TRANSCRIPTIONAL REGULATION

Plant promoter-proximal pausing?

A study of a synthetic auxin response circuit in a heterologous system suggests that hindrance of Mediator complex function by the co-repressor TOPLESS may represent a form of promoter pausing, a mechanism that has not been described in plants before.

Nicholas Morffy and Lucia C. Strader

Promoter pausing is a critical transcriptional regulatory mechanism found in most eukaryotes[1]. Regulating transcriptional elongation via promoter pausing, as opposed to transcriptional initiation, is thought to tune expression levels, coordinate transcript abundance across loci and maintain the potential of gene expression at loci that are not inactive[1]. To date, promoter-pausing systems have been discovered in mammals, flies and fission yeast. However, these have yet to be described in members of the plant lineage[1]. In a recent study published in *eLife*, Leydon and colleagues analysed the mechanism by which the transcriptional co-repressor TOPLESS (TPL) can modulate the expression of target genes, using a synthetic auxin response circuit in yeast[2]. We propose that the authors have discovered a new transcriptional repression mechanism that may provide a regulatory system similar to promoter pausing in plants, allowing for rapid transcriptional initiation following the addition of an important signalling molecule. In this system, the transcriptional co-repressor TPL prevents activity of the Mediator complex, keeping the auxin transcriptional machinery poised for rapid activity.

Auxin signalling proceeds through a relief of repression model. Transcription factors called the AUXIN RESPONSE FACTORS (ARFs) bind DNA and interact with the Auxin/INDOLE-3-ACETIC-ACID (Aux/IAA) proteins, which in turn recruit a class of co-repressors called TPL and TPL-RELATED (TPR) proteins[3]. As auxin levels increase, the Aux/IAA proteins are degraded via the 26S proteasome, removing TPL from ARF-bound DNA and allowing for transcription of auxin-responsive genes.

TPL has been implicated in multiple signalling and developmental pathways and recruits histone deacetylases (HDACs) to generate a repressive chromatin state[4]. In the auxin pathway, TPL recruitment is necessary for Aux/IAA repression of ARF activity[5]. However, auxin treatment results in a rapid increase in responsive transcript abundance, often within 10 minutes of auxin application[6,7]. These rates of increased transcription are likely much faster than the current model for auxin signalling would suggest, as histone acetylation would need to precede active transcription.

Using the synthetic yeast auxin signalling module, Leydon et al. uncover a new mode of TPL repression that results from TPL interacting with components of the Mediator complex and reducing its activity. Interaction studies and directed mutations in both TPL and Mediator subunits result in elevated rates of auxin-induced transcription in this system[2]. These data suggest a model in which TPL prevents full Mediator activity without affecting its recruitment to auxin-responsive loci. Thus, in the presence of TPL, Mediator is present, but inactive. Upon auxin treatment, the

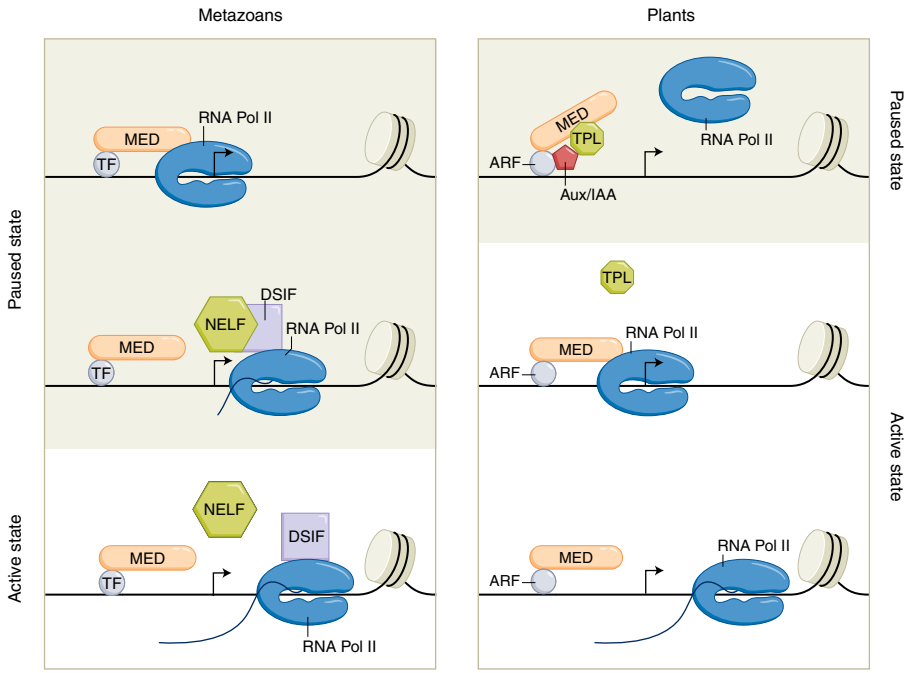

Fig. 1 | Models of promoter pausing in metazoans and plants. In metazoans, DNA-bound transcription factors (TF; light-blue circle) recruit Mediator (MED; orange), which, along with general transcription factors, recruit RNA Pol II (blue) to the transcription start site. At paused loci, transcriptional initiation proceeds with the DSIF complex (lilac square) and the NELF complex (green) bound to RNA Pol II. In the presence of a signal or other factor, the NELF complex is released from the DSIF–RNA Pol II complex and transcription enters the active elongation state[1]. In plants, the paused state is achieved by preventing stable recruitment of RNA Pol II to the transcription start site. DNA-bound ARFs (white) interact with the Aux/IAAs (red) that recruit TPL (green) and interact with components of the Mediator complex, preventing its function as shown by Leydon et al.[2]. When auxin levels increase, the Aux/IAA is degraded and TPL no longer associates with the ARF-bound locus, freeing Mediator to recruit RNA Pol II and initiate active transcription. Similar to promoter pausing in metazoans, the addition of a signal in this system marks the transition from the poised, paused state to the active transcriptional elongation state.

Aux/IAA and therefore TPL disappear from the assembly, allowing for the primed Mediator to recruit RNA polymerase II (RNA Pol II) to auxin-responsive loci and immediately initiate transcription.

The proposed mechanism of TPL repression at auxin-responsive loci would unify several pieces of evidence. First, the Aux/IAAs are generally short lived, with four-minute half-lives[8], which may make HDAC-driven changes to histones difficult to accomplish. Second, auxin-induced transcription proceeds rapidly following auxin application, likely too fast for the recruitment of histone acetylases and other chromatin modifiers.

This proposed model is parallel to promoter-proximal pausing systems found in metazoans and fission yeast. Whilst plants lack the negative elongation factor complex (NELF) homologues found in these other systems, TPL's hindrance of Mediator complex activity may play a similar role. In metazoans, transcriptional machinery components are located at or near the transcriptional start site and are paused by NELF and DRB-sensitivity inducing factor (DSIF). DSIF phosphorylation leads to loss of NELF, liberation of RNA Pol II and onset of transcript elongation[1] (Fig. 1). The plant-based TPL-facilitated system likewise allows for components of the transcriptional machinery to be partially assembled and poised for rapid transcriptional onset. In both systems, promoter pausing is critical for rapid, coordinated responses to environmental or signalling changes, or as a mechanism to ensure signal integration at specific loci.

If the mechanism described by Leydon et al. represents a form of promoter-proximal pausing, plants seemingly rely on a system in which swift recruitment of RNA Pol II, rather than the liberation of paused RNA Pol II, allows for rapid and coordinated transcriptional responses. In this model, auxin-responsive loci rely on TPL-driven repression by blocking Mediator interactions with transcription factors and RNA Pol II (Fig. 1). It remains to be seen if this mechanism is specific to auxin signalling in plants. It is also unclear how much of TPL's function is attributable to this specific repression pathway, as opposed to recruitment of HDACs or other interactions at genomic loci.

It should be noted that these findings of Leydon et al. were conducted primarily in a heterologous system with a mixture of plant and yeast proteins participating in this complex. It is possible that this model is the result of a missing factor in the synthetic yeast auxin signalling system, which would prevent this primed state from occurring. However, yeast two-hybrid experiments, in planta bimolecular fluorescence complementation experiments and cell-specific expression of TPL mutants do point to motif-dependent TPL–MED21 and TPL–MED10 interactions and repression of transcription, suggesting that these results will extend into systems comprised solely of plant proteins. Future work in plants will be needed to fully verify these results.

Overall, these results suggest a model in which plants, and possibly other eukaryotes that lack NELF proteins, such as *Saccharomyces cerevisiae*, rely on primed relief of repression instead of paused RNA Pol II to coordinate rapid transcriptional activation. For example, the Tup1 co-repressor, a homologue of TPL in *S. cerevisiae*, also interacts with members of the Mediator complex[9], raising the possibility that a similar mechanism could exist in yeast.

In plants, auxin signalling is not the only pathway that relies on TPL interactions — ethylene, strigolactone, jasmonate and abscisic acid signalling pathways also do[10]. A rapid activation of Mediator may also be critical for finely tuned responses in one or more of these pathways. The kinetics of TPL binding at hormone-responsive loci, and their ultimate relief of repression, may shed light on some of these questions and improve our understanding of rapid and coordinated transcriptional responses in plants. ❐

Nicholas Morffy[1,2] ✉ and Lucia C. Strader[1,2] ✉

[1]*Department of Biology, Duke University, Durham, NC, USA.* [2]*Center for Science and Engineering Living Systems (CSELS), Washington University, St. Louis, MO, USA.*

✉*e-mail: nicholas.morffy@duke.edu; lucia.strader@duke.edu*

Published online: 1 July 2021
https://doi.org/10.1038/s41477-021-00970-6

References

1. Core, L. & Adelman, K. *Genes Dev.* **33**, 960–982 (2019).
2. Leydon, A. R. et al. *eLife* **10**, e66739 (2021).
3. Morffy, N. & Strader, L. C. *Cold Spring Harb. Perspect. Biol.* https://doi.org/10.1101/cshperspect.a039883 (2021).
4. Long, J. A., Ohno, C., Smith, Z. R. & Meyerowitz, E. M. *Science* **312**, 1520–1523 (2006).
5. Szemenyei, H., Hannon, M. & Long, J. A. *Science* **319**, 1384–1386 (2008).
6. Theologis, A. & Ray, P. M. *Proc. Natl Acad. Sci USA* **79**, 418–421 (1982).
7. Zurfluh, L. L. & Guilfoyle, T. J. *Plant Physiol.* **69**, 332–337 (1982).
8. Abel, S., Oeller, P. W. & Theologis, A. *Proc. Natl Acad. Sci USA* **91**, 326–330 (1994).
9. Papamichos-Chronakis, M., Conlan, R. S., Gounalaki, N., Copf, T. & Tzamarias, D. *J. Biol. Chem.* **275**, 8397–8403 (2000).
10. Causier, B., Ashworth, M., Guo, W. & Davies, B. *Plant Physiol.* **158**, 423–438 (2012).

Competing interests

The authors declare no competing interests.