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Transitions for Regulating Early Transcription

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Gene expression is largely regulated during the initiation of RNA polymerase II (PolII) transcription. In this issue, Kouzine et al. show that control of DNA melting is one of the critical rate-limiting steps for productive mRNA elongation. We discuss these findings in the context of other key energetic transitions.

In higher eukaryotic cells, the transcription machinery undergoes at least five major transitions before productive mRNA elongation occurs (Cheung and Cramer, 2012; Fuda et al., 2009). RNA polymerase II (PolII) is first recruited to promoter DNA and assembles with general transcription factors into a stable closed promoter complex (Figure 1). Next, DNA is melted to form an open promoter complex (DNA melting). The polymerase subsequently synthesizes and releases short RNAs (abortive transcription). When PolII overcomes the abortive phase, it escapes from the promoter but may pause soon thereafter at a promoter-proximal location (promoter escape and polymerase pausing). Release of paused PolII (pause release) finally leads to productive mRNA elongation. Separating these intermediary complexes are energy barriers that must be overcome. Transcriptional regulators may increase or decrease the height of one or more energy barriers, and this may lead to repression or activation, respectively. An activator may lower a barrier in the same way that a catalyst lowers the energy of a transition state in a chemical reaction, and a lowering of the height of all major energy barriers may be required to achieve high levels of transcription. In this issue, Kouzine et al. (2013) reveal that the control of DNA melting is a previously underappreciated point of transcriptional regulation.

In bacterial cells, it has long been known that there are two major barriers to overcome during transcription initiation that depend on the stability of the closed promoter complex and on the rate of promoter DNA melting (Gill et al., 1990). Eukaryotic transcription regulation also occurs when polymerase is recruited during closed complex formation (Ptashne and Gann, 1997). In addition, eukaryotic transcription can be regulated during pause release (Adelman and Lis, 2012). However, whether other barriers in eukaryotic transcription initiation such as DNA melting are targeted for regulation has remained unclear.

Kouzine et al. now examine the role of DNA melting using a well-established cellular system, the activation of resting lymphocytes, which is accompanied by a >10-fold increase in mRNA production. In a first experiment, the authors employ chromatin immunoprecipitation coupled to DNA sequencing to show that PolII occupancy over the genome increases only slightly when cells get activated. In resting cells, about 90% of genes that are involved in lymphocyte activation are preloaded with PolII but exhibit low levels of transcription. Thus, the 10-fold increase in transcription is not due to polymerase recruitment.

The authors assumed that polymerase is recruited to genes in resting cells but that DNA is not melted and thus transcription does not start. To test this, the authors developed an assay to map

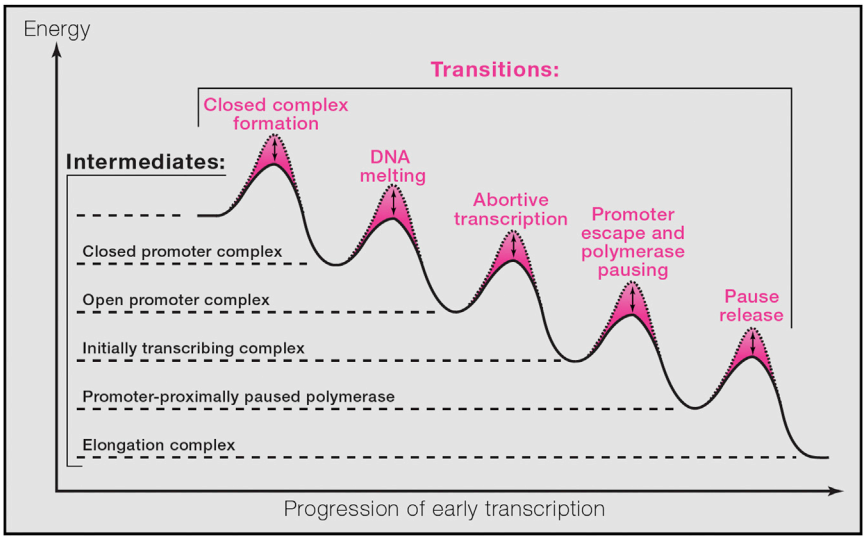

Figure 1. Early Transcription May Involve Five Major Transitions
Early eukaryotic transcription may be regulated at five transitions between polymerase complex intermediates. The horizontal axis describes progression of transcription, whereas the vertical axis depicts the energy of the intermediates. The energy barriers between intermediary states may be increased or decreased by regulators (double-headed arrows).

regions of single-stranded DNA genome wide (ssDNA-seq). The assay is based on potassium permanganate treatment that preferentially modifies DNA bases in ssDNA. The authors find that resting cells exhibit a low level of ssDNA but much higher levels upon activation. Because the increase in ssDNA signals is restricted to PolII genes, the authors investigated the role of the PolII transcription factor TFIIH, which melts DNA, and found that TFIIH levels increase upon activation. Lymphocyte activation also strongly increases phosphorylation of the PolII C-terminal domain (CTD) that depends on TFIIH.

The new study thus shows that transcription regulation can occur during DNA melting and not only during polymerase recruitment and pause release. The work suggests that there are more transitions during early transcription that are potentially targeted for regulation. In the future, we should therefore define all transitions that are targeted for transcription regulation. It may then be investigated how coactivators of transcription such as the Mediator complex influence the transitions.

Transcription regulation not only occurs during early transcription. It also involves alterations in chromatin structure (Henikoff, 2008; Smolle and Workman, 2013) and depends on the genomic context of genes and antisense activity (Wei et al., 2011). Regulation also occurs during transcript elongation, as best studied in bacteria (Landick, 2006). Finally, processing of the nascent RNA may contribute to regulated transcription. Pause release may be coupled to 5′ RNA capping, and cotranscriptional mRNA splicing and 3′ end processing can feed back to transcription. The relative contributions of these diverse regulatory mechanisms to the overall output of gene transcription should also be investigated in the future.

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