1 Cytoplasmic mRNA decay represses RNA polymerase II transcription during early 2 apoptosis

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4 GRAPHICAL ABSTRACT

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SUMMARY

RNA abundance is generally sensitive to perturbations in decay and synthesis rates, but crosstalk between RNA polymerase II transcription and cytoplasmic mRNA degradation often leads to compensatory changes in gene expression. Here, we reveal that widespread mRNA decay during early apoptosis represses RNAPII transcription, indicative of positive (rather than compensatory) feedback. This repression requires active degradation of cytoplasmic mRNA, which leads to impaired recruitment of components of the transcription preinitiation complex to promoter DNA. Importin α/β -mediated nuclear import is critical for this feedback signaling, suggesting that proteins translocating between the cytoplasm and nucleus connect mRNA decay to transcription. We also show that an analogous pathway activated by viral nucleases similarly depends on nuclear protein import. Collectively, these data demonstrate that accelerated mRNA decay leads to the repression of mRNA transcription, thereby amplifying the shutdown of gene expression. This highlights a conserved gene regulatory mechanism by which cells respond to threats.

KEYWORDS

mRNA decay, transcription, apoptosis, nuclear transport

INTRODUCTION

The central dogma of molecular biology depicts gene expression as a unidirectional flow of discrete stages: DNA is first transcribed by RNA polymerase II (RNAPII) into messenger RNA (mRNA), which is processed and exported to the cytoplasm where it is translated and then degraded. However, there is a growing body of work that reveals complex cross talk between the seemingly distal steps of

transcription and decay. For example, the yeast Ccr4-Not deadenylase complex, which instigates basal mRNA decay by removing the poly(A) tails of mRNAs (Tucker et al., 2001), was originally characterized as a transcriptional regulator (Collart and Stmhp, 1994; Denis, 1984). Other components of transcription such as RNAPII subunits and gene promoter elements have been linked to cytoplasmic mRNA decay (Bregman et al., 2011; Lotan et al., 2007, 2005), while the activity of cytoplasmic mRNA degradation machinery such as the cytoplasmic 5'-3' RNA exonuclease Xrn1 can influence the transcriptional response (Haimovich et al., 2013; Sun et al., 2012).

The above findings collectively support a model in which cells engage a buffering response to reduce transcription when mRNA decay is slowed, or reduce mRNA decay when transcription is slowed to preserve the steady state mRNA pool (Haimovich et al., 2013; Hartenian and Glaunsinger, 2019). While much of this research has been performed in yeast, the buffering model is also supported by studies in mouse and human cells (Helenius et al., 2011; Singh et al., 2019). In addition to bulk changes to the mRNA pool, compensatory responses can also occur at the individual gene level to buffer against aberrant transcript degradation. Termed “nonsense-induced transcription compensation” (NITC; Wilkinson, 2019), this occurs when nonsense-mediated mRNA decay leads to transcriptional upregulation of genes with some sequence homology to the aberrant transcript (El-Brolosy et al., 2019; Ma et al., 2019).

A theme that unites much of the research linking mRNA decay to transcription is homeostasis; perturbations in mRNA stability are met with an opposite transcriptional response in order to maintain stable mRNA transcript levels. However, there are cellular contexts in which homeostasis is not beneficial, for example during viral infection. Many

viruses induce widespread host mRNA decay (Narayanan and Makino, 2013) and co-opt the host transcriptional machinery (Harwig et al., 2017) in order to express viral genes. Indeed, infection with mRNA decay-inducing herpesviruses or expression of broad-acting viral ribonucleases in mammalian cells causes RNAPII transcriptional repression in a manner linked to accelerated mRNA decay (Abernathy et al., 2015; Hartenian et al., 2020). It is possible that this type of positive feedback represents a protective cellular shutdown response, perhaps akin to the translational shutdown mechanisms that occur upon pathogen sensing (Walsh et al., 2013). A central question, however, is whether transcriptional inhibition upon mRNA decay is restricted to infection contexts, or whether it is also engaged upon other types of stimuli.

The best-defined stimulus known to broadly accelerate cytoplasmic mRNA decay outside of viral infection is induction of apoptosis. Overall levels of poly(A) RNA are reduced rapidly after the induction of extrinsic apoptosis via accelerated degradation from the 3' ends of transcripts (Thomas et al., 2015). The onset of accelerated mRNA decay occurs coincidentally with mitochondrial outer membrane depolarization (MOMP), and requires release of the mitochondrial exonuclease polyribonucleotide nucleotidyltransferase 1 (PNPT1) into the cytoplasm. PNPT1 then coordinates with other 3' end decay machinery such as DIS3L2 and terminal uridylyltransferases (TUTases; Liu et al., 2018; Thomas et al., 2015). Notably, mRNA decay occurs before other hallmarks of apoptosis including phosphatidylserine (PS) externalization and DNA fragmentation, but likely potentiates apoptosis by reducing the expression of unstable anti-apoptotic proteins such as MCL1 (Thomas et al., 2015).

Here, we used early apoptosis as a model to study the impact of accelerated cytoplasmic mRNA decay on transcription. We reveal that under conditions of increased mRNA decay, there is a coincident decrease in RNAPII transcription, indicative of positive feedback between mRNA synthesis and degradation. Using decay factor depletion experiments, we demonstrate that mRNA decay is required for the transcriptional decrease and further show that transcriptional repression is associated with reduced RNAPII polymerase occupancy on promoters. This phenotype requires ongoing nuclear-cytoplasmic protein transport, suggesting that protein trafficking may provide the signal linking cytoplasmic decay to transcription. Collectively, our findings elucidate a distinct gene regulatory mechanism by which cells sense and respond to threats.

RESULTS

mRNA decay during early apoptosis is accompanied by reduced synthesis of RNAPII transcripts

To induce widespread cytoplasmic mRNA decay, we initiated rapid extrinsic apoptosis in HCT116 colon carcinoma cells by treating them with TNF-related apoptosis inducing ligand (TRAIL). TRAIL treatment causes a well-characterized progression of apoptotic events including caspase cleavage and mitochondrial outer membrane permeabilization or “MOMP” (Albeck et al., 2008; Thomas et al., 2015). It is caspase 8 (CASP8)-induced MOMP that stimulates mRNA decay in response to an apoptosis-inducing ligand (Figure 1A), partly by releasing the mitochondrial 3’-5’ RNA exonuclease PNPT1 into the cytoplasm (Liu et al., 2018; Thomas et al., 2015). A time course experiment in which cells were treated with 100 ng/mL TRAIL for increasing 30 min

increments showed activation of CASP8 and caspase 3 (CASP3) by 1.5 hr, as measured by disappearance of the full-length zymogen upon cleavage (Figure 1B). In agreement with Liu et al. (2018), RT-qPCR performed on total RNA revealed a coincident decrease in the mRNA levels of several housekeeping genes (*ACTB*, *GAPDH*, *EEF1A*, *PPIA*, *CHMP2A*, *DDX6*, *RPB2*, and *RPLP0*) beginning 1.5 hr after TRAIL was applied (Figures 1C, S1A). As expected, this decrease was specific to RNAPII transcripts, as the RNA polymerase III (RNAPIII)-transcribed ncRNAs *U6*, *7SK*, *7SL*, and *5S* did not show a similarly progressive decrease. The *U6* transcript was instead upregulated, possibly suggesting post-transcriptional regulation of *U6* as alluded to in a previous study (Noonberg et al., 1996). These data confirm that mRNA depletion occurs by 1.5-2 hr during TRAIL-induced apoptosis.

To monitor whether apoptosis also influenced transcription, we pulse labeled the cells with 4-thiouridine (4sU) for 20 min at the end of each TRAIL treatment. 4sU is incorporated into actively transcribing RNA and can be subsequently coupled to HPDP-biotin and purified over streptavidin beads, then quantified by RT-qPCR to measure nascent transcript levels (Dölken, 2013). This revealed that in addition to a reduction in steady state mRNA abundance, TRAIL treatment caused a decrease in RNAPII-driven mRNA production, while RNAPIII transcription was either unaffected or enhanced (Figures 1D, S1B). Thus, early apoptosis triggers mRNA decay and decreases nascent mRNA production but does not negatively impact RNAPIII transcript abundance or production.

Transcriptional repression during early apoptosis requires MOMP but is not directly dependent on caspase activity

We next sought to determine whether any hallmark features of apoptosis underlay the observed transcriptional repression. These include limited proteolysis by the “initiator” CASP8, mass proteolysis by the “executioner” CASP3, and the endonucleolytic cleavage of the genome by caspase-activated DNase (CAD), which translocates into the nucleus during late stages of apoptosis (Enari et al.,1998).

To evaluate the contribution of caspases to this phenotype, HCT116 cells were pre-treated with 40 μ M z-VAD-fmk (zVAD, a pan-caspase inhibitor) for 1 hr prior to TRAIL treatment. The effectiveness of zVAD treatment was confirmed by showing it blocked the CASP8-mediated cleavage of BID as well as the degradation of the CASP3 substrate PARP1 (Figure S2A). The decreases in mRNA levels upon TRAIL treatment were rescued in the presence of zVAD (Figure 2A), confirming a role for one or more caspases in activating mRNA decay during TRAIL-induced apoptosis. Nascent mRNA production was also rescued upon zVAD treatment, as measured by 4sU pulse labeling during the last 20 min of treatment (Figure 2B).

Small interfering RNA (siRNA) knock downs of CASP8 and CASP3 (Figure 2C) were then performed to determine if the rescue of mRNA production caused by zVAD was due specifically to the inhibition of the initiator CASP8 or the executioner CASP3. Both accelerated mRNA decay (Figure S2B) and RNAPII transcriptional repression (Figure 2D) required CASP8 but not CASP3, suggesting that mass proteolysis by CASP3 does not significantly contribute to either phenotype. Knock down of CAD did not affect the reduction in 4sU incorporation observed during early apoptosis (Figure 2D), and DNA fragmentation (as measured by TUNEL assay) was not detected in TRAIL-treated HCT116 cells until 4-8 hr post-treatment (Figure S2C). These findings

are in agreement with the prior study showing that MOMP-induced mRNA decay occurs before DNA fragmentation begins during extrinsic apoptosis (Thomas et al., 2015).

Although CASP3 is dispensable for apoptotic transcriptional repression, a previous report suggests that CASP8 may cleave RPB1, the largest subunit of RNAPII (Lu et al., 2002). We therefore measured the protein expression of RPB1 and the 3 next largest RNAPII subunits (RBP2-4) during early apoptosis to determine if degradation of these subunits might explain the observed TRAIL-induced reduction in RNAPII transcription. Western blots probing for RPB1 showed no significant decrease in protein levels upon TRAIL treatment between 1 and 3 hr (Figure S2D). Furthermore, levels of RPB1-4 were relatively unaffected by 2 hr TRAIL treatment in the presence or absence of zVAD, with the exception of a small decrease in the amount of RPB2 that was rescued upon zVAD treatment (Figure S2E).

Finally, to determine if the activity of any caspase was at all required for apoptotic mRNA decay and ensuing transcriptional repression, we used a small molecule, raptinal, that bypasses CASP8 to intrinsically stimulate MOMP (Palchaudhuri et al., 2015). HeLa cells treated with 10 μ M raptinal for 4 hr underwent MOMP, as measured by cytochrome c release into the cytoplasm, in the presence or absence of zVAD (Figure S2E). Steady state levels and transcription of the *ACTB* and *GAPDH* mRNAs were reduced upon raptinal treatment, and this reduction in mRNA abundance and synthesis was maintained upon caspase inhibition by zVAD treatment (Figures 2E-F). Taken together, these data suggest that neither the mRNA degradation nor the concurrent transcriptional repression observed during early apoptosis are a consequence of caspase activation.

Cytoplasmic 3'- but not 5'-RNA exonucleases are required for apoptotic RNAPII transcriptional repression

Based on connections between virus-activated mRNA decay and RNAPII transcription (Abernathy et al., 2015; Gilbertson et al., 2018), we hypothesized that TRAIL-induced mRNA turnover was functionally linked to the concurrent transcriptional repression. Apoptotic mRNA decay occurs from the 3' end by the actions of the cytoplasmic 3'-RNA exonuclease DIS3L2 and the mitochondrial 3'-RNA exonuclease PNPT1, which is released into the cytoplasm by MOMP (Liu et al., 2018; Thomas et al., 2015). This stands in contrast to basal mRNA decay, which occurs predominantly from the 5' end by XRN1 (Jones et al., 2012). We therefore set out to determine if 3' or 5' decay factors were required for apoptosis-linked mRNA decay and the ensuing repression of mRNA transcription. Given the likely redundant nature of the multiple 3' end decay factors (Houseley and Tollervey, 2009), we performed concurrent knockdowns of DIS3L2, the RNA exosome component EXOSC4, and PNPT1. To check the involvement of 5' decay, we also depleted the single cytoplasmic 5'-3' exonuclease XRN1 (Figure 3A). Depletion of the 3'-5' but not the 5'-3' decay machinery increased total RNA levels and largely restored RNAPII transcription (Figure 3B-3C). Importantly, there was only a minor reduction in CASP3 activation in cells depleted of 3'-5' decay factors and this was not significantly different from that observed upon XRN1 knockdown (Figure 3A). These observations suggest that decreased RNAPII transcription occurs as a consequence of accelerated cytoplasmic mRNA degradation during early apoptosis.

Apoptosis causes reduced RNAPII promoter occupancy in an mRNA decay-dependent manner

RNAPII is recruited to promoters in an unphosphorylated state, but its subsequent promoter escape and elongation are governed by a series of phosphorylation events in the heptad (Y₁S₂P₃T₄S₅P₆S₇)_n repeats of the RPB1 C-terminal domain (CTD). To determine the stage of transcription impacted by mRNA decay, we performed RNAPII ChIP-qPCR and western blots using antibodies recognizing the different RNAPII phosphorylation states. Occupancy of hypophosphorylated RNAPII at the *ACTB* and *GAPDH* promoters was significantly reduced after 2 hr TRAIL treatment (Figure 3D). In accordance with the 4sU labeling results, siRNA-mediated knockdowns showed that loss of RNAPII occupancy requires cytoplasmic 3'-5' RNA decay factors but not the 5'-3' exonuclease XRN1 (Figure 3D). Impaired binding of the TATA-binding protein (TBP), which nucleates the formation of the RNAPII pre-initiation complex (PIC) at promoters (Buratowski et al., 1989; Louder et al., 2016), mirrored that of RNAPII (Figure 3E). These changes are not driven by alterations in the stability of RPB1 or TBP, since the expression of these proteins remained relatively constant during early apoptosis regardless of the presence of mRNA decay factors (Figure S3A-B). The relative proportion of initiating RPB1 CTD phosphorylated at the serine 5 position and the ratio of serine 5 to serine 2 phosphorylation, which decreases during transcriptional elongation (Shandilya and Roberts, 2012), also remain unchanged during early apoptosis regardless of the presence of mRNA decay factors (Figure 3F). Taken together, these data suggest that the decay-dependent reduction in mRNA synthesis

occurs at or before PIC formation, rather than during transcriptional initiation and elongation.

PABPC1 translocates into the nucleus coincidentally with apoptotic mRNA decay

Virus-induced mRNA decay causes changes in the nucleocytoplasmic localization numerous proteins, the most abundant classes of which are RNA binding proteins (RBPs) that bind the 3' untranslated regions (UTRs) and poly(A) tails of mRNA (Gilbertson et al., 2018). The nuclear accumulation of one such RBP, PABPC1, has been linked to viral endonuclease-induced restrictions of gene expression (Gilbertson et al., 2018; Kumar and Glaunsinger, 2010). We therefore considered the redistribution of RBPs such as PABPC1 as a potential mechanism by which cytoplasmic mRNA decay is sensed by the RNAPII transcriptional machinery in the nucleus.

In order to probe whether a redistribution of RBPs similar to that observed during viral nuclease expression occurs during early apoptosis, we performed cell fractionations of apoptotic HCT116 cells. Indeed, 2 hr after TRAIL treatment, PABPC1 protein levels increased in the nuclear fraction of cells but not in the cytoplasmic fraction, indicative of relocalization (Figure 4A). This increase in nuclear PABPC was dependent on the presence of CASP8 but not CASP3 (Figure 4A), mirroring the incidence of mRNA decay and reduced RNAPII transcription. Thus, similar to viral infection, PABPC1 relocalization occurs coincidentally with increased mRNA decay and transcriptional repression in the context of early apoptosis.

We next tested the hypothesis that PABPC1 participates in the coordination between mRNA decay and transcription by depleting cells of PABPC1 and its functional homolog PABPC4 using siRNAs (Figure S3A). mRNA transcription after 2 hr TRAIL

treatment was rescued in PABPC1/4 knock downs (Figure S3B), but baseline mRNA levels in the absence of these proteins were generally greatly reduced (Figure S3C). This is likely consistent with the observation RNAPII promoter occupancy is greatly reduced in the absence of PABPC1/4 (Gilbertson et al., 2018). Thus, depletion of PABPC impairs transcriptional repression during early apoptosis, although given its multiple functions in gene expression (Bernstein et al., 1989; Brook and Gray, 2012; Goss and Kleiman, 2013; Ivanov et al., 2016; Tarun et al., 1997; Wang et al., 1999), the role of PABPC in this process could be indirect.

Importin α/β transport links mRNA decay and transcription

We next sought to more directly evaluate the role of protein shuttling in connecting cytoplasmic mRNA decay to transcriptional repression. The majority of proteins ~60 kilodaltons (kDa) and larger cannot passively diffuse through nuclear pores; they require assistance by importins to enter the nucleus from the cytoplasm (Gö, 1998). Classical nuclear transport occurs by importin α binding to a cytoplasmic substrate, which is then bound by an importin β to form a tertiary complex that is able to move through nuclear pore complexes (Stewart, 2007). To test if this mode of nuclear transport is required for transcriptional feedback, HCT116 cells were pretreated with ivermectin, a specific inhibitor of importin α/β transport (Wagstaff et al., 2012), before the 2 hr TRAIL treatment. The efficacy of ivermectin was validated by an observed decrease in the nuclear levels of nucleolin (NCL), a known importin α/β substrate (Figure 4B; Kimura et al., 2013). Ivermectin treatment rescued RNAPII transcription (Figure 4C), suggesting that protein trafficking to the nucleus provides signals connecting cytoplasmic mRNA decay to transcription.

Importantly, ivermectin did not diminish the extent of CASP3 cleavage (Figure 4B) or mRNA decay (Figure S4D) that occurs during early apoptosis, nor did it decrease baseline levels of transcription in non-apoptotic cells (Figure S4E). Interestingly, it also did not prevent PABPC import (Figure 4B), suggesting that PABPC translocation likely occurs via an importin α/β -independent pathway and is necessary but not sufficient to cause repression of RNAPII transcription.

Finally, we evaluated whether importin α/β transport is also required for feedback between viral nuclease-driven mRNA decay and RNAPII transcription, as this would suggest that the underlying mechanisms involved in activating this pathway are conserved. We used the mRNA specific endonuclease muSOX from the gammaherpesvirus MHV68, as muSOX expression has been shown to cause widespread mRNA decay and subsequent transcriptional repression (Abernathy et al., 2015, 2014). HEK-293T cell lines were engineered to stably express a dox-inducible wild-type or a catalytically inactive (D219A; Abernathy et al., 2015) form of muSOX. These cells were treated with ivermectin and the resultant changes in RNAPII promoter occupancy were measured by ChIP-qPCR. As expected, expression of WT but not D219A muSOX caused mRNA decay and transcriptional repression (Figs 4D-E, S4F-G). Notably, inhibiting nuclear import with ivermectin (Figure S4F) rescued RNAPII promoter occupancy (Figure 4E) without altering the extent of mRNA decay in muSOX expressing cells (Figure 4D). Thus, importin α/β nuclear transport plays a key role in linking cytoplasmic mRNA decay to nuclear transcription, both during early apoptosis and upon viral nuclease expression.

DISCUSSION

mRNA decay and synthesis rates are tightly regulated in order to maintain appropriate levels of cellular mRNA transcripts (Braun and Young, 2014). It is well established that when cytoplasmic mRNA is stabilized, for example by the depletion of RNA exonucleases, transcription often slows in order to compensate for increased transcript abundance (Haimovich et al., 2013; Helenius et al., 2011; Singh et al., 2019; Sun et al., 2012). Eukaryotic cells thus have the capacity to “buffer” against reductions in mRNA turnover or synthesis. Here, we revealed that a buffering response does not occur under conditions of elevated cytoplasmic mRNA degradation stimulated during early apoptosis. Instead, cells respond to cytoplasmic mRNA depletion by decreasing RNAPII promoter occupancy and transcript synthesis, thereby amplifying the magnitude of the gene expression shut down. Nuclear import of cytoplasmic proteins is required for this “transcriptional feedback”, suggesting a pathway of gene regulation in which enhanced mRNA decay prompts cytoplasmic proteins to enter the nucleus and halt mRNA production. Notably, similar transcriptional feedback is elicited during virus-induced mRNA decay (Abernathy et al., 2015; Gilbertson et al., 2018; Hartenian et al., 2020), indicating that distinct cellular stresses can converge on this pathway to potentiate a multi-tiered shutdown of gene expression.

Multiple experiments support the conclusion that the TRAIL-induced transcriptional repression phenotype is a consequence cytoplasmic decay triggered by MOMP, rather than caspase activity. XRN1-driven 5'-3' end decay is the major pathway involved in basal mRNA decay (Łabno et al., 2016), but MOMP-induced mRNA decay is primarily driven by 3' exonucleases such as PNPT1 and DIS3L2 (Liu et al., 2018; Thomas et al., 2015). Accordingly, co-depletion of 3' decay factors but not XRN1

restored RNAPII promoter occupancy and transcription during early apoptosis. In contrast to that of 3' mRNA decay factors, depletion of CASP3 (or the caspase activated DNase CAD) did not block mRNA degradation or transcriptional repression, even though CASP3 is responsible for the vast majority of proteolysis that is characteristic of apoptotic cell death (Walsh et al., 2008). Additionally, the initiator CASP8 was required only under conditions where its activity was needed to induce MOMP. These findings reinforce the idea that mRNA decay and transcriptional repression are very early events that are independent of the subsequent cascade of caspase-driven phenotypes underlying many of the hallmark features of apoptosis.

A key open question is what signal conveys cytoplasmic mRNA degradation information to the nucleus to cause transcriptional repression. Our data support a model where the signal during early apoptosis is provided by one or more proteins that are imported into the nucleus in response to accelerated mRNA decay, rather than depletion of transcriptional machinery. Indeed, many RNA binding proteins undergo nuclear-cytoplasmic redistribution under conditions of viral nuclease-induced mRNA decay, including PABPC (Gilbertson et al., 2018; Kumar et al., 2011; Kumar and Glaunsinger, 2010). We propose that a certain threshold of mRNA degradation is necessary to elicit protein trafficking and transcriptional repression. Presumably, normal levels of basal mRNA decay and regular cytoplasmic repopulation result in a balanced level of mRNA-bound versus unbound proteins. However, if this balance is tipped during accelerated mRNA decay, an excess of unbound RNA binding proteins could accumulate and be transported into the nucleus.

PABPC1 is likely among the first proteins released from mRNA transcripts undergoing 3' end degradation, and its nuclear translocation is driven by a poly(A)-masked nuclear localization signal that is exposed upon RNA decay (Kumar et al., 2011). Depletion of PABPC1/4 impairs mRNA decay-driven transcriptional feedback both during apoptosis and upon viral endonuclease expression, suggesting they are a component of this signaling pathway. However, the nuclear accumulation of PABPC1 during early apoptosis is not sufficient to induce transcriptional repression and its depletion also negatively impacts mRNA abundance. It is therefore likely that nuclear import of additional proteins via the importin α/β pathway is critical for reduced mRNA production. The requirement for importin α/β -mediated nuclear transport in both apoptosis-induced and viral nuclease-induced transcriptional repression suggests that these two stimuli activate a conserved pathway of gene regulation. Whether other types of cell stress elicit a similar response remains an important question for future investigation.

The mRNA 3' end decay-dependent decrease in RNA synthesis is accompanied by a reduction in TBP and RNAPII occupancy at promoters, consistent with transcriptional inhibition occurring upstream of RNAPII initiation and elongation (Gilbertson et al., 2018; Hartenian et al., 2020). The mechanism driving such a defect is yet to be defined, but possibilities include changes to transcript processing pathways, transcriptional regulators, or the chromatin state that directly or indirectly impact formation of the preinitiation complex. In this regard, mRNA processing and transcription are functionally linked (Bentley, 2014) and nuclear accumulation of PABPC1 has been shown to affect mRNA processing by inducing hyperadenylation of

nascent transcripts (Kumar and Glaunsinger, 2010; Lee and Glaunsinger, 2009). Furthermore, TBP interacts with the cleavage–polyadenylation specificity factor (Dantonel et al., 1997), providing a possible link between RNAPII preinitiation complex assembly and polyadenylation. Such a mechanism could also explain the specificity of the observed transcriptional repression to RNAPII transcripts. Chromatin architecture could also be influenced by shuttling of RNA binding proteins, as for example the yeast nucleocytoplasmic protein Mrn1 is implicated in the function of chromatin remodeling complexes (Düring et al., 2012). Future work will test these possibilities in order to mechanistically define the connection between nuclear import and RNAPII transcription under conditions of enhanced mRNA decay.

There are several potential benefits to dampening mRNA transcription in response to accelerated mRNA turnover. Debris from apoptotic cells is usually cleared by macrophages, but inefficient clearance of dead cells can lead to the development of autoantibodies to intracellular components such as histones, DNA, and ribonucleoprotein complexes. This contributes to autoimmune conditions such as systemic lupus erythematosus (Caruso and Poon, 2018; Nagata et al., 2010). In the context of infection, many viruses require the host RNAPII transcriptional machinery to express viral genes (Harwig et al., 2017; Rivas et al., 2016; Walker and Fodor, 2019). It may therefore be advantageous for the cell to halt transcription in attempt to pre-empt a viral takeover of mRNA synthesis. That said, as with antiviral translational shutdown mechanisms (Walsh et al., 2013), viruses have evolved strategies to evade transcriptional repression (Hartenian et al., 2020; Harwig et al., 2017), as well as inhibit cell death via apoptosis and/or co-opt apoptotic signaling cascades (Suffert et al., 2011;

Tabtieng et al., 2018; Zhang et al., 2013; Zhou et al., 2017). In either case, the shutdown of transcription in a cell under stress may protect surrounding cells from danger, particularly in an in vivo context. If true, this type of response is likely to be more relevant in multicellular compared with unicellular organisms.

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AUTHOR CONTRIBUTIONS

C.D. conceived and performed the experiments, analyzed and visualized the data, acquired funding, and drafted the manuscript. E.H. created the stable cell lines and drafted the corresponding section of the manuscript. B.G. conceived and supervised the study, acquired funding, and edited the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

FIGURE 1

Figure 1. mRNA decay during early apoptosis is accompanied by reduced synthesis of RNAPII transcripts. (A) Schematic representation of how the extrinsic apoptotic pathway accelerates mRNA decay. (B) Western blot of HCT116 lysates showing caspase 8 (CASP8) and caspase 3 (CASP3) cleavage and activation over a time course of 100 ng/ μ L TRAIL treatment. Vinculin (VCL) serves as a loading control. (C, D) RT-qPCR quantification of total (C) and nascent 4sU pulse-labeled (D) RNA at the indicated times post TRAIL treatment of HCT116 cells. Also see Figure S1. No biotin control quantifies RNA not conjugated to biotin that is pulled down with streptavidin selection beads. Fold changes were calculated from Ct values normalized to 18S rRNA in reference to mock treated cells. Graphs display mean $\pm$ SEM with individual biological replicates represented as dots. Statistically significant deviation from a null hypothesis of 1 was determined using one sample t test; * p <0.05, ** p <0.01, *** p <0.001.

FIGURE 2

Figure 2. Transcriptional repression during early apoptosis requires MOMP but is not directly dependent on caspase activity. (A, B) RT-qPCR measurements of total (A) and nascent 4sU-labeled (B) RNA fold changes after 2 hr TRAIL treatment of HCT116 cells, including a 1 hr pre-treatment with either 40 μ M zVAD or an equal volume of DMSO (“mock”). Also see Figure S2A. (C) Western blots showing the efficiency of CASP3, CASP8, and caspase-activated DNase (CAD) protein depletion following treatment with the indicated siRNAs. α -Tubulin (TUBA1A) serves as a loading control. (D) RT-qPCR measurements of 4sU-labeled nascent transcripts before or after 2 hr TRAIL treatment in the siRNA treated samples shown in (C). Also see Figures S2B-E. (E, F) Total (E) and nascent 4sU-labeled (F) RNA levels measured by RT-qPCR in HeLa cells after 10 μ M raprinal treatment for 4 hr, with or without a 1 hr pre-treatment of 20 μ M zVAD. Also see Figure S2F. RNA Fold changes were calculated from Ct values normalized to 18S or U6 RNA then normalized to non-apoptotic cells under otherwise identical conditions. Graphs display mean $\pm$ SEM with individual biological replicates represented as dots. Statistically significant deviation from a null hypothesis of 1 was determined using one sample t test and indicated with asterisks directly above bars, while multiple t tests with the Holm-Sidak correction for multiple comparisons were performed to compare mean fold change values for mock inhibitor or scramble siRNA-treated cells to those treated with zVAD or a targeting siRNA and indicated with brackets. * p <0.05, ** p <0.01, *** p <0.001.

FIGURE 3

Figure 3. Apoptosis causes reduced RNAPII transcriptional output and promoter occupancy in an mRNA decay-dependent manner. (A) Western blots of lysates of HCT116 cells depleted of the indicated decay factors with and without 2 hr TRAIL treatment. Apoptosis induction was confirmed by disappearance of the full-length CASP3 band, quantified in the graph below by measuring band intensity normalized to an α -tubulin (TUBA1A) loading control. (B, C) Total (B) and nascent 4sU-labeled (C) RNA was quantified by RT-qPCR under the cellular conditions described in (A). Fold changes were calculated from Ct values normalized to 18S rRNA. (D, E) Chromatin immunoprecipitation (ChIP)-qPCR was used to measure occupancy of the indicated promoters by hypophosphorylated RNAPII (D) or TBP (E) under cellular conditions described in (A). Rabbit and mouse IgG antibodies were included in parallel immunoprecipitation reactions with chromatin from scramble siRNA-treated non-apoptotic cells in lieu of TBP and RNAPII antibodies, respectively, as a control. Also see Figures S3A-B, (F) Relative band intensity ratios from 4 replicates of the representative western blots depicted in S3A, using primary antibodies specific to the indicated RPB1 CTD phosphorylation state under cellular conditions described in (A). Band intensity values were first normalized to a vinculin (VCL) loading control in each blot. All bar graphs display mean $\pm$ SEM with individual biological replicates represented as dots. Statistically significant deviation from a null hypothesis of 1 was determined using one sample t test and indicated with asterisks directly above bars, while multiple t tests with the Holm-Sidak correction for multiple comparisons were performed to compare mean values between groups indicated with brackets. * p <0.05, ** p <0.01, *** p <0.001.

FIGURE 4

Figure 4. Importin α/β transport links mRNA decay and transcription. (A) Western blots showing the indicated proteins in the nuclear and cytoplasmic fractions of apoptotic and non-apoptotic HCT116 cells nucleofected with scramble, CASP3, or CASP8 siRNAs. The nuclear and cytoplasmic fractions of PABPC1 were imaged together but cropped and edited separately to account for the large disparity between the nuclear and cytoplasmic band intensities for this traditionally cytoplasmic protein. The graph on the right shows the changes in PABPC1 abundance in the nucleus versus cytoplasm in the indicated siRNA treated samples. Protein abundance was calculated by band intensity in reference to lamin B1 (LMNB1) or α -tubulin (TUBA1A) loading controls, for the nuclear and cytoplasmic fractions, respectively. Also see Figures S4A-C. (B) Nuclear and cytoplasmic expression of the indicated proteins in apoptotic and non-apoptotic cells with a 1 hr pre-treatment with 25 μ M ivermectin or an equal volume of EtOH (“mock”). Nuclear levels of nucleolin (NCL) were quantified by band intensity in reference to a LMNB1 loading control, while disappearance of full-length CASP3 was quantified in the cytoplasm in reference to TUBA1A. The nuclear and cytoplasmic fractions of PABPC1 were imaged together but cropped and edited separately to account for the large disparity between the nuclear and cytoplasmic band intensities for this traditionally cytoplasmic protein. (C) Levels of nascent 4sU-labeled RNA were quantified by RT-qPCR under the cellular conditions described in (B). Also see Figures S4D-E. (D) RT-qPCR quantification of total RNA levels in HEK293T cells stably expression a doxycycline (dox)-inducible form of muSOX endonuclease cultured with or without 1 μ g/mL dox for 48 hr, ending with a 2 hr treatment with 25 μ M ivermectin or an equal volume of EtOH. (E) RNAPII promoter occupancy at the *ACTB* and *GAPDH*

promoters was determined by ChIP-qPCR under cellular conditions described in (D).
 Also see Figures S2D-G. RNA fold changes were calculated from Ct values normalized
 to 18S rRNA. All bar graphs display mean +/- SEM with individual biological replicates
 represented as dots. Statistically significant deviation from a null hypothesis of 1 was
 determined using one sample t test and indicated with asterisks directly above bars,
 while multiple t tests with the Holm-Sidak correction for multiple comparisons were
 performed to compare mean values between groups indicated with brackets. * $p < 0.05$,
 ** $p < 0.01$, *** $p < 0.001$.

FIGURE S1

Figure S1. mRNA decay during early apoptosis is accompanied by reduced synthesis of RNAPII transcripts. (A, B) RT-qPCR quantification of total (C) and nascent 4sU pulse-labeled (D) RNA at the indicated times post TRAIL treatment of HCT116 cells. Fold changes were calculated from Ct values normalized to 18S rRNA in reference to mock treated cells. Graphs display mean +/- SEM with individual biological replicates represented as dots. Statistically significant deviation from a null hypothesis of 1 was determined using one sample t test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

FIGURE S2

Figure S2. Transcriptional repression during early apoptosis requires MOMP but is not dependent on caspase activity. (A) Western blot of HCT116 lysates showing cleavage (or lack thereof) of the caspase 8 (CASP8) targets BID and caspase 3 (CASP3), as well as the CASP3 target PARP1 upon 2 hr 100 ng/ μ L TRAIL treatment in the presence or absence of 40 μ M zVAD. (B) RT-qPCR quantification of the change in total RNA levels in HCT116 cells treated with the indicated siRNAs upon 2 hr TRAIL treatment. Fold change were calculated from Ct values normalized to 18S rRNA. (C) Quantification of BrdUTP-labeled HCT116 cells treated with TRAIL for the indicated times in a TUNEL assay. Percent positive cells calculated by flow cytometry using FloJo imaging software to count the number of cells with elevated FL2 fluorescence. (D) Western blot of HCT116 lysates showing RPB1 levels and activation over a time course of TRAIL treatment. Vinculin (VCL) serves as a loading control. Graph below quantifies mean RPB1 levels by VCL-normalized band intensity from 4 biological replicates and analyzes the resultant curve by simple linear regression with a set x intercept of 1. These data are derived from the same experiment depicted in Figures 1B-D and S1A-B, therefore the VCL and CASP3 panels are identical to that of Figure 1B. (E) Western blot of HCT116 lysates from cells under conditions described in (A) depicting levels of the indicated RNAPII subunits and CASP3 activation. α -Tubulin (TUBA1A) serves as a loading control. Graph below quantifies the change in RNAPII subunit levels upon apoptosis induction by TUBA1A-normalized band density. (F) Western blots of the cytoplasmic and mitochondrial fractions of HeLa cells treated with 10 μ M raptinal or equal volume of DMSO, in the presence of 20 μ M zVAD or additional volume of DMSO. Efficacy of raptinal treatment was demonstrated by cytochrome c (CYTC) release into

the cytoplasm in the presence and absence of zVAD, which effectively blocked the cleavage and activation of CASP3. TUBA1A and VDAC1 served as cytoplasmic and mitochondrial loading controls, respectively. All bar graphs display mean $\pm$ SEM with individual biological replicates represented as dots. Statistically significant deviation from a null hypothesis of 1 was determined using one sample t test and indicated with asterisks directly above bars, while multiple t tests with the Holm-Sidak correction for multiple comparisons were performed to compare mean values between groups indicated with brackets. $*p<0.05$, $**p<0.0.1$, $***p<0.001$.

FIGURE S3

Figure S3. Apoptosis causes reduced RNAPII transcriptional output and promoter occupancy in an mRNA decay-dependent manner. (A) Representative western blots on lysates of HCT116 cells depleted of either 3' DFs (DIS3L2, EXOSC4, and PNPT1) or XRN1 before and after 2 hr TRAIL treatment, probing for the indicated protein or RPB1 C-terminal domain (CTD) phosphorylation state. Apoptosis induction was confirmed by observing CASP3 cleavage. A vinculin (VCL) loading control was imaged for the blots of each phosphorylation state but a single representative panel is shown. Quantification of RPB1 phosphorylation states from 4 replicates displayed in Figure 3F. (B) Band intensity fold changes of hypophosphorylated RPB1 and TBP derived from 4 biological replicates of the experiment described in (A). Bar graph displays mean +/- SEM with individual biological replicates represented as dots. No statistically significant deviations were discovered either from one-sample t tests of fold change values with a null hypothesis of 1 or multiple t tests with the Holm-Sidak correction for multiple comparisons comparing mean values between siRNA treatments.

FIGURE S4

Figure S4. Importin α/β transport links mRNA decay and transcription. (A) Western blot showing the efficacy of siRNA-mediated knock down of PABPC1 and PABPC4 in HCT116 cells before and after 2 hr TRAIL treatment. Caspase 3 (CASP3) cleavage confirmed induction of apoptosis, while α -Tubulin (TUBA1A) served as a loading control. (B) Nascent (4sU) transcription of the indicated genes in HCT116 cells quantified by RT-qPCR. Fold changes calculated in reference to non-apoptotic cells under otherwise identical cellular conditions. (C) RT-qPCR quantification of total RNA levels in cells in (B), but with all conditions normalized to scramble siRNA-treated non-apoptotic cells. (D) Changes in total RNA levels after 2 hr TRAIL treatment in HCT116 cells pretreated with either 25 μ M ivermectin or an equal volume of ethanol for 1 hr. (E) Additional analysis of data presented in Figure 4C, instead quantifying the fold change in nascent transcription in non-apoptotic cells in the presence or absence of 25 μ M ivermectin. (F) Western blot showing nuclear and cytoplasmic expression of the indicated proteins in HEK293T expressing a doxycycline (dox)-inducible form of the viral endonuclease muSOX, with or without a 48 hr 1 μ g/mL dox treatment. In the graph below, nuclear levels of nucleolin (NCL) were quantified by band intensity in reference to a lamin B1 (LMNB1) loading control, while cytoplasmic levels were calculated in reference to α -Tubulin (TUBA1A). (F) RT-qPCR quantification of the change (or lack thereof) in total RNA levels upon dox-inducible expression of the catalytically dead D219A mutant of muSOX. (G) RNAPII occupancy at the *ACTB* and *GAPDH* promoters under cellular conditions described in (F). RNA fold change values were calculated in reference to 18S rRNA. All bar graphs display mean $\pm$ SEM with individual biological replicates represented as dots. Statistically significant deviation from a null hypothesis

of 1 was determined using one sample t test and indicated with asterisks directly above bars, while multiple t tests with the Holm-Sidak correction for multiple comparisons were performed to compare mean values between groups indicated with brackets. $*p<0.05$, $**p<0.01$, $***p<0.001$.

STAR METHODS

Resource Availability

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Britt Glaunsinger (glaunsinger@berkeley.edu).

Materials availability

The dox-inducible muSOX-expressing cell line and rabbit polyclonal anti-muSOX antibody generated in this study are both available upon request.

Data and Code Availability

This study did not generate any datasets or code.

Experimental model and subject details

Cells and culture conditions

Wild-type HCT116, HEK293T, and HeLa cells (all from ATCC) were obtained from the UC Berkeley Tissue Culture Facility. HEK293Ts were made to stably express doxycycline(dox)-inducible wild-type muSOX and its catalytically-dead D219A mutant by PCR amplifying the aforementioned coding sequences from Addgene plasmids 131702 and 131704 using the muSOX F/R primers (see Key Resources Table) and InFusion cloning these fragments into the Lenti-X™ Tet-One™ Inducible Expression System digested with AgeI. Lentivirus was made for both constructs by transfecting 293T cells

with second generation packaging plasmids and spinfected onto 293T cells at a low multiplicity of infection (MOI) as previously described (Hartenian et al., 2020). 24 hr later, 350 μ g/ml zeocin was added to select for transduced cells. HEK293T and HeLa cells were grown in Dulbecco's Modified Eagle Medium (ThermoFisher Scientific) supplemented with 10% fetal bovine serum (FBS) and 1 U/mL penicillin-streptomycin (pen-strep). HCT116 cells were maintained in McCoy's (modified) 5A medium (ThermoFisher Scientific) with 10% FBS and 1 U/mL pen-strep. All cells were incubated at 37 °C with 5% CO₂. Cell lines were authenticated by STR analysis and determined to be free of mycoplasma by PCR screening. Cells were maintained in culture in 10 cm² plates and 1x10⁶ cells were seeded into 6 well plates for all experiments except for chromatin immunoprecipitations, in which 5x10⁶ cells were seeded into 10 cm² plates.

Method Details

siRNA nucleofections

Protein knock downs were performed using the Neon Transfection System (Thermo Fisher Scientific) with Dharmacon ON-TARGETplus siRNA SmartPools for the following targets: Non-targeting Control Pool (scramble or scr siRNA), CASP3, CASP8, DFFB, DIS3L2, EXOSC4, PNPT1, and XRN1. Custom ON-TARGETplus siRNA pools from Dharmacon were also synthesized to target PABPC1 and PABPC4. For all siRNA pools, cells were nucleofected according to manufacturer protocols for HCT116 cells and immediately seeded into plates containing media supplemented with 10% FBS but lacking pen-strep to improve cell viability post-nucleofection. A 50 nM final siRNA concentration was used for pools targeting CASP3, CASP8, and DFFB (CAD), while 200 nM was used for knock down of XRN1 and the concurrent knock downs of

DIS3L2/EXOSC4/PNPT1 (66.7 nM each). For all experiments involving siRNA knock downs, cells were treated and/or harvested once 80-90% confluent in each plate or well, approximately 72 hr post-nucleofection, and protein knock down was confirmed by western blot.

Apoptosis induction

100 ng/mL TNF-related apoptosis-inducing ligand (TRAIL) was used to induce rapid extrinsic apoptosis in HCT116 cells. Where indicated, HCT116 cells were pre-treated for 1 hr with 40 μ M caspase Inhibitor Z-VAD-FMK (zVAD; Promega Corporation) or 25 μ M ivermectin (Millipore Sigma) before TRAIL treatment. Extrinsic apoptosis induction was confirmed by observing CASP8 and CASP3 cleavage on western blots. CASP3/8 cleavage was quantified by disappearance in intensity of the full-sized band normalized to TUBA1A or VCL using BioRad ImageLab software. Intrinsic apoptosis was induced in HeLa cells with a 4 hr treatment of 10 μ M rapitinal (Millipore Sigma), with or without 1 hr pre-treatment with 20 μ M zVAD. Induction was confirmed by evidence of cytochrome c release into the cytoplasm with cell fractionation and western. “Mock” treatments consisted of an equal volume of solvent used to dissolve each reagent: TRAIL storage and dilution buffer, DMSO, and ethanol for TRAIL, zVAD, and ivermectin, respectively.

RNA, DNA, and protein extractions

Total RNA, and protein extractions were performed according to manufacturer’s instructions after cells were harvested with Trizol™ reagent (ThermoFisher Scientific). RNA pellets were dissolved in DEPC water and protein pellets were dissolved in 1% SDS overnight at 50 °C before spinning down insoluble material at 10,000 x g for 10 min.

TUNEL Assay

Terminal deoxynucleotidyl transferase Br-dUTP nick end labeling was performed on TRAIL-treated cells using a TUNEL Assay Kit (Abcam) according to manufacturer protocol. Br-dUTP incorporation was quantified by flow cytometry, analyzing the elevated peak in FL2 fluorescence on a BD Accuri Flow Cytometry System using FlowJo analysis software.

Quantitative reverse transcription PCR (RT-qPCR)

Extracted RNA was DNase-treated treated, primed with random nonamers, and reverse-transcribed to cDNA with Avian Myeloblastosis Virus Reverse Transcriptase (Promega) according to manufacturer protocols. Genes were quantified by RT-qPCR using iTaq Universal SYBR Master Mix (Bio-rad Laboratories) and primers specific to each gene of interest. RNA fold change values were calculated in reference to *18S* or *U6* ncRNAs, as indicated on figure axes.

Western blotting

Protein samples were quantified by Bradford assay according to manufacturer instructions. 12.5-50 µg of protein was mixed with one third volume of 4X Laemmli Sample Buffer (Bio-Rad Laboratories 1610747) and boiled at 100 °C before loading into a polyacrylamide gel alongside either the PageRuler™ or PageRuler™ Plus Prestained Protein Ladder (ThermoFisher Scientific). Proteins were separated by electrophoresis and transferred to a nitrocellulose membrane, which was then cut into sections surrounding the size of the protein of interest, allowing for multiple proteins to be quantified from one gel. Membranes were then blocked with 5% non-fat dry milk in TBST (1X TBS with 0.2% [v/v] Tween 20) at RT for 1 hour then incubated with relevant

primary antibodies diluted with 1% milk in TBST overnight at 4 °C. All primary antibodies were applied at a 1:1000 dilution with the exception of the following (target, dilution): RPB1, 1:500; TUBA1A, 1:500; RPB2, 1:500; RPB3, 1:10000; LMNB1, 1:1000; GAPDH, 1:5000; and CYTC, 1:500. After three five min TBST washes, secondary antibody diluted 1:5000 with 1% milk in TBST was incubated for one hour at RT. Blots were then developed, after three additional five min TBST washes, with Clarity Western ECL Substrate (Bio-rad Laboratories) for five min and imaged using a ChemiDoc™ MP Imaging System. Each membrane section was imaged and processed separately. Band intensity was quantified using Bio-Rad Image Lab software, and relative expression changes were calculated after normalizing to an α -tubulin (TUBA1A), vinculin (VCL), or lamin-B1 (LMNB1) loading control. For all blots that appear in figures, auto-contrast was applied in Image Lab for each membrane section before the resultant image was exported for publication. When appropriate, membrane sections were stripped with 15% hydrogen peroxide and washed 3 times with TBST before being re-probed with a primary antibody targeting a protein of similar size.

Antibody generation

Polyclonal rabbit anti-muSOX antibody was made and purified by YenZym antibodies, LLC from recombinant muSOX protein.

Cell Fractionations

For experiments performed in apoptotic HCT116 and HeLa cells, nuclear, cytoplasmic, and mitochondrial fractions were isolated using the Abcam Cell Fractionation Kit according to manufacturer's instructions. Cytoplasmic and nuclear fractions of samples in experiments that did not involve apoptosis were separated using the REAP method

(Suzuki et al., 2010). 1/5th of the total cell lysate was reserved and diluted to the same volume of the cell and nuclear fractions for whole cell lysate samples. Protein was extracted from 200 µL of each fraction from both methods using Trizol™ LS reagent (ThermoFisher Scientific) and analyzed by western blot as described above.

4-thiouridine- (4sU)- pulse labeling

4sU-pulse labeling was used to measure nascent transcription concurrently with mRNA decay. 50 µM 4sU (Millipore Sigma T4509) was added to cells 20 min before harvesting. Labeled transcripts contained in 25 µg of total extracted RNA were biotinylated as described by Dölken (2013), using 50 µg HPDP biotin (ThermoFisher Scientific). Biotinylated RNA was conjugated to Dynabeads™ MyOne™ Streptavidin C1 (ThermoFisher Scientific) magnetic beads for 1 hour in the dark, then the beads were washed four times (twice at 65 °C and twice at RT) with wash buffer (100 mM Tris-HCl, 10 mM EDTA, 1 M sodium chloride, and 0.1% Tween 20) before eluting RNA off of the beads twice with 5% BME in DEPC water. RNA was precipitated by adding 1/10th volume of 3M sodium acetate and 2.5 volumes of ethanol and spun down at full speed in a 4°C benchtop centrifuge. After a 75% ethanol wash, the 4sU-labelled RNA was resuspended in 20 µL DEPC water for use in in RT-qPCR.

Chromatin immunoprecipitation (ChIP)

HCT116 cells nucleofected with the relevant siRNAs were seeded onto 10 cm² plates. 72 hr post-nucleofection, cells were washed with PBS, trypsinized, washed with PBS again, and fixed in 1% formaldehyde for 2.5 min before quenching with 125 mM glycine. After an additional PBS wash, cells were lysed for 10 min at 4°C in lysis buffer (50 mM HEPES pH 7.9, 140 mM NaCl, 1 mM EDTA, 10% [v/v] glycerol, 0.5% NP40, 0.25%

754 Triton X-100) then washed with wash buffer (10 mM Tris Cl pH 8.1, 100 mM NaCl, 1
755 mM EDTA pH 8.0) at 4 °C for 10 min. Cells were then suspended in 1 mL shearing
756 buffer (50 mM Tris Cl pH 7.5, 10 mM EDTA, 0.1% [v/v] SDS) and sonicated in a Covaris
757 S220 sonicator (Covaris Inc.) with the following parameters: peak power, 140.0; duty
758 factor, 5.0; cycle/burst, 200; and duration, 300 sec. Insoluble material in the shearing
759 buffer was then spun down at full speed in a 4 °C benchtop centrifuge to yield the
760 chromatin supernatant. 10 µg chromatin was rotated overnight at 4 °C with 2.5 µg or 4
761 µg ChIP-grade primary antibodies targeting RPB1 and TBP, respectively, in 500 µL
762 dilution buffer (1.1% [v/v] Triton-X-100, 1.2mM EDTA, 6.7mM Tris-HCl pH 8.0, 167mM
763 NaCl). 5 µL of each IP was reserved as an input sample before antibody was added. 20
764 µL of either Dynabeads™ Protein G or a 1:1 mixture Protein A and Protein G beads, for
765 anti-mouse and anti-rabbit antibodies, respectively, were added to each reaction and
766 rotated at 4 °C for at least 2 hr. The beads were then sequentially washed with low salt
767 immune complex wash buffer (0.1% [v/v] SDS, 1% [v/v] Triton-X-100, 2 mM EDTA, 20
768 mM Tris-HCl pH 8.0, 150 mM NaCl), high low salt immune complex wash buffer (0.1%
769 [v/v] SDS, 1% [v/v] Triton-X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8, 500 mM NaCl),
770 LiCl immune complex buffer (0.25 M LiCl, 1% [v/v] NP40, 1% [v/v] deoxycholic acid,
771 1mM EDTA, 10mM Tris-HCl pH 8.0), and TE buffer (10 mM Tris-HCl pH 8.0, 1 mM
772 EDTA). All washes were 5 min in duration and performed at 4 °C. Beads and input
773 samples were then suspended in 100 µL elution buffer (150 mM NaCl, 50ug/ml
774 proteinase K) and incubated at 55 °C for 2 hr then at 65 °C for 12 hr in a thermal cycler.
775 DNA fragments were purified with an Oligo Clean and Concentrator kit (Zymo

Research) and % input values were quantified by RT-qPCR as previously described using primers complementary to the locus of interest.

Data visualization

Bar graphs were created using GraphPad Prism software and the graphical abstract was made using the Bio-Render online platform.

Quantification and statistical analysis

Biological replicates were defined as experiments performed separately on biologically distinct samples representing identical conditions and/or time points. See figures and figure legends for the number ($n \geq 3$) of biological replicates performed for each experiment. Criteria for the inclusion of data was based on the performance of positive and negative controls within each experiment. No outliers were eliminated in this study. One-sample t-tests were performed on control and experimental groups for which mean fold change values were calculated, comparing these values to the null hypothesis of 1. T tests corrected for multiple comparison with the Holm-Sidak were also performed comparing means between control and experimental groups, signified by brackets spanning the two groups being compared. All statistical analyses were performed using GraphPad Prism 8.

Key Resources Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-RNA polymerase II CTD repeat YSPTSPS antibody (clone 8WG16)	Abcam	Cat#ab817
Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody	Abcam	Cat#ab5095
Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) antibody	Abcam	Cat#ab5131
Anti-alpha Tubulin antibody	Abcam	Cat#ab7291
POLR2B (RPB2) Antibody (clone E-12)	Santa Cruz Biotechnology	Cat#sc-166803

Anti-RPB3 antibody (clone EPR13294B)	Abcam	Cat#ab182150
POLR2D (RPB4) Polyclonal Antibody	ThermoFisher Scientific	Cat#PA5-35954
Anti-Vinculin antibody	Abcam	Cat#ab91459
Anti-PNPT1 antibody	Abcam	Cat#ab96176
Anti-RRP41 (EXOSC4) antibody	Abcam	Cat#ab137250
DIS3L2 Antibody	Novus Biologicals	Cat#NBP184740
Anti-Lamin B1 antibody	Abcam	Cat# ab133741
Anti-GAPDH antibody (clone 6C5)	Abcam	Cat# ab8245
Caspase-8 (clone D35G2) Rabbit mAb	Cell Signaling Technology	Cat#4790
Caspase-3 Antibody	Cell Signaling Technology	Cat#9662
XRN1 Antibody	Bethyl Laboratories	Cat#A400-433A
Anti-DFFB antibody	Abcam	Cat#ab69438
PABP1 Antibody	Cell Signaling Technology	Cat#4992
PABP4 Antibody	Bethyl Laboratories	Cat#A301-466A
C23 (NCL) Antibody (clone MS-3)	Santa Cruz Biotechnology	Cat#sc-8031
PARP Antibody	Cell Signaling Technology	Cat#9542
BID Antibody (clone 5C9)	Santa Cruz Biotechnology	Cat#sc-56025
Anti-TATA binding protein TBP antibody (clone mAbcam 51841)	Abcam	Cat#ab51841
Cytochrome c (clone D18C7) Rabbit mAb	Cell Signaling Technology	Cat#11940
Anti-VDAC1 / Porin antibody	Abcam	Cat# ab15895
Polyclonal rabbit ant-muSOX antibody	This paper	N/A
Chemicals, Peptides, and Recombinant Proteins		
Trizol™ Reagent	ThermoFisher Scientific	Cat#15596026
Trizol™ LS Reagent	ThermoFisher Scientific	Cat#10296028
TURBO™ DNase	ThermoFisher Scientific	Cat#AM2238
Avian Myeloblastosis Virus Reverse Transcriptase	Promega Corporation	Cat#M5108
iTaq Universal SYBR Master Mix	Bio-Rad Laboratories	Cat#1725122
Dynabeads™ Protein G	ThermoFisher Scientific	Cat#10003D

Dynabeads™ Protein A	ThermoFisher Scientific	Cat#10002D
Dynabeads® MyOne™ Streptavidin C1	ThermoFisher Scientific	Cat#
EZ-link™ HPDP-biotin	ThermoFisher Scientific	Cat#21341
Caspase Inhibitor Z-VAD-FMK	Promega Corporation	Cat#G7231
Ivermectin	Millipore Sigma	Cat#I8898
Raptinal	Millipore Sigma	Cat#SML1745
Dulbecco's Modified Eagle Medium	ThermoFisher Scientific	Cat#12800082
McCoy's (modified) 5A medium	ThermoFisher Scientific	Cat#16600082
Fetal Bovine Serum	VWR	Cat#89510-186
PageRuler™ Prestained Protein Ladder	ThermoFisher Scientific	Cat#26616
PageRuler™ Plus Prestained Protein Ladder	ThermoFisher Scientific	Cat#26620
Clarity Western ECL Substrate	Bio-Rad Laboratories	Cat#1705061
Critical Commercial Assays		
TUNEL Assay Kit - BrdU-Red	Abcam	Cat#ab66110
Cell Fractionation Kit	Abcam	Cat#ab109719
Bio-Rad Protein Assay Kit II	Bio-Rad Laboratories	Cat#5000002
Oligo Clean & Concentrator Kit	Zymo Research	Cat#D4060
In-Fusion® HD Cloning Kit	Takara Bio USA	Cat#639650
Lenti-X™ Tet-On® 3G Inducible Expression System	Takara Bio USA	Cat#631187
Neon Transfection System	ThermoFisher Scientific	Cat#MPK5000
Experimental Models: Cell Lines		
Human: HCT116 cells	ATCC	CCL-247
Human: 293T/17 cells	ATCC	CRL-11268
Human: HeLa Cells	ATCC	CCL-2
Oligonucleotides		
PABPC1 siRNA #1	Gilbertson et al. 2018	GAAAGGAGCT CAATGGAA
PABPC1 siRNA #2	Gilbertson et al. 2018	GGACAAATCCA TTGATAAT
PABPC4 siRNA #1	Gilbertson et al. 2018	AGGAGAGAAU UAGUCGAU CAGGG

PABPC4 siRNA #2	Gilbertson et al. 2018	GGAAUUCAAC UCAAGGUUUG AAGAC
muSOX F	This paper	TCCCGTATACA CCGGTATGTG GAGCCACCCC
muSOX R	This paper	ATCCGCCGGC ACCGGTTTAGG GGGTTATGGG
ON-TARGETplus Non-targeting Control Pool	Horizon Discovery Group	Cat#D-001810-10
SMARTpool: ON-TARGETplus DIS3L2 siRNA	Horizon Discovery Group	Cat#L-018715-01
SMARTpool: ON-TARGETplus Human EXOSC4 siRNA	Horizon Discovery Group	Cat#L-013760-00
SMARTpool: ON-TARGETplus Human PNPT1 siRNA	Horizon Discovery Group	Cat#L-019454-01
SMARTpool: ON-TARGETplus XRN1 siRNA	Horizon Discovery Group	Cat#L-013754-01
SMARTpool: ON-TARGETplus CASP3 siRNA	Horizon Discovery Group	Cat#L-004307-00
SMARTpool: ON-TARGETplus CASP8 siRNA	Horizon Discovery Group	Cat#L-003466-00
ON-TARGETplus DFFB siRNA SMARTpool	Horizon Discovery Group	Cat#L-004425-00
See Table S1 for qPCR primers		
Software and Algorithms		
Prism 8	GraphPad	https://www.graphpad.com/scientific-software/prism/
FlowJo	BD	https://www.flowjo.com/solutions/flowjo
Image Lab Software	Bio-Rad Laboratories	Cat#1709690

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Table S1. qPCR primers

Gene/	Forward sequence	Reverse sequence
RT-qPCR		
<i>ACTB</i>	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
<i>GAPDH</i>	CAACGGATTTGGTCGTATTGG	GCAACAATATCCACTTTACCAGAGTTAA
<i>EEF1A</i>	TGTCGTCATTGGACACGTAGA	ACGCTCAGCTTTCAGTTTATCC
<i>PP1A</i>	TCCTGGCATCTTGTCCATG	CCATCCAACCACTCAGTCTTG
<i>CHMP2A</i>	CGCGAGCGACAGAACTAGAG	CCCGCATCAATACAACTTGC
<i>DDX6</i>	CAGGAACATCGAAATCGTG	TCCAATACGATGGAGATAGG
<i>RPB2</i>	CCTGAGGGTGACGAGGATCT	GTGCTTCCATTCCGCATACAG
<i>RPLP0</i>	GCAGCATCTACAACCCTGAAG	CACTGGCAACATTGCGGAC
<i>U6</i>	CGCTTCGGCAGCACATATAC	AAAATATGGAACGCTTCACGA
<i>7SK</i>	CCCCTGCTAGAACCTCCAAAC	CACATGCAGCGCCTCATTT
<i>7SL</i>	ATCGGGTGTCCGCACTAAGTT	CAGCACGGGAGTTTTGACCT
<i>5S</i>	GGCCATACCACCCTGAACGC	CAGCACCCGGTATTCCCAGG
<i>18S</i>	GTAACCCGTTGAACCCCAT	CCATCCAATCGGTAGTAGCG
ChIP		
<i>ACTB</i> promoter	CCGAAAGTTGCCTTTTATGG	GCCGCTGGGTTTTATAGGG
<i>GAPDH</i> promoter	TACTAGCGGTTTTACGGGCG	TCGAACAGGAGGAGCAGAGACCGA