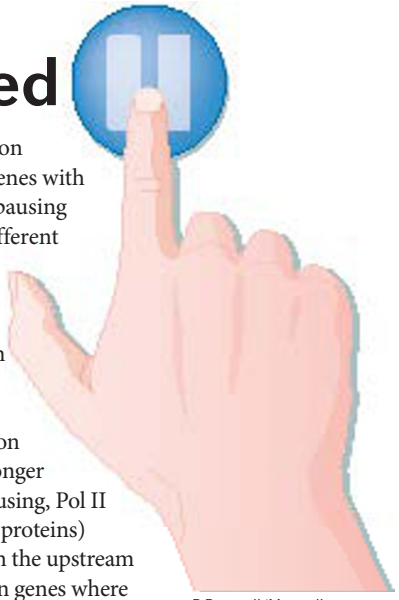
Nature Reviews Molecular Cell Biology | Published online 31 May 2017; doi:10.1038/nrm.2017.57

TRANSCRIPTION

Paused means poised



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RNA polymerase II (Pol II) assembles with basal transcription factors into the transcription pre-initiation complex (PIC) on active promoters. Following transcription initiation, Pol II pauses 30-50 bp downstream of the transcription start site (TSS), and requires further activation to proceed to productive transcription elongation. Some promoters have a greater tendency for Pol II pausing; these promoters better mediate transcriptional responses to developmental or environmental cues, but the relationship between Pol II pausing and gene expression dynamics remains unclear.

A major technical challenge in studying transcription dynamics is that Pol II is not readily detected as part of the PIC *in vivo*. To tackle this, Shao & Zeitlinger used chromatin immunoprecipitation (ChIP)-nexus, which is a high resolution ChIP–sequencing and exonuclease digestion protocol. Using ChIP-nexus in fruit fly cells, they were able to pinpoint the genomic positions of Pol II and other PIC proteins relative to the TSS. The average Pol II footprint (location) was 29 bp downstream of the TSS, but when the cells were treated with the transcription initiation inhibitor triptolide, the footprint mostly shifted to 19 bp upstream of the TSS, in accordance with the pre-initiation location of other PIC proteins. Thus, ChIP-nexus could detect and distinguish between paused Poll II and Pol II at transcription initiation sites.

Next, the authors measured the duration of Pol II pausing genome-wide following triptolide treatment. Pausing half-lives at most genes were measured in minutes, and at some genes Pol II remained paused for almost an hour. Genes with longer pause durations tended to have lower steady-state expression levels. In addition, genes with different degrees of pausing were enriched for different promoter elements.

An inverse correlation was found between the duration of Pol II pausing and Pol II occupancy at transcription initiation sites: in genes with longer duration of Pol II pausing, Pol II (as well as some PIC proteins) were less abundant in the upstream initiation sites than in genes where pausing was of a shorter duration. Importantly, treating the cells with inhibitors of pause–release resulted in a considerable decrease of Pol II at transcription initiation sites. These results indicate that paused Pol II blocks reinitiation of transcription. Other data indicated that pausing-mediated interference with transcription initiation might be caused by differential occupancy of basal transcription factors at promoters.

In summary, Pol II pausing can be persistent and can inhibit transcription reinitiation. This can be associated with genes that have lower steady-state expression levels, such as those that are responsive to external cues. Thus, Pol II pausing at these genes could prevent transcription in the absence of stimuli while simultaneously maintaining the promoters in a poised state for signal-induced activation.

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> Pol II pausing can be persistent and can inhibit transcription reinitiation

ORIGINAL ARTICLE Shao, W. & Zeitlinger, J. Paused RNA polymerase II inhibits new transcriptional initiation. *Nat Genet* http://dx.doi.org/10.1038/ng.3867 (2017)

FURTHER READING Jonkers, I. & Lis, J.T. Getting up to speed with transcription elongation by RNA polymerase II. *Nat. Rev. Mol. Cell Biol.* **16**, 167–177 (2015)