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Opinion Piece

Transcriptional enhancement by acidic activators

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Transcriptional regulatory mechanisms are fundamentally similar in eukaryotic organisms [1,2]. Components of the RNA polymerase II (Pol II) machinery are highly conserved and, in some cases, functionally interchangeable. Transcriptional activators with similar DNA-binding specificities are present from yeast to human, and acidic activation domains stimulate transcription across a wide range of species. Promoters typically contain multiple protein binding sites, and efficient activation generally requires the combinatorial and synergistic action of activators that can function far from the initiation site. This review focuses on molecular mechanisms of transcriptional activation that occur under physiological conditions, with particular emphasis on studies carried out in the yeast *Saccharomyces cerevisiae*.

Activator proteins stimulate gene expression via a transcriptional activation domain that is functionally distinct, and usually physically separate, from the DNA-binding domain. Activation domains often contain short acidic regions that function autonomously when fused to heterologous DNA-binding domains. Negative charge is clearly important, but hydrophobic residues and other features that are poorly understood at the structural level also influence the level of transcriptional activation [3,4]. Because acidic activators function across species, the molecular target(s) of acidic activation domains must be functionally conserved.

It is generally believed that acidic activation domains contact general transcription factors assembled at the TATA and initiator elements. In vitro, acidic activation domains can interact directly with the TATA-binding protein (TBP)[5,6], TBP-associated factors (TAFs) that are components of the TFIID complex [7,8], TFIIA [9], TFIIB [10], TFIIF [11], TFIIH [12], and the C-terminal tail of Pol II [13]. Acidic activation domains can stimulate formation of a TFIID-TFIIA-TATA element complex [14–16], recruitment of TFIIB [17], and recruitment of later-acting components [18]. TAFs have been implicated as being specifically involved in the activation process, because TFIID, but not TBP, can support the response to activators [19]. The physiological significance and relative importance of these interactions is a fundamental issue that has been addressed primarily in studies of the yeast *Saccharomyces cerevisiae*.

Recruitment of TBP (or TFIID) to the TATA element is an important step in transcriptional activation in yeast cells. Measurement of the rate at which TBP can productively access the chromatin template indicates that accessibility of TBP to the his3 TATA element is a limiting step that can be stimulated by the Gcn4 activation domain [20]. Artificial recruitment of TBP by physical connection to a promoter-bound protein activates transcription, thereby bypassing the need for an activation domain [21–23]. This observation suggests that interactions between activation domains and general factors that function after TBP recruitment (e.g. TFIIB, TFIIF, Pol II) can be bypassed for transcriptional activation. The hypothesis that activators stimulate TBP recruitment in vivo is consistent with the very poor binding of TBP to TATA elements in the context of chromatin [24].

The importance of TBP recruitment to transcriptional activation is further supported from analyses of TBP mutants that are specifically defective in the response to acidic activators in vivo. First, several activation-defective derivatives have mutations on the DNA-binding surface of TBP and are unable to bind TATA elements [25,26]. Activation-deficiency does not simply reflect reduced affinity for the TATA element, but rather involves more specific perturbations of the TBP-TATA interface [26]. Conversely, an efficient TBP-TATA interaction is essential for the response to strong activators, because weak TATA elements are functionally saturated at lower levels of activation [27]. Second, another activation-defective TBP mutant is specifically defective in the interaction with TFIIA [28]. It is likely that the role of the TBP-TFIIA interaction in transcriptional activation reflects the ability of TFIIA to

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stabilize the interaction of TBP to the TATA element. Third, some activation-defective TBP mutants interact normally with TATA elements, TFIIA, TFIIB, and activation domains. Presumably, they affect some other protein-protein interaction [29]. The activation defect of some, but not all of these derivatives can be corrected by artificial recruitment of TBP to the promoter. Thus, these TBP mutants define two steps in the response to acidic activators: efficient recruitment to the TATA element and a postrecruitment interaction with the Pol II machinery [29].

Surprisingly, the results of recent experiments strongly suggest that TAFs are not generally required for transcriptional activation in yeast cells [30,31]. Specifically, TAF depletion does not significantly affect activation by four acidic activators: Gcn4, Ace1, Gal4, Hsf. Strikingly, this is the case for TAF130, which provides the scaffold for TAF assembly and without which TFIID is likely to be disrupted. Despite the lack of a general requirement for activation, TAFs are essential for cell growth [32,33]. TAFs (and presumably TFIID) might be required for a subset of activators that affect one or more essential genes, and/or they could subtly affect activation of many genes that cumulatively lead to cell inviability.

The conclusion that TAFs are not generally required for transcriptional activation in yeast contrasts with numerous experiments indicating that TAFs are crucial for activated transcription in vitro [19,34]. One possible explanation for this apparent discrepancy is that yeast TAFs might be less important than their mammalian and Drosophila counterparts. In this regard, the yeast TFIID complex is less stable in extracts. This hypothesis is unlikely, because TAFs are strongly conserved among eukaryotes [34]; TAF-dependent activation in vitro can be achieved with yeast components [32,33]; and activation can occur in hamster cells in which TAF250 (yeast TAF130 homolog) has been thermally inactivated [35]. Instead, we have suggested that TAFs are functionally redundant with other factors that are absent (or inactive) in typical in vitro reactions [30]. In this regard, activated transcription in the apparent absence of TAFs can occur in reactions containing Pol II holoenzyme [36,37] or chromatin templates [38]. Moreover, in vitro transcription reactions are typically reconstituted with core Pol II (the 12 subunit enzyme), and hence are likely to lack components of the Pol II holoenzyme (e.g. Srb proteins, Gal11, Sin4, Swi/Snf complex) that are important for transcription in yeast cells [39,40].

In principle, activator proteins can interact with individual components of the Pol II machinery to stabilize the association of the Pol II machinery with the promoter, thereby permitting increased transcriptional initiation [40]. In this regard, artificial connection of enhancer-bound proteins to TBP [21–23], TAFs (unpublished results), and components of the Pol II holoenzyme [41,42] can bypass the need for an activation domain. If natural activators interact with multiple components of the Pol II machinery, individual components such as TAFs are likely to be non-essential for activation, even if they are potential targets (Struhl and Elkhound, unpublished). Thus, although it is possible to generate conditions in which TAFs are required for activation in vitro, they do not appear to be generally required in vivo. Under physiological conditions, the relative importance of TAFs and other potential targets is unclear. However, it is interesting to note that TAFs have never been identified in the numerous genetic screens for mutations that affect transcription. In contrast, mutations in a variety of Pol II holoenzyme components can affect transcriptional activation, and TBP mutations that alter the interaction with TATA elements or TFIIA can specifically impair the response to acidic activators.

I have proposed a triad model for transcriptional activation [40] involving the three macromolecular entities that correspond to the three kinds of eukaryotic promoter elements: activator proteins bound to enhancer elements; complexes containing TBP and associated proteins bound to the TATA element; and the Pol II holoenzyme bound to the initiator element. Because Pol II is unable to recognize promoters or initiate mRNA synthesis, transcriptional activation can be viewed as recruitment of Pol II to the promoter in an active form. In principle, interactions between any two legs of the triad will lead to increased recruitment of Pol II to the promoter. Because the legs of the triad are connected, protein-protein interactions that strengthen any one connection will increase the overall stability of the complete transcription machinery at the promoter. Thus, enhanced recruitment of the Pol II holoenzyme can be achieved by a variety of protein-protein interactions involving activators. In particular, TBP is associated with numerous proteins, and the Pol II holoenzyme contains at least 25 polypeptides in addition to the core subunits. Activators could contact any of these proteins or could cause a conformational change in one protein that increases interaction with another. Thus, the various protein-protein interactions that underlie distinctions between activator proteins or promoters might reflect a common mechanism of transcriptional activation.

Another aspect of the triad model is the proposal that Pol II can initiate multiple times from a completely or partially assembled complex in vivo. Although the Pol II holoenzyme is required for initiation, the core enzyme disengages from the remainder of the machinery upon the transition to transcriptional elongation. Because highly active promoters in yeast and flies initiate transcripts every 6 seconds and have a Pol II density of 1 molecule/100 base pairs [43,44], it seem unlikely that de novo assembly of the entire triad is required for each initiation. Thus, the overall process of transcriptional activation involves both initial recruitment of TFIID and the Pol II holoenzyme to the promoter, and the ability of metastable subcomplexes on the promoter to permit multiple initiation events. At strong promoters, some of the assembled complex remains intact upon disengagement of the core enzyme, thereby permitting rapid reinitiation by a new Pol II molecule. Con-

versely, promoters involving weak activators or TATA elements are predicted to have lowered triad stability, thereby resulting in fewer rounds of initiation per complex and increased reliance on the slower process of assembling the entire triad on an unoccupied promoter. Overall, the triad reinitiation model provides both a coherent framework for the process of transcriptional activation and the mechanistic basis for the multiplicity of molecular interactions that contributes to the extraordinary diversity of gene regulatory patterns in eukaryotes.

References

[1] Struhl, K. (1995) Ann. Rev. Genet. 29, 651–674.
[2] Zawel, L. and Reinberg, D. (1995) Annu. Rev. Biochem. 64, 533–561.
[3] Struhl, K. (1989) Ann. Rev. Biochem. 58, 1051–1077.
[4] Hahn, S. (1993) Cell 72, 481–483.
[5] Stringer, K.F., Ingles, C.J. and Greenblatt, J. (1990) Nature 345, 783–786.
[6] Ingles, C.J., Shales, M., Cress, W.D., Triezenberg, S.J. and Greenblatt, J. (1991) Nature 351, 588–590.
[7] Goodrich, J.A., Hoey, T., Thut, C.J., Admon, A. and Tjian, R. (1993) Cell 75, 519–530.
[8] Hoey, T., Weinzierl, R.O.J., Gill, G., Chen, J.-L., Dynlacht, B.D. and Tjian, R. (1993) Cell 72, 247–260.
[9] Ozer, J., Moore, P.A., Bolden, A.H., Lee, A., Rosen, C.A. and Lieberman, P.M. (1994) Genes and Dev. 8, 2324–2335.
[10] Lin, Y.-S., Ha, I., Maldonado, E., Reinberg, D. and Green, M.R. (1991) Nature 353, 569–571.
[11] Joliot, V., Demma, M. and Prywes, R. (1995) Nature 373, 632–635.
[12] Xiao, H., Pearson, A., Coulombe, B., Truant, R., Zhang, S., Regier, J.L., Triezenberg, S.J., Reinberg, D., Flores, O., Ingles, C.J. and Greenblatt, J. (1994) Mol. Cell. Biol. 14, 7013–7024.
[13] Usheva, A., Maldonado, E., Goldring, A., Lu, H., Houbavi, C., Reinberg, D. and Aloni, Y. (1992) Cell 69, 871–881.
[14] Wang, W., Gralla, J.D. and Carey, M. (1992) Genes Dev. 6, 1716–1727.
[15] Lieberman, P.M. and Berk, A.J. (1994) Genes Dev. 8, 995–1006.
[16] Chi, T., Lieberman, P., Ellwood, K. and Carey, M. (1995) Nature 377, 254–257.
[17] Lin, Y.-S. and Green, M.R. (1991) Cell 64, 971–981.
[18] Choy, B. and Green, M.R. (1993) Nature 366, 531–536.
[19] Tjian, R. and Maniatis, T. (1994) Cell 77, 5–8.
[20] Klein, C. and Struhl, K. (1994) Science 266, 280–282.
[21] Chatterjee, S. and Struhl, K. (1995) Nature 374, 820–822.
[22] Klages, N. and Strubin, M. (1995) Nature 374, 822–823.
[23] Xiao, H., Friesen, J.D. and Lis, J.T. (1995) Mol. Cell. Biol. 15, 5757–5761.
[24] Imbalzano, A.N., Kwon, H., Green, M.R. and Kingston, R.E. (1994) Nature 370, 481– 485.
[25] Arndt, K.M., Ricupero-Hovasse, S. and Winston, F. (1995) EMBO J. 14, 1490–1497.
[26] Lee, M. and Struhl, K. (1995) Mol. Cell. Biol. 15, 5461–5469.
[27] Iyer, V. and Struhl, K. (1995) Mol. Cell. Biol. 15, 7059–7066.
[28] Stargell, L.A. and Struhl, K. (1995) Science 269, 75–78.
[29] Stargell, L.A. and Struhl, K. (1996) Mol. Cell. Biol. 16, 4456–4464.
[30] Moqtaderi, Z., Bai, Y., Poon, D., Weil, P.A. and Struhl, K. (1996) Nature 382, in press.
[31] Walker, S.S., Reese, J.C., Apone, L.M. and Green, M.R. (1996) Nature 382, in press.
[32] Reese, J.C., Apone, L., Walker, S.S., Griffin, L.A. and Green, M.R. (1994) Nature 371, 523–527.
[33] Poon, D., Bai, Y., Campbell, A.M., Bjorklund, S., Kim, Y.J., Zhou, S., Kornberg, R.D. and Weil, P.A. (1995) Proc. Natl. Acad. Sci. USA 92, 8224–8228.
[34] Burley, S.K. and Roeder, R.G. (1996) Annu. Rev. Biochem. 65, 769–799.
[35] Wang, E.H. and Tjian, R. (1994) Science 263, 811–814.
[36] Koleske, A.J. and Young, R.A. (1994) Nature 368, 466–469.
[37] Kim, Y.-J., Bjorklund, S., Li, Y., Sayre, M.H. and Kornberg, R.D. (1994) Cell 77, 599–608.
[38] Workman, J.L., Taylor, I.C.A. and Kingston, R.E. (1991) Cell 64, 533–544.
[39] Koleske, A.J. and Young, R.A. (1995) Trends Biochem. Sci. 20, 113–116.
[40] Struhl, K. (1996) Cell 84, 179–182.
[41] Barberis, A., Pearlberg, J., Simkovich, N., Farrell, S., Reinagel, P., Bamdad, C., Sigal, G. and Ptashne, M. (1995) Cell 81, 359–368.
[42] Jiang, Y.W. and Stillman, D.J. (1992) Mol. Cell. Biol. 12, 4503–4514.
[43] Iyer, V. and Struhl, K. (1996) Proc. Natl. Acad. Sci. USA 93, 5208–5212.
[44] O'Brien, T. and Lis, J.T. (1993) Mol. Cell. Biol. 13, 3456–3463.