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Commentary

Transcriptional activation: Is it rocket science?

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One of the beautiful things about biology is that you don't have to fully understand it to put it to good use. The engineers who design spacecraft may need a certain working familiarity with all of the parts in their systems. But biologists, and particularly biologists whose mission is to treat human disease, can make do with considerably more ignorance. One reason for this difference is that biological control usually is exerted through linear pathways of protein–protein interactions. Intervention at a single known point in the pathway often is sufficient to capture that pathway in its entirety.

A very elegant and potentially far-reaching example of this phenomenon appears in the current issue of the *Proceedings* (1). The biological pathway in question is the initiation of gene transcription. Transcriptional initiation in eukaryotes requires the assembly of scores of proteins into a superstructure at the gene promoter. The precise identities of these proteins and their order of assembly, while beginning to come into focus, are still quite mysterious. Particularly challenging is the question of how a bound transcription factor attracts these proteins to a promoter and directs their assembly into a preinitiation complex. At least part of the answer is that these factors, through their activation domains, make direct contacts with individual proteins in the complex. Surprisingly, however, reiteration of short peptide motifs that presumably contact only a small subset of these proteins can create very powerful activators (2–4). Thus, it may be necessary to recruit only a small number of these proteins to seed a much larger, fully active transcription complex.

This idea has led Nyanguile *et al.* (1) to ask if the complexity and size of the molecular magnet for the preinitiation complex could be dramatically reduced, perhaps all the way to a small synthetic drug-like molecule. To do this study they placed a chimeric DNA-binding protein at a target promoter. In place of the activation domain that would be present in a natural transcription factor, they substituted the immunophilin protein FKBP12. This protein, by virtue of its high affinity for the immunosuppressant drug FK506, can act as a docking site for chemical entities covalently linked to an FK506 moiety. They synthesized a molecule in which FK506 was fused to a synthetic peptide derived from the activation domain of the viral transcription factor VP16. Two versions were made, one in which the peptide was in its natural L configuration and one in the unnatural D configuration. Both molecules could promote the transcription of a target gene in a cell-free transcription system. And the D enantiomer, which presumably resists proteolytic degradation, could do the same thing in living cells. Thus, the authors have succeeded in creating a semi-synthetic, relatively low-molecular weight molecule that is capable of recruiting the multicomponent preinitiation complex to a target gene.

This observation does not provide new insights into the molecular mechanism of gene transcription. Rather it highlights the inherent flexibility of the transcription process and indicates once again how a complex series of biochemical events—and, consequently, a biological result—can be initiated through a single point of intervention. Nyanguile *et al.* probably would admit that they don't really know how their molecule works. Presumably, the documented ability of this VP16 peptide to interact with TFIIB (5) is required for its activity. But what happens after that is anybody's guess. Nevertheless, these experiments offer the promise of a new generation of small-molecule drugs that could directly modulate gene expression for therapeutic benefit. Such drugs could be targeted to selected genes through the intermediary of a chimeric DNA-bound receptor, as done by Nyanguile *et al*. (1). Or they could be combined with synthetic molecules that have inherent selectivity for specific DNA sequences (6).

How feasible is this prospect? Much of the data in the transcription field suggest that activation of transcription, at least at artificial promoters, is a remarkably flexible process that can be driven by decidedly unnatural means. There is, for example, little evidence for rigid structural requirements or elaborate stereospecific protein contacts in natural activators (7). As we have noted, short peptide motifs as small as eight amino acids can be exceptionally potent activators if present in multiple copies in a DNA-binding protein. Furthermore, the work of Ma and Ptashne (8) suggests that many different peptide sequences have this property—at least 1% of peptides generated from random *Escherichia coli* DNA sequences can activate transcription in yeast. In fact, perfectly adequate activators can be created artificially in the form of simple amphipathic helices (9) and by fortuitous mutagenesis of unrelated protein domains (10). Thus, many different surfaces, which presumably interact with many different targets, can function as activators.

Indeed, it is clear that characterized activators can interact with a number of different proteins in the preinitiation complex, and that each such interaction is sufficient to trigger formation of a preinitiation complex (for review, see ref. 11). Moreover, in yeast at least, it has proven possible to bypass conventional targets entirely and directly recruit the RNA polymerase holoenzyme (10, 12). Thus, there would appear to be a wealth of targets, both known and unknown, on which a small-molecule activator could act.

In addition, where they've been studied, the affinities of natural activators for their presumed targets are not very high. The interaction of the C-terminal peptide of VP16 with human $TAF_{II}31$ is estimated at $>10^{-4}$ M (13). Higher affinities on the order of 10^{-7} M have been reported for the interaction of the GAL4 activation domain with TATA box-binding protein (TBP) and TFIIB (14). But, clearly, nanomolar binders are not needed. In the system used by Nyanguile *et al.* (1), the interaction of an FK506-linked activator to an array of receptors bound to tandemly reiterated sequences would create very high local concentrations of the compound at a single site in the genome. This may well be how real promoters and enhancers work—by acting as templates that display multiple low-affinity ligands for the preinitiation complex, which sum to an irresistible force.

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Thus, there might be reason for optimism. Many different targets, known and unknown, may suffice. Many different surfaces on each of those targets and many different points of contact with these surfaces could work. And the affinities of these interactions need not be high, particularly if multiple copies of the activator could be delivered to the target gene. Finally, it is worth noting that configuring a reporter gene assay for high-throughput screening of compound libraries for novel activators would be a relatively straightforward endeavor with today's technologies.

On the other hand, the Nyanguile *et al.* (1) compounds are far from real drugs. They would need to be reduced in molecular weight by a factor of 10, while retaining sufficient points of contact with their protein targets. Furthermore, the compounds are highly charged, as they are based on an activation domain rich in acidic amino acid residues. As such, they would not readily penetrate cells and in this case were delivered in a lipid formulation. It would be instructive to test analogous peptides containing uncharged amino acids such as glutamine or proline, also commonly found in natural activators.

Ultimately, however, the insurmountable barrier may prove to be biology itself. Natural promoters are highly regulated, requiring multiple combinatorial interactions between bound transcription factors and the initiation machinery. Furthermore, they reside within a complex nucleoprotein structure that imposes specific architecture as well as thermodynamic and kinetic constraints to DNA binding and transcriptional activation. The current experiments use an artificial promoter driven by tandemly reiterated binding sites to which many molecules of the activator can be delivered. It is considerably more difficult to imagine how to deliver many activators to a natural gene sequence. The target gene in these experiments is also episomal and presumably clothed in something less than fully outfitted chromatin. It may prove rather more difficult to deliver a DNA-binding protein or drug to sites embedded in natural chromatin and to activate transcription from such sites even if the activator can get there.

Still, these studies give one much food for thought. The idea that chemical compounds can be used to mobilize complex pathways and functions inside a cell, even with scant knowledge of their components is a compelling one. It is a very different paradigm from the one that drives other modern technologies and emphasizes again that biology is not rocket science. Given our increased capacity for chemical screening, thanks to combinatorial synthesis and robotic assays, the potential for revolutionary new drugs that capture cellular functions is tantalizing.

1. Nyanguile, O., Uesugi, M., Austin, D. J. & Verdine, G. L. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 13402–13406.
2. Seipel, K., Georgiev, O. & Schaffner, W. (1992) *EMBO J.* **11,** 4961–4968.
3. Tanaka, M. & Herr, W. (1994) *Mol. Cell Biol.* **14,** 6056–6067.
4. Blair, W. S., Bogerd, H. P., Madore, S. J. & Cullen, B. R. (1994) *Mol. Cell Biol.* **14,** 7226–7234.
5. Lin, Y.-S., Ha, I., Maldonado, E., Reinberg, D. & Green, M. R. (1991) *Nature (London)* **353,** 569–571.
6. Gottesfeld, J. M., Neely, L., Trauger, J. W., Baird, E. E. & Dervan, P. B. (1997) *Nature (London)* **387,** 202–205.
7. Triezenberg, S. J. (1995) *Curr. Opin. Genet. Dev.* **5,** 190–196.
8. Ma, J. & Ptashne, M. (1987) *Cell* **51,** 113–119.
9. Giniger, E. & Ptashne, M. (1987) *Nature (London)* **330,** 670–672.
10. Barberis, A., Pearlberg, J., Simkovich, N., Farrell, S., Reinagel, P., Bamdad, C., Sigal, G. & Ptashne, M. (1995) *Cell* **81,** 359–368.
11. Ptashne, M. & Gann, A. (1997) *Nature (London)* **386,** 569–577.
12. Farrell, S., Simkovich, N., Wu, Y., Barberis, A. & Ptashne, M. (1996) *Genes Dev.* **10,** 2359–2367.
13. Uesugi, M., Nyanguile, O., Lu, H., Levine, A. J. & Verdine, G. L. (1997) *Science* **277,** 1310–1313.
14. Wu, Y., Reece, R. J. & Ptashne, M. (1996) *EMBO J.* **15,** 3951–3963.