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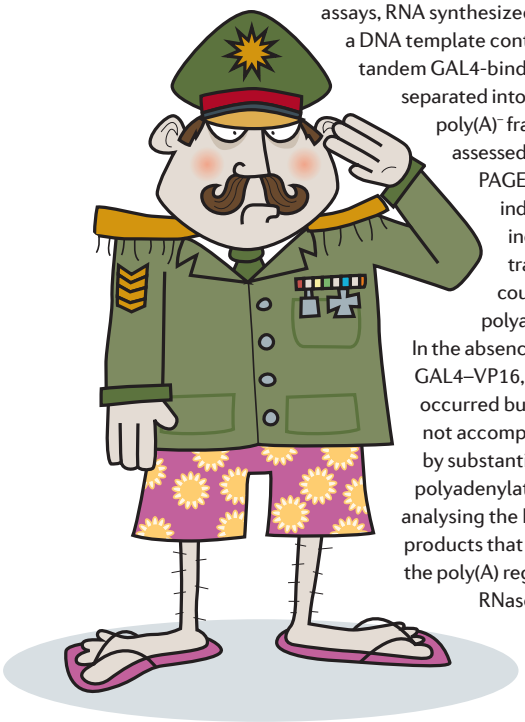
GENE EXPRESSION

Transcriptional activators do double-duty

> a prototypical transcriptional activator can directly stimulate mRNA 3′ processing when it is coupled to transcription.



mRNA processing is coupled to RNA polymerase II (RNAPII) transcription and involves interactions between the polyadenylation machinery, RNAPII and transcriptional activators. However, the mechanism is unclear and the full repertoire of interacting proteins is unknown. In *Molecular Cell*, Nagaike *et al.* report that the transcriptional activator galectin 4 (GAL4) stimulates transcription-coupled 3′ processing of mRNA by interacting with the multisubunit RNAPII-associated factor 1 complex (PAF1C).

Nagaike *et al.* first used coupled transcription 3′-processing assays in HeLa nuclear extracts to test whether the model transcriptional activator GAL4–VP16 activates transcription-coupled polyadenylation. In these assays, RNA synthesized from a DNA template containing tandem GAL4-binding sites was separated into poly(A)$^+$ and poly(A)$^-$ fractions and assessed by denaturing PAGE. GAL4–VP16 indeed strongly induced transcription-coupled polyadenylation. In the absence of GAL4–VP16, transcription occurred but was not accompanied by substantial polyadenylation. By analysing the RNA-cleavage products that arose from the poly(A) region in RNase protection assays, the authors also showed that GAL4–VP16 stimulated 3′ processing. Increased polyadenylation was specific to templates containing GAL4 sites and contingent on active transcription, suggesting that GAL4 must be recruited to DNA and specifically regulates co-transcriptional polyadenylation.

Having previously shown that PAF1C has a role in coupling transcription to 3′ processing, the authors next tested whether GAL4-mediated control of 3′ processing requires PAF1C by depleting PAF1C *in vitro* and *in vivo* and analysing the transcripts produced. Depletion of PAF1C in nuclear extracts using antibodies to one of the complex components, CDC73, resulted in reduced polyadenylation, whereas transcription remained unaltered. Similarly, knockdown of PAF1C using small interfering RNA-mediated depletion of CDC73 or CTR9 (another component of PAF1C) in 293T cells inhibited 3′-end processing. GAL4 was shown to interact directly with PAF1C and to stimulate the recruitment of PAF1C to DNA, thereby providing insight into its mechanism of action.

Thus, this study has identified a functional interaction between GAL4 and PAF1C and shows that a prototypical transcriptional activator can directly stimulate mRNA 3′ processing when it is coupled to transcription.

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ORIGINAL RESEARCH PAPER
Nagaike, T. *et al.* Transcriptional activators enhance polyadenylation of mRNA precursors. *Mol. Cell* **41**, 409–418 (2011)