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Transcriptional ShortCUTs

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Recent work from Kuehner and Brow (2008) and Thiebaut et al. (2008) in *Molecular Cell* and Jenks et al. (2008) in *Molecular and Cellular Biology* reveals that regulated expression of central nucleotide synthesis pathway components directs start site-dependent RNA polymerase II termination.

Transcription by RNA polymerase II (Pol II) comprises cycles of initiation, elongation, and termination. Although RNA synthesis is controlled in many instances at the step of initiation at gene promoters, post-initiation events also contribute essential layers of regulation. A striking example of this is the recent discovery of hundreds of cryptic unstable transcripts (CUTs) that are transcribed by Pol II in yeast (Wyers et al., 2005). CUTs are frequently derived from intergenic regions and normally do not accumulate in significant amounts at steady state. Notably, the mode of termination associated with CUT transcription is a major determinant for their instability.

Two major pathways are known to end processive RNA synthesis by Pol II in yeast (reviewed in Lykke-Andersen and Jensen, 2007). In both cases, the interaction of termination factors with both Pol II and the nascent RNA are important, but distinct classes of transcripts are affected. One pathway produces mostly protein-encoding mRNA, is guided by poly(A) signals, and relies on the canonical pre-mRNA 3′ end formation machinery. The other pathway acts predominately on small nucleolar RNAs (snoRNAs) and CUTs and depends on the Nrd1 complex, which is constituted by the RNA-binding proteins Nrd1 and Nab3 and the helicase Sen1. Interestingly, transcripts terminated via the Nrd1 complex are frequently targeted by the nuclear exosome, a 3′–5′ exonuclease complex. In this way snoRNAs receive some 3′ end trimming, whereas CUTs will be efficiently degraded (Thiebaut et al., 2006; Arigo et al., 2006).

Recent reports by Kuehner and Brow (2008), Thiebaut et al. (2008), and Jenks et al. (2008) established an intriguing concept of transcriptional regulation that integrates essential aspects of initiation and termination in yeast. The Brow and Reines labs dissected the mechanisms governing *IMD2* expression. This gene encodes the inosine monophosphate dehydrogenase (IMPDH) that catalyzes the first step in GMP biosynthesis. Its expression is inversely correlated with the availability of GTP, but how the cell senses nucleotide levels remained obscure. Remarkably, *IMD2* transcripts originate from alternative start sites under control of a single upstream promoter (TATA box; Figure 1A). Kuehner and Brow (2008) demonstrate that RNA obtained in the presence of ample GTP started with guanosine (G) at the first position, whereas reduced levels of GTP gave RNAs starting more distal to the promoter with adenosine (A). This finding nicely fits the previous proposal by the Brow lab that Pol II scans along the *IMD2* promoter and that sequence context as well as GTP concentration constitutes a switch that eventually determines the start site (Steinmetz et al., 2006). The G-site-dependent RNAs contain a Sen1-dependent terminator; consequently, these transcripts are rapidly degraded by the exosome and represent bona fide CUTs. Remarkably, the terminator is excluded when Pol II scanning favors the A-site to start RNA synthesis under low GTP conditions. This facilitates transcription of the entire *IMD2* gene, poly(A)-dependent termination, and production of functional mRNA. Thus, the GTP-dependent selection of the start site regulates the mode of Pol II termination and, with it, the fate of *IMD2*-associated transcripts.

Is this control mechanism of more general relevance? Certain aspects are, e.g., the architecture of the regulatory region: the *URA2* gene encodes an enzyme involved in pyrimidine biosynthesis that is regulated by uracil availability, and Thiebaut et al. (2008) show that a single promoter (TATA box) controls *URA2* start sites, which are separated by a Nrd1 dependent terminator (Figure 1B). In contrast to *IMD2*, however, the authors observed constitutive expression of the upstream CUT independent of uracil levels, whereas *URA2* mRNA transcription was strongly stimulated by low uracil. The identity of the starting nucleotide plays no discernable role in start site choice, arguing against UTP-dependent Pol II initiation. Moreover, Thiebaut et al. (2008) show that reduced CUT expression is not a prerequisite for mRNA transcription. Start site-dependent termination at the *URA2* locus therefore does not direct the mutually exclusive production of either CUTs or mRNA as was observed for *IMD2*. Libri and colleagues suggest instead that the *URA2* CUT might modulate the transcriptional response to reduced nucleotide levels. Activation requires that some scanning Pol II molecules skip the CUT start site and initiate transcription downstream of the terminator. Thiebaut et al. (2008) identified the R box, a T-rich regulatory element, as an essential component of this process. The mechanistic details remain unclear, but the R box might enhance skipping of the CUT site or otherwise promote the usage of the mRNA start site. Although several possible scenarios for activation could be discussed, an important piece of the *URA2* regulation puzzle is still missing: the mechanism of metabolite sensing. Thiebaut et al. (2008) point out, however, that a major function of the *URA2* CUT could be to maintain low basal levels of *URA2* mRNA transcription in the presence of ample uracil by "trapping" initiated Pol II in a cycle of unproductive transcription. Low levels of uracil might interrupt this cycle to facilitate a rapid transcriptional induction of the *URA2* mRNA.

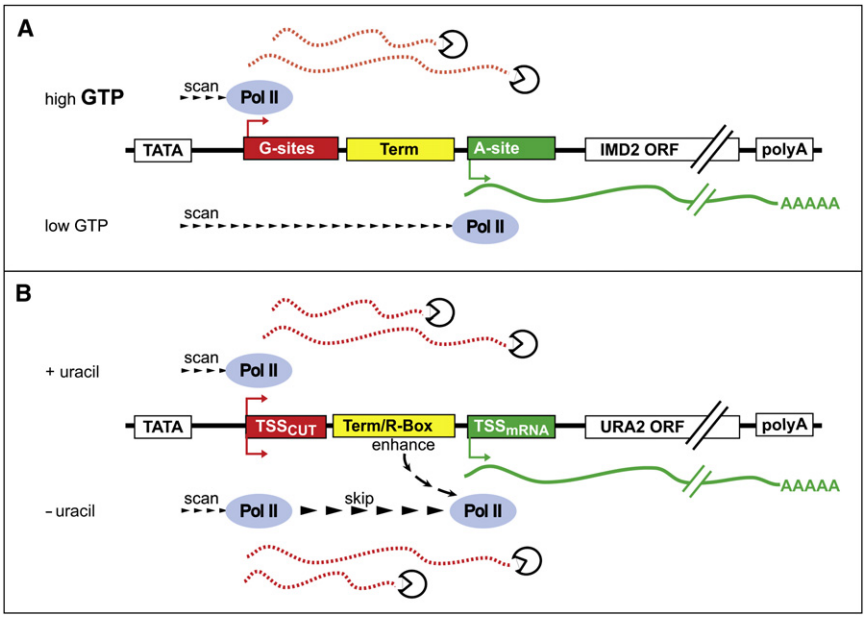

Figure 1. Regulation of *IMD2* and *URA2* Genes Involved in Nucleotide Biosynthesis

Schematic representation (not in scale) of regulatory elements involved in start site-dependent transcriptional termination. Both *IMD2* (A) and *URA2* (B) genes display a similar organization. Alternative transcription start sites, which are under control of a single promoter (TATA), are separated by a transcriptional terminator that is recognized by the Nrd1 complex. GTP levels regulate start selection by scanning Pol II at upstream G sites or a downstream A site of the *IMD2* gene. This results in the synthesis of CUTs that are rapidly degraded by the exosome or of functional mRNA, respectively. By contrast, CUT transcription is constitutive at *URA2*, i.e., independent of uracil level. *URA2* mRNA transcription, however, is dependent on low uracil, CUT transcription, and the R box, a T-rich sequence that is associated with the terminator.

Notably, upstream CUTs are associated with several other genes (*URA8*, *ADE12*, and *IMD3*) involved in nucleotide biosynthesis (Thiebaut et al., 2008), suggesting regulatory mechanisms similar to the examples discussed above. Transcription attenuation, i.e., regulated termination and antitermination, is a widespread strategy to control gene expression in bacteria (Henkin and Yanofsky, 2002). The papers discussed here might point to a prevalence of attenuation also in eukaryotic cells. Start site-dependent Pol II transcription termination is closely associated with CUT metabolism. Considering the central role of the nascent transcript during termination, CUTs could be viewed as RNA elements mediating Pol II termination rather than simply being an "undesired" product of the transcriptional process. Attenuation, therefore, extends the growing functional repertoire of CUTs and CUT transcription, respectively.

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