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Nuclear decay factors crack up mRNA

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In this issue of Molecular Cell, Bresson et al. describe a general function for the nuclear RNA decay factors Nab3 and Mtr4 in reshaping the coding transcriptome during glucose starvation in budding yeast. This places nuclear mRNA metabolism as an important contributor of gene expression regulation.

Cell survival depends on the capacity to effectively adapt gene expression programs to changing environmental conditions. The prime driver of such responses is thought to be adjusting transcription levels, but post-transcriptional processes, like alternative splicing and polyadenylation, or mechanisms altering mRNA availability in the cytoplasm, have also been implicated (Fig. 1, left) (Rabani et al., 2014). In eukaryotes, the bulk of mRNA decay occurs in the cytoplasm, whereas nuclear decay systems appear primarily engaged with non-coding (nc) transcripts and RNA surveillance. The study by Bresson and colleagues challenges this view by suggesting that nuclear RNA decay factors are important regulators of rapid changes in mRNA expression during glucose starvation in the budding yeast *Saccharomyces cerevisiae*.

The authors focus on two proteins: Nab3 and Mtr4, which are components of the Nrd1-Nab3-Sen1 (NNS) and Trf4-Air2-Mtr4-polyadenylation (TRAMP) complexes, respectively. Both complexes are co-factors of the nuclear ribonucleolytic RNA exosome complex and, among various substrates, act on short 200-600 nt RNA polymerase II (RNAPII)-transcribed ncRNAs such as Cryptic Unstable Transcripts (CUTs) and sn/snoRNA (Porrua and Libri, 2015). The NNS complex triggers transcription termination and facilitates the rapid decay of terminated transcripts via interaction with the TRAMP complex, comprising helicase and non-processive polyadenylation activities, which in-turn stimulates RNA decay by the exosome. NNS complex recruitment to RNA occurs via binding of the Nrd1 and Nab3 proteins to short sequence motifs as well as by an interaction of Nrd1 with the Ser5-phosphorylated (Ser5p) C-terminal domain of RNAPII. Target discrimination is achieved by this Ser5p modification, which is prevalent early during transcription (Porrua and Libri, 2015), and the density of NNS complex binding sites, which are generally less frequent in mRNA than ncRNA (Cakiroglu et al., 2016). Despite the indisputable engagement with ncRNA, genome-wide ChIP and PAR-CLIP studies have revealed frequent Nrd1 and Nab3 binding at protein-coding

genes (Kim et al., 2010; Jamonnak et al., 2011; Webb et al., 2014). Yet, during normal growth the consequence of such recruitment has remained elusive as only a restricted set of coding transcripts is affected by NNS complex inactivation. In these cases, NNS complex binding is often observed in promoter proximal regions eliciting downregulation of mRNA production via premature transcription termination, so-called attenuation.

Interestingly, the NNS complex appears to play a specific role in reprogramming gene expression during glucose starvation; the so-called diauxic shift. Here, the NNS complex has been shown to enforce repression of some growth-related genes possibly via its function in transcription termination (Darby et al., 2012). With this knowledge, Bresson et al. used the CRAC technique, a variant of CLIP, to monitor genome-wide changes in recruitment of RNAPII, Nab3 and Mtr4 during the diauxic shift. In agreement with previous studies showing a rapid and global transcriptional response (Galdieri et al., 2010), CRAC also revealed dramatic changes in RNAPII binding to mRNA, including examples of NNS-directed transcription attenuation. Remarkable, however, Bresson et al. observed many cases of striking changes in mRNA binding by Nab3 and Mtr4, that were not attributable to changes in transcription levels (Figure 1, right). That is, transcriptionally repressed mRNAs showed a relatively increased association with Nab3 and/or Mtr4. Curiously, in these cases, Nab3 and Mtr4 binding redistributed from their usual 5'end proximal localizations during normal growth conditions to more distal positions upon glucose starvation. However, RNAPII-occupancy was not reduced downstream of these new Nab3 binding sites, indicating that transcription termination was not triggered. At the same time, sequenced Mtr4-bound RNA fragments derived from gene bodies were often associated with non-templated 3'end adenylation, suggesting TRAMP activity and exosome-mediated degradation of these mRNA. In contrast to these repressed genes, many transcriptionally induced mRNA showed reduced binding to Nab3, Mtr4 or both. Taken together, this suggests that the NNS and TRAMP complexes strengthen the transcriptional response by enhancing or reducing, respectively, the decay of transcriptionally regulated transcripts.

How changed recruitment of Nab3 and Mtr4 during the diauxic shift might work mechanistically remains to be elucidated. Nab3 sequence motifs are presumably indistinguishable during normal growth and after nutrient-depletion, wherefore its differential recruitment might be determined by trans-acting factors. In this respect, it is interesting that Nab3's direct interaction partner Nrd1 is dephosphorylated during nutrient downshift, which may be relevant for altered transcript targeting (Darby et al., 2012). It is also possible that the severe global downregulation of transcript levels liberates a considerable fraction of Nrd1/Nab3 proteins, enabling their recruitment to sites that are

not utilized under normal conditions. However, this does not readily explain the differential binding observed to transcriptionally induced vs. repressed targets (Figure 1, right), which may instead be due to high mRNA production locally exhausting decay factor availability, causing their relatively decreased binding at transcriptionally induced genes. A final possibility is that changes in RNAPII modification, kinetics or chromatin modifications accompanying the transcriptional response serve as signal(s) for NNS/TRAMP complex recruitment.

What are then the consequence(s) of the observed alterations in Nab3/Mtr4 binding to mRNA? Bresson et al. speculate that levels of NNS and TRAMP complex recruitment determine the extent of nuclear decay by the exosome. While this is likely, it remains to be shown experimentally whether mRNAs with enhanced and reduced Nab3 and Mtr4 CRAC signals have higher and lower decay rates, respectively. NNS-linked RNA decay is typically occurring after NNS-triggered transcription termination thereby avoiding canonical 3'end polyadenylation. However, increased NNS binding at distal sites, induced by the diauxic shift, does not seem to cause transcription termination, suggesting termination-independent exosome recruitment. Such post-transcriptional function of Nrd1 was previously suggested to explain the removal of mature snoRNA and tRNA during glucose-starvation (Jamonnak et al., 2011). Perhaps a sufficiently strong NNS-TRAMP binding at protein-coding RNAs would aid the RNA exosome outcompete the polyadenylation machinery. An alternative, but radically different, possibility is that NNS and Mtr4 binding reflects RNA association without consequential decay. In line with this idea, Nrd1 was shown to accumulate in subnuclear foci upon glucose depletion and such foci could serve to store temporarily unneeded RNA, akin to cytoplasmic P-bodies or stress granules (Darby et al., 2012).

Regardless of these issues, the study by Bresson et al. constitutes a pioneering report implying a role for nuclear RNA decay factors in modulating mRNA levels during a dynamic gene expression program. Employment of nuclear decay factors for tuning of gene expression may be used by cells as a means to reinforce transcriptional responses acting prior to any cytoplasmic regulation. Whether nuclear RNA decay factors are of general importance during rapid biological transitions and to what extent this is relevant in higher eukaryotes are important future questions.

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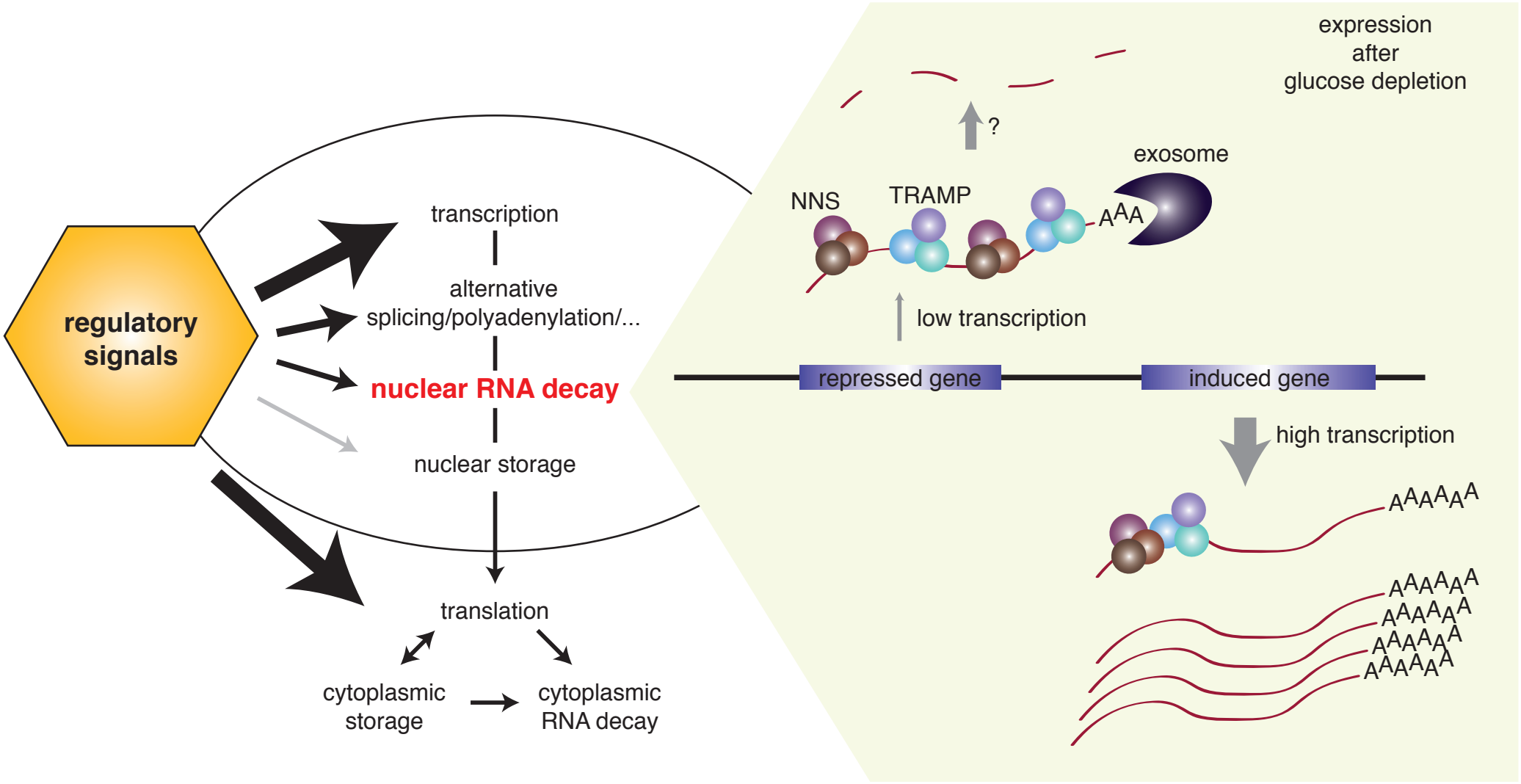
Figure 1

Regulated steps during re-programming of gene expression (left). Bresson et al find relatively increased binding of NNS and TRAMP complexes to mRNA from transcriptionally repressed genes, which may lead to nuclear RNA decay and reciprocally decreased binding to mRNA from transcriptionally induced genes (right). See text for details.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