genes Dev 32**:** 26-41.

Herman-Bachinsky, Y., H. D. Ryoo, A. Ciechanover and H. Gonen, 2007 Regulation of the Drosophila ubiquitin ligase DIAP1 is mediated via several distinct ubiquitin system pathways. Cell Death Differ 14**:** 861-871.

Hernandez, G., P. Vazquez-Pianzola, A. Zurbriggen, M. Altmann, J. M. Sierra *et al.*, 2004 Two functionally redundant isoforms of Drosophila melanogaster eukaryotic initiation factor 4B

are involved in cap-dependent translation, cell survival, and proliferation. Eur J Biochem 271**:** 2923-2936.

Herold, N., C. L. Will, E. Wolf, B. Kastner, H. Urlaub *et al.*, 2009 Conservation of the protein composition and electron microscopy structure of Drosophila melanogaster and human spliceosomal complexes. Mol Cell Biol 29**:** 281-301.

Herranz, H., L. Perez, F. A. Martin and M. Milan, 2008 A Wingless and Notch double-repression mechanism regulates G1-S transition in the Drosophila wing. EMBO J 27**:** 1633-1645.

Herrera, F. J., T. Yamaguchi, H. Roelink and R. Tjian, 2014 Core promoter factor TAF9B regulates neuronal gene expression. Elife 3**:** e02559.

Hirling, H., M. Scheffner, T. Restle and H. Stahl, 1989 RNA helicase activity associated with the human p68 protein. Nature 339**:** 562-564.

Hochheimer, A., S. Zhou, S. Zheng, M. C. Holmes and R. Tjian, 2002 TRF2 associates with DREF and directs promoter-selective gene expression in Drosophila. Nature 420**:** 439-445.

Hogarty, M. D., M. D. Norris, K. Davis, X. Liu, N. F. Evageliou *et al.*, 2008 ODC1 is a critical determinant of MYCN oncogenesis and a therapeutic target in neuroblastoma. Cancer Res 68**:** 9735-9745.

Hsieh, A. L., X. Zheng, Z. Yue, Z. E. Stine, A. Mancuso *et al.*, 2019 Misregulation of Drosophila Myc Disrupts Circadian Behavior and Metabolism. Cell Rep 29**:** 1778-1788 e1774.

Hsouna, A., G. Nallamothu, N. Kose, M. Guinea, V. Dammai *et al.*, 2010 Drosophila von Hippel-Lindau tumor suppressor gene function in epithelial tubule morphogenesis. Mol Cell Biol 30**:** 3779-3794.

Hsu, J. Y., T. Juven-Gershon, M. T. Marr, 2nd, K. J. Wright, R. Tjian *et al.*, 2008 TBP, Mot1, and NC2 establish a regulatory circuit that controls DPE-dependent versus TATA-dependent transcription. Genes Dev 22**:** 2353-2358.

Hsu, T., D. McRackan, T. S. Vincent and H. Gert de Couet, 2001 Drosophila Pin1 prolyl isomerase Dodo is a MAP kinase signal responder during oogenesis. Nat Cell Biol 3**:** 538-543.

Huang, C., F. Yang, Z. Zhang, J. Zhang, G. Cai *et al.*, 2017 Mrg15 stimulates Ash1 H3K36 methyltransferase activity and facilitates Ash1 Trithorax group protein function in Drosophila. Nat Commun 8**:** 1649.

Huijbregts, R. P., A. Svitin, M. W. Stinnett, M. B. Renfrow and I. Chesnokov, 2009 Drosophila Orc6 facilitates GTPase activity and filament formation of the septin complex. Mol Biol Cell 20**:** 270-281.

Hyun, J., H. Jasper and D. Bohmann, 2005 DREF is required for efficient growth and cell cycle progression in Drosophila imaginal discs. Mol Cell Biol 25**:** 5590-5598.

Ibrahim, D., L. Prevaud, N. Faumont, D. Troutaud, J. Feuillard *et al.*, 2022 Alternative c-MYC mRNA Transcripts as an Additional Tool for c-Myc2 and c-MycS Production in BL60 Tumors. Biomolecules 12.

Ichikawa, K., Y. Kubota, T. Nakamura, J. S. Weng, T. Tomida *et al.*, 2015 MCRIP1, an ERK substrate, mediates ERK-induced gene silencing during epithelial-mesenchymal transition by regulating the co-repressor CtBP. Mol Cell 58**:** 35-46.

Ignesti, M., M. Barraco, G. Nallamothu, J. A. Woolworth, S. Duchi *et al.*, 2014 Notch signaling during development requires the function of awd, the Drosophila homolog of human metastasis suppressor gene Nm23. BMC Biol 12**:** 12.

Ihry, R. J., and A. Bashirullah, 2014 Genetic control of specificity to steroid-triggered responses in Drosophila. Genetics 196**:** 767-780.

Ilyin, A. A., S. S. Ryazansky, S. A. Doronin, O. M. Olenkina, E. A. Mikhaleva *et al.*, 2017 Piwi interacts with chromatin at nuclear pores and promiscuously binds nuclear transcripts in Drosophila ovarian somatic cells. Nucleic Acids Res 45**:** 7666-7680.

Immanuel, D., H. Zinszner and D. Ron, 1995 Association of SARFH (sarcoma-associated RNA-binding fly homolog) with regions of chromatin transcribed by RNA polymerase II. Mol Cell Biol 15**:** 4562-4571.

Inoue, H., T. Imamura, Y. Ishidou, M. Takase, Y. Udagawa *et al.*, 1998 Interplay of signal mediators of decapentaplegic (Dpp): molecular characterization of mothers against dpp, Medea, and daughters against dpp. Mol Biol Cell 9**:** 2145-2156.

InterPro Project Members, -. 2004 Gene Ontology annotation through association of InterPro records with GO terms. .

Irion, U., and M. Leptin, 1999 Developmental and cell biological functions of the Drosophila DEAD-box protein abstrakt. Curr Biol 9**:** 1373-1381.

Irion, U., M. Leptin, K. Siller, S. Fuerstenberg, Y. Cai *et al.*, 2004 Abstrakt, a DEAD box protein, regulates Insc levels and asymmetric division of neural and mesodermal progenitors. Curr Biol 14**:** 138-144.

Isabella, A. J., and S. Horne-Badovinac, 2016 Rab10-Mediated Secretion Synergizes with Tissue Movement to Build a Polarized Basement Membrane Architecture for Organ Morphogenesis. Dev Cell 38**:** 47-60.

Ishigaki, Y., Y. Nakamura, T. Tatsuno, M. Hashimoto, K. Iwabuchi *et al.*, 2014 RNA-binding protein RBM8A (Y14) and MAGOH localize to centrosome in human A549 cells. Histochem Cell Biol 141**:** 101-109.

Israeli, D., R. Nir and T. Volk, 2007 Dissection of the target specificity of the RNA-binding protein HOW reveals dpp mRNA as a novel HOW target. Development 134**:** 2107-2114.

Itkonen, H. M., S. Minner, I. J. Guldvik, M. J. Sandmann, M. C. Tsourlakis *et al.*, 2013 O-GlcNAc transferase integrates metabolic pathways to regulate the stability of c-MYC in human prostate cancer cells. Cancer Res 73**:** 5277-5287.

Itkonen, H. M., A. Urbanucci, S. E. Martin, A. Khan, A. Mathelier *et al.*, 2019 High OGT activity is essential for MYC-driven proliferation of prostate cancer cells. Theranostics 9**:** 2183-2197.

Ito, T., M. Bulger, M. J. Pazin, R. Kobayashi and J. T. Kadonaga, 1997 ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. Cell 90**:** 145-155.

Ito, T., M. E. Levenstein, D. V. Fyodorov, A. K. Kutach, R. Kobayashi *et al.*, 1999 ACF consists of two subunits, Acf1 and ISWI, that function cooperatively in the ATP-dependent catalysis of chromatin assembly. Genes Dev 13**:** 1529-1539.

Ito, Y., A. Asada, H. Kobayashi, T. Takano, G. Sharma *et al.*, 2014 Preferential targeting of p39-activated Cdk5 to Rac1-induced lamellipodia. Mol Cell Neurosci 61**:** 34-45.

Jablonowski, C., W. Quarni, S. Singh, H. Tan, D. H. Bostanthirige *et al.*, 2023 Metabolic reprogramming of cancer cells by JMJD6-mediated pre-mRNA splicing is associated with therapeutic response to splicing inhibitor. bioRxiv.

Jackstadt, R., S. Roh, J. Neumann, P. Jung, R. Hoffmann *et al.*, 2013 AP4 is a mediator of epithelial-mesenchymal transition and metastasis in colorectal cancer. J Exp Med 210**:** 1331-1350.

Janody, F., Z. Martirosyan, A. Benlali and J. E. Treisman, 2003 Two subunits of the Drosophila mediator complex act together to control cell affinity. Development 130**:** 3691-3701.

Janssens, D. H., H. Komori, D. Grbac, K. Chen, C. T. Koe *et al.*, 2014 Earmuff restricts progenitor cell potential by attenuating the competence to respond to self-renewal factors. Development 141**:** 1036-1046.

Jasper, H., V. Benes, A. Atzberger, S. Sauer, W. Ansorge *et al.*, 2002 A genomic switch at the transition from cell proliferation to terminal differentiation in the Drosophila eye. Dev Cell 3**:** 511-521.

Jasper, H., and D. Bohmann, 2002 Drosophila innate immunity: a genomic view of pathogen defense. Mol Cell 10**:** 967-969.

JayaNandanan, N., R. Mathew and M. Leptin, 2014 Guidance of subcellular tubulogenesis by actin under the control of a synaptotagmin-like protein and Moesin. Nat Commun 5**:** 3036.

Jeske, M., S. Meyer, C. Temme, D. Freudenreich and E. Wahle, 2006 Rapid ATP-dependent deadenylation of nanos mRNA in a cell-free system from Drosophila embryos. J Biol Chem 281**:** 25124-25133.

Jewett, J. C., and C. R. Bertozzi, 2010 Cu-free click cycloaddition reactions in chemical biology. Chem Soc Rev 39**:** 1272-1279.

Ji, J., X. Tang, W. Hu, K. A. Maggert and Y. S. Rong, 2019 The processivity factor Pol32 mediates nuclear localization of DNA polymerase delta and prevents chromosomal fragile site formation in Drosophila development. PLoS Genet 15**:** e1008169.

Jia, D., M. Soylemez, G. Calvin, R. Bornmann, J. Bryant *et al.*, 2015 A large-scale in vivo RNAi screen to identify genes involved in Notch-mediated follicle cell differentiation and cell cycle switches. Sci Rep 5**:** 12328.

Jia, H., Y. Liu, R. Xia, C. Tong, T. Yue *et al.*, 2010 Casein kinase 2 promotes Hedgehog signaling by regulating both smoothened and Cubitus interruptus. J Biol Chem 285**:** 37218-37226.

Jimeno-Martin, A., E. Sousa, R. Brocal-Ruiz, N. Daroqui, M. Maicas *et al.*, 2022 Joint actions of diverse transcription factor families establish neuron-type identities and promote enhancer selectivity. Genome Res 32**:** 459-473.

Jin, Y., J. Shi, H. Wang, J. Lu, C. Chen *et al.*, 2021 MYC-associated protein X binding with the variant rs72780850 in RNA helicase DEAD box 1 for susceptibility to neuroblastoma. Sci China Life Sci 64**:** 991-999.

Johnston, L. A., D. A. Prober, B. A. Edgar, R. N. Eisenman and P. Gallant, 1999 Drosophila myc regulates cellular growth during development. Cell 98**:** 779-790.

Jung, P., A. Menssen, D. Mayr and H. Hermeking, 2008 AP4 encodes a c-MYC-inducible repressor of p21. Proc Natl Acad Sci U S A 105**:** 15046-15051.

Kadamb, R., S. Mittal, N. Bansal, H. Batra and D. Saluja, 2013 Sin3: insight into its transcription regulatory functions. Eur J Cell Biol 92**:** 237-246.

Kadonaga, J. T., 2002 The DPE, a core promoter element for transcription by RNA polymerase II. Exp Mol Med 34**:** 259-264.

Kadonaga, J. T., 2012 Perspectives on the RNA polymerase II core promoter. Wiley Interdiscip Rev Dev Biol 1**:** 40-51.

Kadrmas, J. L., M. A. Smith, K. A. Clark, S. M. Pronovost, N. Muster *et al.*, 2004 The integrin effector PINCH regulates JNK activity and epithelial migration in concert with Ras suppressor 1. J Cell Biol 167**:** 1019-1024.

Kaguni, L. S., J. M. Rossignol, R. C. Conaway, G. R. Banks and I. R. Lehman, 1983 Association of DNA primase with the beta/gamma subunits of DNA polymerase alpha from Drosophila melanogaster embryos. J Biol Chem 258**:** 9037-9039.

Kakumani, P. K., L. M. Harvey, F. Houle, T. Guitart, F. Gebauer *et al.*, 2020 CSDE1 controls gene expression through the miRNA-mediated decay machinery. Life Sci Alliance 3.

Kal, A. J., T. Mahmoudi, N. B. Zak and C. P. Verrijzer, 2000 The Drosophila brahma complex is an essential coactivator for the trithorax group protein zeste. Genes Dev 14**:** 1058-1071.

Kalverda, B., H. Pickersgill, V. V. Shloma and M. Fornerod, 2010 Nucleoporins directly stimulate expression of developmental and cell-cycle genes inside the nucleoplasm. Cell 140**:** 360-371.

Kaminker, J. S., J. Canon, I. Salecker and U. Banerjee, 2002 Control of photoreceptor axon target choice by transcriptional repression of Runt. Nat Neurosci 5**:** 746-750.

Kang, M. J., J. Chung and H. D. Ryoo, 2012 CDK5 and MEKK1 mediate pro-apoptotic signalling following endoplasmic reticulum stress in an autosomal dominant retinitis pigmentosa model. Nat Cell Biol 14**:** 409-415.

Kanoh, H., L. L. Tong, T. Kuraishi, Y. Suda, Y. Momiuchi *et al.*, 2015 Genome-wide RNAi screening implicates the E3 ubiquitin ligase Sherpa in mediating innate immune signaling by Toll in Drosophila adults. Sci Signal 8**:** ra107.

Kara, E., A. McCambridge, M. Proffer, C. Dilts, B. Pumnea *et al.*, 2023 Mutational analysis of the functional motifs of the DEAD-box RNA helicase Me31B/DDX6 in Drosophila germline development. FEBS Lett 597**:** 1848-1867.

Karandikar, U. C., R. L. Trott, J. Yin, C. P. Bishop and A. P. Bidwai, 2004 Drosophila CK2 regulates eye morphogenesis via phosphorylation of E(spl)M8. Mech Dev 121**:** 273-286.

Karim, F. D., H. C. Chang, M. Therrien, D. A. Wassarman, T. Laverty *et al.*, 1996 A screen for genes that function downstream of Ras1 during Drosophila eye development. Genetics 143**:** 315-329.

Karim, F. D., and G. M. Rubin, 1999 PTP-ER, a novel tyrosine phosphatase, functions downstream of Ras1 to downregulate MAP kinase during Drosophila eye development. Mol Cell 3**:** 741-750.

Katoh, M., M. Igarashi, H. Fukuda, H. Nakagama and M. Katoh, 2013 Cancer genetics and genomics of human FOX family genes. Cancer Lett 328**:** 198-206.

Kedmi, A., Y. Zehavi, Y. Glick, Y. Orenstein, D. Ideses *et al.*, 2014 Drosophila TRF2 is a preferential core promoter regulator. Genes Dev 28**:** 2163-2174.

Kelleher, F. C., A. Rao and A. Maguire, 2014 Circadian molecular clocks and cancer. Cancer Lett 342**:** 9-18.

Kenney, A. M., M. D. Cole and D. H. Rowitch, 2003 Nmyc upregulation by sonic hedgehog signaling promotes proliferation in developing cerebellar granule neuron precursors. Development 130**:** 15-28.

Kharazmi, J., and C. Moshfegh, 2013 Investigation of dmyc Promoter and Regulatory Regions. Gene Regul Syst Bio 7**:** 85-102.

Kharazmi, J., C. Moshfegh and T. Brody, 2012 Identification of cis-Regulatory Elements in the dmyc Gene of Drosophila Melanogaster. Gene Regul Syst Bio 6**:** 15-42.

Khoury, M. J., and D. Bilder, 2020 Distinct activities of Scrib module proteins organize epithelial polarity. Proc Natl Acad Sci U S A 117**:** 11531-11540.

Kiessling, A. A., R. Bletsa, B. Desmarais, C. Mara, K. Kallianidis *et al.*, 2009 Evidence that human blastomere cleavage is under unique cell cycle control. J Assist Reprod Genet 26**:** 187-195.

Kim, C. A., and J. U. Bowie, 2003 SAM domains: uniform structure, diversity of function. Trends Biochem Sci 28**:** 625-628.

Kim, S. H., E. R. Park, E. Cho, W. H. Jung, J. Y. Jeon *et al.*, 2017 Mael is essential for cancer cell survival and tumorigenesis through protection of genetic integrity. Oncotarget 8**:** 5026-5037.

Kim, S. N., A. Jeibmann, K. Halama, H. T. Witte, M. Walte *et al.*, 2014 ECM stiffness regulates glial migration in Drosophila and mammalian glioma models. Development 141**:** 3233-3242.

Kim, S. Y., J. Y. Kim, S. Malik, W. Son, K. S. Kwon *et al.*, 2012 Negative regulation of EGFR/MAPK pathway by Pumilio in Drosophila melanogaster. PLoS One 7**:** e34016.

Kim, Y. O., S. J. Park, R. S. Balaban, M. Nirenberg and Y. Kim, 2004 A functional genomic screen for cardiogenic genes using RNA interference in developing Drosophila embryos. Proc Natl Acad Sci U S A 101**:** 159-164.

King-Jones, K., G. Korge and M. Lehmann, 1999 The helix-loop-helix proteins dAP-4 and daughterless bind both in vitro and in vivo to SEBP3 sites required for transcriptional activation of the Drosophila gene Sgs-4. J Mol Biol 291**:** 71-82.

King, M. W., J. M. Roberts and R. N. Eisenman, 1986 Expression of the c-myc proto-oncogene during development of Xenopus laevis. Mol Cell Biol 6**:** 4499-4508.

Kirilly, D., E. P. Spana, N. Perrimon, R. W. Padgett and T. Xie, 2005 BMP signaling is required for controlling somatic stem cell self-renewal in the Drosophila ovary. Dev Cell 9**:** 651-662.

Kiselev, A. M., I. S. Stepanova, L. S. Adonin, F. M. Batalova, V. N. Parfenov *et al.*, 2017 The exon junction complex factor Y14 is dynamic in the nucleus of the beetle Tribolium castaneum during late oogenesis. Mol Cytogenet 10**:** 41.

Kitadate, Y., S. Shigenobu, K. Arita and S. Kobayashi, 2007 Boss/Sev signaling from germline to soma restricts germline-stem-cell-niche formation in the anterior region of Drosophila male gonads. Dev Cell 13**:** 151-159.

Klymenko, T., B. Papp, W. Fischle, T. Kocher, M. Schelder *et al.*, 2006 A Polycomb group protein complex with sequence-specific DNA-binding and selective methyl-lysine-binding activities. Genes Dev 20**:** 1110-1122.

Knoepfler, P. S., X. Y. Zhang, P. F. Cheng, P. R. Gafken, S. B. McMahon *et al.*, 2006 Myc influences global chromatin structure. EMBO J 25**:** 2723-2734.

Koch, F., R. Fenouil, M. Gut, P. Cauchy, T. K. Albert *et al.*, 2011 Transcription initiation platforms and GTF recruitment at tissue-specific enhancers and promoters. Nat Struct Mol Biol 18**:** 956-963.

Koe, C. T., S. Li, F. Rossi, J. J. Wong, Y. Wang *et al.*, 2014 The Brm-HDAC3-Erm repressor complex suppresses dedifferentiation in Drosophila type II neuroblast lineages. Elife 3**:** e01906.

Koelzer, S., and T. Klein, 2006 Regulation of expression of Vg and establishment of the dorsoventral compartment boundary in the wing imaginal disc by Suppressor of Hairless. Dev Biol 289**:** 77-90.

Kogan, G. L., N. V. Akulenko, Y. A. Abramov, O. A. Sokolova, E. A. Fefelova *et al.*, 2017 [Nascent Polypeptide-Associated Complex as Tissue-Specific Cofactor during Germinal Cell Differentiation in Drosophila Testes]. Mol Biol (Mosk) 51**:** 677-682.

Kogan, G. L., E. A. Mikhaleva, O. M. Olenkina, S. S. Ryazansky, O. V. Galzitskaya *et al.*, 2022 Extended disordered regions of ribosome-associated NAC proteins paralogs belong only to the germline in Drosophila melanogaster. Sci Rep 12**:** 11191.

Koh, C. M., M. Bezzi, D. H. Low, W. X. Ang, S. X. Teo *et al.*, 2015 MYC regulates the core pre-mRNA splicing machinery as an essential step in lymphomagenesis. Nature 523**:** 96-100.

Kohwi-Shigematsu, T., K. Maass and J. Bode, 1997 A thymocyte factor SATB1 suppresses transcription of stably integrated matrix-attachment region-linked reporter genes. Biochemistry 36**:** 12005-12010.

Koizumi, K., H. Higashida, S. Yoo, M. S. Islam, A. I. Ivanov *et al.*, 2007 RNA interference screen to identify genes required for Drosophila embryonic nervous system development. Proc Natl Acad Sci U S A 104**:** 5626-5631.

Kon, C., K. M. Cadigan, S. L. da Silva and R. Nusse, 2005 Developmental roles of the Mi-2/NURD-associated protein p66 in Drosophila. Genetics 169**:** 2087-2100.

Konig, A., and H. R. Shcherbata, 2015 Soma influences GSC progeny differentiation via the cell adhesion-mediated steroid-let-7-Wingless signaling cascade that regulates chromatin dynamics. Biol Open 4**:** 285-300.

Kopecky, B., P. Santi, S. Johnson, H. Schmitz and B. Fritzsch, 2011 Conditional deletion of N-Myc disrupts neurosensory and non-sensory development of the ear. Dev Dyn 240**:** 1373-1390.

Koppen, M., B. G. Fernandez, L. Carvalho, A. Jacinto and C. P. Heisenberg, 2006 Coordinated cell-shape changes control epithelial movement in zebrafish and Drosophila. Development 133**:** 2671-2681.

Kozlova, T., and C. S. Thummel, 2000 Steroid regulation of postembryonic development and reproduction in Drosophila. Trends Endocrinol Metab 11**:** 276-280.

Krenciute, G., S. Liu, N. Yucer, Y. Shi, P. Ortiz *et al.*, 2013 Nuclear BAG6-UBL4A-GET4 complex mediates DNA damage signaling and cell death. J Biol Chem 288**:** 20547-20557.

Kress, T. R., A. Sabo and B. Amati, 2015 MYC: connecting selective transcriptional control to global RNA production. Nat Rev Cancer 15**:** 593-607.

Kristo, I., C. Bajusz, B. N. Borsos, T. Pankotai, J. Dopie *et al.*, 2017 The actin binding cytoskeletal protein Moesin is involved in nuclear mRNA export. Biochim Biophys Acta Mol Cell Res 1864**:** 1589-1604.

Kroiss, M., J. Schultz, J. Wiesner, A. Chari, A. Sickmann *et al.*, 2008 Evolution of an RNP assembly system: a minimal SMN complex facilitates formation of UsnRNPs in Drosophila melanogaster. Proc Natl Acad Sci U S A 105**:** 10045-10050.

Kuan, Y. S., P. Brewer-Jensen, W. L. Bai, C. Hunter, C. B. Wilson *et al.*, 2009 Drosophila suppressor of sable protein [Su(s)] promotes degradation of aberrant and transposon-derived RNAs. Mol Cell Biol 29**:** 5590-5603.

Kuan, Y. S., P. Brewer-Jensen and L. L. Searles, 2004 Suppressor of sable, a putative RNA-processing protein, functions at the level of transcription. Mol Cell Biol 24**:** 3734-3746.

Kubickova, A., J. B. De Sanctis and M. Hajduch, 2023 Isoform-Directed Control of c-Myc Functions: Understanding the Balance from Proliferation to Growth Arrest. Int J Mol Sci 24.

Kugler, S. J., E. M. Gehring, V. Wallkamm, V. Kruger and A. C. Nagel, 2011 The Putzig-NURF nucleosome remodeling complex is required for ecdysone receptor signaling and innate immunity in Drosophila melanogaster. Genetics 188**:** 127-139.

Kugler, S. J., and A. C. Nagel, 2007 putzig is required for cell proliferation and regulates notch activity in Drosophila. Mol Biol Cell 18**:** 3733-3740.

Kumar, J. P., M. Tio, F. Hsiung, S. Akopyan, L. Gabay *et al.*, 1998 Dissecting the roles of the Drosophila EGF receptor in eye development and MAP kinase activation. Development 125**:** 3875-3885.

Kuroda, K., R. Kagiyama-Takahashi and T. Shinomiya, 1990 Immunoaffinity purification and properties of Drosophila melanogaster DNA polymerase alpha-primase complex. J Biochem 108**:** 926-933.

Kusch, T., L. Florens, W. H. Macdonald, S. K. Swanson, R. L. Glaser *et al.*, 2004 Acetylation by Tip60 is required for selective histone variant exchange at DNA lesions. Science 306**:** 2084-2087.

Kutach, A. K., and J. T. Kadonaga, 2000 The downstream promoter element DPE appears to be as widely used as the TATA box in Drosophila core promoters. Mol Cell Biol 20**:** 4754-4764.

Lacoste, J., S. Codani-Simonart, M. Best-Belpomme and F. Peronnet, 1995 Characterization and cloning of p11, a transrepressor of Drosophila melanogaster retrotransposon 1731. Nucleic Acids Res 23**:** 5073-5079.

LaJeunesse, D., and A. Shearn, 1995 Trans-regulation of thoracic homeotic selector genes of the Antennapedia and bithorax complexes by the trithorax group genes: absent, small, and homeotic discs 1 and 2. Mech Dev 53**:** 123-139.

Lambertsson, A., 1998 The minute genes in Drosophila and their molecular functions. Adv Genet 38**:** 69-134.

Lancaster, O. M., C. F. Cullen and H. Ohkura, 2007 NHK-1 phosphorylates BAF to allow karyosome formation in the Drosophila oocyte nucleus. J Cell Biol 179**:** 817-824.

Langst, G., and P. B. Becker, 2001 ISWI induces nucleosome sliding on nicked DNA. Mol Cell 8**:** 1085-1092.

Laranjeiro, R., T. K. Tamai, E. Peyric, P. Krusche, S. Ott *et al.*, 2013 Cyclin-dependent kinase inhibitor p20 controls circadian cell-cycle timing. Proc Natl Acad Sci U S A 110**:** 6835-6840.

Larochelle, S., J. Chen, R. Knights, J. Pandur, P. Morcillo *et al.*, 2001 T-loop phosphorylation stabilizes the CDK7-cyclin H-MAT1 complex in vivo and regulates its CTD kinase activity. EMBO J 20**:** 3749-3759.

Lascu, I., A. Chaffotte, B. Limbourg-Bouchon and M. Veron, 1992 A Pro/Ser substitution in nucleoside diphosphate kinase of Drosophila melanogaster (mutation killer of prune) affects stability but not catalytic efficiency of the enzyme. J Biol Chem 267**:** 12775-12781.

Lasko, P., 2000 The drosophila melanogaster genome: translation factors and RNA binding proteins. J Cell Biol 150**:** F51-56.

Lasko, P., 2003 Ribosomes rule: translation, not transcription, is the primary target of two major intercellular signaling pathways. Dev Cell 5**:** 671-672.

Lasko, P., 2013 The DEAD-box helicase Vasa: evidence for a multiplicity of functions in RNA processes and developmental biology. Biochim Biophys Acta 1829**:** 810-816.

Lavoie, C. A., P. E. Lachance, N. Sonenberg and P. Lasko, 1996 Alternatively spliced transcripts from the Drosophila eIF4E gene produce two different Cap-binding proteins. J Biol Chem 271**:** 16393-16398.

Le Hir, H., and G. R. Andersen, 2008 Structural insights into the exon junction complex. Curr Opin Struct Biol 18**:** 112-119.

Lecuit, T., W. J. Brook, M. Ng, M. Calleja, H. Sun *et al.*, 1996 Two distinct mechanisms for long-range patterning by Decapentaplegic in the Drosophila wing. Nature 381**:** 387-393.

Lee, J. E., N. J. Cranna, A. S. Chahal and L. M. Quinn, 2012 Genetic systems to investigate regulation of oncogenes and tumour suppressor genes in Drosophila. Cells 1**:** 1182-1196.

Lee, L. A., and T. L. Orr-Weaver, 2003 Regulation of cell cycles in Drosophila development: intrinsic and extrinsic cues. Annu Rev Genet 37**:** 545-578.

Lee, M. P., M. Sander and T. S. Hsieh, 1989 Single strand DNA cleavage reaction of duplex DNA by Drosophila topoisomerase II. J Biol Chem 264**:** 13510-13518.

Lehner, C. F., and P. H. O'Farrell, 1990 The roles of Drosophila cyclins A and B in mitotic control. Cell 61**:** 535-547.

Lei, J. X., Q. Y. Liu, C. Sodja, J. LeBlanc, M. Ribecco-Lutkiewicz *et al.*, 2005 S/MAR-binding properties of Sox2 and its involvement in apoptosis of human NT2 neural precursors. Cell Death Differ 12**:** 1368-1377.

Leitner, A., T. Walzthoeni, A. Kahraman, F. Herzog, O. Rinner *et al.*, 2010 Probing native protein structures by chemical cross-linking, mass spectrometry, and bioinformatics. Mol Cell Proteomics 9**:** 1634-1649.

Levanon, D., R. E. Goldstein, Y. Bernstein, H. Tang, D. Goldenberg *et al.*, 1998 Transcriptional repression by AML1 and LEF-1 is mediated by the TLE/Groucho corepressors. Proc Natl Acad Sci U S A 95**:** 11590-11595.

Levine, J. D., C. I. Casey, D. D. Kalderon and F. R. Jackson, 1994 Altered circadian pacemaker functions and cyclic AMP rhythms in the Drosophila learning mutant dunce. Neuron 13**:** 967-974.

Levine, M., 2010 Transcriptional enhancers in animal development and evolution. Curr Biol 20**:** R754-763.

Li, C., J. Lv, G. Wumaier, Y. Zhao, L. Dong *et al.*, 2023 NDRG1 promotes endothelial dysfunction and hypoxia-induced pulmonary hypertension by targeting TAF15. Precis Clin Med 6**:** pbad024.

Li, L., S. Anderson, J. Secombe and R. N. Eisenman, 2013 The Drosophila ubiquitin-specific protease Puffyeye regulates dMyc-mediated growth. Development 140**:** 4776-4787.

Li, L., P. Williams, Z. Gao and Y. Wang, 2020 VEZF1-guanine quadruplex DNA interaction regulates alternative polyadenylation and detyrosinase activity of VASH1. Nucleic Acids Res 48**:** 11994-12003.

Li, L. Y., J. F. Yang, F. Rong, Z. P. Luo, S. Hu *et al.*, 2021 ZEB1 serves an oncogenic role in the tumourigenesis of HCC by promoting cell proliferation, migration, and inhibiting apoptosis via Wnt/beta-catenin signaling pathway. Acta Pharmacol Sin 42**:** 1676-1689.

Li, Q., S. Li, S. Mana-Capelli, R. J. Roth Flach, L. V. Danai *et al.*, 2014 The conserved misshapen-warts-Yorkie pathway acts in enteroblasts to regulate intestinal stem cells in Drosophila. Dev Cell 31**:** 291-304.

Li, S., Y. S. Cho, T. Yue, Y. T. Ip and J. Jiang, 2015 Overlapping functions of the MAP4K family kinases Hppy and Msn in Hippo signaling. Cell Discov 1**:** 15038.

Li, W., E. M. Skoulakis, R. L. Davis and N. Perrimon, 1997 The Drosophila 14-3-3 protein Leonardo enhances Torso signaling through D-Raf in a Ras 1-dependent manner. Development 124**:** 4163-4171.

Liang, H. L., C. Y. Nien, H. Y. Liu, M. M. Metzstein, N. Kirov *et al.*, 2008 The zinc-finger protein Zelda is a key activator of the early zygotic genome in Drosophila. Nature 456**:** 400-403.

Lim, A. K., and T. Kai, 2007 Unique germ-line organelle, nuage, functions to repress selfish genetic elements in Drosophila melanogaster. Proc Natl Acad Sci U S A 104**:** 6714-6719.

Lim, Y. M., S. Hayashi and L. Tsuda, 2012 Ebi/AP-1 suppresses pro-apoptotic genes expression and permits long-term survival of Drosophila sensory neurons. PLoS One 7**:** e37028.

Lim, Y. M., K. Nishizawa, Y. Nishi, L. Tsuda, Y. H. Inoue *et al.*, 1999 Genetic analysis of rolled, which encodes a Drosophila mitogen-activated protein kinase. Genetics 153**:** 763-771.

Lin, Y. H., H. Currinn, S. M. Pocha, A. Rothnie, T. Wassmer *et al.*, 2015 AP-2-complex-mediated endocytosis of Drosophila Crumbs regulates polarity by antagonizing Stardust. J Cell Sci 128**:** 4538-4549.

Lindhorst, D., and M. S. Halfon, 2023 Reporter gene assays and chromatin-level assays define substantially non-overlapping sets of enhancer sequences. BMC Genomics 24**:** 17.

Lindner, J. R., P. R. Hillman, A. L. Barrett, M. C. Jackson, T. L. Perry *et al.*, 2007 The Drosophila Perlecan gene trol regulates multiple signaling pathways in different developmental contexts. BMC Dev Biol 7**:** 121.

Liu, H., X. Tang, A. Srivastava, T. Pecot, P. Daniel *et al.*, 2015 Redeployment of Myc and E2f1-3 drives Rb-deficient cell cycles. Nat Cell Biol 17**:** 1036-1048.

Liu, J., F. Kouzine, Z. Nie, H. J. Chung, Z. Elisha-Feil *et al.*, 2006 The FUSE/FBP/FIR/TFIIH system is a molecular machine programming a pulse of c-myc expression. EMBO J 25**:** 2119-2130.

Liu, N., T. Konuma, R. Sharma, D. Wang, N. Zhao *et al.*, 2023a Histone H3 lysine 27 crotonylation mediates gene transcriptional repression in chromatin. Mol Cell 83**:** 2206-2221 e2211.

Liu, S., G. H. Baeg, Y. Yang, F. G. Goh, H. Bao *et al.*, 2023b The Integrator complex desensitizes cellular response to TGF-beta/BMP signaling. Cell Rep 42**:** 112007.

Liu, X., and J. Secombe, 2015 The Histone Demethylase KDM5 Activates Gene Expression by Recognizing Chromatin Context through Its PHD Reader Motif. Cell Rep 13**:** 2219-2231.

Liu, Y., Y. Hu, X. Li, L. Niu and M. Teng, 2010 The crystal structure of the human nascent polypeptide-associated complex domain reveals a nucleic acid-binding region on the NACA subunit. Biochemistry 49**:** 2890-2896.

Liu, Y., J. Mattila and V. Hietakangas, 2020 Systematic Screen for Drosophila Transcriptional Regulators Phosphorylated in Response to Insulin/mTOR Pathway. G3 (Bethesda) 10**:** 2843-2849.

Liu, Y., J. Mattila, S. Ventela, L. Yadav, W. Zhang *et al.*, 2017 PWP1 Mediates Nutrient-Dependent Growth Control through Nucleolar Regulation of Ribosomal Gene Expression. Dev Cell 43**:** 240-252 e245.

Liu, Y. I., M. V. Chang, H. E. Li, S. Barolo, J. L. Chang *et al.*, 2008 The chromatin remodelers ISWI and ACF1 directly repress Wingless transcriptional targets. Dev Biol 323**:** 41-52.

Lloret-Llinares, M., C. Carre, A. Vaquero, N. de Olano and F. Azorin, 2008 Characterization of Drosophila melanogaster JmjC+N histone demethylases. Nucleic Acids Res 36**:** 2852-2863.

Lobbardi, R., G. Lambert, J. Zhao, R. Geisler, H. R. Kim *et al.*, 2011 Fine-tuning of Hh signaling by the RNA-binding protein Quaking to control muscle development. Development 138**:** 1783-1794.

Loncle, N., M. Boube, L. Joulia, C. Boschiero, M. Werner *et al.*, 2007 Distinct roles for Mediator Cdk8 module subunits in Drosophila development. EMBO J 26**:** 1045-1054.

Loo, L. W., J. Secombe, J. T. Little, L. S. Carlos, C. Yost *et al.*, 2005 The transcriptional repressor dMnt is a regulator of growth in Drosophila melanogaster. Mol Cell Biol 25**:** 7078-7091.

Lu, J., Y. Wang, X. Wang, D. Wang, G. O. Pflugfelder *et al.*, 2022 The Tbx6 Transcription Factor Dorsocross Mediates Dpp Signaling to Regulate Drosophila Thorax Closure. Int J Mol Sci 23.

Lu, Y., L. Yang, M. Shen, Z. Zhang, S. Wang *et al.*, 2023 Tespa1 facilitates hematopoietic and leukemic stem cell maintenance by restricting c-Myc degradation. Leukemia 37**:** 1039-1047.

Luanpitpong, S., J. Poohadsuan, P. Klaihmon, X. Kang, K. Tangkiettrakul *et al.*, 2021 Metabolic sensor O-GlcNAcylation regulates megakaryopoiesis and thrombopoiesis through c-Myc stabilization and integrin perturbation. Stem Cells 39**:** 787-802.

Luo, S., N. B. Wehr and R. L. Levine, 2006 Quantitation of protein on gels and blots by infrared fluorescence of Coomassie blue and Fast Green. Anal Biochem 350**:** 233-238.

Luo, W., M. S. Friedman, K. D. Hankenson and P. J. Woolf, 2011 Time series gene expression profiling and temporal regulatory pathway analysis of BMP6 induced osteoblast differentiation and mineralization. BMC Syst Biol 5**:** 82.

Luo, Y., S. Yang, X. Wu, S. Takahashi, L. Sun *et al.*, 2021 Intestinal MYC modulates obesity-related metabolic dysfunction. Nat Metab 3**:** 923-939.

Lupo, R., A. Breiling, M. E. Bianchi and V. Orlando, 2001 Drosophila chromosome condensation proteins Topoisomerase II and Barren colocalize with Polycomb and maintain Fab-7 PRE silencing. Mol Cell 7**:** 127-136.

Luscher-Firzlaff, J., I. Gawlista, J. Vervoorts, K. Kapelle, T. Braunschweig *et al.*, 2008 The human trithorax protein hASH2 functions as an oncoprotein. Cancer Res 68**:** 749-758.

Luscher, B., 2001 Function and regulation of the transcription factors of the Myc/Max/Mad network. Gene 277**:** 1-14.

Lyman, D. F., and B. Yedvobnick, 1995 Drosophila Notch receptor activity suppresses Hairless function during adult external sensory organ development. Genetics 141**:** 1491-1505.

Ma, J., H. Plesken, J. E. Treisman, I. Edelman-Novemsky and M. Ren, 2004 Lightoid and Claret: a rab GTPase and its putative guanine nucleotide exchange factor in biogenesis of Drosophila eye pigment granules. Proc Natl Acad Sci U S A 101**:** 11652-11657.

Mahajan, S. S., K. M. Johnson and A. C. Wilson, 2003 Molecular cloning of Drosophila HCF reveals proteolytic processing and self-association of the encoded protein. J Cell Physiol 194**:** 117-126.

Maier, D., P. Kurth, A. Schulz, A. Russell, Z. Yuan *et al.*, 2011 Structural and functional analysis of the repressor complex in the Notch signaling pathway of Drosophila melanogaster. Mol Biol Cell 22**:** 3242-3252.

Maines, J. Z., L. M. Stevens, X. Tong and D. Stein, 2004 Drosophila dMyc is required for ovary cell growth and endoreplication. Development 131**:** 775-786.

Mallik, M., M. Catinozzi, C. B. Hug, L. Zhang, M. Wagner *et al.*, 2018 Xrp1 genetically interacts with the ALS-associated FUS orthologue caz and mediates its toxicity. J Cell Biol 217**:** 3947-3964.

Manohar, C. F., H. R. Salwen, G. M. Brodeur and S. L. Cohn, 1995 Co-amplification and concomitant high levels of expression of a DEAD box gene with MYCN in human neuroblastoma. Genes Chromosomes Cancer 14**:** 196-203.

Marenda, D. R., C. B. Zraly and A. K. Dingwall, 2004 The Drosophila Brahma (SWI/SNF) chromatin remodeling complex exhibits cell-type specific activation and repression functions. Dev Biol 267**:** 279-293.

Marenda, D. R., C. B. Zraly, Y. Feng, S. Egan and A. K. Dingwall, 2003 The Drosophila SNR1 (SNF5/INI1) subunit directs essential developmental functions of the Brahma chromatin remodeling complex. Mol Cell Biol 23**:** 289-305.

Marinho, J., F. Casares and P. S. Pereira, 2011 The Drosophila Nol12 homologue viriato is a dMyc target that regulates nucleolar architecture and is required for dMyc-stimulated cell growth. Development 138**:** 349-357.

Marinkovic, T., and D. Marinkovic, 2021 Obscure Involvement of MYC in Neurodegenerative Diseases and Neuronal Repair. Mol Neurobiol 58**:** 4169-4177.

Markesich, D. C., K. M. Gajewski, M. E. Nazimiec and K. Beckingham, 2000 bicaudal encodes the Drosophila beta NAC homolog, a component of the ribosomal translational machinery*. Development 127**:** 559-572.

Marmorstein, R., 2003 Structure of SET domain proteins: a new twist on histone methylation. Trends Biochem Sci 28**:** 59-62.

Marquez, R. M., M. A. Singer, N. T. Takaesu, W. R. Waldrip, Y. Kraytsberg *et al.*, 2001 Transgenic analysis of the Smad family of TGF-beta signal transducers in Drosophila melanogaster suggests new roles and new interactions between family members. Genetics 157**:** 1639-1648.

Marsden, M., and D. W. DeSimone, 2001 Regulation of cell polarity, radial intercalation and epiboly in Xenopus: novel roles for integrin and fibronectin. Development 128**:** 3635-3647.

Martin-Bermudo, M. D., 2000 Integrins modulate the Egfr signaling pathway to regulate tendon cell differentiation in the Drosophila embryo. Development 127**:** 2607-2615.

Martin, D. D., E. Beauchamp and L. G. Berthiaume, 2011 Post-translational myristoylation: Fat matters in cellular life and death. Biochimie 93**:** 18-31.

Martin, S. R., and P. M. Bayley, 2004 Calmodulin bridging of IQ motifs in myosin-V. FEBS Lett 567**:** 166-170.

Martins, T., F. Meghini, F. Florio and Y. Kimata, 2017 The APC/C Coordinates Retinal Differentiation with G1 Arrest through the Nek2-Dependent Modulation of Wingless Signaling. Dev Cell 40**:** 67-80.

Marygold, S. J., J. Roote, G. Reuter, A. Lambertsson, M. Ashburner *et al.*, 2007 The ribosomal protein genes and Minute loci of Drosophila melanogaster. Genome Biol 8**:** R216.

Marygold, S. J., C. Walker, M. Orme and S. Leevers, 2011 Genetic characterization of ebi reveals its critical role in Drosophila wing growth. Fly (Austin) 5**:** 291-303.

Mashtalir, N., H. Suzuki, D. P. Farrell, A. Sankar, J. Luo *et al.*, 2020 A Structural Model of the Endogenous Human BAF Complex Informs Disease Mechanisms. Cell 183**:** 802-817 e824.

Masri, S., M. Cervantes and P. Sassone-Corsi, 2013 The circadian clock and cell cycle: interconnected biological circuits. Curr Opin Cell Biol 25**:** 730-734.

Matharu, N. K., T. Hussain, R. Sankaranarayanan and R. K. Mishra, 2010 Vertebrate homologue of Drosophila GAGA factor. J Mol Biol 400**:** 434-447.

Mathews, K. W., M. Cavegn and M. Zwicky, 2017 Sexual Dimorphism of Body Size Is Controlled by Dosage of the X-Chromosomal Gene Myc and by the Sex-Determining Gene tra in Drosophila. Genetics 205**:** 1215-1228.

Matias-Barrios, V. M., and X. Dong, 2023 The Implication of Topoisomerase II Inhibitors in Synthetic Lethality for Cancer Therapy. Pharmaceuticals (Basel) 16.

Matsui, M., K. C. Sharma, C. Cooke, B. T. Wakimoto, M. Rasool *et al.*, 2011 Nuclear structure and chromosome segregation in Drosophila male meiosis depend on the ubiquitin ligase dTopors. Genetics 189**:** 779-793.

Matsuo, T., S. Yamaguchi, S. Mitsui, A. Emi, F. Shimoda *et al.*, 2003 Control mechanism of the circadian clock for timing of cell division in vivo. Science 302**:** 255-259.

Matsuzawa, S. I., and J. C. Reed, 2001 Siah-1, SIP, and Ebi collaborate in a novel pathway for beta-catenin degradation linked to p53 responses. Mol Cell 7**:** 915-926.

Matyash, A., N. Singh, S. D. Hanes, H. Urlaub and H. Jackle, 2009 SAP18 promotes Kruppel-dependent transcriptional repression by enhancer-specific histone deacetylation. J Biol Chem 284**:** 3012-3020.

Mazur, P. K., H. Einwachter, M. Lee, B. Sipos, H. Nakhai *et al.*, 2010 Notch2 is required for progression of pancreatic intraepithelial neoplasia and development of pancreatic ductal adenocarcinoma. Proc Natl Acad Sci U S A 107**:** 13438-13443.

McGrail, M., and T. S. Hays, 1997 The microtubule motor cytoplasmic dynein is required for spindle orientation during germline cell divisions and oocyte differentiation in Drosophila. Development 124**:** 2409-2419.

Meng, Z., T. Moroishi, V. Mottier-Pavie, S. W. Plouffe, C. G. Hansen *et al.*, 2015 MAP4K family kinases act in parallel to MST1/2 to activate LATS1/2 in the Hippo pathway. Nat Commun 6**:** 8357.

Methot, N., and K. Basler, 2000 Suppressor of fused opposes hedgehog signal transduction by impeding nuclear accumulation of the activator form of Cubitus interruptus. Development 127**:** 4001-4010.

Michalak, W., V. Tsiamis, V. Schwammle and A. Rogowska-Wrzesinska, 2019 ComplexBrowser: A Tool for Identification and Quantification of Protein Complexes in Large-scale Proteomics Datasets. Mol Cell Proteomics 18**:** 2324-2334.

Miles, W. O., E. Jaffray, S. G. Campbell, S. Takeda, L. J. Bayston *et al.*, 2008 Medea SUMOylation restricts the signaling range of the Dpp morphogen in the Drosophila embryo. Genes Dev 22**:** 2578-2590.

Militti, C., S. Maenner, P. B. Becker and F. Gebauer, 2014 UNR facilitates the interaction of MLE with the lncRNA roX2 during Drosophila dosage compensation. Nat Commun 5**:** 4762.

Miller, C. L. W., J. L. Warner and F. Winston, 2023 Insights into Spt6: a histone chaperone that functions in transcription, DNA replication, and genome stability. Trends Genet 39**:** 858-872.

Miller, M., A. Chen, V. Gobert, B. Auge, M. Beau *et al.*, 2017 Control of RUNX-induced repression of Notch signaling by MLF and its partner DnaJ-1 during Drosophila hematopoiesis. PLoS Genet 13**:** e1006932.

Mishra, A. K., N. Sachan, M. Mutsuddi and A. Mukherjee, 2015 Kinase active Misshapen regulates Notch signaling in Drosophila melanogaster. Exp Cell Res 339**:** 51-60.

Mitchell, N. C., E. B. Tchoubrieva, A. Chahal, S. Woods, A. Lee *et al.*, 2015 S6 Kinase is essential for MYC-dependent rDNA transcription in Drosophila. Cell Signal 27**:** 2045-2053.

Mitchell, S. A., E. C. Brown, M. J. Coldwell, R. J. Jackson and A. E. Willis, 2001 Protein factor requirements of the Apaf-1 internal ribosome entry segment: roles of polypyrimidine tract binding protein and upstream of N-ras. Mol Cell Biol 21**:** 3364-3374.

Mitchelson, A., M. Simonelig, C. Williams and K. O'Hare, 1993 Homology with Saccharomyces cerevisiae RNA14 suggests that phenotypic suppression in Drosophila melanogaster by suppressor of forked occurs at the level of RNA stability. Genes Dev 7**:** 241-249.

Mizuguchi, G., A. Vassilev, T. Tsukiyama, Y. Nakatani and C. Wu, 2001 ATP-dependent nucleosome remodeling and histone hyperacetylation synergistically facilitate transcription of chromatin. J Biol Chem 276**:** 14773-14783.

Mohan, M., M. Bartkuhn, M. Herold, A. Philippen, N. Heinl *et al.*, 2007 The Drosophila insulator proteins CTCF and CP190 link enhancer blocking to body patterning. EMBO J 26**:** 4203-4214.

Mohrmann, L., K. Langenberg, J. Krijgsveld, A. J. Kal, A. J. Heck *et al.*, 2004 Differential targeting of two distinct SWI/SNF-related Drosophila chromatin-remodeling complexes. Mol Cell Biol 24**:** 3077-3088.

Moller, A., F. W. Avila, J. W. Erickson and H. Jackle, 2005 Drosophila BAP60 is an essential component of the Brahma complex, required for gene activation and repression. J Mol Biol 352**:** 329-337.

Molnar, C., and J. F. de Celis, 2013 Tay bridge is a negative regulator of EGFR signalling and interacts with Erk and Mkp3 in the Drosophila melanogaster wing. PLoS Genet 9**:** e1003982.

Moore, R., K. Vogt, A. E. Acosta-Martin, P. Shire, M. Zeidler *et al.*, 2020 Integration of JAK/STAT receptor-ligand trafficking, signalling and gene expression in Drosophila melanogaster cells. J Cell Sci 133.

Morales-Mulia, S., and J. M. Scholey, 2005 Spindle pole organization in Drosophila S2 cells by dynein, abnormal spindle protein (Asp), and KLP10A. Mol Biol Cell 16**:** 3176-3186.

Morelli, E., M. Fulciniti, M. K. Samur, C. F. Ribeiro, L. Wert-Lamas *et al.*, 2023 A MIR17HG-derived long noncoding RNA provides an essential chromatin scaffold for protein interaction and myeloma growth. Blood 141**:** 391-405.

Morera, S., M. Chiadmi, G. LeBras, I. Lascu and J. Janin, 1995 Mechanism of phosphate transfer by nucleoside diphosphate kinase: X-ray structures of the phosphohistidine intermediate of the enzymes from Drosophila and Dictyostelium. Biochemistry 34**:** 11062-11070.

Morgan, J. L., Y. Song and E. Barbar, 2011 Structural dynamics and multiregion interactions in dynein-dynactin recognition. J Biol Chem 286**:** 39349-39359.

Morillo Prado, J. R., S. Srinivasan and M. T. Fuller, 2013 The histone variant His2Av is required for adult stem cell maintenance in the Drosophila testis. PLoS Genet 9**:** e1003903.

Moser, M., A. Pscherer, C. Roth, J. Becker, G. Mucher *et al.*, 1997 Enhanced apoptotic cell death of renal epithelial cells in mice lacking transcription factor AP-2beta. Genes Dev 11**:** 1938-1948.

Mount, S. M., and H. K. Salz, 2000 Pre-messenger RNA processing factors in the Drosophila genome. J Cell Biol 150**:** F37-44.

Muller, B., B. Hartmann, G. Pyrowolakis, M. Affolter and K. Basler, 2003 Conversion of an extracellular Dpp/BMP morphogen gradient into an inverse transcriptional gradient. Cell 113**:** 221-233.

Muller, D., S. J. Kugler, A. Preiss, D. Maier and A. C. Nagel, 2005 Genetic modifier screens on Hairless gain-of-function phenotypes reveal genes involved in cell differentiation, cell growth and apoptosis in Drosophila melanogaster. Genetics 171**:** 1137-1152.

Murphy, T. D., 2003 Drosophila skpA, a component of SCF ubiquitin ligases, regulates centrosome duplication independently of cyclin E accumulation. J Cell Sci 116**:** 2321-2332.

Myster, S. H., and M. Peifer, 2001 Wingless can't fly so it hitches a ride with dynein. Bioessays 23**:** 869-872.

Nagel, A. C., A. Krejci, G. Tenin, A. Bravo-Patino, S. Bray *et al.*, 2005 Hairless-mediated repression of notch target genes requires the combined activity of Groucho and CtBP corepressors. Mol Cell Biol 25**:** 10433-10441.

Nagel, A. C., D. Maier and A. Preiss, 2000 Su(H)-independent activity of hairless during mechano-sensory organ formation in Drosophila. Mech Dev 94**:** 3-12.

Nair, M., A. Teng, V. Bilanchone, A. Agrawal, B. Li *et al.*, 2006 Ovol1 regulates the growth arrest of embryonic epidermal progenitor cells and represses c-myc transcription. J Cell Biol 173**:** 253-264.

Narayanan, R., and M. Ramaswami, 2001 Endocytosis in Drosophila: progress, possibilities, prognostications. Exp Cell Res 271**:** 28-35.

Neto-Silva, R. M., S. de Beco and L. A. Johnston, 2010 Evidence for a growth-stabilizing regulatory feedback mechanism between Myc and Yorkie, the Drosophila homolog of Yap. Dev Cell 19**:** 507-520.

Neves, A., and R. N. Eisenman, 2019 Distinct gene-selective roles for a network of core promoter factors in Drosophila neural stem cell identity. Biol Open 8.

Nguyen, M. B., L. T. Vuong and K. W. Choi, 2016 Ebi modulates wing growth by ubiquitin-dependent downregulation of Crumbs in Drosophila. Development 143**:** 3506-3513.

Nicholson, S. C., B. N. Nicolay, M. V. Frolov and K. H. Moberg, 2011 Notch-dependent expression of the archipelago ubiquitin ligase subunit in the Drosophila eye. Development 138**:** 251-260.

Nicolas, E., N. Chenouard, J. C. Olivo-Marin and A. Guichet, 2009 A dual role for actin and microtubule cytoskeleton in the transport of Golgi units from the nurse cells to the oocyte across ring canals. Mol Biol Cell 20**:** 556-568.

Nie, M., Y. Xie, J. A. Loo and A. J. Courey, 2009 Genetic and proteomic evidence for roles of Drosophila SUMO in cell cycle control, Ras signaling, and early pattern formation. PLoS One 4**:** e5905.

Nikalayevich, E., and H. Ohkura, 2015 The NuRD nucleosome remodelling complex and NHK-1 kinase are required for chromosome condensation in oocytes. J Cell Sci 128**:** 566-575.

Nilkanta, C., and A. Bagchi, 2018 Comparative analysis of prokaryotic and eukaryotic transcription factors using machine-learning techniques. Bioinformation 14**:** 315-326.

Nir, R., R. Grossman, Z. Paroush and T. Volk, 2012 Phosphorylation of the Drosophila melanogaster RNA-binding protein HOW by MAPK/ERK enhances its dimerization and activity. PLoS Genet 8**:** e1002632.

Nollet, F., G. Berx and F. van Roy, 1999 The role of the E-cadherin/catenin adhesion complex in the development and progression of cancer. Mol Cell Biol Res Commun 2**:** 77-85.

Norbury, C., and P. Nurse, 1992 Animal cell cycles and their control. Annu Rev Biochem 61**:** 441-470.

Noura, M., S. Tomita, T. Yasuda, S. Tsuzuki, H. Kiyoi *et al.*, 2024 NUP98-BPTF promotes oncogenic transformation through PIM1 upregulation. Cancer Med 13**:** e7445.

Nowak, A. J., C. Alfieri, C. U. Stirnimann, V. Rybin, F. Baudin *et al.*, 2011 Chromatin-modifying complex component Nurf55/p55 associates with histones H3 and H4 and polycomb repressive complex 2 subunit Su(z)12 through partially overlapping binding sites. J Biol Chem 286**:** 23388-23396.

Ntwasa, M., M. Egerton and N. J. Gay, 1997 Sequence and expression of Drosophila myristoyl-CoA: protein N-myristoyl transferase: evidence for proteolytic processing and membrane localisation. J Cell Sci 110 (Pt 2)**:** 149-156.

Nurminsky, D. I., M. V. Nurminskaya, E. V. Benevolenskaya, Y. Y. Shevelyov, D. L. Hartl *et al.*, 1998 Cytoplasmic dynein intermediate-chain isoforms with different targeting properties created by tissue-specific alternative splicing. Mol Cell Biol 18**:** 6816-6825.

O'Keefe, D. D., E. Gonzalez-Nino, M. Burnett, L. Dylla, S. M. Lambeth *et al.*, 2009 Rap1 maintains adhesion between cells to affect Egfr signaling and planar cell polarity in Drosophila. Dev Biol 333**:** 143-160.

O'Neill, E. M., I. Rebay, R. Tjian and G. M. Rubin, 1994 The activities of two Ets-related transcription factors required for Drosophila eye development are modulated by the Ras/MAPK pathway. Cell 78**:** 137-147.

Oegema, K., C. Wiese, O. C. Martin, R. A. Milligan, A. Iwamatsu *et al.*, 1999 Characterization of two related Drosophila gamma-tubulin complexes that differ in their ability to nucleate microtubules. J Cell Biol 144**:** 721-733.

Oellers, N., and E. Hafen, 1996 Biochemical characterization of rolledSem, an activated form of Drosophila mitogen-activated protein kinase. J Biol Chem 271**:** 24939-24944.

Ogirenko, A. A., D. A. Karagodin, N. V. Pavlova, S. A. Fedorova, M. A. Voloshina *et al.*, 2008 [Molecular and genetic description of a new hypomorphic mutation of Trithorax-like gene and analysis of its effect on Drosophila melanogaster oogenesis]. Ontogenez 39**:** 134-142.

Ogiyama, Y., B. Schuettengruber, G. L. Papadopoulos, J. M. Chang and G. Cavalli, 2018 Polycomb-Dependent Chromatin Looping Contributes to Gene Silencing during Drosophila Development. Mol Cell 71**:** 73-88 e75.

Ogura, Y., F. L. Wen, M. M. Sami, T. Shibata and S. Hayashi, 2018 A Switch-like Activation Relay of EGFR-ERK Signaling Regulates a Wave of Cellular Contractility for Epithelial Invagination. Dev Cell 46**:** 162-172 e165.

Oh, S., M. Kato, C. Zhang, Y. Guo and P. A. Beachy, 2015 A Comparison of Ci/Gli Activity as Regulated by Sufu in Drosophila and Mammalian Hedgehog Response. PLoS One 10**:** e0135804.

Ohkuma, Y., H. Sumimoto, A. Hoffmann, S. Shimasaki, M. Horikoshi *et al.*, 1991 Structural motifs and potential sigma homologies in the large subunit of human general transcription factor TFIIE. Nature 354**:** 398-401.

Ohler, U., G. C. Liao, H. Niemann and G. M. Rubin, 2002 Computational analysis of core promoters in the Drosophila genome. Genome Biol 3**:** RESEARCH0087.

Ohler, U., and D. A. Wassarman, 2010 Promoting developmental transcription. Development 137**:** 15-26.

Ohshiro, T., Y. Emori and K. Saigo, 2002 Ligand-dependent activation of breathless FGF receptor gene in Drosophila developing trachea. Mech Dev 114**:** 3-11.

Okada, M., and Y. B. Shi, 2018 The balance of two opposing factors Mad and Myc regulates cell fate during tissue remodeling. Cell Biosci 8**:** 51.

Okamoto, T., S. Yamamoto, Y. Watanabe, T. Ohta, F. Hanaoka *et al.*, 1998 Analysis of the role of TFIIE in transcriptional regulation through structure-function studies of the TFIIEbeta subunit. J Biol Chem 273**:** 19866-19876.

On, K., G. Crevel, S. Cotterill, M. Itoh and Y. Kato, 2021 Drosophila telomere capping protein HOAP interacts with DSB sensor proteins Mre11 and Nbs. Genes Cells 26**:** 219-229.

Orian, A., J. J. Delrow, A. E. Rosales Nieves, M. Abed, D. Metzger *et al.*, 2007 A Myc-Groucho complex integrates EGF and Notch signaling to regulate neural development. Proc Natl Acad Sci U S A 104**:** 15771-15776.

Orian, A., S. S. Grewal, P. S. Knoepfler, B. A. Edgar, S. M. Parkhurst *et al.*, 2005 Genomic binding and transcriptional regulation by the Drosophila Myc and Mnt transcription factors. Cold Spring Harb Symp Quant Biol 70**:** 299-307.

Orian, A., B. van Steensel, J. Delrow, H. J. Bussemaker, L. Li *et al.*, 2003 Genomic binding by the Drosophila Myc, Max, Mad/Mnt transcription factor network. Genes Dev 17**:** 1101-1114.

Oster, S. K., W. W. Marhin, C. Asker, L. M. Facchini, P. A. Dion *et al.*, 2000 Myc is an essential negative regulator of platelet-derived growth factor beta receptor expression. Mol Cell Biol 20**:** 6768-6778.

Ota, R., and S. Kobayashi, 2020 Myc plays an important role in Drosophila P-M hybrid dysgenesis to eliminate germline cells with genetic damage. Commun Biol 3**:** 185.

Pai, C. Y., E. P. Lei, D. Ghosh and V. G. Corces, 2004 The centrosomal protein CP190 is a component of the gypsy chromatin insulator. Mol Cell 16**:** 737-748.

Pan, D., and G. M. Rubin, 1995 cAMP-dependent protein kinase and hedgehog act antagonistically in regulating decapentaplegic transcription in Drosophila imaginal discs. Cell 80**:** 543-552.

Panda, D., P. Pascual-Garcia, M. Dunagin, M. Tudor, K. C. Hopkins *et al.*, 2014 Nup98 promotes antiviral gene expression to restrict RNA viral infection in Drosophila. Proc Natl Acad Sci U S A 111**:** E3890-3899.

Pankotai, T., Z. Ujfaludi, E. Vamos, K. Suri and I. M. Boros, 2010 The dissociable RPB4 subunit of RNA Pol II has vital functions in Drosophila. Mol Genet Genomics 283**:** 89-97.

Papoulas, O., G. Daubresse, J. A. Armstrong, J. Jin, M. P. Scott *et al.*, 2001 The HMG-domain protein BAP111 is important for the function of the BRM chromatin-remodeling complex in vivo. Proc Natl Acad Sci U S A 98**:** 5728-5733.

Pardee, A. B., 1989 G1 events and regulation of cell proliferation. Science 246**:** 603-608.

Park, J. M., J. M. Kim, L. K. Kim, S. N. Kim, J. Kim-Ha *et al.*, 2003a Signal-induced transcriptional activation by Dif requires the dTRAP80 mediator module. Mol Cell Biol 23**:** 1358-1367.

Park, J. W., K. Parisky, A. M. Celotto, R. A. Reenan and B. R. Graveley, 2004 Identification of alternative splicing regulators by RNA interference in Drosophila. Proc Natl Acad Sci U S A 101**:** 15974-15979.

Park, Y., C. Rangel, M. M. Reynolds, M. C. Caldwell, M. Johns *et al.*, 2003b Drosophila perlecan modulates FGF and hedgehog signals to activate neural stem cell division. Dev Biol 253**:** 247-257.

Parker, D. S., Y. Y. Ni, J. L. Chang, J. Li and K. M. Cadigan, 2008 Wingless signaling induces widespread chromatin remodeling of target loci. Mol Cell Biol 28**:** 1815-1828.

Parkhurst, S. M., and V. G. Corces, 1986 Mutations at the suppressor of forked locus increase the accumulation of gypsy-encoded transcripts in Drosophila melanogaster. Mol Cell Biol 6**:** 2271-2274.

Parkinson, W. M., M. Dookwah, M. L. Dear, C. L. Gatto, K. Aoki *et al.*, 2016 Synaptic roles for phosphomannomutase type 2 in a new Drosophila congenital disorder of glycosylation disease model. Dis Model Mech 9**:** 513-527.

Parrott, B. B., Y. Chiang, A. Hudson, A. Sarkar, A. Guichet *et al.*, 2011 Nucleoporin98-96 function is required for transit amplification divisions in the germ line of Drosophila melanogaster. PLoS One 6**:** e25087.

Pascual-Garcia, P., J. Jeong and M. Capelson, 2014 Nucleoporin Nup98 associates with Trx/MLL and NSL histone-modifying complexes and regulates Hox gene expression. Cell Rep 9**:** 433-442.

Patalano, S., M. Mihailovich, Y. Belacortu, N. Paricio and F. Gebauer, 2009 Dual sex-specific functions of Drosophila Upstream of N-ras in the control of X chromosome dosage compensation. Development 136**:** 689-698.

Patel, J. H., Y. Du, P. G. Ard, C. Phillips, B. Carella *et al.*, 2004 The c-MYC oncoprotein is a substrate of the acetyltransferases hGCN5/PCAF and TIP60. Mol Cell Biol 24**:** 10826-10834.

Pearson, J. C., and S. T. Crews, 2014 Enhancer diversity and the control of a simple pattern of Drosophila CNS midline cell expression. Dev Biol 392**:** 466-482.

Peck, V. M., E. W. Gerner and A. E. Cress, 1992 Delta-type DNA polymerase characterized from Drosophila melanogaster embryos. Nucleic Acids Res 20**:** 5779-5784.

Peissert, S., A. Schlosser, R. Kendel, J. Kuper and C. Kisker, 2020 Structural basis for CDK7 activation by MAT1 and Cyclin H. Proc Natl Acad Sci U S A 117**:** 26739-26748.

Pek, J. W., A. K. Lim and T. Kai, 2009 Drosophila maelstrom ensures proper germline stem cell lineage differentiation by repressing microRNA-7. Dev Cell 17**:** 417-424.

Pek, J. W., B. F. Ng and T. Kai, 2012 Polo-mediated phosphorylation of Maelstrom regulates oocyte determination during oogenesis in Drosophila. Development 139**:** 4505-4513.

Pennetier, D., J. Oyallon, I. Morin-Poulard, S. Dejean, A. Vincent *et al.*, 2012 Size control of the Drosophila hematopoietic niche by bone morphogenetic protein signaling reveals parallels with mammals. Proc Natl Acad Sci U S A 109**:** 3389-3394.

Perez-Lluch, S., E. Blanco, A. Carbonell, D. Raha, M. Snyder *et al.*, 2011 Genome-wide chromatin occupancy analysis reveals a role for ASH2 in transcriptional pausing. Nucleic Acids Res 39**:** 4628-4639.

Perez-Roger, I., S. H. Kim, B. Griffiths, A. Sewing and H. Land, 1999 Cyclins D1 and D2 mediate myc-induced proliferation via sequestration of p27(Kip1) and p21(Cip1). EMBO J 18**:** 5310-5320.

Perrin, L., C. Benassayag, D. Morello, J. Pradel and J. Montagne, 2003 Modulo is a target of Myc selectively required for growth of proliferative cells in Drosophila. Mech Dev 120**:** 645-655.

Perrin, L., O. Demakova, L. Fanti, S. Kallenbach, S. Saingery *et al.*, 1998 Dynamics of the sub-nuclear distribution of Modulo and the regulation of position-effect variegation by nucleolus in Drosophila. J Cell Sci 111 (Pt 18)**:** 2753-2761.

Perrin, L., P. Romby, P. Laurenti, H. Berenger, S. Kallenbach *et al.*, 1999 The Drosophila modifier of variegation modulo gene product binds specific RNA sequences at the nucleolus and interacts with DNA and chromatin in a phosphorylation-dependent manner. J Biol Chem 274**:** 6315-6323.

Petit, V., U. Nussbaumer, C. Dossenbach and M. Affolter, 2004 Downstream-of-FGFR is a fibroblast growth factor-specific scaffolding protein and recruits Corkscrew upon receptor activation. Mol Cell Biol 24**:** 3769-3781.

Petruk, S., Y. Sedkov, K. M. Riley, J. Hodgson, F. Schweisguth *et al.*, 2006 Transcription of bxd noncoding RNAs promoted by trithorax represses Ubx in cis by transcriptional interference. Cell 127**:** 1209-1221.

Peyrefitte, S., D. Kahn and M. Haenlin, 2001 New members of the Drosophila Myc transcription factor subfamily revealed by a genome-wide examination for basic helix-loop-helix genes. Mech Dev 104**:** 99-104.

Pflugfelder, G. O., F. Eichinger and J. Shen, 2017 T-Box Genes in Drosophila Limb Development. Curr Top Dev Biol 122**:** 313-354.

Phillips, J. W., Y. Pan, B. L. Tsai, Z. Xie, L. Demirdjian *et al.*, 2020 Pathway-guided analysis identifies Myc-dependent alternative pre-mRNA splicing in aggressive prostate cancers. Proc Natl Acad Sci U S A 117**:** 5269-5279.

Phizicky, E. M., and S. Fields, 1995 Protein-protein interactions: methods for detection and analysis. Microbiol Rev 59**:** 94-123.

Piazzi, M., A. Bavelloni, A. Gallo, I. Faenza and W. L. Blalock, 2019 Signal Transduction in Ribosome Biogenesis: A Recipe to Avoid Disaster. Int J Mol Sci 20.

Pojer, J. M., S. A. Manning, B. Kroeger, S. Kondo and K. F. Harvey, 2021 The Hippo pathway uses different machinery to control cell fate and organ size. iScience 24**:** 102830.

Polesello, C., I. Delon, P. Valenti, P. Ferrer and F. Payre, 2002 Dmoesin controls actin-based cell shape and polarity during Drosophila melanogaster oogenesis. Nat Cell Biol 4**:** 782-789.

Port, F., M. Kuster, P. Herr, E. Furger, C. Banziger *et al.*, 2008 Wingless secretion promotes and requires retromer-dependent cycling of Wntless. Nat Cell Biol 10**:** 178-185.

Prioleau, M. N., J. Huet, A. Sentenac and M. Mechali, 1994 Competition between chromatin and transcription complex assembly regulates gene expression during early development. Cell 77**:** 439-449.

Prober, D. A., and B. A. Edgar, 2002 Interactions between Ras1, dMyc, and dPI3K signaling in the developing Drosophila wing. Genes Dev 16**:** 2286-2299.

Prochownik, E. V., 2022 Regulation of Normal and Neoplastic Proliferation and Metabolism by the Extended Myc Network. Cells 11.

Qian, S., J. Y. Zhang, M. A. Kay and M. Jacobs-Lorena, 1987 Structural analysis of the Drosophila rpA1 gene, a member of the eucaryotic 'A' type ribosomal protein family. Nucleic Acids Res 15**:** 987-1003.

Qiao, F., B. Harada, H. Song, J. Whitelegge, A. J. Courey *et al.*, 2006 Mae inhibits Pointed-P2 transcriptional activity by blocking its MAPK docking site. EMBO J 25**:** 70-79.

Qiu, H., C. Hu, N. A. Gaur and A. G. Hinnebusch, 2012 Pol II CTD kinases Bur1 and Kin28 promote Spt5 CTR-independent recruitment of Paf1 complex. EMBO J 31**:** 3494-3505.

Quinn, L. M., J. Secombe and G. R. Hime, 2013 Myc in stem cell behaviour: insights from Drosophila. Advances in experimental medicine and biology 786**:** 269-285.

Radimerski, T., J. Montagne, F. Rintelen, H. Stocker, J. van der Kaay *et al.*, 2002 dS6K-regulated cell growth is dPKB/dPI(3)K-independent, but requires dPDK1. Nat Cell Biol 4**:** 251-255.

Raffeiner, P., A. Schraffl, T. Schwarz, R. Rock, K. Ledolter *et al.*, 2017 Calcium-dependent binding of Myc to calmodulin. Oncotarget 8**:** 3327-3343.

Rafti, F., D. Scarvelis and P. F. Lasko, 1996 A Drosophila melanogaster homologue of the human DEAD-box gene DDX1. Gene 171**:** 225-229.

Raha, D., M. Hong and M. Snyder, 2010a ChIP-Seq: a method for global identification of regulatory elements in the genome. Curr Protoc Mol Biol Chapter 21**:** Unit 21 19 21-14.

Raha, D., Z. Wang, Z. Moqtaderi, L. Wu, G. Zhong *et al.*, 2010b Close association of RNA polymerase II and many transcription factors with Pol III genes. Proc Natl Acad Sci U S A 107**:** 3639-3644.

Rajan, A., and N. Perrimon, 2010 Steroids make you bigger? Fat chance says Myc. Cell Metab 12**:** 7-9.

Ramanujam, P. L., S. Mehrotra, R. P. Kumar, S. Verma, G. Deshpande *et al.*, 2021 Global chromatin organizer SATB1 acts as a context-dependent regulator of the Wnt/Wg target genes. Sci Rep 11**:** 3385.

Ramdas Nair, A., P. Singh, D. Salvador Garcia, D. Rodriguez-Crespo, B. Egger *et al.*, 2016 The Microcephaly-Associated Protein Wdr62/CG7337 Is Required to Maintain Centrosome Asymmetry in Drosophila Neuroblasts. Cell Rep 14**:** 1100-1113.

Rauskolb, C., S. Sun, G. Sun, Y. Pan and K. D. Irvine, 2014 Cytoskeletal tension inhibits Hippo signaling through an Ajuba-Warts complex. Cell 158**:** 143-156.

Ravens, S., C. Yu, T. Ye, M. Stierle and L. Tora, 2015 Tip60 complex binds to active Pol II promoters and a subset of enhancers and co-regulates the c-Myc network in mouse embryonic stem cells. Epigenetics Chromatin 8**:** 45.

Ray-Jones, H., and M. Spivakov, 2021 Transcriptional enhancers and their communication with gene promoters. Cell Mol Life Sci 78**:** 6453-6485.

Ray, P., X. Luo, E. J. Rao, A. Basha, E. A. Woodruff, 3rd *et al.*, 2010 The splicing factor Prp31 is essential for photoreceptor development in Drosophila. Protein Cell 1**:** 267-274.

Reaume, A. G., D. A. Knecht and A. Chovnick, 1991 The rosy locus in Drosophila melanogaster: xanthine dehydrogenase and eye pigments. Genetics 129**:** 1099-1109.

Reddy, B. V., and K. D. Irvine, 2011 Regulation of Drosophila glial cell proliferation by Merlin-Hippo signaling. Development 138**:** 5201-5212.

Reich, A., and B. Z. Shilo, 2002 Keren, a new ligand of the Drosophila epidermal growth factor receptor, undergoes two modes of cleavage. EMBO J 21**:** 4287-4296.

Reim, I., and M. Frasch, 2005 The Dorsocross T-box genes are key components of the regulatory network controlling early cardiogenesis in Drosophila. Development 132**:** 4911-4925.

Reiter, F., B. P. de Almeida and A. Stark, 2023 Enhancers display constrained sequence flexibility and context-specific modulation of motif function. Genome Res 33**:** 346-358.

Ren, F., Q. Shi, Y. Chen, A. Jiang, Y. T. Ip *et al.*, 2013 Drosophila Myc integrates multiple signaling pathways to regulate intestinal stem cell proliferation during midgut regeneration. Cell Res 23**:** 1133-1146.

Ren, F., B. Wang, T. Yue, E. Y. Yun, Y. T. Ip *et al.*, 2010 Hippo signaling regulates Drosophila intestine stem cell proliferation through multiple pathways. Proc Natl Acad Sci U S A 107**:** 21064-21069.

Ren, M., E. Coutavas, P. D'Eustachio and M. G. Rush, 1994 Effects of mutant Ran/TC4 proteins on cell cycle progression. Mol Cell Biol 14**:** 4216-4224.

Riese, J., X. Yu, A. Munnerlyn, S. Eresh, S. C. Hsu *et al.*, 1997 LEF-1, a nuclear factor coordinating signaling inputs from wingless and decapentaplegic. Cell 88**:** 777-787.

Rincon-Limas, D. E., C. H. Lu, I. Canal and J. Botas, 2000 The level of DLDB/CHIP controls the activity of the LIM homeodomain protein apterous: evidence for a functional tetramer complex in vivo. EMBO J 19**:** 2602-2614.

Roberts, D. M., M. I. Pronobis, J. S. Poulton, E. G. Kane and M. Peifer, 2012 Regulation of Wnt signaling by the tumor suppressor adenomatous polyposis coli does not require the ability to enter the nucleus or a particular cytoplasmic localization. Mol Biol Cell 23**:** 2041-2056.

Rodriguez, C. F., and O. Llorca, 2020 RPAP3 C-Terminal Domain: A Conserved Domain for the Assembly of R2TP Co-Chaperone Complexes. Cells 9.

Roignant, J. Y., K. Legent, F. Janody and J. E. Treisman, 2010 The transcriptional co-factor Chip acts with LIM-homeodomain proteins to set the boundary of the eye field in Drosophila. Development 137**:** 273-281.

Rosales-Vega, M., D. Resendez-Perez, M. Zurita and M. Vazquez, 2023 TnaA, a trithorax group protein, modulates wingless expression in different regions of the Drosophila wing imaginal disc. Sci Rep 13**:** 15162.

Rospert, S., Y. Dubaquie and M. Gautschi, 2002 Nascent-polypeptide-associated complex. Cell Mol Life Sci 59**:** 1632-1639.

Rosselot, C., S. Baumel-Alterzon, Y. Li, G. Brill, L. Lambertini *et al.*, 2021 The many lives of Myc in the pancreatic beta-cell. J Biol Chem 296**:** 100122.

Roux, P. P., and I. Topisirovic, 2018 Signaling Pathways Involved in the Regulation of mRNA Translation. Mol Cell Biol 38.

Roy, F., G. Laberge, M. Douziech, D. Ferland-McCollough and M. Therrien, 2002 KSR is a scaffold required for activation of the ERK/MAPK module. Genes Dev 16**:** 427-438.

Royou, A., C. Field, J. C. Sisson, W. Sullivan and R. Karess, 2004 Reassessing the role and dynamics of nonmuscle myosin II during furrow formation in early Drosophila embryos. Mol Biol Cell 15**:** 838-850.

Rust, K., M. D. Tiwari, V. K. Mishra, F. Grawe and A. Wodarz, 2018 Myc and the Tip60 chromatin remodeling complex control neuroblast maintenance and polarity in Drosophila. EMBO J 37.

Ryoo, H. D., A. Bergmann, H. Gonen, A. Ciechanover and H. Steller, 2002 Regulation of Drosophila IAP1 degradation and apoptosis by reaper and ubcD1. Nat Cell Biol 4**:** 432-438.

Sabino, D., N. H. Brown and R. Basto, 2011 Drosophila Ajuba is not an Aurora-A activator but is required to maintain Aurora-A at the centrosome. J Cell Sci 124**:** 1156-1166.

Samuels, T. J., A. I. Jarvelin, D. Ish-Horowicz and I. Davis, 2020 Imp/IGF2BP levels modulate individual neural stem cell growth and division through myc mRNA stability. Elife 9.

Sanchez-Arevalo Lobo, V. J., M. Doni, A. Verrecchia, S. Sanulli, G. Faga *et al.*, 2013 Dual regulation of Myc by Abl. Oncogene 32**:** 5261-5271.

Sarikas, A., T. Hartmann and Z. Q. Pan, 2011 The cullin protein family. Genome Biol 12**:** 220.

Sato, M., and T. B. Kornberg, 2002 FGF is an essential mitogen and chemoattractant for the air sacs of the drosophila tracheal system. Dev Cell 3**:** 195-207.

Satou, A., T. Taira, S. M. Iguchi-Ariga and H. Ariga, 2001 A novel transrepression pathway of c-Myc. Recruitment of a transcriptional corepressor complex to c-Myc by MM-1, a c-Myc-binding protein. J Biol Chem 276**:** 46562-46567.

Savitsky, M., M. Kim, O. Kravchuk and Y. B. Schwartz, 2016 Distinct Roles of Chromatin Insulator Proteins in Control of the Drosophila Bithorax Complex. Genetics 202**:** 601-617.

Sazer, S., and M. Dasso, 2000 The ran decathlon: multiple roles of Ran. J Cell Sci 113 (Pt 7)**:** 1111-1118.

Scantlebury, N., X. L. Zhao, V. G. Rodriguez Moncalvo, A. Camiletti, S. Zahanova *et al.*, 2010 The Drosophila gene RanBPM functions in the mushroom body to regulate larval behavior. PLoS One 5**:** e10652.

Schaub, C., M. Rose and M. Frasch, 2019 Yorkie and JNK revert syncytial muscles into myoblasts during Org-1-dependent lineage reprogramming. J Cell Biol 218**:** 3572-3582.

Scheer, E., F. Delbac, L. Tora, D. Moras and C. Romier, 2012 TFIID TAF6-TAF9 complex formation involves the HEAT repeat-containing C-terminal domain of TAF6 and is modulated by TAF5 protein. J Biol Chem 287**:** 27580-27592.

Schmidt, E. V., 2004 The role of c-myc in regulation of translation initiation. Oncogene 23**:** 3217-3221.

Schneider, M., A. A. Khalil, J. Poulton, C. Castillejo-Lopez, D. Egger-Adam *et al.*, 2006 Perlecan and Dystroglycan act at the basal side of the Drosophila follicular epithelium to maintain epithelial organization. Development 133**:** 3805-3815.

Schnepp, B., T. Donaldson, G. Grumbling, S. Ostrowski, R. Schweitzer *et al.*, 1998 EGF domain swap converts a drosophila EGF receptor activator into an inhibitor. Genes Dev 12**:** 908-913.

Schweitzer, R., M. Shaharabany, R. Seger and B. Z. Shilo, 1995 Secreted Spitz triggers the DER signaling pathway and is a limiting component in embryonic ventral ectoderm determination. Genes Dev 9**:** 1518-1529.

Schweizer, L., D. Nellen and K. Basler, 2003 Requirement for Pangolin/dTCF in Drosophila Wingless signaling. Proc Natl Acad Sci U S A 100**:** 5846-5851.

Sears, R., G. Leone, J. DeGregori and J. R. Nevins, 1999 Ras enhances Myc protein stability. Mol Cell 3**:** 169-179.

Secombe, J., L. Li, L. Carlos and R. N. Eisenman, 2007 The Trithorax group protein Lid is a trimethyl histone H3K4 demethylase required for dMyc-induced cell growth. Genes Dev 21**:** 537-551.

Serafini, G., G. Giordani, L. Grillini, D. Andrenacci, G. Gargiulo *et al.*, 2020 The Impact of Drosophila Awd/NME1/2 Levels on Notch and Wg Signaling Pathways. Int J Mol Sci 21.

Sethi, M. K., F. F. Buettner, A. Ashikov, V. B. Krylov, H. Takeuchi *et al.*, 2012 Molecular cloning of a xylosyltransferase that transfers the second xylose to O-glucosylated epidermal growth factor repeats of notch. J Biol Chem 287**:** 2739-2748.

Shalaby, N. A., A. L. Parks, E. J. Morreale, M. C. Osswalt, K. M. Pfau *et al.*, 2009 A screen for modifiers of notch signaling uncovers Amun, a protein with a critical role in sensory organ development. Genetics 182**:** 1061-1076.

Sharma, A., S. Halder, M. Felix, K. Nisaa, G. Deshpande *et al.*, 2018 Insulin signaling modulates border cell movement in Drosophila oogenesis. Development 145.

Shchepinov, M. S., I. A. Udalova, A. J. Bridgman and E. M. Southern, 1997 Oligonucleotide dendrimers: synthesis and use as polylabelled DNA probes. Nucleic Acids Res 25**:** 4447-4454.

Shcherbata, H. R., C. Althauser, S. D. Findley and H. Ruohola-Baker, 2004 The mitotic-to-endocycle switch in Drosophila follicle cells is executed by Notch-dependent regulation of G1/S, G2/M and M/G1 cell-cycle transitions. Development 131**:** 3169-3181.

Shi, J., W. A. Whyte, C. J. Zepeda-Mendoza, J. P. Milazzo, C. Shen *et al.*, 2013 Role of SWI/SNF in acute leukemia maintenance and enhancer-mediated Myc regulation. Genes Dev 27**:** 2648-2662.

Shi, Q., S. Li, S. Li, A. Jiang, Y. Chen *et al.*, 2014 Hedgehog-induced phosphorylation by CK1 sustains the activity of Ci/Gli activator. Proc Natl Acad Sci U S A 111**:** E5651-5660.

Shidlovskii, Y. V., A. N. Krasnov, J. V. Nikolenko, L. A. Lebedeva, M. Kopantseva *et al.*, 2005 A novel multidomain transcription coactivator SAYP can also repress transcription in heterochromatin. EMBO J 24**:** 97-107.

Shilatifard, A., 2012 The COMPASS family of histone H3K4 methylases: mechanisms of regulation in development and disease pathogenesis. Annu Rev Biochem 81**:** 65-95.

Shima, S., T. Aigaki, T. Nojima and D. Yamamoto, 2007 Identification of trf2 mutants of Drosophila with defects in anterior spiracle eversion. Arch Insect Biochem Physiol 64**:** 157-163.

Shimamura, M., A. Kyotani, Y. Azuma, H. Yoshida, T. Binh Nguyen *et al.*, 2014 Genetic link between Cabeza, a Drosophila homologue of Fused in Sarcoma (FUS), and the EGFR signaling pathway. Exp Cell Res 326**:** 36-45.

Shir-Shapira, H., A. Sloutskin, O. Adato, A. Ovadia-Shochat, D. Ideses *et al.*, 2019 Identification of evolutionarily conserved downstream core promoter elements required for the transcriptional regulation of Fushi tarazu target genes. PLoS One 14**:** e0215695.

Shohayeb, B., N. Mitchell, S. S. Millard, L. M. Quinn and D. C. H. Ng, 2020 Elevated levels of Drosophila Wdr62 promote glial cell growth and proliferation through AURKA signalling to AKT and MYC. Biochim Biophys Acta Mol Cell Res 1867**:** 118713.

Siddiqui-Jain, A., C. L. Grand, D. J. Bearss and L. H. Hurley, 2002 Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. Proc Natl Acad Sci U S A 99**:** 11593-11598.

Siebel, C. W., R. Kanaar and D. C. Rio, 1994 Regulation of tissue-specific P-element pre-mRNA splicing requires the RNA-binding protein PSI. Genes Dev 8**:** 1713-1725.

Sienski, G., D. Donertas and J. Brennecke, 2012 Transcriptional silencing of transposons by Piwi and maelstrom and its impact on chromatin state and gene expression. Cell 151**:** 964-980.

Sim, D. Y., H. J. Lee, C. H. Ahn, J. Park, S. Y. Park *et al.*, 2024 Negative Regulation of CPSF6 Suppresses the Warburg Effect and Angiogenesis Leading to Tumor Progression Via c-Myc Signaling Network: Potential Therapeutic Target for Liver Cancer Therapy. Int J Biol Sci 20**:** 3442-3460.

Simoes, S., B. Denholm, D. Azevedo, S. Sotillos, P. Martin *et al.*, 2006 Compartmentalisation of Rho regulators directs cell invagination during tissue morphogenesis. Development 133**:** 4257-4267.

Sinenko, S. A., T. Hung, T. Moroz, Q. M. Tran, S. Sidhu *et al.*, 2010 Genetic manipulation of AML1-ETO-induced expansion of hematopoietic precursors in a Drosophila model. Blood 116**:** 4612-4620.

Sinenko, S. A., L. Mandal, J. A. Martinez-Agosto and U. Banerjee, 2009 Dual role of wingless signaling in stem-like hematopoietic precursor maintenance in Drosophila. Dev Cell 16**:** 756-763.

Singh, A. M., and S. Dalton, 2009 The cell cycle and Myc intersect with mechanisms that regulate pluripotency and reprogramming. Cell Stem Cell 5**:** 141-149.

Siomi, M. C., K. Higashijima, A. Ishizuka and H. Siomi, 2002 Casein kinase II phosphorylates the fragile X mental retardation protein and modulates its biological properties. Mol Cell Biol 22**:** 8438-8447.

Slack, C., N. Alic, A. Foley, M. Cabecinha, M. P. Hoddinott *et al.*, 2015 The Ras-Erk-ETS-Signaling Pathway Is a Drug Target for Longevity. Cell 162**:** 72-83.

Sloan, R. S., C. I. Swanson, L. Gavilano, K. N. Smith, P. Y. Malek *et al.*, 2012 Characterization of null and hypomorphic alleles of the Drosophila l(2)dtl/cdt2 gene: Larval lethality and male fertility. Fly (Austin) 6**:** 173-183.

Sloutskin, A., D. Itzhak, G. Vogler, H. Pozeilov, D. Ideses *et al.*, 2024 From promoter motif to cardiac function: a single DPE motif affects transcription regulation and organ function in vivo. Development 151.

Smale, S. T., and J. T. Kadonaga, 2003 The RNA polymerase II core promoter. Annu Rev Biochem 72**:** 449-479.

Small, S., and D. N. Arnosti, 2020 Transcriptional Enhancers in Drosophila. Genetics 216**:** 1-26.

Smelkinson, M. G., Q. Zhou and D. Kalderon, 2007 Regulation of Ci-SCFSlimb binding, Ci proteolysis, and hedgehog pathway activity by Ci phosphorylation. Dev Cell 13**:** 481-495.

Smibert, C. A., Y. S. Lie, W. Shillinglaw, W. J. Henzel and P. M. Macdonald, 1999 Smaug, a novel and conserved protein, contributes to repression of nanos mRNA translation in vitro. RNA 5**:** 1535-1547.

Smith-Bolton, R. K., M. I. Worley, H. Kanda and I. K. Hariharan, 2009 Regenerative growth in Drosophila imaginal discs is regulated by Wingless and Myc. Dev Cell 16**:** 797-809.

Smith, G. D., W. H. Ching, P. Cornejo-Paramo and E. S. Wong, 2023 Decoding enhancer complexity with machine learning and high-throughput discovery. Genome Biol 24**:** 116.

Smylla, T. K., M. Meier, A. Preiss and D. Maier, 2019 The Notch repressor complex in Drosophila: in vivo analysis of Hairless mutants using overexpression experiments. Dev Genes Evol 229**:** 13-24.

Solinet, S., K. Mahmud, S. F. Stewman, K. Ben El Kadhi, B. Decelle *et al.*, 2013 The actin-binding ERM protein Moesin binds to and stabilizes microtubules at the cell cortex. J Cell Biol 202**:** 251-260.

Soltani-Bejnood, M., S. E. Thomas, L. Villeneuve, K. Schwartz, C. S. Hong *et al.*, 2007 Role of the mod(mdg4) common region in homolog segregation in Drosophila male meiosis. Genetics 176**:** 161-180.

Somma, M. P., B. Fasulo, G. Cenci, E. Cundari and M. Gatti, 2002 Molecular dissection of cytokinesis by RNA interference in Drosophila cultured cells. Mol Biol Cell 13**:** 2448-2460.

Song, H., P. Hasson, Z. Paroush and A. J. Courey, 2004 Groucho oligomerization is required for repression in vivo. Mol Cell Biol 24**:** 4341-4350.

Song, H., C. Spichiger-Haeusermann and K. Basler, 2009 The ISWI-containing NURF complex regulates the output of the canonical Wingless pathway. EMBO Rep 10**:** 1140-1146.

Song, S., H. Herranz and S. M. Cohen, 2017 The chromatin remodeling BAP complex limits tumor-promoting activity of the Hippo pathway effector Yki to prevent neoplastic transformation in Drosophila epithelia. Dis Model Mech 10**:** 1201-1209.

Spain, M. M., J. A. Caruso, A. Swaminathan and L. A. Pile, 2010 Drosophila SIN3 isoforms interact with distinct proteins and have unique biological functions. J Biol Chem 285**:** 27457-27467.

Spencer, C. A., and M. Groudine, 1991 Control of c-myc regulation in normal and neoplastic cells. Adv Cancer Res 56**:** 1-48.

Squire, J. A., P. S. Thorner, S. Weitzman, J. D. Maggi, P. Dirks *et al.*, 1995 Co-amplification of MYCN and a DEAD box gene (DDX1) in primary neuroblastoma. Oncogene 10**:** 1417-1422.

St Clair, S. L., H. Li, U. Ashraf, J. A. Karty and J. M. Tennessen, 2017 Metabolomic Analysis Reveals That the Drosophila melanogaster Gene lysine Influences Diverse Aspects of Metabolism. Genetics 207**:** 1255-1261.

Stern, B., G. Ried, N. J. Clegg, T. A. Grigliatti and C. F. Lehner, 1993 Genetic analysis of the Drosophila cdc2 homolog. Development 117**:** 219-232.

Strick, T. R., V. Croquette and D. Bensimon, 2000 Single-molecule analysis of DNA uncoiling by a type II topoisomerase. Nature 404**:** 901-904.

Stroebele, E., and A. Erives, 2016 Integration of Orthogonal Signaling by the Notch and Dpp Pathways in Drosophila. Genetics 203**:** 219-240.

Su, Y. C., J. E. Treisman and E. Y. Skolnik, 1998 The Drosophila Ste20-related kinase misshapen is required for embryonic dorsal closure and acts through a JNK MAPK module on an evolutionarily conserved signaling pathway. Genes Dev 12**:** 2371-2380.

Suganuma, T., A. Mushegian, S. K. Swanson, L. Florens, M. P. Washburn *et al.*, 2012 A metazoan ATAC acetyltransferase subunit that regulates mitogen-activated protein kinase signaling is related to an ancient molybdopterin synthase component. Mol Cell Proteomics 11**:** 90-99.

Suganuma, T., and J. L. Workman, 2008 Crosstalk among Histone Modifications. Cell 135**:** 604-607.

Swarup, S., and E. M. Verheyen, 2011 Drosophila homeodomain-interacting protein kinase inhibits the Skp1-Cul1-F-box E3 ligase complex to dually promote Wingless and Hedgehog signaling. Proc Natl Acad Sci U S A 108**:** 9887-9892.

Symes, A. J., M. Eilertsen, M. Millar, J. Nariculam, A. Freeman *et al.*, 2013 Quantitative analysis of BTF3, HINT1, NDRG1 and ODC1 protein over-expression in human prostate cancer tissue. PLoS One 8**:** e84295.

Szentirmay, M. N., and M. Sawadogo, 1991 Transcription factor requirement for multiple rounds of initiation by human RNA polymerase II. Proc Natl Acad Sci U S A 88**:** 10691-10695.

Takahashi, S., and I. Takada, 2023 Recent advances in prostate cancer: WNT signaling, chromatin regulation, and transcriptional coregulators. Asian J Androl 25**:** 158-165.

Takai, A., T. Chiyonobu, I. Ueoka, R. Tanaka, T. Tozawa *et al.*, 2020 A novel Drosophila model for neurodevelopmental disorders associated with Shwachman-Diamond syndrome. Neurosci Lett 739**:** 135449.

Takayama, M., T. Taira, S. M. Iguchi-Ariga and H. Ariga, 2000 CDC6 interacts with c-Myc to inhibit E-box-dependent transcription by abrogating c-Myc/Max complex. FEBS Lett 477**:** 43-48.

Takemura, M., and H. Nakato, 2017 Drosophila Sulf1 is required for the termination of intestinal stem cell division during regeneration. J Cell Sci 130**:** 332-343.

Takeuchi, H., R. C. Fernandez-Valdivia, D. S. Caswell, A. Nita-Lazar, N. A. Rana *et al.*, 2011 Rumi functions as both a protein O-glucosyltransferase and a protein O-xylosyltransferase. Proc Natl Acad Sci U S A 108**:** 16600-16605.

Taliaferro, J. M., D. Marwha, J. L. Aspden, D. Mavrici, N. E. Cheng *et al.*, 2013 The Drosophila splicing factor PSI is phosphorylated by casein kinase II and tousled-like kinase. PLoS One 8**:** e56401.

Tang, R., W. Huang, J. Guan, Q. Liu, B. T. Beerntsen *et al.*, 2021 Drosophila H2Av negatively regulates the activity of the IMD pathway via facilitating Relish SUMOylation. PLoS Genet 17**:** e1009718.

Taniguchi, K., S. Hozumi, R. Maeda, T. Okumura and K. Matsuno, 2007 Roles of type I myosins in Drosophila handedness. Fly (Austin) 1**:** 287-290.

Tarayrah, L., Y. Li, Q. Gan and X. Chen, 2015 Epigenetic regulator Lid maintains germline stem cells through regulating JAK-STAT signaling pathway activity. Biol Open 4**:** 1518-1527.

Tariq, A., L. Green, J. C. G. Jeynes, C. Soeller and J. G. Wakefield, 2020 In vitro reconstitution of branching microtubule nucleation. Elife 9.

Tavares, L., A. Correia, M. A. Santos, J. B. Relvas and P. S. Pereira, 2017 dMyc is required in retinal progenitors to prevent JNK-mediated retinal glial activation. PLoS Genet 13**:** e1006647.

Taylor, J. P., R. H. Brown, Jr. and D. W. Cleveland, 2016 Decoding ALS: from genes to mechanism. Nature 539**:** 197-206.

Terriente-Felix, A., and J. F. de Celis, 2009 Osa, a subunit of the BAP chromatin-remodelling complex, participates in the regulation of gene expression in response to EGFR signalling in the Drosophila wing. Dev Biol 329**:** 350-361.

Teufel, A., T. Maass, S. Strand, S. Kanzler, T. Galante *et al.*, 2010 Liver-specific Ldb1 deletion results in enhanced liver cancer development. J Hepatol 53**:** 1078-1084.

Thao, D. T., H. Seto and M. Yamaguchi, 2008 Drosophila Myc is required for normal DREF gene expression. Exp Cell Res 314**:** 184-192.

Thevenon, D., I. Seffouh, C. Pillet, X. Crespo-Yanez, M. O. Fauvarque *et al.*, 2020 A Nucleolar Isoform of the Drosophila Ubiquitin Specific Protease dUSP36 Regulates MYC-Dependent Cell Growth. Front Cell Dev Biol 8**:** 506.

Thomas, M. C., and C. M. Chiang, 2006 The general transcription machinery and general cofactors. Crit Rev Biochem Mol Biol 41**:** 105-178.

Thompson, E. B., 1998 The many roles of c-Myc in apoptosis. Annu Rev Physiol 60**:** 575-600.

Tokamov, S. A., T. Su, A. Ullyot and R. G. Fehon, 2021 Negative feedback couples Hippo pathway activation with Kibra degradation independent of Yorkie-mediated transcription. Elife 10.

Toledano-Katchalski, H., R. Nir, G. Volohonsky and T. Volk, 2007 Post-transcriptional repression of the Drosophila midkine and pleiotrophin homolog miple by HOW is essential for correct mesoderm spreading. Development 134**:** 3473-3481.

Toth, J., M. Kovacs, F. Wang, L. Nyitray and J. R. Sellers, 2005 Myosin V from Drosophila reveals diversity of motor mechanisms within the myosin V family. J Biol Chem 280**:** 30594-30603.

Tran, D. D., S. Saran, A. J. Williamson, A. Pierce, O. Dittrich-Breiholz *et al.*, 2014 THOC5 controls 3'end-processing of immediate early genes via interaction with polyadenylation specific factor 100 (CPSF100). Nucleic Acids Res 42**:** 12249-12260.

Treiber, T., N. Treiber, U. Plessmann, S. Harlander, J. L. Daiss *et al.*, 2017 A Compendium of RNA-Binding Proteins that Regulate MicroRNA Biogenesis. Mol Cell 66**:** 270-284 e213.

Tritto, P., V. Palumbo, L. Micale, M. Marzulli, M. P. Bozzetti *et al.*, 2015 Loss of Pol32 in Drosophila melanogaster causes chromosome instability and suppresses variegation. PLoS One 10**:** e0120859.

Tsai, C. C., H. Y. Kao, A. Mitzutani, E. Banayo, H. Rajan *et al.*, 2004 Ataxin 1, a SCA1 neurodegenerative disorder protein, is functionally linked to the silencing mediator of retinoid and thyroid hormone receptors. Proc Natl Acad Sci U S A 101**:** 4047-4052.

Tsuda, L., M. Kaido, Y. M. Lim, K. Kato, T. Aigaki *et al.*, 2006 An NRSF/REST-like repressor downstream of Ebi/SMRTER/Su(H) regulates eye development in Drosophila. EMBO J 25**:** 3191-3202.

Tsuda, L., R. Nagaraj, S. L. Zipursky and U. Banerjee, 2002 An EGFR/Ebi/Sno pathway promotes delta expression by inactivating Su(H)/SMRTER repression during inductive notch signaling. Cell 110**:** 625-637.

Tu, L. C., X. Yan, L. Hood and B. Lin, 2007 Proteomics analysis of the interactome of N-myc downstream regulated gene 1 and its interactions with the androgen response program in prostate cancer cells. Mol Cell Proteomics 6**:** 575-588.

Turnage, M. A., P. Brewer-Jensen, W. L. Bai and L. L. Searles, 2000 Arginine-rich regions mediate the RNA binding and regulatory activities of the protein encoded by the Drosophila melanogaster suppressor of sable gene. Mol Cell Biol 20**:** 8198-8208.

Tyler, J. K., M. Bulger, R. T. Kamakaka, R. Kobayashi and J. T. Kadonaga, 1996 The p55 subunit of Drosophila chromatin assembly factor 1 is homologous to a histone deacetylase-associated protein. Mol Cell Biol 16**:** 6149-6159.

Uemura, T., K. Shiomi, S. Togashi and M. Takeichi, 1993 Mutation of twins encoding a regulator of protein phosphatase 2A leads to pattern duplication in Drosophila imaginal discs. Genes Dev 7**:** 429-440.

Ukken, F. P., I. Aprill, N. JayaNandanan and M. Leptin, 2014 Slik and the receptor tyrosine kinase Breathless mediate localized activation of Moesin in terminal tracheal cells. PLoS One 9**:** e103323.

Valanne, S., H. Myllymaki, J. Kallio, M. R. Schmid, A. Kleino *et al.*, 2010 Genome-wide RNA interference in Drosophila cells identifies G protein-coupled receptor kinase 2 as a conserved regulator of NF-kappaB signaling. J Immunol 184**:** 6188-6198.

Van Dyke, M. W., M. Sawadogo and R. G. Roeder, 1989 Stability of transcription complexes on class II genes. Mol Cell Biol 9**:** 342-344.

van Meyel, D. J., D. D. O'Keefe, S. Thor, L. W. Jurata, G. N. Gill *et al.*, 2000 Chip is an essential cofactor for apterous in the regulation of axon guidance in Drosophila. Development 127**:** 1823-1831.

van Riggelen, J., A. Yetil and D. W. Felsher, 2010 MYC as a regulator of ribosome biogenesis and protein synthesis. Nat Rev Cancer 10**:** 301-309.

van Tienen, L. M., J. Mieszczanek, M. Fiedler, T. J. Rutherford and M. Bienz, 2017 Constitutive scaffolding of multiple Wnt enhanceosome components by Legless/BCL9. Elife 6.

Varga-Weisz, P. D., M. Wilm, E. Bonte, K. Dumas, M. Mann *et al.*, 1997 Chromatin-remodelling factor CHRAC contains the ATPases ISWI and topoisomerase II. Nature 388**:** 598-602.

Vasquez, C. G., M. Tworoger and A. C. Martin, 2014 Dynamic myosin phosphorylation regulates contractile pulses and tissue integrity during epithelial morphogenesis. J Cell Biol 206**:** 435-450.

Verni, F., R. Gandhi, M. L. Goldberg and M. Gatti, 2000 Genetic and molecular analysis of wings apart-like (wapl), a gene controlling heterochromatin organization in Drosophila melanogaster. Genetics 154**:** 1693-1710.

Vichas, A., M. T. Laurie and J. A. Zallen, 2015 The Ski2-family helicase Obelus regulates Crumbs alternative splicing and cell polarity. J Cell Biol 211**:** 1011-1024.

Villani, G., B. Sauer and I. R. Lehman, 1980 DNA polymerase alpha from Drosophila melanogaster embryos. Subunit structure. J Biol Chem 255**:** 9479-9483.

Vinayagam, A., Y. Hu, M. Kulkarni, C. Roesel, R. Sopko *et al.*, 2013 Protein complex-based analysis framework for high-throughput data sets. Sci Signal 6**:** rs5.

Voelker, R. A., W. Gibson, J. P. Graves, J. F. Sterling and M. T. Eisenberg, 1991 The Drosophila suppressor of sable gene encodes a polypeptide with regions similar to those of RNA-binding proteins. Mol Cell Biol 11**:** 894-905.

Volohonsky, G., G. Edenfeld, C. Klambt and T. Volk, 2007 Muscle-dependent maturation of tendon cells is induced by post-transcriptional regulation of stripeA. Development 134**:** 347-356.

Vorobyeva, N. E., N. V. Soshnikova, J. L. Kuzmina, M. R. Kopantseva, J. V. Nikolenko *et al.*, 2009 The novel regulator of metazoan development SAYP organizes a nuclear coactivator supercomplex. Cell Cycle 8**:** 2152-2156.

Vriz, S., M. Taylor and M. Mechali, 1989 Differential expression of two Xenopus c-myc proto-oncogenes during development. EMBO J 8**:** 4091-4097.

Vuilleumier, R., M. Miao, S. Medina-Giro, C. M. Ell, S. Flibotte *et al.*, 2022 Dichotomous cis-regulatory motifs mediate the maturation of the neuromuscular junction by retrograde BMP signaling. Nucleic Acids Res 50**:** 9748-9764.

Vyas, S., C. Bechade, B. Riveau, J. Downward and A. Triller, 2002 Involvement of survival motor neuron (SMN) protein in cell death. Hum Mol Genet 11**:** 2751-2764.

Wagner, C. R., K. Hamana and S. C. Elgin, 1992 A high-mobility-group protein and its cDNAs from Drosophila melanogaster. Mol Cell Biol 12**:** 1915-1923.

Wakefield, L. M., and C. Stuelten, 2007 Keeping order in the neighborhood: new roles for TGFbeta in maintaining epithelial homeostasis. Cancer Cell 12**:** 293-295.

Walsh, E. P., and N. H. Brown, 1998 A screen to identify Drosophila genes required for integrin-mediated adhesion. Genetics 150**:** 791-805.

Wan, M., J. Liang, Y. Xiong, F. Shi, Y. Zhang *et al.*, 2013 The trithorax group protein Ash2l is essential for pluripotency and maintaining open chromatin in embryonic stem cells. J Biol Chem 288**:** 5039-5048.

Wang, C., Z. Liu and X. Huang, 2012 Rab32 is important for autophagy and lipid storage in Drosophila. PLoS One 7**:** e32086.

Wang, G., K. Amanai, B. Wang and J. Jiang, 2000 Interactions with Costal2 and suppressor of fused regulate nuclear translocation and activity of cubitus interruptus. Genes Dev 14**:** 2893-2905.

Wang, H., C. Dienemann, A. Stutzer, H. Urlaub, A. C. M. Cheung *et al.*, 2020 Structure of the transcription coactivator SAGA. Nature 577**:** 717-720.

Wang, J., M. Michel, L. Bialas, G. Pierini and C. Dahmann, 2023 Preferential recruitment and stabilization of Myosin II at compartment boundaries in Drosophila. J Cell Sci 136.

Wang, J. W., J. R. Brent, A. Tomlinson, N. A. Shneider and B. D. McCabe, 2011 The ALS-associated proteins FUS and TDP-43 function together to affect Drosophila locomotion and life span. J Clin Invest 121**:** 4118-4126.

Wang, L., G. Lam and C. S. Thummel, 2010 Med24 and Mdh2 are required for Drosophila larval salivary gland cell death. Dev Dyn 239**:** 954-964.

Wang, M., M. Ly, A. Lugowski, J. D. Laver, H. D. Lipshitz *et al.*, 2017 ME31B globally represses maternal mRNAs by two distinct mechanisms during the Drosophila maternal-to-zygotic transition. Elife 6.

Wang, X., S. K. Hansen, R. Ratts, S. Zhou, A. J. Snook *et al.*, 1997 Drosophila TFIIE: purification, cloning, and functional reconstitution. Proc Natl Acad Sci U S A 94**:** 433-438.

Wang, X., X. Huang, C. Wu and L. Xue, 2018a Pontin/Tip49 acts as a novel regulator of JNK pathway. Cell Death Dis 9**:** 978.

Wang, X., X. Huang, C. Wu and L. Xue, 2018b Pontin/Tip49 negatively regulates JNK-mediated cell death in Drosophila. Cell Death Discov 4**:** 8.

Wang, Y. L., S. H. Duttke, K. Chen, J. Johnston, G. A. Kassavetis *et al.*, 2014 TRF2, but not TBP, mediates the transcription of ribosomal protein genes. Genes Dev 28**:** 1550-1555.

Wang, Z., S. Buratowski, J. Q. Svejstrup, W. J. Feaver, X. Wu *et al.*, 1995 The yeast TFB1 and SSL1 genes, which encode subunits of transcription factor IIH, are required for nucleotide excision repair and RNA polymerase II transcription. Mol Cell Biol 15**:** 2288-2293.

Wang, Z. H., Y. Liu, V. Chaitankar, M. Pirooznia and H. Xu, 2019 Electron transport chain biogenesis activated by a JNK-insulin-Myc relay primes mitochondrial inheritance in Drosophila. Elife 8.

Weake, V. M., J. O. Dyer, C. Seidel, A. Box, S. K. Swanson *et al.*, 2011 Post-transcription initiation function of the ubiquitous SAGA complex in tissue-specific gene activation. Genes Dev 25**:** 1499-1509.

Weake, V. M., S. K. Swanson, A. Mushegian, L. Florens, M. P. Washburn *et al.*, 2009 A novel histone fold domain-containing protein that replaces TAF6 in Drosophila SAGA is required for SAGA-dependent gene expression. Genes Dev 23**:** 2818-2823.

Wei, Y., D. Resetca, Z. Li, I. Johansson-Akhe, A. Ahlner *et al.*, 2019 Multiple direct interactions of TBP with the MYC oncoprotein. Nat Struct Mol Biol 26**:** 1035-1043.

Weiss, A., E. Charbonnier, E. Ellertsdottir, A. Tsirigos, C. Wolf *et al.*, 2010 A conserved activation element in BMP signaling during Drosophila development. Nat Struct Mol Biol 17**:** 69-76.

Wickens, M., D. S. Bernstein, J. Kimble and R. Parker, 2002 A PUF family portrait: 3'UTR regulation as a way of life. Trends Genet 18**:** 150-157.

Wiersdorff, V., T. Lecuit, S. M. Cohen and M. Mlodzik, 1996 Mad acts downstream of Dpp receptors, revealing a differential requirement for dpp signaling in initiation and propagation of morphogenesis in the Drosophila eye. Development 122**:** 2153-2162.

Willemsen, M. H., B. Nijhof, M. Fenckova, W. M. Nillesen, E. M. Bongers *et al.*, 2013 GATAD2B loss-of-function mutations cause a recognisable syndrome with intellectual disability and are associated with learning deficits and synaptic undergrowth in Drosophila. J Med Genet 50**:** 507-514.

Willert, K., M. Brink, A. Wodarz, H. Varmus and R. Nusse, 1997 Casein kinase 2 associates with and phosphorylates dishevelled. EMBO J 16**:** 3089-3096.

Willy, P. J., R. Kobayashi and J. T. Kadonaga, 2000 A basal transcription factor that activates or represses transcription. Science 290**:** 982-985.

Windler, S. L., and D. Bilder, 2010 Endocytic internalization routes required for delta/notch signaling. Curr Biol 20**:** 538-543.

Wolf, D., T. K. Smylla, J. Reichmuth, P. Hoffmeister, L. Kober *et al.*, 2019 Nucleo-cytoplasmic shuttling of Drosophila Hairless/Su(H) heterodimer as a means of regulating Notch dependent transcription. Biochim Biophys Acta Mol Cell Res 1866**:** 1520-1532.

Wolfe, A. L., K. Singh, Y. Zhong, P. Drewe, V. K. Rajasekhar *et al.*, 2014 RNA G-quadruplexes cause eIF4A-dependent oncogene translation in cancer. Nature 513**:** 65-70.

Wolfe, S. A., L. Nekludova and C. O. Pabo, 2000 DNA recognition by Cys2His2 zinc finger proteins. Annu Rev Biophys Biomol Struct 29**:** 183-212.

Wong, J. J., S. Li, E. K. Lim, Y. Wang, C. Wang *et al.*, 2013 A Cullin1-based SCF E3 ubiquitin ligase targets the InR/PI3K/TOR pathway to regulate neuronal pruning. PLoS Biol 11**:** e1001657.

Wong, K. H., Y. Jin and K. Struhl, 2014 TFIIH phosphorylation of the Pol II CTD stimulates mediator dissociation from the preinitiation complex and promoter escape. Mol Cell 54**:** 601-612.

Wong, L. C., A. Costa, I. McLeod, A. Sarkeshik, J. Yates, 3rd *et al.*, 2011 The functioning of the Drosophila CPEB protein Orb is regulated by phosphorylation and requires casein kinase 2 activity. PLoS One 6**:** e24355.

Woolworth, J. A., G. Nallamothu and T. Hsu, 2009 The Drosophila metastasis suppressor gene Nm23 homolog, awd, regulates epithelial integrity during oogenesis. Mol Cell Biol 29**:** 4679-4690.

Worringer, K. A., and B. Panning, 2007 Zinc finger protein Zn72D promotes productive splicing of the maleless transcript. Mol Cell Biol 27**:** 8760-8769.

Wright, K. J., M. T. Marr, 2nd and R. Tjian, 2006 TAF4 nucleates a core subcomplex of TFIID and mediates activated transcription from a TATA-less promoter. Proc Natl Acad Sci U S A 103**:** 12347-12352.

Wu, D., L. Wu, H. An, H. Bao, P. Guo *et al.*, 2019 RanGAP-mediated nucleocytoplasmic transport of Prospero regulates neural stem cell lifespan in Drosophila larval central brain. Aging Cell 18**:** e12854.

Wu, D. C., and L. A. Johnston, 2010 Control of wing size and proportions by Drosophila myc. Genetics 184**:** 199-211.

Wu, J., C. Capp, L. Feng and T. S. Hsieh, 2008 Drosophila homologue of the Rothmund-Thomson syndrome gene: essential function in DNA replication during development. Dev Biol 323**:** 130-142.

Xia, P., S. Wang, G. Huang, P. Zhu, M. Li *et al.*, 2014 WASH is required for the differentiation commitment of hematopoietic stem cells in a c-Myc-dependent manner. J Exp Med 211**:** 2119-2134.

Xia, R., Y. Liu, L. Yang, J. Gal, H. Zhu *et al.*, 2012 Motor neuron apoptosis and neuromuscular junction perturbation are prominent features in a Drosophila model of Fus-mediated ALS. Mol Neurodegener 7**:** 10.

Xu, J., K. Hopkins, L. Sabin, A. Yasunaga, H. Subramanian *et al.*, 2013 ERK signaling couples nutrient status to antiviral defense in the insect gut. Proc Natl Acad Sci U S A 110**:** 15025-15030.

Xu, X. Z., P. D. Wes, H. Chen, H. S. Li, M. Yu *et al.*, 1998 Retinal targets for calmodulin include proteins implicated in synaptic transmission. J Biol Chem 273**:** 31297-31307.

Xu, Y., Z. Lei, H. Huang, W. Dui, X. Liang *et al.*, 2009 dRecQ4 is required for DNA synthesis and essential for cell proliferation in Drosophila. PLoS One 4**:** e6107.

Xue, C., D. M. Yu, S. Gherardi, J. Koach, G. Milazzo *et al.*, 2016 MYCN promotes neuroblastoma malignancy by establishing a regulatory circuit with transcription factor AP4. Oncotarget 7**:** 54937-54951.

Yager, J., S. Richards, D. S. Hekmat-Scafe, D. D. Hurd, V. Sundaresan *et al.*, 2001 Control of Drosophila perineurial glial growth by interacting neurotransmitter-mediated signaling pathways. Proc Natl Acad Sci U S A 98**:** 10445-10450.

Yamashita, T., A. D. Agulnick, N. G. Copeland, D. J. Gilbert, N. A. Jenkins *et al.*, 1998 Genomic structure and chromosomal localization of the mouse LIM domain-binding protein 1 gene, Ldb1. Genomics 48**:** 87-92.

Yan, D., and N. Perrimon, 2015 spenito is required for sex determination in Drosophila melanogaster. Proc Natl Acad Sci U S A 112**:** 11606-11611.

Yang, L., S. Paul, K. G. Trieu, L. G. Dent, F. Froldi *et al.*, 2016 Minibrain and Wings apart control organ growth and tissue patterning through down-regulation of Capicua. Proc Natl Acad Sci U S A 113**:** 10583-10588.

Yang, Y., D. A. Primrose, A. C. Leung, R. B. Fitzsimmons, M. C. McDermand *et al.*, 2012 The PP1 phosphatase flapwing regulates the activity of Merlin and Moesin in Drosophila. Dev Biol 361**:** 412-426.

Yano, M., S. Nakamuta, X. Wu, Y. Okumura and H. Kido, 2006 A novel function of 14-3-3 protein: 14-3-3zeta is a heat-shock-related molecular chaperone that dissolves thermal-aggregated proteins. Mol Biol Cell 17**:** 4769-4779.

Yao, B., Y. Li, Z. Wang, L. Chen, M. Poidevin *et al.*, 2018a Active N(6)-Methyladenine Demethylation by DMAD Regulates Gene Expression by Coordinating with Polycomb Protein in Neurons. Mol Cell 71**:** 848-857 e846.

Yao, J., M. B. Ardehali, C. J. Fecko, W. W. Webb and J. T. Lis, 2007 Intranuclear distribution and local dynamics of RNA polymerase II during transcription activation. Mol Cell 28**:** 978-990.

Yao, L. C., I. L. Blitz, D. A. Peiffer, S. Phin, Y. Wang *et al.*, 2006 Schnurri transcription factors from Drosophila and vertebrates can mediate Bmp signaling through a phylogenetically conserved mechanism. Development 133**:** 4025-4034.

Yao, W. D., and C. F. Wu, 2001 Distinct roles of CaMKII and PKA in regulation of firing patterns and K(+) currents in Drosophila neurons. J Neurophysiol 85**:** 1384-1394.

Yao, Y., X. Li, W. Wang, Z. Liu, J. Chen *et al.*, 2018b MRT, Functioning with NURF Complex, Regulates Lipid Droplet Size. Cell Rep 24**:** 2972-2984.

Yau, T. Y., O. Molina and A. J. Courey, 2020 SUMOylation in development and neurodegeneration. Development 147.

Yavatkar, A. S., Y. Lin, J. Ross, Y. Fann, T. Brody *et al.*, 2008 Rapid detection and curation of conserved DNA via enhanced-BLAT and EvoPrinterHD analysis. BMC Genomics 9**:** 106.

Yeh, E., M. Cunningham, H. Arnold, D. Chasse, T. Monteith *et al.*, 2004 A signalling pathway controlling c-Myc degradation that impacts oncogenic transformation of human cells. Nat Cell Biol 6**:** 308-318.

Yeh, E. S., B. O. Lew and A. R. Means, 2006 The loss of PIN1 deregulates cyclin E and sensitizes mouse embryo fibroblasts to genomic instability. J Biol Chem 281**:** 241-251.

Yeh, T. H., S. Y. Huang, W. Y. Lan, G. J. Liaw and J. Y. Yu, 2015 Modulation of cell morphogenesis by tousled-like kinase in the Drosophila follicle cell. Dev Dyn 244**:** 852-865.

Yeom, E., S. T. Hong and K. W. Choi, 2015 Crumbs interacts with Xpd for nuclear division control in Drosophila. Oncogene 34**:** 2777-2789.

Yokomori, K., J. L. Chen, A. Admon, S. Zhou and R. Tjian, 1993 Molecular cloning and characterization of dTAFII30 alpha and dTAFII30 beta: two small subunits of Drosophila TFIID. Genes Dev 7**:** 2587-2597.

Yokoshi, M., K. Kawasaki, M. Cambon and T. Fukaya, 2022 Dynamic modulation of enhancer responsiveness by core promoter elements in living Drosophila embryos. Nucleic Acids Res 50**:** 92-107.

Yoon, J. W., M. Gallant, M. L. Lamm, S. Iannaccone, K. F. Vieux *et al.*, 2013 Noncanonical regulation of the Hedgehog mediator GLI1 by c-MYC in Burkitt lymphoma. Mol Cancer Res 11**:** 604-615.

Yu, H., H. Takeuchi, M. Takeuchi, Q. Liu, J. Kantharia *et al.*, 2016 Structural analysis of Notch-regulating Rumi reveals basis for pathogenic mutations. Nat Chem Biol 12**:** 735-740.

Yuen, H. F., V. K. Gunasekharan, K. K. Chan, S. D. Zhang, A. Platt-Higgins *et al.*, 2013 RanGTPase: a candidate for Myc-mediated cancer progression. J Natl Cancer Inst 105**:** 475-488.

Zacarias-Fluck, M. F., L. Soucek and J. R. Whitfield, 2024 MYC: there is more to it than cancer. Front Cell Dev Biol 12**:** 1342872.

Zaessinger, S., I. Busseau and M. Simonelig, 2006 Oskar allows nanos mRNA translation in Drosophila embryos by preventing its deadenylation by Smaug/CCR4. Development 133**:** 4573-4583.

Zaman, S., K. Sukhodolets, P. Wang, J. Qin, D. Levens *et al.*, 2014 FBP1 Is an Interacting Partner of Menin. Int J Endocrinol 2014**:** 535401.

Zanet, J., S. Pibre, C. Jacquet, A. Ramirez, I. M. de Alboran *et al.*, 2005 Endogenous Myc controls mammalian epidermal cell size, hyperproliferation, endoreplication and stem cell amplification. J Cell Sci 118**:** 1693-1704.

Zavortink, M., L. N. Rutt, S. Dzitoyeva, J. C. Henriksen, C. Barrington *et al.*, 2020 The E2 Marie Kondo and the CTLH E3 ligase clear deposited RNA binding proteins during the maternal-to-zygotic transition. Elife 9.

Zaytseva, O., and L. M. Quinn, 2017 Controlling the Master: Chromatin Dynamics at the MYC Promoter Integrate Developmental Signaling. Genes (Basel) 8.

Zehring, W. A., J. M. Lee, J. R. Weeks, R. S. Jokerst and A. L. Greenleaf, 1988 The C-terminal repeat domain of RNA polymerase II largest subunit is essential in vivo but is not required for accurate transcription initiation in vitro. Proc Natl Acad Sci U S A 85**:** 3698-3702.

Zeitler, J., C. P. Hsu, H. Dionne and D. Bilder, 2004 Domains controlling cell polarity and proliferation in the Drosophila tumor suppressor Scribble. J Cell Biol 167**:** 1137-1146.

Zeke, A., M. Misheva, A. Remenyi and M. A. Bogoyevitch, 2016 JNK Signaling: Regulation and Functions Based on Complex Protein-Protein Partnerships. Microbiol Mol Biol Rev 80**:** 793-835.

Zeng, X., S. R. Singh, D. Hou and S. X. Hou, 2010 Tumor suppressors Sav/Scrib and oncogene Ras regulate stem-cell transformation in adult Drosophila malpighian tubules. J Cell Physiol 224**:** 766-774.

Zenvirt, S., Y. Nevo-Caspi, S. Rencus-Lazar and D. Segal, 2008 Drosophila LIM-only is a positive regulator of transcription during thoracic bristle development. Genetics 179**:** 1989-1999.

Zhan, W., X. Liao, Y. Wang, L. Li, J. Li *et al.*, 2019 circCTIC1 promotes the self-renewal of colon TICs through BPTF-dependent c-Myc expression. Carcinogenesis 40**:** 560-568.

Zhang, J., Y. Liu, K. Jiang and J. Jia, 2017 SUMO regulates the activity of Smoothened and Costal-2 in Drosophila Hedgehog signaling. Sci Rep 7**:** 42749.

Zhang, P., Y. Wu, T. Y. Belenkaya and X. Lin, 2011 SNX3 controls Wingless/Wnt secretion through regulating retromer-dependent recycling of Wntless. Cell Res 21**:** 1677-1690.

Zhang, X., J. Wang, Y. Jia, T. Liu, M. Wang *et al.*, 2019 CDK5 neutralizes the tumor suppressing effect of BIN1 via mediating phosphorylation of c-MYC at Ser-62 site in NSCLC. Cancer Cell Int 19**:** 226.

Zhang, Y., R. Cai, R. Zhou, Y. Li and L. Liu, 2016 Tousled-like kinase mediated a new type of cell death pathway in Drosophila. Cell Death Differ 23**:** 146-157.

Zhang, Y. W., and D. N. Arnosti, 2011 Conserved catalytic and C-terminal regulatory domains of the C-terminal binding protein corepressor fine-tune the transcriptional response in development. Mol Cell Biol 31**:** 375-384.

Zhao, L. J., P. M. Loewenstein and M. Green, 2018 Identification of a panel of MYC and Tip60 co-regulated genes functioning primarily in cell cycle and DNA replication. Genes Cancer 9**:** 101-113.

Zheng, Y., J. Wildonger, B. Ye, Y. Zhang, A. Kita *et al.*, 2008 Dynein is required for polarized dendritic transport and uniform microtubule orientation in axons. Nat Cell Biol 10**:** 1172-1180.

Zhou, H., I. Grubisic, K. Zheng, Y. He, P. J. Wang *et al.*, 2013 Taf7l cooperates with Trf2 to regulate spermiogenesis. Proc Natl Acad Sci U S A 110**:** 16886-16891.

Zhou, Z. Q., and P. J. Hurlin, 2001 The interplay between Mad and Myc in proliferation and differentiation. Trends Cell Biol 11**:** S10-14.

Zhu, J. Y., J. van de Leemput and Z. Han, 2024 Distinct roles of COMPASS subunits to Drosophila heart development. Biol Open 13.

Zhu, W., M. Foehr, J. B. Jaynes and S. D. Hanes, 2001 Drosophila SAP18, a member of the Sin3/Rpd3 histone deacetylase complex, interacts with Bicoid and inhibits its activity. Dev Genes Evol 211**:** 109-117.

Zoranovic, T., L. Grmai and E. A. Bach, 2013 Regulation of proliferation, cell competition, and cellular growth by the Drosophila JAK-STAT pathway. JAKSTAT 2**:** e25408.