GENE EXPRESSION

More roles and details for polymerase pausing

RNA polymerase II (Pol II) pausing is a widespread mechanism of metazoan gene regulation. Two new studies have proposed a revised model for its overall cellular role and have provided intriguing molecular insights.

The prevailing view of the role of Pol II pausing is that pausing poises genes that are targets of stimulus–response signal transduction pathways for a rapid transcriptional response. To characterize the genes that are regulated by pausing globally, Gilchrist *et al.* analysed genome-wide data sets from *Drosophila melanogaster* cells, including Pol II genome occupancy data and gene expression changes caused by knockdown of the negative elongation factor (NELF) pausing complex. Focusing on genes involved in the well-characterized immune response to lipopolysaccharide (LPS), they found that it was primarily the genes encoding the signal transduction components of this pathway, rather than highly induced transcriptional targets of the pathway, that were subjected to regulation by Pol II pausing.

These observations led the authors to propose that a major role of Pol II pausing may be to set the basal expression levels of components of stimulus–response pathways to allow an appropriate signalling amplitude following stimulation. In support of this, NELF depletion attenuated the induction of both paused and non-paused target genes after LPS challenge, but induction of target genes could be restored if the signalling cascade was bypassed (using a constitutively active form of nuclear factor κ B).

As evidence that Pol II pausing may be relevant for signal transduction in other contexts, Gilchrist *et al.* showed that paused Pol II was enriched in multiple genes encoding signalling components of the JNK and JAK–STAT signalling pathways in *D. melanogaster* and self-renewal pathways in mouse embryonic stem cells.

A separate study focused on heat shock protein 90 (HSP90). This molecular chaperone is thought to function principally by regulating protein folding and assembly, but various roles for HSP90 in transcriptional regulation have been proposed. To investigate these transcriptional roles, Sawarkar *et al.* characterized the genome-wide occupancy of HSP90 in *D. melanogaster* cells and found enrichment at sites of Pol II pausing, including at sites of intergenic or microRNA transcription.

Small-molecule inhibitors of HSP90 resulted in HSP90-bound loci producing more full-length transcripts and harbouring elongating phospho-forms of Pol II, thus providing evidence that HSP90 is functionally involved in Pol II pausing. Furthermore, various experiments showed physical and functional interactions between HSP90 and the NELF complex, including studies *in vivo* in *D. melanogaster* and in mouse and human cells.

Demonstrating the physiological relevance of HSP90 in Pol II pausing, Sawarkar *et al.* found that HSP90 inhibition impaired the LPS-mediated target gene induction in *D. melanogaster* cells and mouse cells as well as ecdysone-induced pupation in *D. melanogaster*.

Given that Pol II pausing seems to have roles both in the immediate regulation of target gene expression and in the longer-term regulation of signal transduction protein levels, it will be interesting to see whether timecourses of HSP90 inhibition will allow the relative importance of these roles to be dissected.

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ORIGINAL RESEARCH PAPERS Gilchrist, D. A. *et al.* Regulating the regulators: the pervasive effects of Pol II pausing on stimulus-responsive gene networks. *Genes Dev.* **26**, 933–944 (2012) | Sawarkar, R., Sievers, C. & Paro, R. Hsp90 globally targets paused RNA polymerase to regulate gene expression in response to environmental stimuli. *Cell* **149**, 807–818 (2012)

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