The total mRNA concentration buffering system in yeast is global rather than gene-specific

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Abstract

Gene expression in eukaryotes does not follow a linear process from transcription to translation and mRNA degradation. Instead it follows a circular process in which cytoplasmic mRNA decay crosstalks with nuclear transcription. In many instances this crosstalk contributes to buffer mRNA at a roughly constant concentration. Whether the mRNA buffering concept operates on the total mRNA concentration or at the gene-specific level, and if the mechanism to do so is a global or a specific one, remain unknown. Here we assessed changes in mRNA concentrations and their synthesis rates (SRs) along the transcriptome of aneuploid strains of the yeast *Saccharomyces cerevisiae*. We also assessed mRNA concentrations and their SRs in non sense-mediated decay (NMD) targets in euploid strains. We found that the altered SRs in the genes from the aneuploid chromosome and the changes in their mRNA stabilities were not balanced. In addition, the stability of NMD targets was not specifically counterbalanced by the changes in SR. We conclude that there is no genetic compensation of NMD mRNA targets, and total mRNA buffering uses mostly a global system rather than a gene-specific one in yeast.

Key words: mRNA buffering, crosstalk, transcription, mRNA decay, yeast, genetic compensation, aneuploidy, NMD

Abbreviations: SR (Synthesis Rate); NMD (Non sense-Mediated Decay); DR (Degradation Rate); $[mRNA]_t$ (total mRNA concentration); PTC (Premature Stop Codon); HL (mRNA Half Life); RBP (RNA Binding Protein); BF (Buffering Factor); RA (mRNA amount or level); GRO (Genomic Run-On); cDTA (comparative Dynamic Transcriptome Analysis); TRO (Transcription Run-On); NITC (nonsense-induced transcriptional compensation)

Introduction

Cell homeostasis requires the total concentrations of proteins and RNAs to be remain within a certain range [1]. In the yeast *Saccharomyces cerevisiae*, it has been shown that total mRNA concentration ($[mRNA]_t$) is buffered [2-4]. Indeed several reports in strains with mutations in proteins related to either mRNA synthesis or decay machineries, indicate the primary defect caused by mutation: a drop in either global synthesis rates (SR) or degradation rates (DR) is compensated by a roughly comparable increase in the reciprocal rate [3-6]. In principle, buffering can also apply to specific mRNAs or groups of functionally-related mRNAs. For instance, $[mRNA]_t$ buffering in a series of yeast mutants showed that the buffering effect varies between individual mRNA species [3-8]. Yet despite the indirect effects of mutations, it is difficult to unequivocally conclude whether gene-specific buffering exists or not.

In mammalian cells, the attenuation of global transcription leads to the widespread stabilization of mRNAs [9-10], which thus demonstrates the existence of $[mRNA]_t$ buffering in higher eukaryotes [11]. In these organisms, specific mRNA buffering has also been demonstrated in one particular case called genetic compensation, where the defective mRNAs bearing premature stop codons (PTC) are destabilized by the non sense-mediated decay (NMD) pathway. However, a rise in the SRs of sequence-related mRNAs partially compensates by increasing their mRNA levels [12,13]. This mechanism has been called nonsense-induced transcriptional compensation (NITC) and is enacted by up-frameshift proteins 1, 2 and 3 (Upf1, 2 and 3) broadly present in eukaryotes [14]. Therefore, NITC could also exist in other organisms [15]. It could have conceivably evolved to buffer transcription and mRNA splicing errors in the gene families of higher eukaryotes.

Global or gene-specific buffering are likely driven by distinct molecular mechanisms, and the purpose of each process might be completely different. Total mRNA buffering may have evolved to keep $[mRNA]_t$ within the physiological limits that maintain processes in the cytoplasm efficient, such as translation [1,16], and to also maintain normal physico-chemical cytoplasm behavior and the solubility of cellular proteins, which very much depend on RNA concentration [17,18]. However, gene-specific mRNA buffering would likely have an entirely different purpose. For instance, it could be used to transiently adjust the timing of the transcriptional response. In fact it has been shown that the genes with or without antisense transcription have different

average mRNA half lives (HL), and that antisense transcription inactivation can lead to an increase in sense SR and a decrease in HL, which would help to make the mRNA level constant [19]. In this case, the specific buffering effect is obtained through chromatin signatures that are established by antisense transcription, and affect the initiation and elongation of sense transcription. The influence of transcription elongation on mRNA stability has been recently established [6,20,21]. Hence the control by antisense transcription, which reduces production and increases stability, but maintains the same final transcript level, can be a regulatory process for specific genes and a useful one when rapidly varying conditions are expected [19].

$[mRNA]_t$ buffering is based on the crosstalk between the transcription and the mRNA decay machineries [3,4,22]. This crosstalk functions from the nucleus to the cytoplasm (direct) [23], and from the cytoplasm to the nucleus (reverse) [3]. As such, it has been suggested that the gene expression process in eukaryotes is circular [4], and that direct crosstalk may occur by mRNA imprinting [24] with RNA binding proteins (RBP) or by the co-transcriptional methylation of specific nucleotides [10]. The reverse crosstalk mechanism is less clear, but may be based on the titration of general RBPs in the cytoplasm with mRNA molecules. Titration by mRNAs limits the number of RBP molecules that are imported back to the nucleus and, therefore, senses $[mRNA]_t$ [11,25,26]. However, these studies did not conclusively answer the question as to whether $[mRNA]_t$ buffering is the additive consequence of gene-specific regulations of many individual mRNA species, or if it operates as a global mechanism.

Here we used the model eukaryote *S. cerevisiae* to investigate whether widespread gene-specific mRNA buffering exists. To achieve this, we used a set of aneuploid strains with either excess or a defect in a single copy of a chromosome. This allowed us to assess if the presumed increase/decrease in gene transcription of the genes belonging to that particular chromosome would provoke a compensatory effect on their mRNAs stabilities. We also studied NMD targets in euploid strains (of both the wild type and an *upf1* mutant) to check both the existence of genetic compensation in yeast and if an increase in mRNA stabilities of a selected group of mRNAs would provoke a specific compensatory effect on their SRs. We conclude from our obtained results that, at least in this unicellular eukaryote, most genes do not undergo gene-specific mRNA buffering, which seems mostly a global process. Nor did we find evidence for a genetic compensation mechanism in yeast.

Materials & Methods

Yeast strains and culture media

Yeast cells were grown in YPD (1% yeast extract, 2% peptone, 2% glucose) or YPGal (1% yeast extract, 2% peptone, 2% galactose) media at 30ºC. Pre-cultures were grown overnight in 250 mL flasks and agitated at 190 rpm. The next day, pre-cultures were diluted to OD_{600} = 0.05 and grown until an OD_{600} of ~0.5 was reached. Cells were recovered by centrifugation and flash-frozen in liquid nitrogen. Yeast strains are listed in Supplementary Table S1. For the experiments described in Figure 1, strains were transformed with either centromeric plasmid pSCh212 [27] containing the in-frame translational *PHO5-lacZ* fusion or a derivative plasmid including a *PHO5-STOP-lacZ* fusion.

Genomic methods

Genomic run-on (GRO) was performed as described in [8]. Their datasets are available at the Gene Expression Omnibus (GEO) with accession numbers: GSE29519, GSE57467 and GSE155372. cDTA was performed as described in [3] and was deposited in ArrayExpress with accession number: E-MTAB-1525.

mRNA half life calculations

To determine mRNA half lives (HL), we used a transcription shut-off assay by collecting samples at 0, 5, 10 and 15 min after glucose addition. This method was the same as that described in [6], except that Northern blot hybridization was used instead of RT-PCR. Half lives were estimated by calculating the time it takes for half the initial amount of mRNA to be degraded. mRNA extraction and Northern blots were carried out following the protocols detailed in [27].

Transcription run-on and RNApol II-chromatin immunoprecipitation assays

The transcription run-on (TRO) assays of *PHO5-lacZ* fusions and *GAL1* were performed as in [28]. *PHO5-lacZ* signals were normalized against the plasmid copy

number as determined by Q-PCR. RNApol II ChIP experiments were run using anti-Rpb3 antibodies (ab81859; Abcam) as in [6]. Q-PCR was carried out employing SYBR Green Premix Ex Taq (Takara) in a Light Cycler 480 II (Roche). Oligonucleotide sequences are available upon request.

Results

For our search to find the mechanism behind $[mRNA]_t$ buffering, we designed two strategies to test the existence of gene-specific mRNA buffering in the yeast *S. cerevisiae*. Both are based on the idea that if one of the two buffering parameters, SRs or HLs, of a selected group of non-functionally related genes changes in relation to the whole genome, this will cause a similar relative change in its mRNA levels unless a gene-specific crosstalk buffers it by acting on the reciprocal parameter.

To illustrate HL alteration, we used the NMD system that destabilizes mRNAs with premature stop codons [29]. This strategy also allowed us to check the existence of NITC in yeast. For the SR change, we employed stable aneuploid strains in which a single chromosome has a different copy number because this would bring about a change in the synthesis rates only in the set of genes contained in that chromosome.

Alteration of mRNA stability via NMD: effect of a premature STOP codon

We first investigated if the NMD pathway can direct the specific crosstalk that buffers the increased decay of the mRNAs containing a premature stop codon (PTC) in the same way as in metazoa [12,13]. To check whether this behavior is also present in yeast, we engineered a copy of a fusion gene having or not a PTC (Fig. 1A). We measured the stability of both mRNAs and found a significant decrease in the half-life of the allele containing the PTC (Fig. 1B, left panel). As an internal control, we measured the stability of the *GAL1* gene, which is driven by the same promoter, and found no significant change (Fig 1B, left panel; Fig S1A). To further confirm that the diminished stability of the PTC-containing mRNA was due to the action of the NMD system, we repeated the experiments with an *upf1*Δ mutant, which lacked one of the fundamental factors of NMD machinery. As expected, the mRNA stability of the PTC-containing

allele significantly increased, while no changes were detected in the internal *GAL1* control (Fig 1B, right panel; Fig S1A).

Next we assessed whether RNA pol II SR changed in the PTC-containing allele in parallel to mRNA stability. We measured the SR, by transcription run-on (TRO, measuring active elongating RNA pol II, Fig. 1C) and chromatin immunoprecipitation (ChIP, measuring total RNA pol II, Fig. 1D). The results showed that the SR in the two alleles was similar, which indicates the absence of NITC in yeast. No significant differences were found in the SR between the two alleles in the *upf1*Δ background (Fig. S1B-C).

Crosstalk for post-transcriptional regulons: study of the *upf1*Δ mutant

We used the NMD system to determine if specific buffering regulated non engineered mRNAs at the decay level. We used the same *upf1*Δ mutant as that employed in the previous experiment. Upf1 is known to be cytoplasmatic in yeast, where it degrades both mRNAs with PTCs and normal-appearance mRNAs but that have either a tendency to increase out-of-frame translation or to have a lower average codon optimality plus a biased distribution pattern of non optimal codons. This set comprised about 900 targets, shared with factors Upf2/Upf3 [29,30]. This group of mRNAs comprises genes with no evident functional relationship. By genomic run-on (GRO), we first analyzed global yeast transcriptome behavior in the *upf1*Δ mutant by comparing the relative changes in both HL and SR. As expected, we found a group of mRNAs (mostly coinciding with known Upf1 targets; not shown), in which HLs were longer. However, no significant change in the SR of these genes took place (red circle in Figure 2A). Next we studied the behavior of the whole set of known Upf1 targets by analyzing these genes in comparison with the rest of the *upf1*Δ mutant transcriptome. Figure 2B depicts a clear and statistically significant increase in the HLs in targets in relation to the rest of mRNAs that, by not being compensated by a change in the SR, provokes a statistically significant relative increase in the mRNA levels (RA) of NMD targets. This result indicates the absence of gene-specific buffering when HLs from a group of genes are altered.

Transcriptomic study of a set of aneuploid yeast strains

To investigate the reciprocal case, the alteration of a group of SR, we took advantage of the fact that some yeast strains have an extra copy of one chromosome (disomic) in haploid backgrounds, or only one copy of a chromosome (monosomic) in diploid strains. In these strains, we would expect an increase or decrease in the SR, respectively, of the genes belonging to the aneuploid chromosome in relation to the rest of the genome. The buffering effect cannot be investigated by just looking at the mRNA level, as done previously by other researchers [31,32], because the possible influence of both SR and HL on the actual mRNA level. The growth phenotype of many single-gene mutant yeast strains is partially suppressed by the over-/under-expression of a set of genes contained in a given chromosome [33]. If the average SR and RA in the aneuploid chromosome vary in line with the other chromosomes (e.g. no change in HLs), then we can reject the gene-specific buffering hypothesis.

We first employed the mutant strains that had been determined as aneuploids by two different genomic methods in two different laboratories: GRO [4] and comparative Dynamic Transcriptome Analysis (cDTA, [34]). As seen in Figure 3A, the haploid strains with a disomy all showed an average increase in the SR of about 1.71-fold (except for mutant *edc1*) in relation to the whole genome. This is similar to that of the mRNA levels (1.76-fold) for aneuploid chromosomes as regards the whole transcriptome (Tables 1 and S2). The diploid strains with one monosomy showed an average decrease of 0.56-fold in both the SR and mRNA levels for aneuploid chromosomes (Figure 3B-C and Table 1). Thus we conclude that no change occurred in the HLs of the mRNAs of the chromosome with an altered ploidy.

The previous strains were mutants in the genes associated with mRNA decay. Despite the fact that the deleted gene had no direct relation with most of the genes in the altered copy chromosome, an indirect effect of the mutation occurring on the buffering phenomenon could be argued. To address this issue, we carried out another experiment with a series of stable aneuploids that had no mutations requiring compensation. We specifically used a series of haploid or diploid strains with an extra chromosome constructed in A. Amon's laboratory [31]. Once again, as shown in Figure 3D, the higher SR values (1.64 for the disomic chromosome in haploids and 1.39-fold for the trisomic chromosome in diploid strains) were comparable to those for the increase in

mRNA: 1.64 and 1.33-fold, respectively (Tables 1 and S2). This indicates that no specific buffering for mRNAs with an increased synthesis rate exists.

From these experiments, we conclude that when the copy number of a group of genes was altered compared to the rest of the genome, the SR increased or decreased accordingly. However, this increase or decrease took place to lesser extent than we expected (see Discussion). As this change in the SR was comparable to that observed in the RA in all cases, we conclude that no specific mechanism exists to detect altered levels of individual mRNAs and to correct them by HL change compensation.

Discussion

The mRNA buffering concept is often applied to the general set of mRNAs, i.e. to $[mRNA]_t$, but whether it acts through a global system or the mechanism is gene-specific was previously unknown. The results obtained to date by transcriptomic methods show that buffering is global, albeit with important differences between individual mRNAs or between groups of mRNAs in yeast [3-5]. This means that both global and specific mechanisms may exist at the same time.

In order to determine the possible existence of global or gene-specific processes for mRNA buffering in yeast, we devised three experiments. These strategies were based on the idea that if a specific buffering mechanism exists, it should be detectable in single genes or in a group of non functionally-related mRNAs by a change in either the HL or the SR. In our first experiment, we compared one gene to an engineered version of that gene, which was converted into an NMD target via the inclusion of a PTC. The stability of the two resulting mRNAs clearly differed, but had identical SRs. This provides evidence against gene-specific mRNA buffering and a NITC mechanism in yeast.

In our second experiment, we assessed how a group of non functionally-related mRNAs, controlled by NMD [29], performed when the main NMD factor, Upf1, was lacking. Once again, our results did not support the existence of gene-specific reverse crosstalk based on NMD. Interestingly, this contrasts with what has been observed in metazoa, where NMD mediates a mechanism (NITC) of transcriptional compensation for specifically destabilized mRNAs [12,13]. This difference could be due to the much shorter genes and fewer introns in yeast compared to metazoa. Because of this, the

probability of transcription and pre-mRNA splicing errors that cause non functional mRNAs with PTCs is much lower in yeast and, consequently, there may be no need for compensation at this level.

In our third experiment, we investigated groups of genes belonging to a given chromosome. In the yeast genome, genes mostly occupy random positions with no functional clustering [35]. This strategy allows checks to be made of what happens with the mRNA stability of functionally unrelated transcripts when changing the chromosome copy number as regards genome content. We obtained our results with different sets of aneuploids (in mutant or wild type strains), which showed that SR and mRNA levels underwent parallel variations in the genes of the altered chromosome in relation to the other chromosomes. This indicated lack of gene-specific buffering.

While we expected an increase of 2-fold or a decrease of 0.5-fold in the SR of the genes in aneuploid strains, the actual increase and decrease were lower at 1.71 and 0.56, respectively (except for *edc1*Δ: see Supp. Table S2 and Fig. 3A-C). This has also been observed by other labs [31,32]. Indeed in the set of stable aneuploids from A. Amon's laboratory, the expected increase of the SR in haploid strains was 2-fold and 1.5-fold in diploid strains, but the actual results were lower than expected with 1.64 and 1.39, respectively (see Supp. Table S2 and Fig. 3D). Thus in all cases of extra chromosome copies, the obtained increases in SR were between 22 and 35% lower than expected. If the copy number of a chromosome lowered in a diploid strain, the observed decrease was also 12% lower than expected. We hypothesize this because the titration of specific transcription factors becomes limiting when their targets increase in copy number. Alternatively these yeast cultures could have a leaking percentage of euploid cells [36], which would make the average lower than expected. In any case, the differences of the observed changes in mRNA levels (1.76 and 0.56, see Table 2) with regard the expected ones (2-fold and 0.5-fold, assuming a trivial translation of the increase in gene copy number to final mRNA level) reinforces the need of evaluating both SR and HLs of all the mRNAs to verify the existence, or not, of gene-specific buffering.

Our results support a model in which $[mRNA]_t$ buffering does not result from the addition of individual gene-specific buffering phenomena but, instead, is the product of the global crosstalk between mRNA decay and gene transcription machineries using direct and reverse branches (Figure 4). Based on these results, we propose a model for transcription-degradation crosstalk grounded on the co-transcriptional imprinting of

mRNAs with general factors, such as RNA pol II subunits (Rpb4/7), mRNA decay factors (Xrn1, Ccr4-NOT), or others. This model is similar to those proposed by Gilbertson et al. [25] and Schmid and Jennsen [26], which are based on polyA-binding proteins (PABP, Nab2), but assume that the factor binds mRNA during transcription elongation, and not necessarily in the poly(A) tail. These factors cannot be sequence-specific in order to be capable of bind most mRNAs. The binding, however, can have different strengths and be biased among mRNA species. We call them "buffering factors" (BF). They would travel from the nucleus to the cytoplasm only when bound to mRNA, and would then return to the nucleus by active nuclear import after being released from their targets.

This model would explain the $[mRNA]_t$ buffering in situations where the SR or DR globally increases or decreases compensatorily to each other. The existence of transcription-mRNA decay crosstalk even in mutants lacking suspected RBPs [3], and many different publications on Rpb4/7 [22-24], decaysome [4], Ccr4-NOT [22,37-39] polyA-binding proteins [11,26], all indicate that there are many redundant pathways that make the process very robust. We also recently found that Xrn1 and Ccr4, two decay factors also capable of influencing transcription elongation, act in parallel with transcription [6].

To summarize, our results support the existence of global systems involved in the compensation of total mRNA levels to maintain $[mRNA]_t$ within the required ribostasis limits. This is likely because the precise balance of molecules and macromolecules required for the correct functioning of cytoplasmic cell processes includes, albeit perhaps not exclusively, translation [1]. The existence of a global buffering system does not impede the particular regulation of specific mRNAs because the impact of variation in a few mRNA species would be very low in $[mRNA]_t$. Our results obtained with aneuploid strains and the NMD system exclude the possibility of an all-purpose general mechanism based on specific mRNA recognition, and devoted to compensate alterations in the proportion of cytoplasmic mRNAs species one by one. Nor did we find any clear evidence for a genetic compensation mechanism (NITC) in yeast, but we cannot rule out that specific mRNA buffering could still exist in other specific situations.

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Declaration of interest statement

The authors declare no financial interest.

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Figure legends

Figure 1.- Effect of the premature stop codon (PTC) on mRNA stability and the synthesis rate. A). Scheme of the two plasmidic gene fusions used for these experiments. The *GAL1* promoter directs the synthesis of an mRNA that contains the yeast *PHO5* and *E. coli lacZ* open reading frames fused in-frame until the natural *lacZ* stop (bottom), or containing a PTC between the two ORFs (top). The *CYC1* terminator is placed at the end of constructs. The three probes (1-3) used for transcription run-on (TRO) and RNA polymerase II chromatin immunoprecipitation (ChIP) with the anti-Rbp3 antibody are shown. B) The mRNA half life (HL) was determined by transcription shut-off of the *GAL1* promoter, by changing cells from galactose to glucose-containing media, and a northern blot of the extracted RNAs, which was successively tested with probes *PHO5*, *GAL1* and 18S rRNA. Note that the presence of a PTC (pink bars) provokes a one third reduction in the HL in the fusion transcript, but no decrease in the endogenous *GAL1* mRNA in the wild-type cells. Conversely, the PTC-containing fusion transcript was significantly stabilized in an *upf1* mutant. The greater stability of the PTC-containing transcript *versus* the non containing one in *upf1*Δ could be related to the much shorter translated region of the former. The shown HL values correspond to the average of at least three independent experiments. C) The TRO experiment with the wild-type strain shows no significant difference in elongating RNA pol II (SR) in the fusion genes containing (pink bars) a PTC, or not (blue bars). Values were presented after normalizing to the plasmid copy number measured by Q-PCR. The results are similar using probes 1-3. Bars represent the average and SD of three independent replicates of the experiment. D) ChIP using anti-Rpb3 shows similar results as the TRO experiment. See Suppl. Figure S1 for additional information on these experiments.

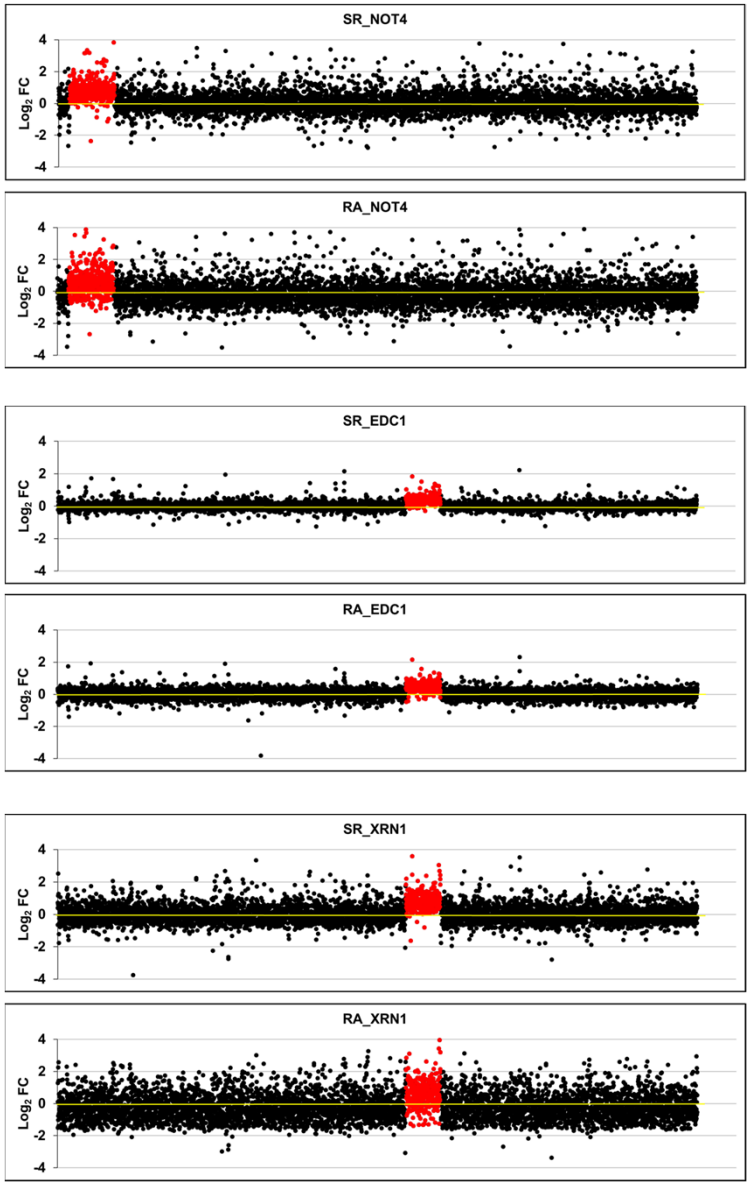
Figure 2.- Transcriptomic analysis of the *upf1* mutant. We performed a GRO analysis of the *upf1* mutant compared to its wild-type (WT) strain (BY4741). A) Fold change (FC) in the mutant as regards the WT of half lives (HL) vs. the FC of the synthesis rates (SRs) on the $\log_2$ scale. The red circle marks the most stabilized mRNAs in the absence of the Upf1 protein. B) Boxplot showing the fold-changes (FC) between the *upf1* mutant and the wild type strain for the group of 499 Upf1 targets and the rest of the transcriptome. The medians of the data values for the synthesis rate (SR), mRNA

amounts (RA) and HL are shown in boxes. The experiment was run in triplicate. Statistical significance using the Kolmogorov-Smirnov test of differences to compare distributions. ** is a p-value of <0.0005.

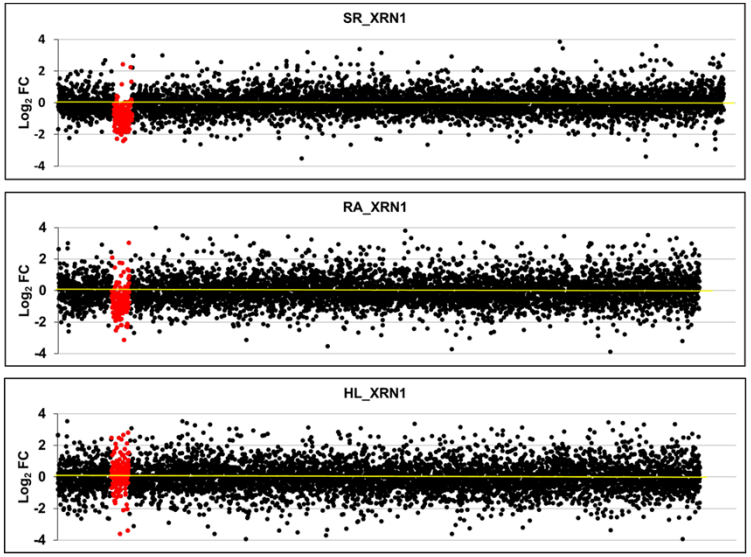
Figure 3.- Transcriptomic analysis of aneuploid strains. We performed GRO or a cDTA analysis of aneuploid strains compared to their wild-type strain (BY4741). Then we compared the fold changes of the synthesis rates (SR), mRNA amounts (RA) and mRNA half lives (HL) of the genes aligned from left to right telomeres in each chromosome ordered from chromosome I (left) to chromosome XVI (right) on a $\log_2$ scale. Genes from the aneuploid chromosome (marked as a roman numeral) are highlighted in red. A) The results of the cDTA analysis of haploid strains *not4*, *edc1* and *xrn1*. B) The results of the GRO analysis of the *xrn1* diploid strain from [4] showing the SR, RA and HL data. The calculated mRNA stabilities (HL) from the RA and SR data indicate how chromosome III has no average HL that differs from the other chromosomes. C) The results of the cDTA analysis of diploid strains *cbp20* and *hpr1*. D) The results of the GRO analysis of a set of diploid (left) and haploid (right) strains from A. Amon's aneuploid collection [31]. Note that the HL plot is shown only in the *xrn1* mutant for simplification. In all cases, the HL plot indicates no difference for the aneuploid chromosome HLs (see Tables 1 and S2).

Figure 4.- Model for mRNA buffering mediated by the nucleo-cytoplasmic shuttling of RNA binding factors. Cartoon model showing the crosstalk pathways from the nucleus to the cytoplasm (direct) and from the cytoplasm to the nucleus (reverse). Buffering factors (BF) (colored ovals) shuttle between the cytoplasm and the nucleus. They are imported to the nucleus when they become free from mRNAs, either when mRNAs are degraded (bottom) by decay machineries (green and orange *pacman*) or before an equilibrium is struck between the bound and free states (top). BF can travel back to the cytoplasm by co-transcriptional imprinting in either mRNA sequences (black ovals) or poly(A) tails (red ovals). Synthesis (SR) and degradation (DR) rates are adjusted for $[mRNA]_t$ buffering due to the stimulating activity of the nuclear BF in RNA pol II transcription and the (direct or indirect) relation of BF to mRNA degradation in the cytoplasm. mRNAs may have a cap (c) or phosphate (P) on their 5'-end, and may be translated by the ribosomes in the cytoplasm. Figure created with the help of *BioRender*.

A)



B)



C)

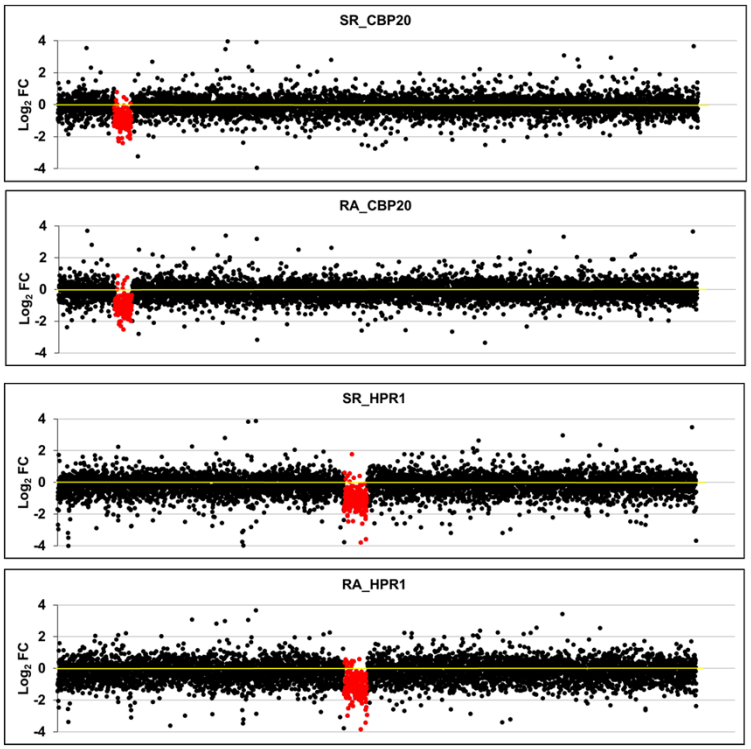


Figure 3A-C

D)

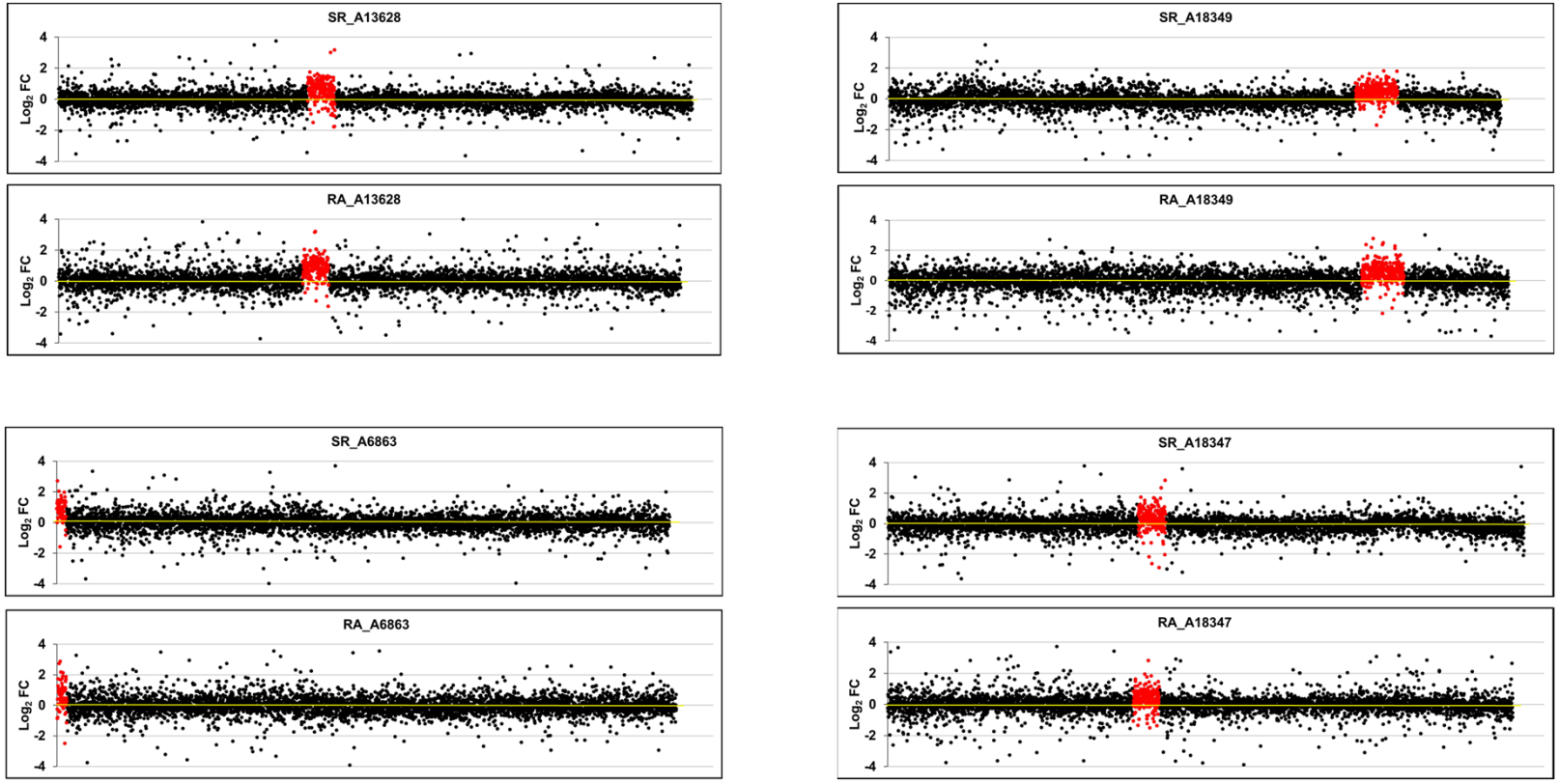

SR_A13628
RA_A13628
SR_A18349
RA_A18349
SR_A6863
RA_A6863
SR_A18347
RA_A18347
Log2 FC
4
2
0
-2
-4

Figure 3D

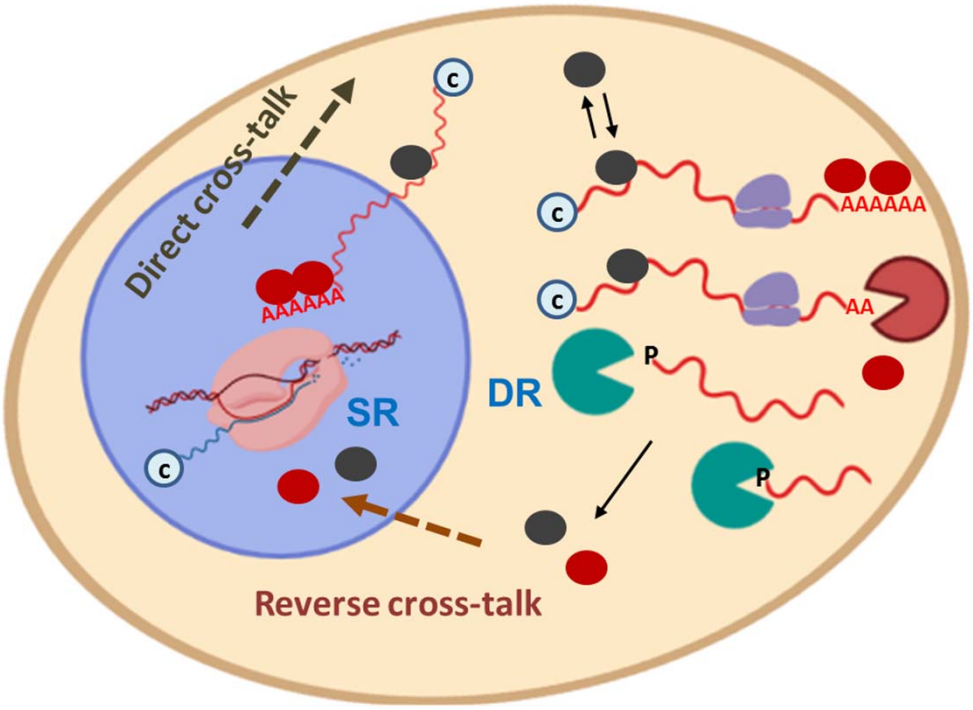

Direct cross-talk
c
AAAAAA
SR
c
c
AAAAAA
c
AA
DR
P
P
Reverse cross-talk

Figure 4

Table 1: Averages of increases or decreases in the ratios of synthesis rates (SR), mRNA amounts (RA) and mRNA half-lives (HL) of the genes from the chromosome altered in copy number *vs* the rest of the genome in different series of aneuploid strains.

n + 1 strains (mutants)	**SR change**	**RA change**	**HL change**
Average increase	**1.71**	**1.76**	**1.05**
2n – 1 strains (mutants)			
Average decrease	**0.56**	**0.56**	**1.01**
n + 1 strains			
Average increase	**1.64**	**1.64**	**1.00**
2n + 1 strains			
Average increase	**1.39**	**1.33**	**0.97**

See Table S2 for details on specific mutants and chromosomes

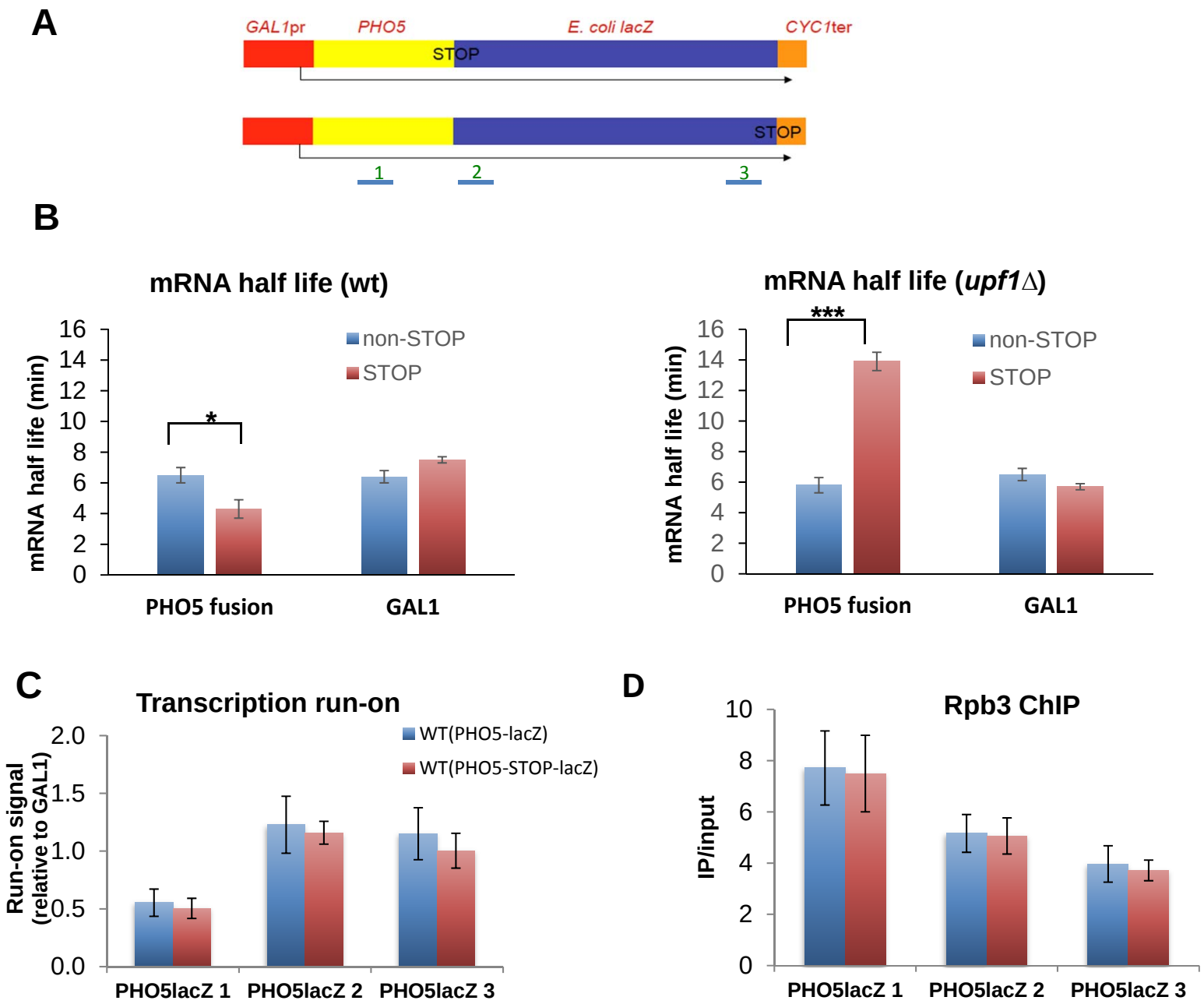
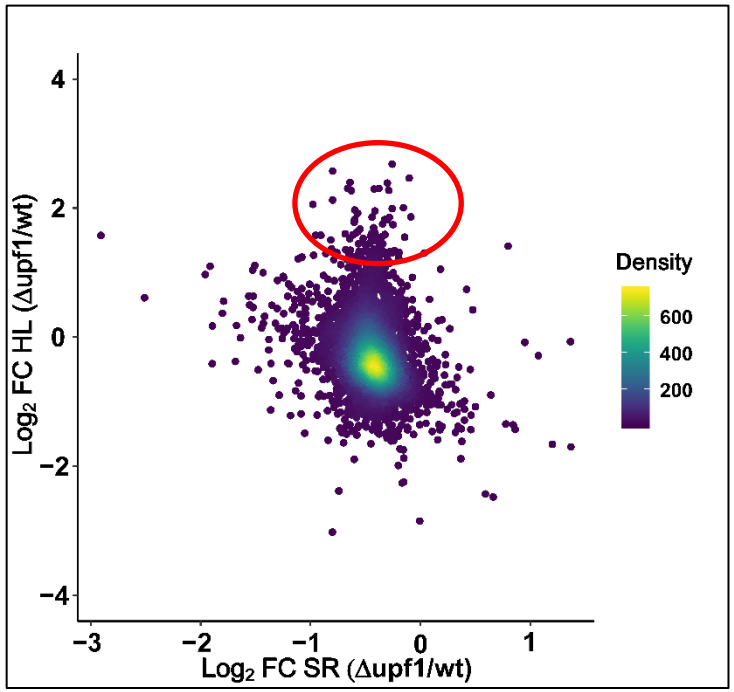

A
GAL1pr
PHO5
E. coli lacZ
CYC1ter
STOP
STOP
1
2
3
B
mRNA half life (wt)
mRNA half life (min)
non-STOP
STOP
*
PHO5 fusion
GAL1
mRNA half life (upf1Δ)
***
C
Transcription run-on
Run-on signal (relative to GAL1)
WT(PHO5-lacZ)
WT(PHO5-STOP-lacZ)
PHO5lacZ 1
PHO5lacZ 2
PHO5lacZ 3
D
Rpb3 ChIP
IP/input

Figure 1

A


Log2 FC HL (Δupf1/wt)
Log2 FC SR (Δupf1/wt)
Density
600
400
200
4
2
0
−2
−4
−3
−2
−1
0
1

B

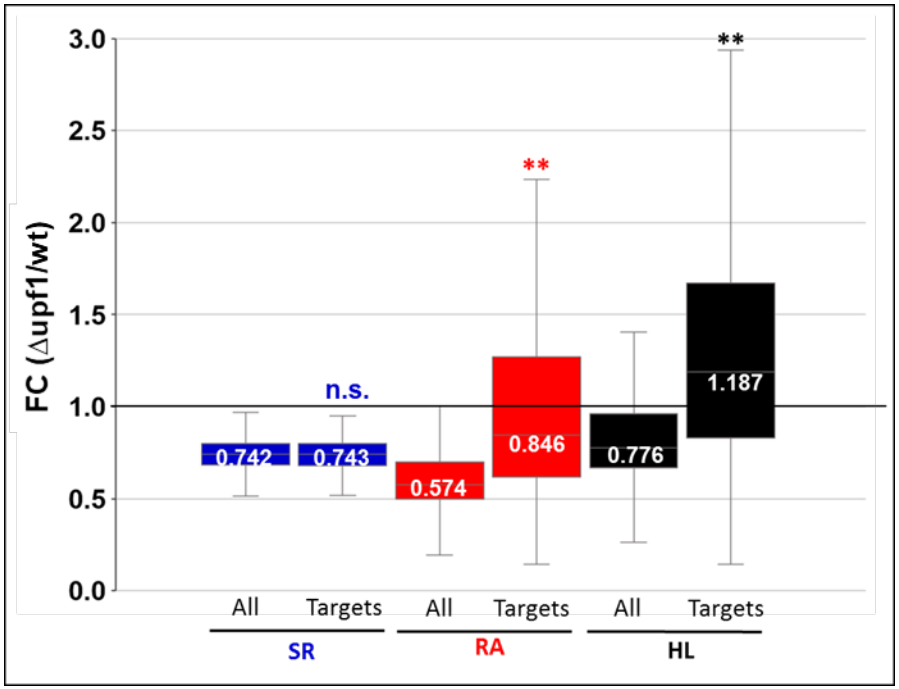

FC (Δupf1/wt)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
n.s.
**
**
0.742
0.743
0.574
0.846
0.776
1.187
All
Targets
All
Targets
All
Targets
SR
RA
HL

Figure 2