Transcriptional activation by recruitment

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The recruitment model for gene activation stipulates that an activator works by bringing the transcriptional machinery to the DNA. Recent experiments in bacteria and yeast indicate that many genes can be activated by this mechanism. These findings have implications for our understanding of the nature of activating regions and their targets, and for the role of histones in gene regulation.

For some years now we have understood, in broad outline, the principles of gene regulation in eukaryotes. That picture might be sketched as follows. Eukaryotic transcriptional activators stimulate transcription of otherwise silent genes. The typical activator, like its bacterial counterpart, activates by binding to specific sites on DNA and, with its 'activating region', contacting the multiprotein machinery that directs transcription (Fig. 1). For many activators and genes the specificity of activation is determined solely by the DNA-binding address of the activator. For example, the activator Gal4 ordinarily activates genes required for galactose metabolism in yeast. But when any of a wide array of genes is modified so as to bear Gal4 binding sites nearby, Gal4 activates that gene as well. Gal4 will also work in higher eukaryotes, and, as in yeast, can do so cooperatively with other DNA-binding activators. These observations suggest that the underlying mechanisms of activation are similar in different eukaryotes, and that regulatory networks—which include specific DNA-bound repressors that counter the effects of activators—can evolve by distributing binding sites for regulatory proteins near the genes that are to be regulated (for a review, see ref. 1).

Further illustrations and elaborations of this scheme have emerged over the past few years, but still unresolved are an array of questions concerning the nature of activating regions and how they work. For example, how do activating regions—families of peptides with little obvious similarities, even fragments of which still retain function—exert their effects on their target proteins? What are those targets? Are specialized co-activator proteins required to link activating regions to the transcriptional machinery? What complications are introduced by the fact that the typical gene is wrapped on histones to form nucleosomes, which present a formidable barrier to the transcriptional machinery?

We begin by summarizing recent findings in bacteria that characterize a basic mechanism for activation called recruitment. We describe how a large variety of activator–target interactions can effect gene activation by this mechanism. We then describe yeast experiments indicating that a similar mechanism works in that eukaryote. The model that emerges at least partly explains activating region design, the nature of the targets of those peptides, and how disparate activators can work cooperatively. The model also suggests a role for histones in the process.

Bacteria

The machinery

All genes in *Escherichia coli* are transcribed by an RNA polymerase with four essential subunits (Fig. 2). Most genes are transcribed by an enzyme bearing the sigma 70 (σ^{70}) subunit, and certain other genes, an example of which we will encounter, are transcribed by a form of the enzyme bearing a different σ subunit. The polymerase recognizes characteritstic promoter sequences found upstream of the transcription start site. Certain promoter sequences direct efficient transcription initiation in the absence of activators, whereas others require assistance from activators. A considerable

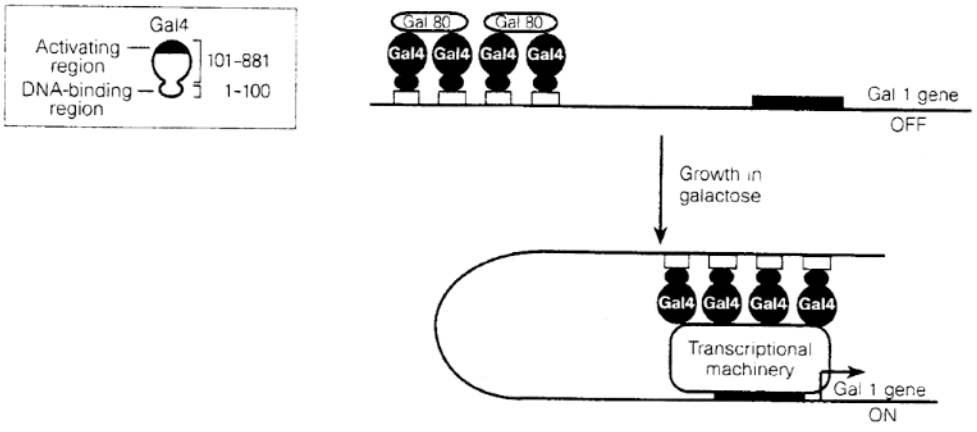

Figure 1 The yeast activator Gal4 binds to sites located approximately 250 base pairs upstream of *GAL1*, one of the genes required for galactose metabolism in yeast. Similar Gal4 binding sites are found upstream of other *GAL* genes in yeast. In the absence of galactose from the growth medium, the activating regions of Gal4 are covered by the inhibitor Gal80. Growth in galactose releases that inhibition, and Gal4's activating region contacts the transcriptional machinery to trigger activation. The DNA between the activators and the gene loops out to accommodate the reaction. The representation is highly schematic: there are actually four Gal4 sites upstream of *GAL1*, each of which is recognized by a Gal4 dimer. The DNA-binding and activating regions are found on separable domains, as indicated.

BOX Revisiting activation in bacteria

In the text we draw parallels between the action of typical yeast activators and that of the bacterial activators CAP and lambda repressor, and we contrast this shared mechanism with that of another bacterial activator, NTRC. We say that the first group of bacterial activators (CAP and lambda repressor) recruit polymerase to DNA, whereas NTRC works on a stably bound polymerase. This distinction, the significance of which we expand upon here, partly reflects differences between the promoters at which these activators work. At all bacterial promoters, polymerase binds in at least two steps: in the first, it binds to helical DNA to form a 'closed' complex, and in the second, the complex isomerizes to form the 'open' complex in which the DNA strands are locally opened[88]. But there is a crucial difference in the stabilities of the closed complexes, and in the requirements for the transition from the closed to the open complexes, at these two classes of promoters.

Consider first the typical promoter (for example, that of gene *glnAp2*) stimulated by NTRC. Experiments *in vitro* show that polymerase (bearing σ^{54}) forms a highly stable *closed* complex with DNA in the absence of activator, and *in vivo* polymerase is found bound to the promoter before the action of activator[89,90]. At that promoter, therefore, the activator's role is not to stimulate formation of a stable polymerase–DNA complex (that is, to recruit polymerase), but rather to stimulate isomerization from one stable complex (closed) to another (open). In this case, therefore, isomerization, a reaction that occurs extremely rarely in the absence of activator at this promoter, is a post-recruitment step. Consistent with the idea that the activator works on a prebound polymerase, NTRC (and its relative NifA), expressed at sufficiently high levels, will activate its target genes even if it (the activator) cannot bind DNA[31,32].

Now consider, in contrast, promoters stimulated by CAP and lambda repressor. At these promoters, polymerase (bearing σ^{70}) is not stably associated before activation[91–93] and so we say that these activators work by recruitment. The closed complex is less stable than that formed at *glnAp2*, but once formed, the transition to the open complex can occur more readily than at the *glnAp2* promoter. Because polymerase is bound stably only upon formation of the open complex, in these cases isomerization is part of the recruitment process. The activators recruit by stimulating one or another, or both, of the steps that lead to formation of the stable open complex. For example, CAP working at the *lac* promoter primarily stimulates the initial binding of polymerase[94], lambda repressor primarily stimulates isomerization at its own promoter (called P_{RM})[95], and CAP stimulates both steps at the *gal* promoter[11]. The stimulation of either or both steps has the effect of moving the promoter from one state, in which polymerase is largely absent, to another, in which polymerase is bound and can initiate transcription.

Strikingly, despite these apparent differences in activation at the *lac* promoter and at P_{RM}, artificial tethering of polymerase to DNA results in significant activation at both promoters (Fig. 4). It is obvious how this might work at the lac promoter: in that case the artificial tethering would mimic the effect of CAP in holding the polymerase on the DNA, where it spontaneously isomerizes. The explanation for the effect at P_{RM} is less obvious, but the phenomenon can readily be accounted for in our scheme as follows. Polymerase, which binds only intermittently to this promoter in the absence of activator, typically dissociates before isomerizing. DNA-bound lambda repressor stimulates the isomerization step, and thus activated levels of transcription are achieved by ensuring that even a fleetingly bound polymerase gets pushed into the stable open complex. A plausible explanation for activation by artificial tethering at P_{RM}, therefore, would be that the tethering increases the fraction of time the promoter is occupied by polymerase and thereby increases the likelihood that isomerization would occur. A similar explanation–increased formation of the closed complex–would explain the observation[96] that, *in vitro* high concentrations of polymerase obviate the need for stimulation by repressor for activated levels of transcription[36], and the finding that, *in vivo*, a suitably positioned CAP molecule can activate P_{RM}, presumably by stimulating formation of the closed complex[22].

We have noted that, in recruiting polymerase to a stable complex with DNA, lambda repressor and CAP stimulate different steps in the reaction at P_{RM} and the *lac* promoter, respectively. These steps leading to recruitment are analogous to steps in a typical enzymatic reaction (K. Ebright, personal communication) an analogy suggesting that similar protein–protein interactions can account for the action of both activators. Thus, in the enzyme-substrate example, the same interactions between enzyme and modified forms of substrate can facilitate different steps of the reaction (K_m, k_{cat}, or both). By analogy, the step in initiation affected by a given activator–polymerase–promoter interaction would depend on the positioning of the components, the identity of the promoter sequence, and the properties of the polymerase. A recent finding indicates that lambda repressor behaves in accord with that suggestion. Thus, as already mentioned lambda repressor, working at its own promoter with wild-type polymerase, primarily stimulates the isomerization step[95]; but with a polymerase bearing a single amino-acid change (in σ^{70}), the effect is primarily on the initial binding step[97]. For other examples in which similar activator–polymerase interactions are believed to have context-dependent effects, see refs 98–100. Activator–polymerase interactions that affect some step beyond recruitment–such as that between NTRC and polymerase–would be expected to be qualitatively different from those that simply recruit. The fact that activation by NTRC requires ATP but that by lambda repressor and CAP does not, is consistent with that expectation[27–29].

body of evidence shows that the typical activator works by binding to specific sites on DNA and contacting RNA polymerase.

Recruitment

We begin with a discussion of two *Escherichia coli* activators—CAP (for catabolite activator protein), and the phage-lambda repressor working in its guise as an activator—that help polymerase bind stably to promoter DNA. This characterization (see Box 1), identifies these activators as working by recruitment, and distinguishes their action from that of another bacterial activator described below. A generalization that emerges from a large body of work is that any of a wide array of activator–polymerase interactions (Fig. 3), including a totally artificial one, can effect recruitment, and multiple interactions can work synergistically. The following sections summarize results of experiments supporting this generalization.

Activator–polymerase interactions

Lambda-repressor dimers bind cooperatively to adjacent DNA sites, one of which positions repressor very near the polymerase binding site at the promoter (called P_{RM}) of the repressor gene[2]. At this position, repressor contacts polymerase and thereby activates transcription of that gene. The following genetic experiments indicate that repressor activates by contacting the σ (in this case σ^{70}) subunit of polymerase. Repressor mutants specifically deficient in the activation function (called PC for positive control) have been described[3–5]; these mutants bear single amino-acid substitutions on their surface that most closely approaches RNA polymerase when both proteins are bound at the promoter. Certain mutations in σ^{70} specifically reduce the response to wild-type repressor[6], whereas another restores response to a PC mutant repressor[7].

CAP, in the presence of the cofactor cyclic AMP, binds to and activates several promoters, including those of the Lac and Gal genes. CAP contacts a subunit of polymerase different from that contacted by lambda repressor—namely α—and the precise interaction differs at different promoters[8,9]. When bound very near polymerase (as at the *Gal* promoter), CAP makes two contacts with α using two different activating surfaces: one with the amino part of α and the other with the carboxyl domain of

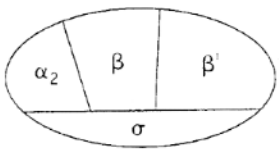

Figure 2 Each of the essential four subunits of *E. coli* RNA polymerase is present as a single copy, except for α of which there are two. The σ subunit directs the enzyme to specific promoters. Thus σ^{70}, the most commonly used form, recognizes characteristic sequences centred around positions 10 and 35 base pairs upstream of the transcription start site. The enzyme bearing σ^{54} recognizes different promoter sequences.

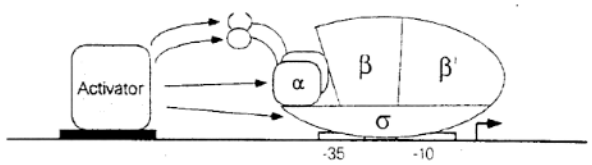

Figure 3 Activators can contact any of a variety of sites on RNA polymerase to recruit the enzyme and trigger gene activation. As discussed in the text, the precise contact or contacts made depend upon the identity of the activator and its position on DNA. The α-subunit is shown in greater detail than in Fig. 2. The α carboxy-terminal domain, a peptide of some 80 amino acids, is linked to the remainder of the molecule by a flexible linker.

that subunit[10]. When positioned upstream of polymerase (for example, 10 base pairs as at the Lac promoter), CAP contacts only the C-terminal domain of α. CAP need not work only through α: a mutant CAP has been described that replaces an α contact by a new contact with σ^{70} (refs 8, 11). As with lambda repressor, these contacts have been defined genetically: PC mutations have been isolated in both activating regions of CAP[10–12], and specific mutations in α abolish interaction with CAP[13–15]. Chemical cross-linking provides additional evidence for these CAP–polymerase interactions[10,16]. At least 12 activators in addition to CAP touch the α carboxyl domain[17], and each of these activators contacts one of several different sites on this domain.

The fact that, as we have seen, a variety of different activator–polymerase interactions can activate transcription suggests that no special protein–protein interaction is required for recruitment. This idea is supported by two further observations. First, some of these activator–polymerase interactions can be replaced by a protein–DNA interaction: the promoters of ribosomal RNA genes work at a high level in the absence of activators, an effect attributable to a DNA 'up' element that is specifically recognized by the α carboxyl domain[18]. (For an example in which a polymerase–activator interaction compensates for a loss of a protein–DNA interaction, see ref. 20.) Second, as we will now describe, transcription can be activated by a protein–protein interaction not normally involved in gene activation.

Artificial recruitment

The following experiment[21] shows that at promoters activated by both CAP and lambda repressor, the various activator–polymerase interactions described above can be replaced by a heterologous protein–protein interaction. This is an example of an 'activator bypass' experiment, in which activation is effected in the absence of a natural activating region (Fig. 4); similar experiments will play an important role in our analysis of activation of eukaryotic genes as well. In this instance, the carboxyl domain of α (present in two copies in the polymerase) was replaced by the carboxyl domain of lambda repressor, a domain that can form dimers and tetramers (Fig. 4a). When a lambda repressor dimer was bound upstream (at a position where it cannot activate using the natural activating region located on its amino domain), interaction between the carboxyl domains of that repressor and the carboxyl domains displayed by the modified polymerase (that is, tetramerization) triggers gene activation (Fig. 4b). Moreover, among a series of lambda mutants, the degree of tetramerization, measured in a separate experiment, roughly predicted the degree of activation.

Synergy

Other experiments in bacteria reveal an additional important principle of activation: multiple (in these cases two) activator–polymerase contacts have synergistic effects. That is, the level of transcription elicited by two contacts is significantly greater than the sum of the levels elicited by each single contact. One example uses the experimental system just described in which polymerase bears the modified α subunit. When lambda repressor is positioned very near polymerase (as it ordinarily is at P_{RM}), it can make two contacts: the natural contact between its amino domain and σ^{70}, and the artificial contact involving the lambda repressor tetramerization interaction. Together these two interactions elicit a much greater level of activation than either single interaction[21]. In this case (as in the previously discussed case of CAP working at the Gal promoter), the two contacts are made by a single DNA-bound activator. A synergistic effect can also be achieved by two DNA-bound activators each making a single contact. In one example, one molecule of CAP and another of lambda repressor work synergistically on an artificial construct[22]. In another, two CAP molecules (both in an artificial and a natural setting) work synergistically[23,24].

A contrasting example

A family of bacterial activators has been described whose members, unlike CAP and lambda repressor, work at promoters that bear stably prebound polymerase. In *E. coli*, for example, polymerase (bearing σ^{54}) binds stably but inertly to the promoter of the *glnAp2* gene[25,26]. As described in Box 1, the activator NTRC converts the complex to a form capable of initiating transcription. This effect, which, unlike activation by CAP and lambda repressor requires hydrolysis of ATP[27–29], evidently involves a more elaborate activator–polymerase interaction than those we have described so far. For example, NTRC might, by interacting with one or more special sites on polymerase, trigger a conformational change that stimulates the initiation of transcription. Another *E. coli* activator, the phage protein N4SSB, is also suspected to interact with polymerase that has already bound stably to DNA. In this case, the polymerase bears σ^{70}, and the activator target is the β′ subunit[30]. One interesting consequence of the fact that these activators work on prebound polymerases is that, when present at sufficiently high levels, they can activate without themselves binding to DNA[30–32], a matter we return to below.

Yeast

In yeast the transcriptional apparatus is more complicated than in bacteria, and in addition, genes are evidently less accessible because they are wrapped in histones. Nevertheless, we shall argue that, as in bacteria, any of a variety of activator–target interactions, including artificial ones, will activate transcription by recruiting the machinery to DNA. According to this picture, histones present a barrier against which polymerase recruitment is effected.

The transcriptional machinery

Most genes encoding messenger RNA are transcribed by one of three RNA polymerases, RNA polymerase II (Pol II), an enzyme comprising twelve subunits. Unlike the bacterial polymerase, Pol II requires many additional proteins—at least 30—to recognize a promoter

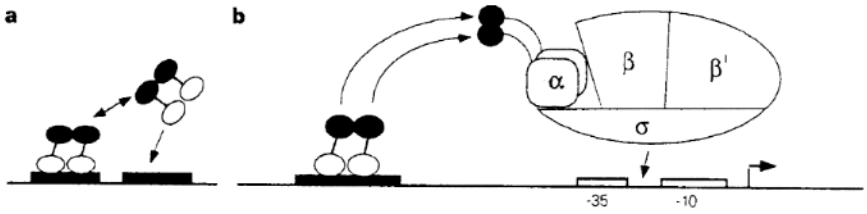

Figure 4 A heterologous protein–protein interaction can trigger gene activation in bacteria. **a**, Two lambda-repressor dimers bind DNA cooperatively by tetramerization of their carboxy termini, here represented as black circles. **b**, The tetramerization reaction can be used to recruit a hybrid polymerase in which the α carobxy-terminal domains are replaced by the carboxy-terminal domains of lambda repressor. In this experiment lambda repressor is bound some 20 base pairs upstream from its usual position at the promoter normally activated by repressor. At this upstream position, the only contact with polymerase is the artificial one with α shown here. As discussed in the text, when it is positioned closer to polymerase, repressor's amino-terminal domain (white circles) makes its natural contact with σ in addition.

and initiate transcription. Our knowledge of promoter structure would seem to be rudimentary: typical promoters are characterized as having an (A + T)-rich sequence (the TATA element) positioned some 50–70 base pairs upstream of the transcriptional start[33], and there is some evidence for initiator sequences around the start sites. Binding sites for yeast activators are typically found 100–250 base pairs upstream of the genes they regulate (for reviews, see refs 33 and 34).

The proteins that direct transcription, including PolII, are believed to be organized into a small number of complexes that work together[35,36] (Fig. 5). This picture of the transcriptional machinery is a relatively new one that has arisen from the results of genetic and biochemical experiments. Early experiments, performed with mammalian cell extracts, revealed a series of general transcription factors (GTFs) which were required, in addition to polymerase itself, for promoter recognition and transcription initiation *in vitro*. At least some of these original factors contain more than one component: for example, transcription factor TFIID contains TBP, the protein that binds to the TATA sequence, and in addition a group of proteins called TAFs (for TBP-associated factors). The separated GTF's were found to assemble in a unique order at the promoter: TFIID first, followed by TFIIB, and then, in a defined order, the remaining factors attach (for a review, see ref. 37). Similar results were found by analysis of yeast cell extracts, and many of the components are interchangeable between the yeast and mammalian systems.

Genetic analysis in yeast revealed, however, that a group of proteins in addition to the GTFs are required for transcription *in vivo*. Thus, starting with a yeast strain bearing a damaged polymerase, Young and colleagues isolated mutations in nine different genes called *SRBs* (for suppressor of RNA polymerase B, the B referring to an alternative name for polymerase II) that restored normal growth to those cells[35]. It was subsequently revealed that the SRB proteins can be isolated in a large complex that includes polymerase and certain other GTFs, including TFIIB[38,39]. This holoenzyme (Fig. 5) bears all the factors known to be involved in transcription initiation except for TFIID, which we discuss in greater detail below, and TFIIE. The aggregation state of the holoenzyme *in vitro* depends upon the isolation conditions. For example, RNA polymerase can be separated from a 'mediator' subcomplex which contains certain SRB proteins, TFIIF and Gal11, a protein we will discuss later. There is therefore room for debate as to which is the physiologically relevant form of the holoenzyme, before DNA binding, *in vivo*. We have only limited knowledge of the roles played by many of the components of the transcriptional machinery. One case for which we have structural information involves TBP. This protein binds to and distorts DNA bearing the TATA sequence so that a platform is presented for subsequent binding of TFIIB[40]. How most of the other components are arranged at the promoter is not known.

Despite the increased complexity of the transcriptional machinery, the basic design of eukaryotic activators is similar to that of prokaryotic activators: each bears an activating region and a DNA-binding region. One salient difference, illustrated by typical yeast activators such as Gal4 (ref. 41), and GCN4 (ref. 42) is that the activating regions can be physically separated from the DNA-binding regions; each of these activating regions functions when tethered to DNA by fusion to a heterologous DNA-binding domain. Certain natural activators (such as the herpes virus protein VP16) do not themselves bear DNA-binding domains, but rather attach to DNA by contacting other DNA-binding proteins.

Activation

A series of recent activator bypass experiments demonstrate gene activation by recruitment in yeast. The experiments are similar in concept to that already discussed, in which an artificial protein–protein interaction between a DNA-tethered protein and RNA polymerase triggers gene activation in bacteria (Fig. 4). We begin this section by describing examples of such experiments in yeast, and then, in the light of these, consider the action of natural activators.

Artificial recruitment. The first activator bypass experiment we consider involves a protein–protein interaction, created by spontaneous mutation, that enables an otherwise inert DNA-tethered peptide to activate transcription (Fig. 6a). The experiment shows explicitly that interaction between a DNA-tethered protein and the transcriptional machinery can trigger gene activation. The mutation changes a single amino acid in Gal11, the component of the holoenzyme mentioned above. (Gal11's role in transcription is not limited to the *GAL* genes, and its name is a historical artefact; deletion of *GAL11* decreases transcription generally some five- to-tenfold.) In an ordinary yeast strain, the dimerization region of Gal4 (residues 58–97) has no detectable transcriptional activating function when tethered to DNA. In contrast, in cells bearing the mutant *GAL11* (called *GAL11P* for potentiator), the Gal4 fragment works as an activating region (provided that it is tethered to DNA by fusion to a DNA-binding domain) nearly as powerfully as does that of Gal4 itself. *In vitro* Gal11P (but not Gal11) interacts with Gal4(58–97), and among a series of *GAL11P* alleles, the strength of this interaction is proportional to the level of gene activation observed *in vivo*. Peptide fragments bearing the interacting regions of these proteins can be swapped without loss of function. Overexpression of a fragment comprising either interacting component, unattached to a DNA-binding domain, inhibits activation, evidently because the fragment titrates its interacting partner. These and other experiments argue strongly against the possibility that the Gal11P/Gal4(58–97) interaction conveys some special conformational change that triggers activation; rather, they suggest that this arbitrary interaction activates gene expression to the extent of the binding energy involved, a result that is to be expected if the interaction serves to recruit the holoenzyme to DNA[43,44].

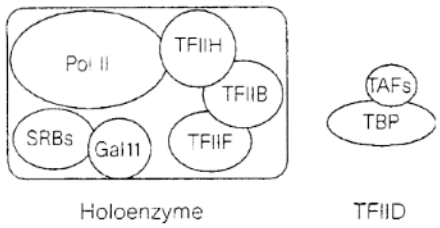

Figure 5 The yeast machinery that initiates transcription is organized in complexes. Some of the depicted components of the holoenzyme themselves comprise multiple proteins. For example, Pol II has 12 subunits and there are nine different SRBs; only some of the known holoenzyme components are shown. Not all of the Pol II is found in the holoenzyme, nor is all of the TBP complexed with TAFs, of which there are about 12. An essential factor that is not shown, TFIIE, is not strongly associated with either complex.

The Gal4(58–97)/Gal11P interaction can also drive gene activation in bacteria in an experiment similar to that of Fig. 4: in a strain bearing a polymerase fused through its α carboxyl domain to a fragment of Gal11, Gal4(58–97), tethered to DNA, activates transcription, provided the Gal11 fragment bears the P mutation[21]. Thus an identical protein–protein interaction will trigger gene activation in bacteria and in yeast.

The idea that the Gal4(58–97)/Gal11P interaction activates by recruiting the holoenzyme leads to the following prediction: fusion of a DNA-binding domain to a holoenzyme component would allow transcription at genes bearing the appropriate DNA binding site in the absence of any activator. This prediction is realized: yeast cells containing, for example, Gal11 fused to the DNA-binding domain of the bacterial protein LexA transcribe at a high level genes bearing LexA binding sites (Fig. 6b)[43]. Various SRB proteins, including SRB2, SRB7, and SRB11, have all been shown to have a similar effect when fused to a DNA-binding domain (ref. 44 and L. Gaudreau, J. Nevado and M.P., unpublished results). In some cases, the level of gene activation is very high: LexA–Gal11, for example, works when the LexA binding sites are positioned quite far upstream (1,000 base pairs away for example) of the gene; when assayed at these large distances, LexA–Gal11 activates even more strongly than Gal4, itself a very efficient activator in yeast[43].

It is imagined that, in these experiments, each fusion protein is incorporated into the holoenzyme, and that the attached DNA-binding domain tethers the complex to DNA as depicted in Fig. 6b. Any required components that are not part of the holoenzyme (for example, TBP and TFIIE) would then bind cooperatively with it and trigger transcription. A similar picture would explain activation by TAF[45] and TBP[46–48] fusions; in these cases the holoenzyme would bind cooperatively with TBP that is tethered directly or by association with a tethered TAF.

There is further evidence supporting the idea that the holoenzyme fusion proteins insert into the holoenzyme, as shown in Fig. 6b. A plausible interpretation of the genetic experiments of Koeleske *et al.*[49] is that certain SRB mutants more readily associate with a mutant polymerase (that used in the original selection for the SRB mutants) than do the wild-type forms of these SRB proteins. One such mutant is *SRB2-1* (ref. 49) and, as expected on the basis of the scheme just described, LexA–*SRB2-1* activates transcription in a strain bearing the damaged polymerase more efficiently than does a LexA fusion bearing the wild-type form of SRB2 (ref. 44). Also, holoenzyme has been isolated from a strain bearing, in place of wild-type Gal11, a Gal11–PHO4 fusion protein. In this fusion protein PHO4's activating region is replaced by Gal11. Template DNA bearing PHO4 binding sites is transcribed more efficiently than is template lacking those sites in a reaction containing, in addition to the purified holoenzyme, purified TBP and TFIIE. (L. Gaudreau and M.P., manuscript in preparation).

Natural activation. We have seen that in yeast, as in bacteria, recruitment of the transcriptional machinery can trigger gene activation. We now consider whether natural yeast activators work in this way. The evidence suggests that activating regions can contact a variety of targets, and the recruitment model suggests that any of those interactions could trigger gene activation.

An early observation suggesting that the typical yeast activator does not work on a stably prebound transcriptional machinery arose from experiments in which activators that were damaged in their DNA-binding functions were expressed in cells at unusually high levels. To understand the implication of such experiments, consider the expectations based on the two models for gene activation we have discussed. First, consider the case in which the activator works on a polymerase stably bound to DNA. Ordinarily, activation requires that the activator binds DNA so as to raise its concentration to an appropriate level in the vicinity of the promoter. We would imagine that high activator concentrations would obviate that requirement, and the activator could work on the bound polymerase without itself binding to DNA. In fact, that is the effect observed with mutant forms of NTRC and with N4SSB, the bacterial activators that work on prebound polymerases[29–31]. Second, consider activators that work by recruitment. We would anticipate that such activators could not activate were they unable to bind DNA, because in this case DNA tethering is required to bring the target to the DNA.

In fact, the typical result of overexpressing natural activating regions in yeast is inhibition, not activation. For example, overexpression of *GAL4* inhibits activation by GCN4 of a gene bearing GCN4 sites[50]. This 'squelching' phenomenon, widely observed with various activators in disparate organisms, is explained by assuming that the two activators have common target sites, and titration of those sites by one activator (at high concentration) prevents interaction with the second, DNA-bound, activator. We saw a demonstration of squelching (actually an example of 'self-squelching') in experiments with the Gal4(58–97)/Gal11P system[44]. In that case, overexpression of Gal4(58–97), unattached to a DNA binding domain, inhibited activation by DNA-tethered Gal4(58–97).

Also consistent with the idea that yeast activators do not work on a prebound machinery are experiments showing that at several promoters activation is accompanied by marked changes in sensitivity to nucleases and chemical probes, a simple interpretation of which is that histones have been replaced by the transcriptional machinery[51,52].

Activating regions and their targets. Much of what we know about natural activating regions, otherwise quite puzzling, is at least partly explained by the assumption that their role is merely to stick to the transcriptional machinery and thereby recruit it to DNA. For example, no elaborate structure is required for the activating function[53]. Anecdotal evidence suggests that typical activating regions—such as those found on Gal4 (ref. 41), GCN4 (ref. 54) and PHO4 (ref. 55), which bear an excess of negatively charged residues—are largely unstructured in the absence of an interacting partner. For both GCN4 and Gal4, fragments of the activating region function when tethered to DNA. In the Gal4 case, for example, a recent study found that, among a series of fragments extending from 17 to 41 amino acids, activation was approximately proportional to length[56]. There must of course be some specificity determinants in activating regions that distinguish them from random peptides, but we do not have a clear idea as to what they are. The apparent lack of a structural requirement, however, would seem to be most readily explained by invoking simple interactions[57] that recruit. Some activating regions perform disparate functions with different sequence requirements. For example, the 41-amino-acid activating fragment of Gal4 alluded to above also recognizes the inhibitor Gal80 (ref. 58); introduced proline mutations have, in several instances, little effect on the activation function but strongly

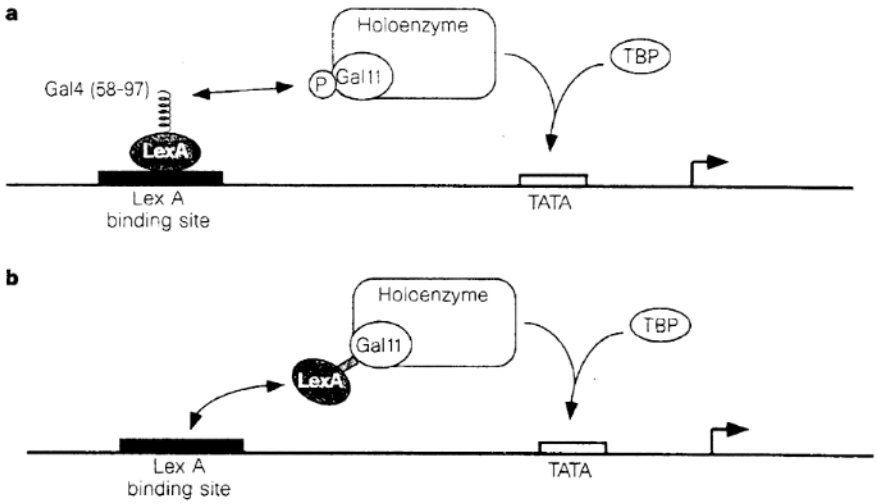

Figure 6 Gene activation in yeast can be effected in the absence of a natural activator by artificial recruitment of the holoenzyme. These are two examples of activator bypass experiments. **a**, The peptide Gal4(58-97), tethered to DNA by fusion to the DNA-binding domain of the bacterial repressor LexA, binds to the mutant holoenzyme component Gal11P and thereby recruits the holoenzyme to the promoter. TBP binds cooperatively, presumably because it interacts both with DNA and with the holoenzyme. **b**, The LexA DNA-binding domain is fused directly to Gal11. The holoenzyme is recruited to DNA bearing a LexA-binding site near the beginning of a gene.

decrease Gal80 recognition, a result consistent with the idea that no very elaborate structure is required for the activation function (refs 41, 59, and A. Ansari and M.P., unpublished results).

An implication of this review is that, as in bacteria, there need be no specialized targets for activators; any of a large array of interactions would in principle suffice. In fact, many activator–putative target interactions have been detected *in vitro* in experiments with yeast and mammalian activators, although few of these have been quantified or extensively challenged with mutant forms of the interacting proteins[53]. Showing relevance in any given case has been difficult, however, in part because the interactions may be redundant. Those proteins under serious consideration as potential activating region targets include the following.

TBP and TFIIB. An array of mammalian and yeast activators have been shown to interact with these proteins (for example, refs 56, 60, 61). In the quantitative study mentioned above, in which activation was proportional to the length of a fragment excised from Gal4, each individual fragment interacted indistinguishably with TBP and with TFIIB[56]. Furthermore, the affinities of these interactions predicted the degree of gene activation. That relationship between affinity measured *in vitro* and activation measured *in vivo* was found to hold for a series of point mutants of activating fragments as well. A further suggestion that the measured affinities are physiologically relevant is provided by the observation that, for two unrelated interactions that give comparable levels of activation *in vivo* (that between the largest Gal4 fragment and TBP/TF11B, and that between Gal4(58–97) and Gal11P), the measured affinities are approximately equal ($\sim 10^{-7}$ M) (refs 44, 56, 60). This energy of interaction is orders of magnitude greater than that measured for the bacterial activator CAP and *E. coli* RNA polymerase in the absence of DNA (R. H. Ebright, personal communication). That difference is what one would expect: the eukaryotic activators work when bound to sites positioned much further away from the transcriptional start than does this bacterial activator.

These and other results provide a strong, if not completely compelling, argument identifying TBP and TFIIB as activating region targets. At the same time they raise two questions, the resolution of which might require further analysis of the relevant structures. First, why is the affinity for either putative target protein approximately proportional to the length of the activating region fragment? Might, for example, the activating region contain reiterations of some sequence pattern that is not evident upon inspection? Second, what common surface features of TBP and TFIIB dictate indistinguishable affinities for activator fragments in these experiments?

TAFs. It has been suggested that TAFs (TBP-associated factors) are required to transmit the effects of activators to the transcriptional machinery, and for this reason they were called coactivators. The simplest form of the recruitment model would seem not to require such specialized proteins and, as we have seen, such proteins are not required in bacteria. In addition to experiments already described, recent experiments suggest that in yeast as well there is no strong reason to assume that coactivators, in the sense defined here, are required. To understand this we must briefly review experiments that led to the coactivator hypothesis.

The proposed role of TAFs as coactivators was based on the findings that they were required for response to activators in mammalian and yeast cell extracts, and because they interacted with various activating regions. In those early experiments with crude extracts, removal of TFIID (that is, of TBP and TAFs) abolished transcription. Subsequent addition of purified TBP restored transcription, but that activity—called 'basal' transcription—was unaffected by activator[62]. In mammalian[62] and subsequently in yeast experiments[63], response to activator was reestablished by adding a fraction containing TAFs to the reaction. These results were interpreted as showing that TAFs conveyed the activation signal from the activator to the basal transcriptional machinery. But subsequent analysis with mammalian extracts showed that a number of additional components—including PC4, an HMG protein, and other uncharacterized proteins—are also required for activation and are therefore by that criterion, coactivators[64].

Elimination of TAFs from yeast, although ultimately lethal, does not have a significant effect on activation of a wide array of genes[65,66]. Therefore although the early *in vitro* results remain unexplained, if TAFs are targets of transcriptional activating regions in yeast, they are dispensable at most genes. Recent experiments with purified holoenzyme show that with at least one test promoter there is activation in the absence of TAFs[58].

In the TAF elimination experiments, although most genes were

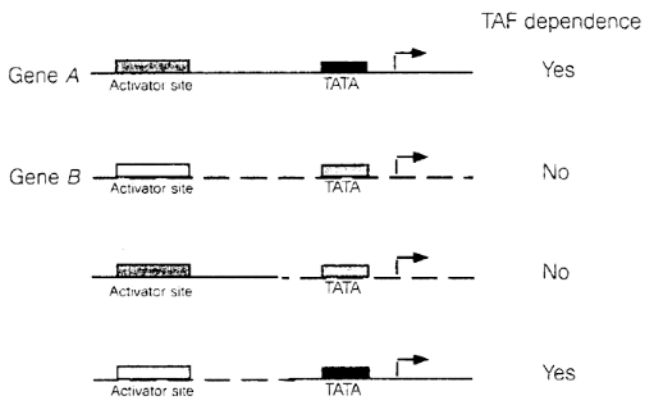

Figure 7 Certain genes cease transcription upon elimination of TAFs. This experiment shows that TAF dependence is determined by sequences around the TATA region and not by the nature of the activator. Each construct was assayed in dying yeast cells from which TAFs were eliminated as described[55]. This experiment has been done with several pairs of genes. An example of gene *A* is *RPS5* and of gene *B* *ADH1* (W. Shen, S. Walker and M. Green, manuscript submitted).

unaffected, certain genes were found to cease transcription. Two scenarios might be imagined for the role of TAFs at those genes: they could be coactivators whose sole role is to transmit the activation signal, or they could be part of the transcriptional apparatus required at these promoters. That the latter is correct is suggested by the following experiment involving two genes, here called *A* and *B* (Fig. 7). The genes have upstream activating sequences that bind specific but different activators; transcription of *A* is TAF-dependent, but that of *B* is not. Hybrid genes were constructed in which the activator-binding elements were swapped. In the swapped configuration, the gene bearing the activating sequence from *A* was transcribed in a TAF-independent manner, whereas transcription of the gene bearing the corresponding sequence from *B* required TAFs. Thus, the TAF requirement of certain genes is specified by the sequence flanking the TATA element, and not by the nature of the activator. The simplest explanation for these results is that the TAF requirement arises not because certain activators must work through TAFs, but rather because at certain genes TAFs constitute an essential part of the transcription apparatus. This surmise is consistent with the finding that TAFs are DNA-binding proteins which could contribute binding energy required at certain promoters[67]. This picture does not of course exclude the possibility that TAFs, like other components of the machinery, are activating-region targets. Evidence that TAFs might play such a role in higher eukaryotes has been presented[68].

In retrospect, an impetus for entertaining the coactivator hypothesis was the observation that, as we have seen, the machinery that directs activated transcription *in vitro* is apparently different from that which directs basal transcription. We suggest that this distinction is not physiologically relevant, and that basal transcription, should it occur in yeast, would best be described as we envisage basal transcription in bacteria. In bacteria, the term 'basal' transcription refers to the low level of transcription observed at certain promoters in the absence of activator. For example, the lambda-repressor gene, which is stimulated by lambda repressor, is transcribed at a low level *in vivo* in the absence of repressor. That basal transcription is understood to result from the spontaneous and infrequent stable binding of the intact polymerase to the promoter. A corresponding example of basal transcription in yeast may be that observed, in the absence of activator, when histones are removed from the cell (see below). We imagine that, as in bacteria, this basal transcription arises from the spontaneous stable binding of the intact transcriptional machinery to the promoter.

Others. There is no reason to exclude other components of the transcriptional machinery from consideration as activating region targets. For example, both the 'mediator' subcomplex of the holoenzyme and TFIIH interact with certain mammalian activators[69,70] and perhaps these are also bona fide targets in mammalian and yeast cells.

Multiple interactions and synergy

We have seen that in bacteria multiple activator–polymerase interactions can work synergistically, and we would expect this to be true in yeast as well. The idea that yeast activating regions can touch multiple targets would provide an obvious way to achieve this effect, but there may be an additional consideration. In bacteria the entire machinery essential for the initiation of transcription is present in a single complex (the RNA polymerase) and so each contact simply adds energy to the binding reaction. However, as already pointed out, the yeast holoenzyme as currently isolated lacks at least one or two essential components, including TBP, and it is possible that, as suggested by Struhl[34], the different subcomplexes could be recruited independently. The following experiments are consistent with that view.

In the first experiment, Strubin *et al.*[101] found that, in an artificial tethering experiment, a promoter bearing a weakened TATA sequence requires tethering of both the holoenzyme and, independently, TBP. In this case, recruitment of both components has a synergistic effect compared with the effect of recruitment of either component alone. In the second experiment, as noted earlier, at a wild-type promoter LexA–Gal11 is a stronger activator than Gal4. The reverse is the case, however, at a promoter bearing a damaged TATA sequence (A. Barberis and M.P., unpublished results). The results are plausibly explained by the observation that whereas LexA–Gal11 directly recruits only the holoenzyme, intact Gal4 molecules can touch both TBP and TFIIB.

It is unlikely that the activator–target interactions described here account for the full synergistic effects of activators. Experiments by Tanaka[71], for example, suggest an important additional contribution from cooperative DNA binding. That effect evidently does not depend on direct interaction between the binding proteins, nor upon an indirect interaction mediated by interaction of activating regions with the transcriptional machinery (at least, not entirely). Rather, as he has proposed, some significant aspect of cooperativity may involve the concerted effect of two or more activators competing with histones for access to DNA (see also ref. 72).

Nucleosomes

The various examples of 'activator bypass' experiments described here suggest that holoenzyme recruitment is sufficient to overcome whatever obstacles to transcription are presented by nucleosomes and any other general inhibitors. An explicit test of this idea has been described using the *PHO5* gene in yeast, which is activated by PHO4. An array of nucleosomes is positioned at the promoter of this gene, and, as assayed by nuclease sensitivity, histones are removed or modified upon gene activation[59]. When the activating region of PHO4 was replaced with a holoenzyme component (such as Gal11), the *PHO5* gene was efficiently transcribed and chromatin remodelled as usual[73]. The experiment demonstrates that penetration of the nucleosomal barrier requires no special effect of an activator other than recruitment of the holoenzyme. Nucleosome remodelling is evidently a direct consequence of recruiting the transcriptional machinery: remodelling is observed even at a promoter deleted of its TATA sequence and thus unable to support transcription.

The simplest interpretation of these experiments, and of a related experiment performed with a yeast Pol III gene by Sentenac and colleagues[74], is that the holoenzyme competes for DNA access with histones. Histones would thus constitute an important part of the regulatory apparatus by presenting a barrier against which holoenzyme recruitment must be effected. Consistent with that idea is the

finding that histone depletion allows, to varying extents, spontaneous transcription of many genes[75]. Weakening the histone barrier—by the binding of additional proteins and/or by histone modification—could therefore increase gene expression. Strengthening the barrier could decrease or even prevent transcription altogether. Widom and colleagues[76,77] have measured the dissociation of histones from DNA *in vitro*, and on the basis of these results have proposed a model in which histones and regulatory proteins compete for common DNA sites[72].

At least three protein complexes have been proposed to help in histone removal in yeast: SNF/SWI (ref. 78), GCN5/ADA (ref. 79) and RSC (ref. 80). One of these complexes (SNF/SWI) is reported (but see ref. 80) to be associated with the holoenzyme[81], a finding that would account for the abilities of certain LexA–SNF fusion proteins to activate transcription[82]. One of these proteins, GCN5, is a histone acetylase, and active genes in higher eukaryotes have been characterized as being associated with hyperacetylated histones[79] (but see ref. 83). Mutation of components of the GCN5/ADA and SNF/SWI complexes has been reported to decrease expression of certain genes[78,79]. In the experiments with the *PHO5* promoter described above, however, no significant effect on transcription was observed by mutation of either the SNF/SWI or GCN5/ADA complex. Full expression of the *PHO5* and *GAL1* promoters, activated by their natural activators, is similarly unaffected by mutation of either component. At the *GAL1* promoter, however, weakening activation (by, for example, using a promoter bearing only two Gal4 sites in place of the usual four sites) renders transcription elicited by either natural or holoenzyme-fusion activators sensitive to mutation of SNF/SWI or ADA/GCN5 (refs 73, 78, and L. Gaudreau and M.P., unpublished results). It is possible that as the total DNA–protein and protein–protein interactions are weakened, a greater contribution is required from these accessory factors. Whatever these complexes may do, the fact that their effects can be observed in activator bypass experiments shows that they can be called into play simply by recruiting the holoenzyme.

Higher eukaryotes

It would be surprising if the mechanism described here for gene activation in bacteria and yeast were absent from higher organisms. Mechanisms other than the one emphasized in our review are sure to be found in higher eukaryotes and perhaps in yeast as well (see refs 84–86 for example). Transcription of many genes, particularly in higher eukaryotes, is dependent upon multiple physiological signals. The following discussion shows how the recruitment mechanism we have described can help effect this integration.

The typical yeast gene discussed here is instructed to be transcribed at a high level by only one or two extracellular signals. Thus a few copies of DNA-bound Gal4, freed of the inhibitor Gal80 by the presence of galactose (and the absence of glucose), maximally activate the *GAL* genes. In the simplest elaboration of the recruitment mechanism, multiple activator–machinery contacts would be required for efficient transcription, and these contacts would be provided by disparate DNA-binding activators, each of which is controlled by a different physiological signal. Binding sites for these various activators, suitably positioned, would allow these activators to work together (synergistically as we have described) to activate the gene. In a further refinement we might imagine that, as already suggested[87], certain activators in higher eukaryotes differ from Gal4 in that they would be specialized to recognize more limited sets of potential targets. According to that idea, only certain combinations of such activators would work together at certain promoters because, at those promoters, two or more subcomplexes of the transcriptional machinery would have to be recruited independently. Other eukaryotic activators might be designed, like Gal4, to work independently. It is clear, however, that strong activation in higher eukaryotes does not intrinsically require such a complexity. Thus, for example, VP16, the strong activator expressed by herpes simplex virus, works very efficiently without help from other activators[87]. □

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