and a simple method to transform rice will undoubtedly speed up identification of the genes that determine important agricultural traits of rice—flower and leaf development, photoperiod sensitivity of flowering, and resistance to various pests and pathogens. For instance, we know little about flower development of rice at present. If the determinants of the number of flowers were known, we could increase the number of seeds. Similarly, if we understood how the flower is induced by a light signal, we may be able to generate rice with much shorter growth periods. There will likely be much excitement about the molecular biology of rice in the near future.

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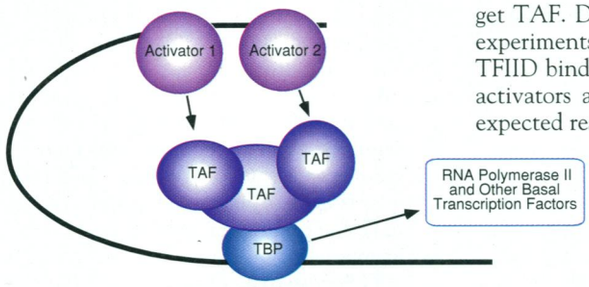
Mechanisms of Gene Activation

Stephen Buratowski

The main question facing the eukaryotic gene expression field is the same today as it has been for many years. How do regulatory transcription factors (which include the products of many proto-oncogenes, genes essential for proper differentiation and development, and key components of signal transduction pathways) increase the amount of messenger RNA produced by their target gene promoters? Clear answers to this question have been waiting for a better understanding of the basal transcription machinery—RNA polymerase II and the accessory factors that position polymerase at the transcription initiation site. A great deal of recent evidence points to the basal transcription factor complex TFIID as a key component in the response to transcription regulators. Some of the most persuasive experiments yet that show TFIID is a target of activators are presented in two papers by Sauer *et al.* in this issue (*1*).

Transcription regulatory proteins typically contain two functional domains: a DNA binding domain that recognizes specific sequences within its target promoters and an activation domain that is required for transcription stimulation. On the basis of the prokaryotic transcription paradigm, activation domains of regulatory proteins are predicted to make direct protein-protein contacts with one or more components of the basal transcription machinery. This contact could stabilize binding of the basal machinery to the promoter or increase the rate of a kinetically slow step (as some prokaryotic activators stabilize association of RNA polymerase with the promoter DNA or increase the rate of DNA strand separation within the transcription complex). A great deal of effort has gone into identifying the targets of eukaryotic activation domains, and certainly no shortage of candidates exists. In vitro protein interaction studies have detected activator interactions with nearly every component of the basal transcription complex. However, very few of these contacts have been shown to occur in the context of the transcription complex or are even correlated with transcription stimulation.

The case for activator-TFIID interactions is much stronger. TFIID consists of the TATA binding protein (TBP) and approximately 10 other TBP-associated factors (TAFs). Whereas TBP is sufficient for promoter binding and basal transcription, the presence of the TAFs confers the ability to respond to activators. Thus, TAFs have been postulated to be coactivators in transcription. In vitro protein interaction assays show that different activators can contact specific TAFs. The caveat of these results is that the interactions are seen between two proteins removed from the context of the transcription complex. In the biochemical tour-de-force leading up to the two papers in this issue (both from the same set of authors), Sauer *et al.* (*1*) have expressed each of the individual TAFs and reconstituted the TFIID complex from recombinant subunits. More importantly, they have assembled subcomplexes of TFIID to test whether the absence of a particular TAF affects activation. As predicted, in vitro transcription stimulation by an activator absolutely requires the presence of the contacted TAF subunit. This correlation strongly supports the hypothesis that the TFIID subunits are targets for transcription activation domains.



One mechanism of synergistic transcription activation. Two simultaneous contacts by activators can cause synergistic activation of transcription, leading to stabilized binding of TFIID to the promoter and, possibly, conformation changes in the complex. As a result of this interaction, association of the remaining basal transcription factors is enhanced.

The experiments of Sauer *et al.* (*1*) extend earlier results by reproducing a key feature of eukaryotic transcription: synergistic activation of transcription by multiple activator proteins. Multiple activators at a promoter in vivo or in crude in vitro systems lead to a greater than additive increase in the amount of transcription. Sauer *et al.* show that the ability of two activators to work synergistically correlates with simultaneous contacts between each activator and its target TAF. Deoxyribonuclease I footprinting experiments show a dramatic increase of TFIID binding to the promoter when both activators are present. This is exactly the expected result if the two activators are stabilizing the same complex. The additive free energy of the individual protein contacts leads to an exponential increase in the equilibrium binding constant ($\Delta G = -RT\ln K$, where ΔG is the change in the Gibbs free energy, R is the gas constant, T is the absolute temperature in degrees Kelvin, and K is the equilibrium binding constant). Therefore, at least some transcription activation appears to be simply the result of cooperative binding and recruitment of TFIID. This idea is supported by in vivo experiments in which a protein fusion between a regulatory factor DNA binding domain and TBP bypasses the need for an activation domain (*2*).

So, are the TAFs merely passive targets

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for activators, necessary only to escort TBP to the promoter? This possibility seems unlikely, considering how highly conserved the TAFs are over evolution and their number (8 to 10 at least). It is likely that there are other structural and functional roles for TAFs, and further experiments will undoubtedly uncover these functions. Indeed, some TAFs make DNA contacts and contribute to promoter recognition. Upcoming genetic experiments in *Drosophila* and yeast will be critical for evaluating the in vivo roles of the TAF proteins.

Several reports suggest that TFIID responds to activators with conformation changes that occur after binding to the promoter. These changes are thought to facilitate subsequent interactions of other basal factors such as TFIIB and polymerase (*3*). These models of activator function are not inconsistent with increased TFIID recruitment. In fact, both recruitment and conformation changes could be mediated by the same activator-TAF contacts.

If the TAFs are essential for response to activators, are they sufficient? The experiments of Sauer *et al.* are done with highly purified factors, suggesting that only TAFs and the basal factors are necessary for activator responsiveness. Other factors can potentiate the response to activators in vitro, including TFIIA and several positive and negative cofactors (*3*, *4*). The activity of these factors is apparently still dependent on TAFs. In addition, a substantial body of genetic and, more recently, biochemical evidence points to chromatin as an essential regulator of gene expression. Repression and derepression, as opposed to an actual increase in the rate of transcription, probably account for many of the observed effects of these additional factors. Nevertheless, it is essential to determine whether these other transcription regulators modulate the TAF-based activation process or function in independent pathways of gene activation.

Another set of factors important for gene regulation are those recently identified as components of a yeast mediator (or holoenzyme) complex (*5*). This set of proteins was originally identified genetically as suppressors of mutations in RNA polymerase II, and biochemical analysis revealed that the complex was associated with RNA polymerase II and one or more of the basal transcription factors. The holoenzyme contains several proteins implicated in gene regulation by yeast genetics. Surprisingly, this complex responds in vitro to transcription activators in the presence of TBP, implying the existence of a TAF-independent mechanism for transcription regulation. Like TAF-dependent activation, holoenzyme-mediated activation may be due to stabilizing contacts between the transcription regulators and holoenzyme components (*6*).

How are the basal trascription factors delivered to a promoter? Until recently, it was assumed that a stepwise assembly of basal factors would provide multiple points for regulation. In another view, the holoenzyme model, some or all basal factors are preassociated before reaching the promoter. If an entire transcription complex was assembled off the DNA, it would present only a single, extremely large target for activators. A recently described mammalian holoenzyme preparation apparently carries all the essential basal transcription factors (*7*) but, unexpectedly, does not respond to activators.

Many prokaryotic activators stimulate transcription either by recruiting the holoenzyme to the promoter or by increasing DNA strand separation at the initiation site. It is likely that eukaryotic activators can also affect multiple steps in initiation. The TAF-dependent activation described by Sauer *et al.* (*1*) is mechanistically similar to the first class of bacterial activation. Activator-induced conformation changes in the eukaryotic transcription complex also seem likely, but more work is required. Although partial answers are emerging, deciphering the mechanisms of activator function will remain a central goal of the field for some time.

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UPDATE

Ethylene Sensors: How Perceptive!

Athanasios Theologis

Ethylene (C_2H_4), a multipurpose signaling molecule in plants (*1*), orchestrates vital growth stages such as fruit and flower senescence and defense against pathogens. As reviewed previously in *Science*, its receptor and transduction apparatus are just emerging into view (*2*); now two new studies on ethylene sensors (*3*, *4*) put the receptor into even sharper focus.

Plants sense ethylene by a protein kinase cascade (*2*, *5*). *CTR1* and *ETR1*, two *Arabidopsis* genes essential for ethylene signaling, encode a putative RAF-like serine-threonine protein kinase (*6*) and a putative histidine protein kinase similar to the prokaryotic, two-component sensors. Without functional ETR1, plants do not bind ethylene effectively; ETR1 acts upstream of CTR1 and other components in the pathway (*2*). So the ETR1 protein has been proposed as the ethylene receptor (*7*). And indeed it is—as shown by Schaller and Bleecker on page 1809, where they demonstrate that ETR1 binds ethylene.

When ETR1 was first described, it seemed to be the only ethylene receptor; but then a second ethylene sensor, ERS, was isolated from *Arabidopsis* (*8*). An ERS mutation confers dominant ethylene insensitivity to wild-type *Arabidopsis* (*8*). But the excitement does not end there. On page 1807 of this issue, H. Klee's laboratory reveals that the old and forgotten tomato ripening mutant *Nr* is the result of a dominant mutation in the transmembrane domain of the NR protein (an ERS-like gene product). More importantly, a transgenic yeast strain expressing the wild-type NR protein also binds ethylene (*9*). At the same time, M. Tucker's laboratory at the USDA in Beltsville, Maryland, cloned an ETR1 homolog from tomato (*10*). The tomato eTAE1 (*ETR1*) mRNA is expressed during flower and fruit senescence, whereas the NR (*ERS*) mRNA is developmentally regulated during fruit ripening (*4*). The picture that emerges is that ethylene sensors are encoded by multigene families with members that are differentially expressed during plant growth and development.

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