Functional interaction between the RNA exosome and the sirtuin deacetylase Hst3 maintains transcriptional homeostasis

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Eukaryotic cells maintain an optimal level of mRNAs through unknown mechanisms that balance RNA synthesis and degradation. We found that inactivation of the RNA exosome leads to global reduction of nascent mRNA transcripts, and that this defect is accentuated by loss of deposition of histone variant H2A.Z. We identify the mRNA for the sirtuin deacetylase Hst3 as a key target for the RNA exosome that mediates communication between RNA degradation and transcription machineries. These findings reveal how the RNA exosome and H2A.Z function together to control a deacetylase, ensuring proper levels of transcription in response to changes in RNA degradation.

Supplemental material is available for this article.

Received August 10, 2021; revised version accepted November 22, 2021.

Eukaryotic cells rely on dynamic, multifaceted regulation at each step of RNA biogenesis to maintain mRNA pools and ensure normal protein synthesis. Loss of regulation at any step of this process can lead to aberrant gene expression and deleterious effects on cell fitness. Studies in S. cerevisiae suggest a buffering phenomenon that maintains global mRNA transcript levels through the balance of nuclear transcriptional control and cytoplasmic RNA decay [Haimovich et al. 2013; Sun et al. 2013]. For instance, global decreases in transcription caused by depletion of general transcription factors are associated with a compensatory increase in mRNA half-lives, such that steady-state RNA levels remain largely unaffected [Rodríguez-Molina et al. 2016; Baptista et al. 2017]. It has been proposed that expression of the cytoplasmic ribonuclease Xrn1 may play a key role in regulating RNA half-lives in response to decreases in transcription [Sun et al. 2013], but it remains unclear how a decrease in RNA degradation efficiency feeds back to control transcription.

Although previous work focused on cytoplasmic RNA degradation pathways [Haimovich et al. 2013; Sun et al. 2013], inactivation of the nuclear RNA exosome also leads to increased RNA half-lives as well as decreases in RNA synthesis, indicating that nuclear RNA decay can also activate a buffering mechanism [Sun et al. 2013]. The RNA exosome is a 3′-to-5′ exonuclease that functions within the nucleus or cytoplasm depending on which catalytic subunit is incorporated; yeast Rrp6 functions within the nuclear RNA exosome, while Dis3 is the nuclease for the cytoplasmic enzyme [Liu et al. 2006]. The RNA exosome is highly conserved across eukaryotes and is considered a key RNA quality control pathway that regulates cryptic unstable transcripts (CUTs), noncoding RNAs, and abnormal coding transcripts [Mitchell et al. 1997; Torchet et al. 2002; Kiss and Andrulis 2010, Schneider et al. 2012, Delan-Forino et al. 2017]. In S. pombe, the RNA exosome also functions with RNA polymerase II (RNAPII) to facilitate transcription termination [Lemay et al. 2014].

Recently, we found that the RNA exosome masks changes in the steady-state pools of both coding and noncoding transcripts following dysregulation of key promoter-proximal, nucleosomal features. For instance, loss of histone H3-K56 acetylation [H3-K56Ac] through inactivation of the Rtt109 acetylase and chaperone Asf1 results in a global decrease in nascent RNAPII transcripts, while hyperacetylation of H3-K56, following depletion of the Hst3 sirtuin deacetylase, leads to a global increase in nascent transcripts. However, in both cases, changes in the steady-state pools are only detected following depletion of the RNA exosome [Rege et al. 2015; Feldman and Peterson 2019, Topal et al. 2019]. In addition to H3-K56Ac, nucleosomes flanking RNAPII gene promoters are enriched for the histone variant H2A.Z [Albert et al. 2007; Ruffiange et al. 2007]. Similar to H3-K56Ac, histone H2A.Z functions with the RNA exosome to regulate divergent noncoding RNAs in both yeast and mouse embryonic stem cells, and yeast strains lacking both the nuclear RNA exosome and H2A.Z show synthetic growth phenotypes [Rege et al. 2015].

We found that the sirtuin histone deacetylase Hst3 plays a key role in a feedback circuit in which nuclear RNA degradation is coupled to RNA synthesis. Using native elongating transcript sequencing [NET-seq] [Churchman and Weissman 2011], we confirmed that conditional depletion of the nuclear RNA exosome leads to a global reduction in RNAPII nascent transcription. Interestingly, depletion of both the RNA exosome and an essential component of the histone H2A.Z deposition machinery, the Swr1 ATPase, leads to a greater decrease in nascent transcription, suggesting two partially redundant mechanisms that buffer transcript levels. Analysis of steady-state mRNA pools shows that the RNA exosome regulates levels of the Hst3 mRNA, and overexpression of Hst3 in wild-type cells is sufficient to drive a global decrease in nascent transcription. Thus, these findings support a novel model in which the sirtuin deacetylase Hst3 is a key player in a regulatory circuit containing both the nuclear RNA exosome and the histone variant H2A.Z that connects RNA degradation and transcription initiation.

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[Keywords: transcription, RNA decay, chromatin, H2A.Z, sirtuin, Hst3]

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Article published online ahead of print. Article and publication date are online at http://www.genesdev.org/cgi/doi/10.1101/gad.348923.121.
Results and Discussion

Depletion of the RNA exosome stabilizes RNA pools and reduces transcription

Strains harboring a deletion of RRP6, encoding the catalytic subunit of the nuclear RNA exosome, exhibit a global increase in mRNA half-lives and decreased mRNA synthesis rates compared with a wild-type strain (Sun et al. 2013). Given this global impact on the transcriptome, we worried that growth of rrp6 deletion cells for many generations may lead to indirect effects that either mask or augment the impact on mRNA biogenesis. To investigate more immediate impacts of RNA exosome loss on transcription, the anchor away system (Haruki et al. 2008) was used to conditionally deplete the Rrp6 catalytic subunit from the nucleus.

To evaluate the effectiveness of the nuclear depletion strategy, RNA-seq was used to monitor changes in the steady-state mRNA pool following a 1-h treatment of RRP6-FRB cells with rapamycin, which triggers Rrp6 nuclear depletion. For library normalization, a S. pombe spike-in control was added to each sample. Previous studies reported varying impacts of the RNA exosome on protein-coding transcripts, though loss of the RNA exosome has a large impact on the abundance of small structural RNAs and noncoding RNAs, such as cryptic unstable transcripts (CUTs) (Gudipati et al. 2012; Schneider et al. 2012). CUTs are 5′ capped and polyadenylated noncoding RNAs that harbor multiple Nrd1–Nab1–Sen1 [NNS] binding motifs. Binding of the NNS complex leads to the recruitment of the RNA exosome and subsequent degradation prior to capping and polyadenylation (Arigo et al. 2006; Thiebaut et al. 2006; Vasiljeva and Buratowski 2006; Schulz et al. 2013). Importantly, a 1-h depletion of Rrp6 was sufficient to cause an increase for 369 of the 929 CUTs by >1.5-fold (Fig. 1A). By comparison, we previously found that a 3-h rapamycin treatment led to up-regulation of 868 CUTs (Feldman and Peterson 2019), nearly identical to the impact of an rrp6 deletion allele, as assayed by microarray analyses (765 CUTs increased 1.5x or more) (Rege et al. 2015). In addition to changes in CUT expression, consistent with its reported cytoplasmic localization (Supplemental Fig. S1D).

Previously, we reported that double mutants lacking both Rrp6 and Swr1, the ATPase subunit of the SWR1 complex (SWR1C), have a synthetic slow-growth phenotype (Rege et al. 2015). Likewise, studies have used epistatic miniarray profiling (E-MAP) to reveal negative genetic interactions between genes encoding Rrp6, multiple subunits of SWR1C, and histone H2A.Z (Collins et al. 2007; Wilmes et al. 2008; Zheng et al. 2010). These genetic interactions led us to investigate the interplay between these two complexes and their influence on transcription by performing RNA-seq and NET-seq analyses for SWR1-FRB and SWR1-FRB RRP6-FRB strains. Consistent with previous deletion allele

Figure 1. Loss of the RNA exosome stabilizes RNAs and reduces nascent transcripts. (A) RNA-seq analyses comparing coding [left panel] and CUT [right panel] RNAs, RRP6-FRB to WT. Density scatter plots of mean log2 RNA abundance of two biological replicates of WT and two biological replicates of RRP6-FRB normalized with S. pombe. Black line denotes x = y (no change). FDR was determined by edgeR. Log2(fold change mutant/WT) is denoted as LFC. Average fold change ± standard error and standard deviation (stdev) are displayed. (B) NET-seq analyses of asynchronous WT and RRP6-FRB strains treated with rapamycin for 1-h and normalized with S. pombe spike-in. Density scatter plots of coding nascent transcripts show the log2 mean intensity for three biological replicates of RRP6-FRB cells plotted against six WT replicates. The black line indicates x = y (no change). P-value was determined by Mann-Whitney U-test. Average fold change ± standard error and standard deviation (stdev) are displayed. (C) Normalized RNAPII density for all replicates of WT and RRP6-FRB cells. NET-seq reads for each gene, scaled to 500 bp, are normalized to total reads. Shaded region represents 95% confidence interval.

Redundant functional roles of H2A.Z and the RNA exosome in maintaining transcription

Previously, we reported that double mutants lacking both Rrp6 and Swr1, the ATPase subunit of the SWR1 complex (SWR1C), have a synthetic slow-growth phenotype (Rege et al. 2015). Likewise, studies have used epistatic miniarray profiling (E-MAP) to reveal negative genetic interactions between genes encoding Rrp6, multiple subunits of SWR1C, and histone H2A.Z (Collins et al. 2007; Wilmes et al. 2008; Zheng et al. 2010). These genetic interactions led us to investigate the interplay between these two complexes and their influence on transcription by performing RNA-seq and NET-seq analyses for SWR1-FRB and SWR1-FRB RRP6-FRB strains. Consistent with previous deletion allele
studies (Rege et al. 2015), a 1-h depletion of Swr1 had no significant impact on the stable coding or noncoding RNA pools (Supplemental Fig. S1E, top panels). Notably, a recent study has found that a 1-h depletion of Swr1 is sufficient to cause the global loss of H2A.Z from chromatin (Ranjian et al. 2020). The codepletion of both Swr1 and Rp6 led to a similar increase in the stable pool of mRNA transcripts as compared with the sole depletion of Rp6 (1793 of 5302, LFC > 0.58, edgeR) (Supplemental Fig. S1E, bottom left panel). Likewise, the increased levels of noncoding CUT transcripts were nearly identical between the RRP6-FRB and RRP6-FRB SWR1-FRB strains (363 of 920) (Supplemental Fig. S1E, bottom right panel).

Whereas the single depletion of Swr1 had no significant impact on nascent transcription (Fig. 2A), codepletion of Rp6 and Swr1 resulted in a dramatic decrease (Fig. 2B; see also Supplemental Fig. S1B,C). When further analysis of the data is applied, 3504 nascent transcripts were decreased >1.5-fold (Fig. 2C). In contrast, this same type of analysis revealed that depletion of Rp6 led to a 1.5-fold decrease of only 15 genes (Fig. 2C; see also Supplemental Fig. S2). Furthermore, the codepletion of Rp6 and Swr1 had the largest impact on highly expressed genes, specifically the top 25% of genes transcribed in WT (Fig. 2D). Interestingly, nascent transcription of CUTs remained relatively unchanged following codepletion of Rp6 and Swr1 (Supplemental Fig. S1G). Codepletion of Swr1 and the cytoplasmic RNA exosome component Dis3 resulted in no change in transcription, further confirming a nuclear mechanism of transcriptional homeostasis (Supplemental Fig. S1F). We also analyzed the distribution of RNAPII after normalizing to the overall nascent transcription for a given gene. Whereas depletion of Swr1 had little impact on RNAPII (Fig. 2E), loss of Rp6 led to an increase in RNAPII distribution past the 3′ end, resulting in readthrough transcripts (Fig. 1C). In the absence of both Swr1 and Rp6, there is an accentuation of RNAPII distribution at the transcription termination site, suggesting a deficiency in termination, specifically in longer-length genes (Fig. 2E; Supplemental Fig. S1B). These alterations are consistent with previous reports of extended transcript 3′ ends in mutants defective in the RNA exosome, as well as work in fission yeast implicating both H2A.Z and the RNA exosome in transcription termination (Hilleren et al. 2001; Torchet et al. 2002; Zofall et al. 2009; Lemay et al. 2014).

To investigate whether Rp6 and Swr1 might also impact expression of an inducible gene set, nascent transcript levels were monitored for genes that are transcriptionally regulated by short exposure to diamide chemical stress (Supplemental Fig. S3A–E; Gasch et al. 2000). In wild-type cells, exposure to diamide leads to the induction of 689 genes and the repression of 644 genes. Similar to constitutively expressed genes, depletion of both Rp6 and Swr1 led to decreases in nascent transcript levels for both the induced and repressed gene sets.

Our NET-seq analysis indicates that loss of the RNA exosome decreases the number of RNAPII molecules along genes, suggesting that it may alter transcription initiation and elongation. To more directly evaluate how the RNA exosome impacts the levels of actively transcribing RNAPII, we used transient transcripome sequencing (TT-seq) (Schwalb et al. 2016). In this assay, cells were incubated with 4-thiouridine (4su) for 10 min to label newly transcribed RNAs, followed by biotin capture of labeled RNAs and sequencing library preparation (Fig. 3A). Similar to the results from NET-seq, depletion of Swr1 has little impact on transcription, but loss of Rp6 leads to a global ∼1.25-fold reduction in TT-seq signals (Fig. 3B). Furthermore, depletion of both the Rp6 and Swr1 results in a further decrease in transcription, with reductions nearly 1.5-fold compared with WT (Fig. 3B, right panel; see also Supplemental Fig. S4). Taken together, these findings suggest that the RNA exosome and deposition of the histone variant H2A.Z function together to maintain transcriptional homeostasis.

Hst3 transcript levels are regulated by the RNA exosome

Previous studies suggest that transcriptional buffering involves the cytoplasmic RNA degradation factor Xrn1. In a
Interestingly, inactivation of the nuclease activity of Xrn1 does not alter HST3 expression levels in RNA exosome depletion (Supplemental Fig. S5C). The Hst3 transcript contains several consensus binding sites for the NNS complex near the 5’ end, and previous work found that the Nrd1 and NAB components of the NNS complex bind to the Hst3 transcript in vivo [Supplemental Fig. S5D; Creamer et al. 2011]. A simple model predicts that the RNA exosome regulates HST3 transcript levels by promoting early termination of transcription by the NNS complex. To test this possibility, we analyzed the 5’ to 3’ ratio of TT-seq reads at the HST3 gene for WT, RRP6-FRB, and RRP6-FRB SWR1-FRB strains. In wild-type cells, the 5’ and 3’ read counts are nearly equal (5’ to 3’ ratio of 1.12 ± 0.03), whereas this ratio is decreased in RRP6-FRB and RRP6-FRB SWR1-FRB strains (0.49 ± 0.22 and 0.67 ± 0). This is consistent with less stalling of RNA PolII at the 5’ end of HST3 and more productive elongation to the 3’ end. Overall, these data indicate that the RNA exosome regulates HST3 mRNA abundance, likely through NNS-dependent transcription termination.

Hst3 and Hst4 are two yeast paralogs of mammalian Sirt6 that target the deacetylation of H3-K56 [Celic et al. 2006]. Previously, we showed that depletion of both Hst3 and Hst4 resulted in hyperacetylation of H3-K56 and a global, ~2x increase in nascent RNA transcription [Maas et al. 2006; Feldman and Peterson 2019]. In contrast to HST3, the HST4 mRNA was not significantly stabilized by loss of Rrp6, and thus this sirtuin does not appear to be involved in this feedback mechanism. These data are consistent with a model in which increased levels of Hst3, due to Rrp6 depletion, might lead to a global decrease in RNA synthesis. To test this idea, we performed NET-seq analyses in triple depletion ([HST3-FRB RRP6-FRB SWR1-FRB] and double depletion ([HST3-FRB RRP6-FRB SWR1-FRB]) strains. Strikingly, whereas depletion of both Rrp6 and Swr1 led to a global reduction in nascent transcripts,
the additional depletion of Hst3 restored RNA synthesis and RNApolyI distribution to wild-type levels (Fig. 4B, Supplemental Fig. S6A). Specifically, whereas ~3500 genes were significantly decreased following depletion of both Rtp6 and Swr1 (Fig. 2C), the codepletion of Rtp6, Swr1, and Hst3 results in only 12 genes significantly decreased (Fig. 4C). Violin plots of nascent transcripts, with the mean denoted in red, shows this shift in nascent transcription to WT levels (Fig. 4D).

If deacetylation of H3-K56 is key for the role of the RNA exosome in transcriptional regulation, then decreases in transcription due to loss of H3-K56Ac should be epistatic with RNA exosome depletion. Asf1 is a histone chaperone that functions with the Rtt109 acetyltransferase to catalyze H3-K56Ac (Han et al. 2007; Tsubota et al. 2007), and nuclear depletion of Asf1 leads to a global decrease in transcription (see also Supplemental Fig. S6B; Topal et al. 2019). However, codepletion of both Asf1 and Rtp6 did not result in a further reduction in nascent transcription, suggesting that these two factors function within the same pathway (Supplemental Fig. S6B).

To test whether increased levels of Hst3 are sufficient to induce a global decrease in transcription, Hst3 was transiently overexpressed from a galactose-inducible promoter, followed by NET-seq analysis. Consistent with the model, Hst3 overexpression is sufficient to reduce nascent transcription of nearly all genes >1.5-fold (Fig. 4E). A representative genome browser view reveals the increase in Hst3 transcription and the global reduction in transcription across several genes (Fig. 4F). These findings are consistent with a feedback model whereby the RNA exosome regulates the levels of the Hst3 mRNA, which in turn impacts RNA synthesis.

The cellular mechanism that maintains transcriptional homeostasis appears to depend on the appropriate regulation of histone acetylases and deacetylases. Our previous results suggest that H3-H56Ac may function as a transcriptional rheostat in which a loss of this mark leads to an approximately twofold decrease in global transcription while hyperacetylation of H3-K56 causes an approximately twofold increase in transcription (Feldman and Peterson 2019; Topal et al. 2019). Here, our data indicate that smaller modulations in H3-K56Ac, mediated by changes in Hst3 mRNA abundance, also cause global transcriptional defects. These transcriptional changes are further augmented by loss of H2A.Z deposition, even though loss of H2A.Z by itself has little impact on transcription. This suggests a new, key role for H2A.Z in buffering promoters against changing levels of histone deacetylases, such as Hst3. Given that H2A.Z is often enriched at the same promoter-proximal nucleosomes that harbor H3-K56Ac, one attractive possibility is that nucleosomes harboring H2A.Z impact the deacetylation activity of Hst3. Together, our work uncovers new roles for H2A.Z and uncovers a feedback circuit in which nuclear mRNA decay is linked to transcription initiation.

Materials and methods

Strains and media

The S. cerevisiae strains used here were derived from MATa tor1-1 fpr1::LoxP-LEU2 loxP RPL13A-2xKRP12-loxP (H1Y22I) bar1Δ::HIS5 RPB3::FLAG:NAT. Unless noted otherwise, cells were cultivated in YPD [10% yeast extract, 20% bacterial peptone, 2% glucose]. Diamide stress analysis was performed in YPD media with treatment for 30 min of diaminobenzidine (DAB).

Feedback regulation of transcription

RNA-seq

Asynchronous cells were grown in 50 mL of YPD media at 30°C to midlog phase, treated with rapamycin at a final concentration of 8 µg/mL for 1 h, and harvested, and total RNA was isolated through hot acid phenol extraction. Strand-specific RNA-seq library preparation was done as described previously with a S. pombe spike-in for normalization (Feldman and Peterson 2019; Topal et al. 2019). Briefly, cells were collected and spiked with S. pombe at a 1:6 ratio. Following hot phenol extraction and ethanol precipitation, RNA was further purified with RNAeasy miniprep kit, DNA was digested, and then RNA was removed using RiboZero magnetic beads (illumina) from 3 µg of RNA. Two biological replica libraries for WT and each anchor away mutant were then prepared as described by Zhang et al. [2012] and sequenced using paired-end sequencing on an Illumina NextSeq 500 with read length of 75 bp. Data analysis is described in the Supplemental Material.

NET-seq

NET-seq libraries were prepared as described in Churchman and Weissman (2011) for three biological replicates for each anchor away mutant (unlabeled state otherwise) and three biological replicates and three technical replicates for WT. A comparison of replicates is in Supplemental Figure S7. As previously described, we implemented a 1:10 S. pombe spike-in, which contained a Flag-tagged Rpd3 subunit (JY7741), to normalize reads (Feldman and Peterson 2019; Topal et al. 2019). Overnight cultures were diluted to OD 0.05 in 1 L of YPD unless stated otherwise. Once cultures reached OD 0.45, cells were treated for 1 h with rapamycin (final concentration 8 µg/mL). S. pombe was added, and cells were collected. RNA Pol II IP, RNA purification, and library construction were carried out as described (Churchman and Weissman 2011); 3′end sequencing was performed on an Illumina NextSeq 500 with a read length of 75 bp. Detailed analysis is described in the Supplemental Material.

TT-seq

TT-seq was performed as described [Schwalb et al. 2016] with an ERCC spike-in (three unlabeled and three 4sU-labeled) for normalization (Topal et al. 2019). Two biological replicates for each strain were grown to 1.5 x 10⁶ cells and labeled with 2.5 mM 4-thiouracil (4su) (Sigma-Aldrich) for 10 min. Cells were harvested by centrifugation at 3000g for 2 min and RNA was extracted by hot acid phenol. RNA was fragmented to <1.5 kb, which contained a Flag-tagged Rpd3 subunit (JY7741), and sequenced using paired-end sequencing on an Illumina NextSeq 500 with read length of 75 bp. Library preparation and sequencing were completed as described for RNA-seq. TT-seq data were processed and analyzed as described in the Supplemental Material.

Western blot and flow cytometry are described in the Supplemental Material.

Competing interest statement

The authors declare no competing interests.

Acknowledgments

We thank Jessica Feldman (University of Massachusetts Medical School), Salih Topal (University of Massachusetts Medical School), and Ozean Aydemir (University of Massachusetts Medical School) for help with bioinformatics analyses, and other members of the Peterson laboratory for helpful discussions. This work was supported by a grant from the National Institutes of Health (R35-GM122519) to C.L.P., and a University of Massachusetts Medical School General Medical Sciences Medical Scientist Training Program Training Grant (MSTP T32GM107000) to A.R.B. Author contributions: A.R.B. and C.L.P. designed the experiments. A.R.B. performed all experiments and analyzed the data. A.R.B. and C.L.P. wrote and edited the manuscript.
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