Cell

Taking a Pause to Reflect on Morphogenesis

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Enhancers have been intensely studied as the sequences determining spatial and temporal gene expression during development. Lagha et al. now put the focus back on the promoter as the critical element coordinating gene expression across a cell population.

Following transcription initiation, RNA polymerase II (Pol II) can pause downstream from the promoter, where it requires additional activation signals before proceeding into productive elongation. Although this promoter-proximal pausing was initially thought to be a peculiarity of just a handful of genes, recent studies have shown that it is a major regulatory mechanism associated with transcriptional activation of 30%–50% of genes (Adelman and Lis, 2012). Previously, Levine and colleagues showed that genes with paused polymerase exhibit synchronous activation in the *Drosophila* embryo, in contrast to the stochastic activation of nonpaused genes (Boettiger and Levine, 2009). However, questions remained as to whether these different modes of activation are important for developmental processes and how pausing is established. In this issue, by uncoupling pausing at promoters from other gene sequences, Lagha et al. (2013) show that loss of synchronous gene expression in the absence of paused polymerase leads to morphogenesis defects during embryogenesis.

The authors employ quantitative nascent transcript imaging methods to measure transcriptional activation of different genes in the *Drosophila* embryo and employ BAC recombineering to manipulate sequence elements. They find that replacement of a short promoter sequence at a nonpaused gene with the equivalent region from a paused gene (~200 bp centered around the transcriptional start site, +1) is sufficient to establish paused polymerase. In addition, this replacement alters the mode of reporter activation from slow and stochastic to rapid and synchronous. This synchronous reporter activation is disrupted when the levels of known pausing factors are reduced, providing further evidence that the heterologous pause is required for synchrony. The reciprocal promoter exchange has the opposite effect in that reduced pausing promotes stochastic activation. Therefore, the promoter, and not the enhancer, confers the level of promoter-associated Pol II and the synchrony of gene activation.

Having ascertained that minimal promoter sequences can recapitulate paused Pol II levels of the endogenous gene, the authors identify a spectrum of promoters with varying degrees of pausing in the different tissues of the embryo. One highly paused gene that shows rapid, synchronous activation is *snail* (*sna*), which encodes a transcriptional repressor that is expressed in the presumptive mesoderm of the early *Drosophila* embryo. Shortly after *sna* transcriptional activation, these mesodermal cells undergo coordinated invagination during gastrulation. Given the conserved role of Sna family proteins in mediating epithelial-mesenchymal transitions during development, the authors select the *sna* gene to determine whether altering activation timing generates phenotypes. Following BAC recombineering to replace the promoter sequences in the *sna* gene, the ability of the transgenes to rescue mesoderm morphogenesis defects in *sna* mutant embryos is tested. The selected replacement promoters have reduced or no pausing but remain capable of directing transcription rates similar to that of the *sna* promoter within a given cell. The *sna* BAC transgene carrying the endogenous highly paused *sna* promoter directs robust invagination of the mesoderm in *sna* mutant embryos, as expected. However, the *sna* transgenes containing promoters with reduced pausing show defects in *sna* activation and cell invagination (Figure 1). The defects apparent with weak pausing are exacerbated in the absence of pausing. A mathematical model developed by the authors explains the different gastrulation phenotypes, revealing the time to synchrony of expression as a key parameter, in addition to other features such as *sna* autoregulation.

It is clear that high pausing leads to synchronous activation, but what does "high pausing" actually mean? Pausing levels are the average density of promoter polymerases at a particular gene across a large population of cells. Typically, one paused polymerase occupies each uninduced gene promoter (Lis, 1998); therefore, higher pausing most likely reflects more cells with promoter-paused polymerase, which ultimately results in more cells with activated transcription. Paused Pol II may increase the probability of activation by occluding promoter nucleosomes (Adelman and Lis, 2012), as the chance of activation will be far greater when the need for chromatin remodeling is bypassed. Alternatively, the insulator function of paused polymerase (Chopra et al., 2009) could help to direct enhancer interactions with the desired promoter through the formation of a higher-order chromatin structure. This could increase a promoter's probability of activation and could prevent promiscuous, nonproductive enhancer-promoter interactions (Chopra et al., 2009). However, Lagha

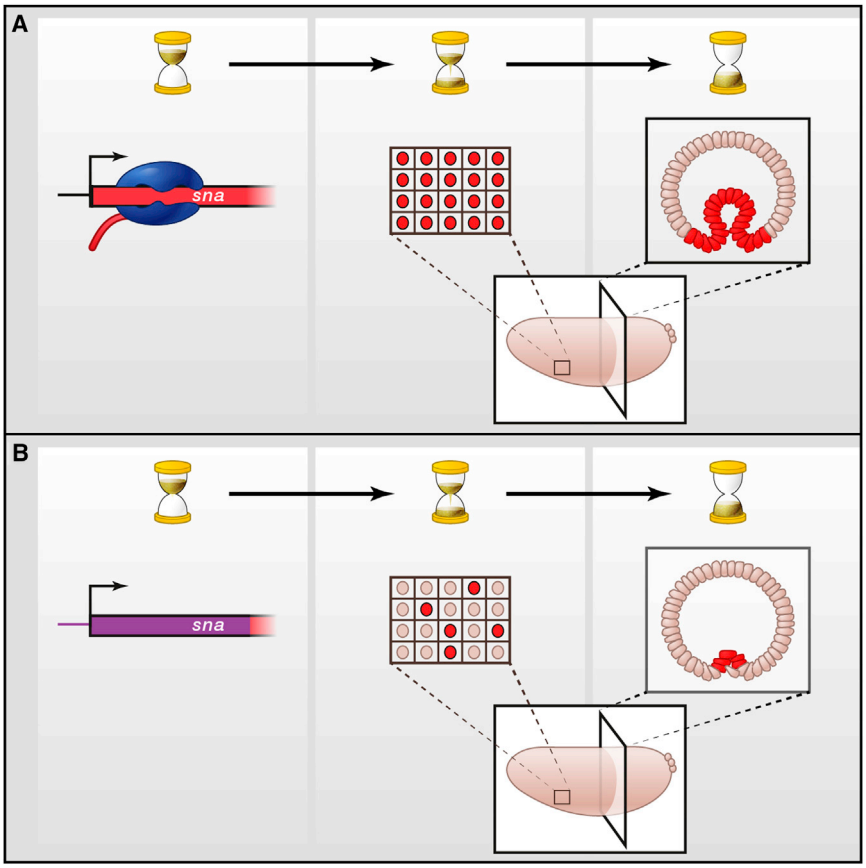

Figure 1. Promoter-Proximal Pausing Coordinates Cell Behavior
(A) Promoter-proximal pausing promotes synchronous gene activation across a population of cells within the short time frame that is required for developmental processes such as mesodermal invagination in the *Drosophila* embryo.
(B) In the absence of pausing, gene activation occurs in a more stochastic manner so that, within a given time frame, only a few cells have activated transcription, leading to developmental defects.

et al. (2013) suggest a trade-off between the ability to activate genes in all cells versus the total levels of mRNA produced in those cells, as above a threshold, higher pausing results in weaker expression. It would seem that genes need to establish a balance between promoter elements that are strong enough to establish promoter-proximal pausing in most cells but are still weak enough to allow efficient escape from the pause.

A next step will be to further refine the exact sequences that dictate the pausing level of promoters used by Lagha et al. (2013) and to then manipulate pausing levels on the same promoter. Will removal of the pausing sequence motif, the "pause button," single handedly disrupt synchrony, or will additional sequences also be important? Other types of promoters could also be studied with respect to synchrony versus stochasticity. For example, some genes feature a localized pause in which the polymerase consistently resides at around +40, whereas others have more dispersed pausing in which the polymerase can sit anywhere up to around +100 (Kwak et al., 2013). Does the exact location of the paused polymerase influence synchrony? Furthermore, pausing can also be observed on genes that are regulated by Pol II recruitment (Saunders et al., 2013). Therefore, it will be interesting to take promoters from genes with equivalent pause levels but whose main point of regulation is through Pol II recruitment versus pause release to determine whether regulation by these different mechanisms affects synchrony. Similar strategies to those employed by Lagha et al. (2013) for modifying pausing on genes will also be useful to test other features associated with pausing. For example, disruption of pausing can delay gene repression (Ghosh et al., 2011); does the absence of a pause favor stochastic repression? Furthermore, it was recently shown that transiently inactivated genes tend to maintain promoter-associated polymerases upon gene repression, whereas silenced genes that do not reactivate transcription lose them (Saunders et al., 2013). Whether a transiently repressed gene will be able to reactivate transcription at a later stage in development with a nonpaused promoter can now be addressed.

The most highly paused gene examined by Lagha et al. (2013), *tailup*, encodes a transcription factor activated by BMP signaling in the dorsal ectoderm, whereas the gene at the opposite end of the pausing spectrum, *pannier*, is a second BMP target, again encoding a transcription factor. What might be the advantage of having rapid versus slow activation of these two transcription factors in the dorsal ectoderm? Perhaps the temporal order of transcription factor activation in the dorsal ectoderm is key to cell fate specification, similar to the bursts of transcription factor activity regulating neuronal identities during postembryonic neurogenesis in *Drosophila* (Maurange et al., 2008). Would synchronous expression of normally stochastic genes, such as *pannier*, disrupt development in the same way that stochastic expression of synchronous genes does? The evidence would suggest so. Though synchrony is a clear advantage for certain developmental processes, there are some situations that favor more stochastic events. Examples include cell fate choices within the mouse embryo inner cell mass, the diversity of receptor expression across sensory neurons, and the ability of cell populations to adapt to environmental situations (Eldar and Elowitz, 2010). Overall, despite a perception for the last couple of decades that the promoter was subordinate to the enhancer in terms of regulating temporal gene expression, the findings of Lagha et al. (2013) put the promoter back in the spotlight as a key regulatory element in coordinating gene expression during development.

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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