Activating Transcription in Bacteria

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Abstract

Bacteria use a variety of mechanisms to direct RNA polymerase to specific
promoters in order to activate transcription in response to growth signals or
environmental cues. Activation can be due to factors that interact at specific
promoters, thereby increasing transcription directed by these promoters.
We examine the range of architectures found at activator-dependent pro-
moters and outline the mechanisms by which input from different factors
is integrated. Alternatively, activation can be due to factors that interact
with RNA polymerase and change its preferences for target promoters. We
summarize the different mechanistic options for activation that are focused
directly on RNA polymerase.

Contents

PREAMBLE

In his autobiography (62), Francois Jacob recalls the Sunday afternoon, late in July 1958, when he suddenly realized that a host of experimental observations concerning bacteriophage development and enzyme induction in *Escherichia coli* could be accounted for simply by postulating the existence of repressors that "acted on the DNA." This delightful eureka moment marks the start of the scientific age of transcription factors, and most readers will know how it led immediately to the discovery of the lactose operon repressor; the discovery of the bacteriophage λ cI repressor; and the subsequent establishment of transcription factors as key players in just about every area of biological regulation, adaptation, and development. However, many readers will be unaware that, at the time, most people thought that all this regulation would be done by repressors, and little credence was given to the existence of transcription activators (123). In their seminal 1984 review (106), Raibaud & Schwartz recall two decades of meticulous research, based mainly on genetics with the *E. coli* arabinose, maltose, and cyclic AMP systems, that overturned the prejudice and placed transcription activators on an even footing with transcription repressors.

Right from the start, it was clear how transcription repressors could work, simply by behaving as blocking agents at their targets. In contrast, up to the 1990s, most transcription activators were regarded as mysterious agents that could somehow, as if by magic, turn on the expression of their target genes. Although there are still a few areas where the magic remains, we now understand the mechanism by which many transcription activators do their job. This is due mainly to big advances in our knowledge of how the bacterial transcription apparatus is organized, and one of the major tasks of this review is to explain the modus operandi of some of these activators and

how they interface with the transcription apparatus. Later sections are concerned with the variety of mechanisms by which transcription can be activated, and how promoters can act as integrators of different activator signals.

BACTERIAL TRANSCRIPTION APPARATUS AND ECONOMICS

All the transcription activation mechanisms discussed in this chapter are concerned with changing the distribution of RNA polymerase molecules between the different transcription units in a bacterium. Transcription in all bacteria is due to the multisubunit DNA-dependent RNA polymerase, originally characterized in the 1960s by Stevens, Burgess, and others (17, 18). The core enzyme subunit composition is $\beta\beta\alpha_2\omega$, and this form of polymerase is capable of DNA-dependent RNA synthesis but is unable to locate promoters and direct specific transcript initiation. The key factor for specific transcript initiation at promoters is the σ subunit, which carries the major determinants for promoter recognition, and the $\beta\beta\alpha_2\omega\sigma$ form of polymerase is known as the holoenzyme and is competent for transcription initiation (19). Many of the functions of RNA polymerase core and holoenzyme are now well understood at the structural level following high-resolution structural studies using polymerase from thermophilic bacteria (97, 98). These functions have been reviewed in detail elsewhere, but some aspects of promoter recognition and transcription initiation are summarized schematically in **Figure 1**.

Concerning *E. coli*, the principal σ factor is the 613-amino-acid σ^{70}, the founder member of the largest group of σ factors (102). σ^{70} is often called the housekeeping sigma factor because it is responsible for most transcription, including all essential genes in *E. coli* (60). Like all members of this family, σ^{70} contains four distinct domains, and determinants in these conserved domains recognize the different promoter elements (50, 96). Many groups have attempted to quantify the levels of σ^{70} and RNA polymerase core enzymes in vivo and there is a wide variation in the measurements reported (49, 60, 105). Most recent reports, including a comprehensive analysis from Carol Gross's laboratory (49), show that several thousand core enzyme molecules are present per cell (the exact number depends upon growth conditions) with 1.3- to 1.6-fold more σ^{70} molecules. One consequence of this is that the activities of alternative σ factors are limited by competition, and this is especially critical in *E. coli*, where σ^{70} has a higher affinity for core RNA polymerase than do any of the six alternative σ factors (86). Another important consideration is that the number of RNA polymerase molecules per cell exceeds the number of growing RNA chains, and so cells contain a pool of unemployed polymerase. Most of this polymerase is probably sequestered either at random chromosomal targets or by RNA, and this acts as a reserve to supply polymerase during transcription activation (49).

TRANSCRIPT INITIATION AND THE OPTIONS FOR ACTIVATION

The first step in transcription initiation is promoter recognition in which holo RNA polymerase recognizes promoter elements located upstream of a transcript start point. This is followed by open complex formation in which a short segment of promoter DNA including the start point is unwound and the template strand is inserted into the active site of the polymerase (96). These open complexes are competent for transcript formation provided that appropriate nucleoside triphosphates are present. As illustrated in **Figure 1**, the main promoter elements that facilitate specific transcript initiation by RNA polymerase are the UP (upstream) element, the −35 element, the extended −10 element, and the −10 element, with other elements located in the spacer region between the −10 and −35 elements and the discriminator element from position −4 to −6. Determinants in conserved domains 4, 3, 2, and 1 of the σ factor are responsible for recognition of

a Binding

αNTD

αCTD

ω

DNA strand

UP element | −35 | Extended −10 | −10 | +1

σ⁴ σ³ σ² σ¹

β′ β

TTGACA   TGnTATAAT   +1

b Isomerization

αNTD

αCTD

ω

DNA strand

UP element | −35 | −10

σ⁴ σ³ σ² σ¹

β′ β

NTPs

c Transcript formation

αNTD

αCTD

ω

DNA strand

UP element | −35 | −10

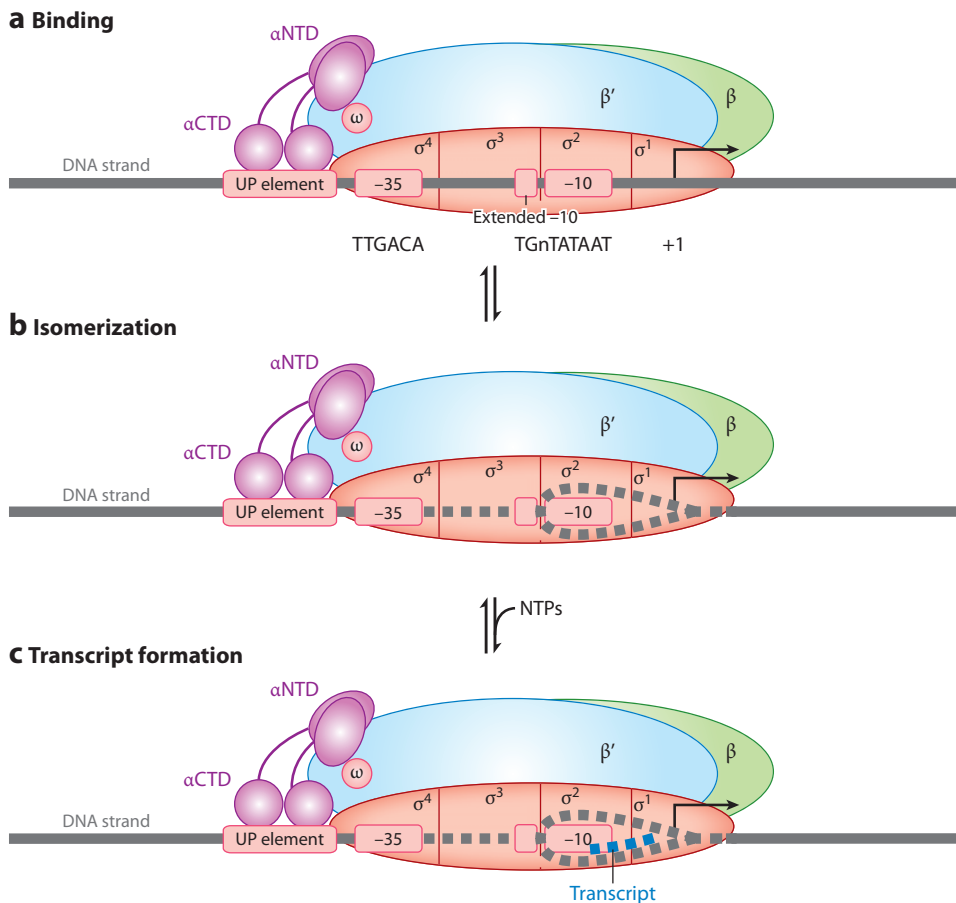σ⁴ σ³ σ² σ¹

β′ β

Transcript

Figure 1

RNA polymerase interactions at a promoter and the steps to transcription initiation. (*a*) A schematic
representation of RNA polymerase bound to a promoter. The location of the transcription start site is
indicated by the bent arrow. (*b*) Isomerization to the open complex is signified by unwinding of the duplex
DNA around the transcription start site. (*c*) Formation of transcripts (*blue dashed line*) ensues in the presence
of NTPs. Abbreviations: NTD, N-terminal domain; CTD, C-terminal domain; UP, upstream;
NTP, nucleoside triphosphate.

the −35 element, certain bases in the spacer, the extended −10 element, the −10 element, and the
discriminator element, respectively. Additional promoter recognition determinants are provided
by the C-terminal domains of the two RNA polymerase α subunits (αCTDs) that interact with
UP elements, located upstream of the −35 element (39, 44).

An important aspect of αCTD is that it is separated from the α subunit N-terminal domain
(αNTD) by an unstructured linker, and this confers a degree of flexibility in its positioning at
promoters. The two αCTDs can be thought of as antennae for RNA polymerase, because they are
peripheral to the core enzyme and play no role in its assembly or in the catalysis of RNA synthesis.
However, at many promoters, a big contribution is made by the promoter-proximal αCTD, which
binds to the minor groove of promoter DNA at position −41 and interacts directly with Domain
4 of the σ subunit bound to the −35 element (44, 113). The contribution of the different elements

differs greatly from one promoter to another, and bacterial promoters appear to have evolved by a pick-and-mix mechanism that has resulted in hierarchies of promoter activities that permit a 1000-fold dynamic range in transcript initiation frequencies (55, 92). Although to date the time course of recognition of the different elements has been studied at very few promoters, it seems likely that for many cases the initial encounter of polymerase involves the promoter UP element and −35 element, with interactions with the downstream elements coming later (16, 30, 116, 124). In particular, recent structural studies from Feklistov & Darst (40) and Zhang & Ebright (personal communication) show that interactions between σ and the −10 element take place only after promoter melting.

Irrespective of differences in the functional elements and the pathway to the open complex, the overall organization at the heart of most final open RNA polymerase holoenzyme-promoter complexes appears to be very similar. At many promoters, these complexes, which are competent for transcript initiation, can form without help from any other factor. A key point, established by early studies with the *lac* operon promoter, is that both activator-dependent and activator-independent promoters use the same overall pathway to transcript initiation (1, 130). Hence, the different determinants for promoter recognition and open complex formation outlined above are the same at activator-dependent promoters. Thus, it is useful to consider activator-dependent promoters as activator-independent promoters that have become handicapped in some way. For example, a promoter may be activator dependent because it carries one or more defective elements so that the initial binding of polymerase is reduced, and it is easy to see how the degree of dependence on an activator could be set by the precise nature of these elements (115). Put simply, reducing the intrinsic activity of a promoter leads to the possibility of increasing its activity by another factor, thereby introducing a step at which promoter activity can be regulated by some environmental factor. Of course, some promoters are so defective that they exhibit no factor-independent activity.

Bacteria use two distinct sets of mechanisms for activation of transcript initiation and these are focused either on the promoter or on the RNA polymerase. In promoter-centric mechanisms, factors interact with the promoter to improve its ability to guide RNA polymerase to initiate transcription, either by providing additional functional determinants to the promoter or by reversing the action of a repressor. In RNA polymerase–centric mechanisms, factors interact with RNA polymerase to alter its promoter preference. The simplest scenario is when the housekeeping sigma factor of a subpopulation of polymerase molecules is replaced by an alternative sigma factor (50, 60). In other cases, holoenzyme containing the housekeeping factor is altered in order to direct it to certain promoters, and this is often referred to as RNA polymerase appropriation (52).

SIMPLE TRANSCRIPTION ACTIVATION

In this section we consider the simplest scenario of transcription activation in which a single activator molecule binding at a bacterial target promoter is essential for it to be served by holo-RNA polymerase containing σ^{70}. Most simple activators function either by stabilizing the initial polymerase-promoter complex or by accelerating the transition to the open complex. An increase in the rate of polymerase passage through the initiation pathway will result if the free energy of the initial polymerase-promoter complex is reduced, or if the energy barrier for the transition to the open complex is reduced (115). In principle, there are two ways in which a transcription factor could directly influence these parameters. The activator might alter the conformation of the promoter DNA to improve the promoter quality, or the activator could interact directly with RNA polymerase to compensate for the defects in a promoter.
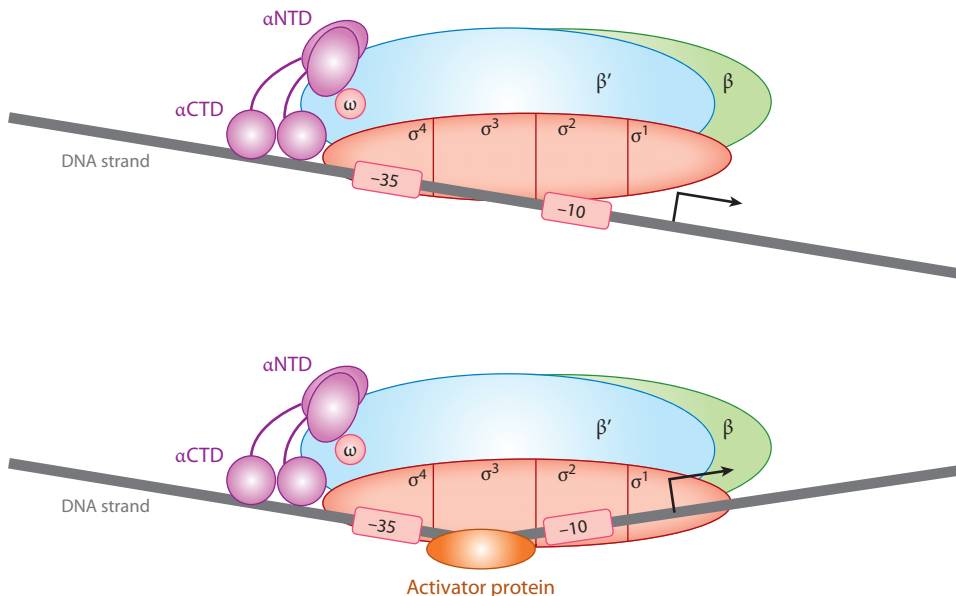
Figure 2

Simple activation by conformational change. The activator protein binds between the −10 and −35
elements and induces a conformational change in the DNA, allowing RNA polymerase to fully engage with
the promoter. Abbreviations: CTD, C-terminal domain; NTD, N-terminal domain.

Activation by Altering Promoter Conformation

A few well-characterized bacterial regulatory factors work by inducing a conformation change in
promoter DNA. The best-understood cases involve members of the MerR family of transcrip-
tion factors that mostly bind between the −10 and −35 elements at target promoters (11, 107).
Examples include the Tn*901* MerR protein at the *merP* promoter, which controls expression of a
mercury resistance determinant, and BmtR, a MerR family member found in *Bacillus subtilis* that
controls expression of efflux pumps and whose activity is triggered by xenometabolites. Target
promoters for these and other related activators are defective because of nonoptimal spacing be-
tween the −35 and −10 elements. Thus, after initial binding of RNA polymerase to the promoter
UP element and −35 element, the −10 element is misplaced and promoter melting, unwinding,
and interaction with Domain 2 of the σ subunit are hindered (**Figure 2**). This hindrance is over-
come by activated MerR or BmtR, which causes a twist in the spacer that results in the promoter
−10 element being brought into register with the −35 element, thereby triggering transcription
activation. For many years, it was believed that the twist was a smooth change in the winding of
the spacer element, but high-resolution structural studies from Brennan and colleagues (51, 99)
have now shown how MerR family activators cause a specific localized distortion that aligns the
−10 element with the −35 element. This mechanism was originally thought to be restricted to the
MerR family, but a recent report has suggested that other unrelated activators may use a similar
mechanism (61).

The fact that a promoter can be activated by altering its conformation suggests the possi-
bility of regulation by supercoiling without the direct intervention of transcription factors. In
E. coli and related bacteria, it appears that supercoiling plays a global role in setting hierarchies of
promoter activity, but it is rarely responsible for activation at specific promoters in response to

specific signals (33). In contrast, in *Mycoplasma* species that contain very few transcription factors, changes in DNA topology may be responsible for the osmoregulation of certain genes, although it is unclear how promoter specificity is set (147). One possibility is that specific sequences located near a promoter transcript start point make it more or less sensitive to modulation by supercoiling. In this context, results from Hatfield and colleagues (101, 128) and Travers and colleagues (2, 103, 136) have shown how specific binding of certain *E. coli* nucleoid-associated proteins adjacent to a promoter can induce local changes in topology that can modulate promoter activity. Recall that the bacterial nucleoid-associated proteins are a small group of abundant proteins whose main functions are thought to be the folding and compaction of bacterial chromosomes, but many of these proteins also play key roles in transcriptional regulation (14). Hence, working with the *E. coli ilvG* promoter, Hatfield and colleagues (128) showed that upstream-bound IHF (integration host factor; a nucleoid-associated protein) influences the conformation of the neighboring promoter sequences, and defined the specific DNA base sequence that rendered the promoter susceptible to this activation. Here, IHF binding sets the conformation of neighboring sequences, thereby triggering promoter activation. Similarly, at the *tyrT* promoter, Travers and colleagues (2) found that upstream binding of another nucleoid-associated protein, FIS (factor for inversion stimulation), constrains DNA supercoiling, thereby activating the formation of transcriptionally competent complexes.

Activation by Direct Contact with RNA Polymerase

Genetic and biochemical studies at many different bacterial promoters have shown that a direct activator–RNA polymerase contact is essential for activation. At the majority of promoters that are dependent on a single activator, the activator binds to a DNA target either upstream of or overlapping the promoter −35 element (**Figure 3a,b**), so the bound activator can make a direct interaction with RNA polymerase when it engages with the promoter (20, 53, 104). Remarkably, irrespective of whether polymerase binding is dependent on or independent of an activator, the final organization of the open complex appears to be similar. Thus, most activator-dependent promoters are simply defective activator-independent promoters, with activator protein–polymerase interactions replacing promoter DNA–polymerase interactions (12, 53). The key evidence for this comes from the existence of positive control mutations that result in single amino acid substitutions that reduce or destroy the ability of an activator to interact with RNA polymerase without affecting its other functions such as DNA binding or regulation. Positive control mutations reduce or stop transcription activation and have been used to identify the amino acid side chains in activators that are essential for the direct interaction with polymerase. Analysis of positive control mutations in several bacterial transcription factors has shown that these side chains are often clustered and form small surface-exposed patches known as activating regions (20, 53, 80).

The discovery of direct contacts between bacterial transcription activators and RNA polymerase that are essential for promoter activation leads to interesting mechanistic questions. Are the contacts essential for the initial recruitment of polymerase to the promoter, required for isomerization to the open complex, or needed for promoter escape? Alternatively, could the contact push some activatory button in polymerase that, for example, somehow tweaks the active site? Although examples of all these possibilities are mentioned below, two important strands of evidence suggest that many (if not most) activators intervene early in the pathway to transcription initiation at most target promoters. First, in the two cases for which we have a high-resolution structure of a ternary complex between promoter DNA and an activator making contact with its cognate target in RNA polymerase (see below), it is clear that the structure of the polymerase target is unaltered by interaction with the activator (7, 64). Thus, the interaction appears to
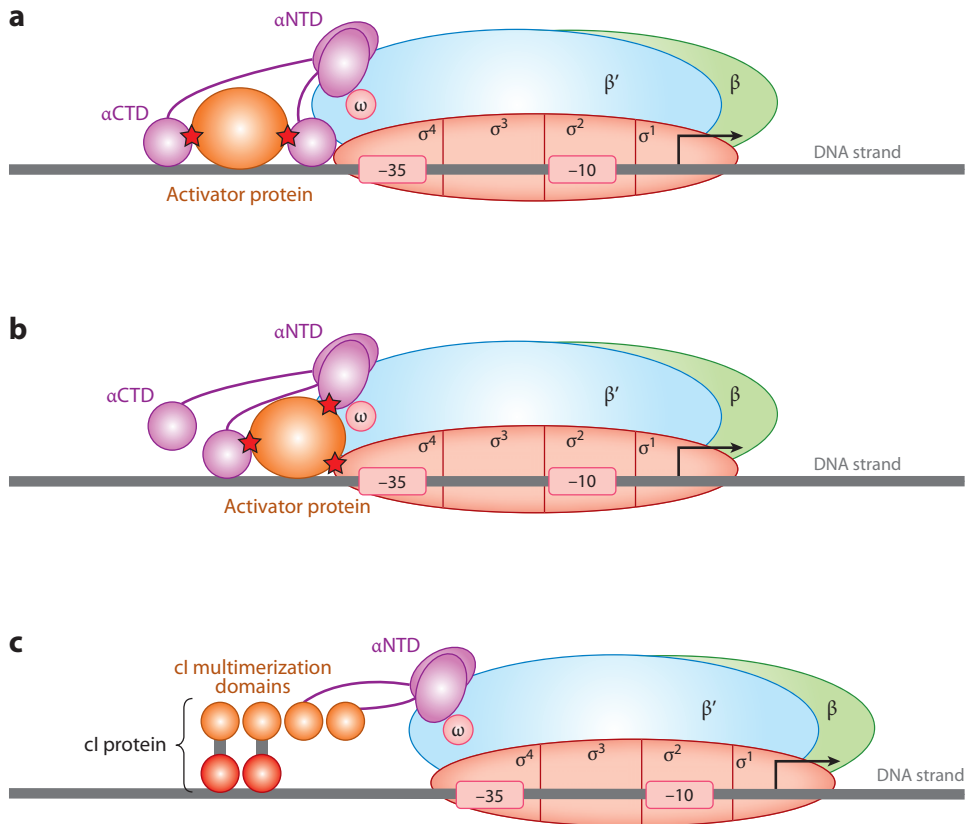
Figure 3

Simple activation by recruitment. (*a*) The activator protein binds upstream of RNA polymerase and contacts (*red stars*) one or both α subunit C-terminal domains (CTDs). (*b*) The activator binds close to RNA polymerase and interacts with different polymerase subunits. (*c*) Artificial activation in which the DNA-bound cI (bacteriophage λ repressor) protein activates transcription by interacting with the cI multimerization domains fused to the RNA polymerase α subunit N-terminal domain (NTD).

function merely as molecular velcro. Second, Hochschild and colleagues (37) have demonstrated that RNA polymerase can be recruited to promoters by nonnative contacts, and deduced that this recruitment is sufficient for promoter activation. The Hochschild experiments work by engineering *E. coli* to contain polymerase with a supplementary protein domain that can then be used to target the polymerase to specific promoters. One set of experiments exploit the fact that bacteriophage λ repressor (cI) consists of separate DNA-binding and multimerization domains, and use a synthetic test promoter with an upstream DNA site for cI located at a position where cI alone has no effect on transcription with normal polymerase (35, 37). However, if the cI multimerization domain is attached to the RNA polymerase α or ω subunits, then the test promoter can be activated by cI because the cI multimerization domain associates with the multimerization domain attached to the RNA polymerase α or ω subunits (**Figure 3c**). Hence, an arbitrary nonnative interaction can activate transcription. Another study from Hochschild and colleagues (46) exploits the bacteriophage T4 AsiA protein, which normally inactivates RNA polymerase holoenzyme by interacting with Domain 4 of σ[70] and changing its conformation. AsiA was fused

to a DNA-binding module and the hybrid protein was able to direct inactivated polymerase to promoters that carried the cognate target sequence for the DNA-binding module, resulting in activation at these promoters. Taken together, these experiments define the minimum requirement for transcription activation as any contact that recruits RNA polymerase to a target promoter, and they show that complex mechanisms involving activatory buttons and extensive conformational changes may often be unnecessary.

ACTIVATOR–RNA POLYMERASE INTERACTIONS

In this section, we describe the best-understood examples of simple transcription activation that involve direct activator–RNA polymerase interactions. Some of the examples cited have been studied for over 30 years and, rightly or wrongly, are taken as the paradigms for our understanding of less-well-established systems.

Activation by Bacteriophage λ cI Protein at the λ P_{RM} Promoter

Although bacteriophage λ cI protein is primarily a repressor, it also activates its own expression at the λ rightward promoter for the maintenance of lysogeny (P_{RM}). Activation results from the binding of a single cI dimer to a DNA site that abuts the P_{RM} −35 hexamer element. Activation is due to direct interactions between λ cI residues E34 and D38, which are immediately adjacent to the cI DNA recognition helix, and σ^{70} residues R588 and R596, located in Domain 4, just next to the determinant that contacts the −35 element. Residues E34 and D38 cI were originally found as the sites of positive control substitutions, and the cognate target in σ^{70} Domain 4 was identified from suppression genetic experiments in which allele-specific suppressors of positive control mutations in cI were found and characterized (82). These interactions have been confirmed by high-resolution structural studies (64) of the ternary complex between the DNA-binding domain of cI, a segment of DNA carrying the cI operator sequence and the P_{RM} −35 element, and Domain 4 of the housekeeping σ factor from *Thermus aquaticus* (which was used in preference to its *E. coli* counterpart to facilitate the structural analysis). From these studies, it is clear that the key activator–RNA polymerase contact takes place when both partners are DNA bound and is mediated by interactions between small clusters of amino acid side chains on cI and σ Domain 4 that are positioned immediately adjacent to the DNA. In addition, the interaction causes little or no conformation change in either protein partner, and this is a theme that is repeated in other systems (see below).

The action of cI at P_{RM} is of special interest, not only because it is a key component of the first genetic switch to be understood in detail, but also because, against all expectations, cI at P_{RM} has no effect on the initial binding of RNA polymerase but rather accelerates the formation of the open complex. Because the structural analysis shows that the cI–σ Domain 4 interaction has no effect on the conformation of σ Domain 4, the conclusion must be that the cI-activating region makes contact with its cognate target only after both cI and RNA polymerase have bound to the promoter, thereby driving RNA polymerase forward through the pathway to the transcriptionally competent open complex (64). Interestingly, with RNA polymerase containing σ^{70} with the RH596 suppressor substitution in Domain 4, cI-dependent activation at P_{RM} takes place via accelerated formation of the initial binding of RNA polymerase (81). Hence, an interaction between the same two surfaces can have different kinetic consequences, depending on the stage in the initiation pathway at which the interaction comes into play.

Key lessons of the λ cI story are that molecular velcro is sufficient for activation and that there is no need for activator-induced conformational changes in RNA polymerase, even to drive open

complex formation. These lessons were underscored by another series of elegant experiments by Hochschild and colleagues (36) showing that the cI–σ Domain 4 contact in a different context could be used to drive RNA polymerase recruitment to a promoter. These experiments used the synthetic promoter described above, with a DNA site for cI located upstream of the promoter −35 element. At this promoter, the activating region of cI is unable to contact its target in σ Domain 4, and hence cI cannot activate transcription with normal RNA polymerase. However, with RNA polymerase carrying an ectopic σ Domain 4 fused to one of its α subunits, cI can activate, because the flexible α subunit linker permits formation of the cI–σ Domain 4 interaction that then recruits the polymerase to the promoter.

Another key lesson from the λ cI story is that, even though cI is a homodimer, only one of the two subunits makes contact with σ Domain 4 during activation. This is unsurprising because each RNA polymerase carries only one σ factor, but it underscores that, although a single protein-protein interaction is sufficient for transcription activation at a promoter, binding specificity of a transcription factor is dependent on reading 8–10 base pairs. This requires two recognition helix determinants that, in cI and in many other homodimeric transcription factors, are carried by the two subunits. During activation at P_{RM}, cI binds to the same face of the DNA helix as σ Domain 4, and hence it is the downstream subunit of the cI dimer that makes the activatory contact. This was shown by Kim & Hu (72), who engineered an asymmetric version of the cI dimer that bound in a specific orientation to an asymmetric DNA site for cI. Positive control substitutions could then be targeted to either cI subunit to identify which subunit makes the key contact with σ Domain 4.

Domain 4 of σ Factors as a Target for Transcription Activators

The positively charged residues in σ^{70} Domain 4 that interact with λ cI during activation at the λ P_{RM} promoter are located on one face of an α-helix that is immediately adjacent to the interface between Domain 4 and the promoter −35 element. As might be expected, many other transcription activators have evolved to exploit this target (34, 84). Examples include bacteriophage Mu Mor protein, *E. coli* FNR (regulator of fumarate and nitrate reduction) protein, many response regulators, and most members of the AraC family of transcription activators. In order to make contact with this target, activators have to be precisely positioned and bind to the same face of the promoter as σ Domain 4. This is because the positioning of Domain 4 with respect to the rest of RNA polymerase at promoters is critical, with little room for flexibility (96). Thus, for many AraC family members, transcription activation at target promoters is contingent on binding to a target that overlaps the upstream end of the −35 hexamer, and this binding is triggered by an activatory ligand (90). Examples include the action of arabinose with AraC (121), melibiose with MelR (45), and rhamnose with RhaS and RhaR (142). As with all AraC family members, these factors all contain a ∼100-amino-acid module that carries two helix-turn-helix DNA-binding determinants that insert into two adjacent major grooves. The bound module is oriented by this interaction. Genetic analyses with RhaR, RhaS, and MelR have shown that the C-terminal helix-turn-helix binds adjacent to σ Domain 4 bound at target promoter −35 elements and identified a conserved negatively charged amino acid residue (D261 in MelR and D241 in RhaS) that interacts with σ^{70} residues R588 and R596 (8, 45). A similar set of interactions appears to be involved with MarA, SoxS, and AraC (and doubtless dozens of other AraC family members). Hence, here we have a rerun of activation by cI at P_{RM}, and it is interesting to note that AraC promotes both the initial binding of RNA polymerase at the *araBAD* promoter and passage to the open complex (148). This should not be surprising because we know that σ Domain 4 engagement with −35 elements is central to promoter recognition and that evolution uses the same mechanisms over and over again.

The key point is that for an activator to contact σ^{70} residues such as R588 and R596, it has to be carefully positioned. This is neatly illustrated by the Ada transcription factor, whose activity is triggered by methylation damage. Hence, at the *aidB* and *ada* promoters, the principal Ada-activating region interacts with a target in σ Domain 4 that overlaps the target for λ cI and AraC family members (76). However, at the *alkA* promoter, the Ada-binding target is offset upstream by a few base pairs, and thus completely different residues in Ada and σ supply the activatory contact (77). Note that some activators contact DNA targets that overlap target promoters' −35 element, but are unable to contact σ Domain 4 (discussed below).

Activation by CRP at the *lac* Promoter

Transcription initiation at the *E. coli lac* operon promoter is highly dependent on the cyclic AMP receptor protein (CRP), a global regulator whose activity is dependent on cyclic AMP (23, 129, 149). Thus, even when the *lac* repressor is induced, the *lac* promoter requires activation by the binding of homodimeric CRP to a 22-bp DNA target centered between base pairs 61 and 62 upstream from the transcript start point (i.e., position −61.5). When bound at this target it is clear that direct interaction with σ^{70} Domain 4 would be impossible unless there were a massive conformation change in the promoter DNA (38). The solution to the puzzle came from Ishihama and colleagues (58, 59), who worked with holo-RNA polymerase reconstituted with α subunits lacking the C-terminal domain (residues 235–329). They found that, whereas this form of RNA polymerase was competent for transcription initiation at many promoters, it was defective for CRP-dependent activation at the *lac* promoter. The subsequent discovery that the RNA polymerase αCTD could fold into an autonomous DNA-binding unit, and that it was joined to the αNTD by a flexible linker (9, 65, 112), prompted the suggestion that CRP activated the *lac* promoter by interacting with αCTD. This has been proved by work from Ebright and colleagues (78) that culminated with the structure of the ternary CRP–holo-RNA polymerase–promoter complex, deduced from electron microscopy, exploiting high-resolution X-ray structures of the component parts (56). The structure shows one αCTD sandwiched between the bound CRP dimer and σ Domain 4. Strikingly, αCTD, σ Domain 4, and the other parts of RNA polymerase bind to the promoter almost exactly as if it were a factor-independent promoter.

CRP provides a supplemental functional determinant to the *lac* promoter that stabilizes the formation of the initial RNA polymerase–promoter complex (22, 78). Genetic studies defined this activatory determinant as a small surface-exposed patch (known as activating region 1, AR1), comprising amino acid side chains in a β-turn in the DNA-binding domain, immediately adjacent to the DNA recognition. High-resolution structural analysis (7) of the ternary CRP-αCTD-DNA complex shows that this determinant interacts directly with specific residues of αCTD near position 287 (known as the 287 determinant). Thus, αCTD in the ternary CRP–holo-RNA polymerase–promoter complex makes direct contact with AR1 of CRP via the 287 determinant, with the *lac* promoter UP element via the 265 determinant (residues near R265), and with σ Domain 4 via the 261 determinant (residues near D261) (27, 56, 119). Significantly, the interaction between CRP and αCTD causes no detectable change in the conformation of either CRP or αCTD, and again, this argues that CRP, like cI, activates by a simple recruitment mechanism involving molecular velcro.

An interesting aspect of CRP-dependent activation at the *lac* promoter is that, although each subunit of the CRP homodimer contains AR1 and RNA polymerase contains two αCTDs, activation requires only one AR1-αCTD interaction. Experiments with CRP dimers containing one wild-type AR1 and one mutated nonfunctional AR1 show that only the AR1 in the downstream

subunit is required for activation (150), and this AR1 contacts the αCTD that interacts with σ Domain 4 (56, 78). In these experiments, CRP heterodimers were oriented by using derivatives carrying substitutions in the DNA recognition helix and asymmetric 22-bp DNA sites for CRP. The location of the second αCTD during *lac* promoter activation is less well defined, but it appears to bind upstream and to make no functional interaction with AR1 in the upstream subunit of the CRP dimer. This result was confirmed using RNA polymerase reconstituted with one full-length α subunit and one α subunit lacking αCTD (83). This one-armed RNA polymerase is fully functional for CRP-dependent activation at promoters with an architecture similar to that of the *lac* promoter. Hence, as with cI, although the binding specificity of CRP requires two subunits, the activating region in only one subunit is required for the activation function.

αCTD as a Target for Transcription Activators

The RNA polymerase αCTD acts as an antenna for RNA polymerase, by interacting either with UP elements or with transcription activators such as CRP, and although the number of proven and completely characterized cases is still quite small, it is likely to be a target for scores of activators in *E. coli* and other bacteria (**Figure 3a**). Because αCTD is joined to αNTD, and hence the rest of RNA polymerase, by a nonstructured linker, there is considerable flexibility with respect to where an activator can be positioned for productive activation (39). An interesting aspect of the *lac* promoter is that, if the DNA site for CRP is moved upstream by 11 base pairs from position −61.5 to −72.5, CRP still can make an activatory interaction with RNA polymerase (133). Systematic studies showed that CRP could activate transcription from a single site at positions −72.5 and −82.5, and some activation was found from position −93.5 (42, 137). Because the action of CRP from all these locations is stopped by substitutions that render AR1 nonfunctional, activation from each of these locations must depend on AR1 interacting with αCTD.

A similar set of observations was reported for FNR (145), which is the global transcription regulator for adaptation to anaerobic conditions and is structurally and functionally related to CRP (see sidebar CRP and FNR). The helical periodicity for activation in these cases must be due to the need for the activator to be lined up on the same face of promoter DNA as RNA polymerase, and it is now clear that this causes wrapping of upstream promoter sequences. Note, however, that it is possible for factors to make activatory interactions with one or more αCTDs without binding to the same face of the DNA as the rest of RNA polymerase. Such activators interact with a different surface of αCTD. This is likely to be the case for some response regulators [e.g., NarL at the *E. coli ogt* promoter (131) or BvgA at the *Bordetella pertussis fim3* promoter (31)], but the best-understood case is the activation of the bacteriophage λ P$_{RE}$ promoter by the cII protein.

CRP AND FNR

The cyclic AMP receptor protein (also known as CAP, catabolite gene activator protein) was originally discovered as an essential activator of the *E. coli lac* promoter but was subsequently found to modulate transcription at hundreds of promoters (129, 149). In 1983, Guest and colleagues (127) reported homologies between CRP and FNR, the global transcription activator that controls fumarate and nitrate reduction and other genes induced during anaerobic growth. Since then, the CRP family has continued to grow. CRP was the first transcription activator to be purified and characterized structurally and, together with FNR, has proved to be a useful paradigm for understanding transcription activation (78). Members of the CRP family of transcription factors play diverse roles in many different bacteria.

CLASS I AND CLASS II ACTIVATION

The terms Class I and Class II were introduced in the 1990s, following studies on CRP, to describe activation by upstream-bound CRP that interacts with the C-terminal domain of the RNA polymerase α subunit (Class I), or activation by CRP bound to a target that overlaps the promoter −35 element, where αCTD is dispensable (Class II) (58, 137) (as in **Figure 4a,b**). These terms have proven useful and are often applied loosely to describe the actions of other bacterial transcription activators. However, it is now clear that many activator-dependent promoters do not fit with such a simple classification. The term Class III is often used to describe promoters in which two transcription factors make independent contacts with polymerase (as in **Figure 4c,d** and **Figure 7a①,②**).

At λ P$_{RE}$, cII binds as a tetramer that recognizes two four-base elements that flank the promoter −35 hexamer element. Although it appears at first sight that cII might interact directly with σ Domain 4, model building, based on high-resolution structures of cII bound to its target, argues for a contact between the cII subunit bound to the upstream four-base element and the αCTD bound at position −41 (63), and this is supported by a vast amount of mutational analysis (70). The significance of this is that even transcription factors that bind to targets overlapping with promoter −35 elements can function by interacting with αCTD. Note too that the αCTD that contacts cII is located exactly where it would be at an activator-independent promoter, and again, it is unnecessary to postulate any activator-induced conformational changes in RNA polymerase.

Organization of Class II CRP- and FNR-Dependent Promoters

Early in the study of transcription activation by CRP and FNR, it became apparent that the majority of their target promoters did not follow the *lac* promoter model (58). Such promoters are often referred to as Class II promoters, and these are defined by their target site for CRP or FNR overlapping the promoter −35 element (21) (in contrast to Class I promoters such as *lac* for which the activator binding site is upstream of the −35 element) (see sidebar Class I and Class II Activation; **Figure 4a,b**). In fact, at these Class II promoters, the location of bound CRP or FNR is similar to that of cI protein at λ P$_{RM}$. Extensive genetic and biochemical studies have shown that bound CRP (or FNR) prevents αCTD from contacting σ Domain 4, that αCTD is displaced to bind just upstream of the activator, and that the displaced αCTD makes a direct interaction with the upstream subunit of the activator homodimer (5, 144, 151). For CRP, this interaction involves AR1 and the 287 determinant of αCTD, and FNR contains an equivalent to AR1 (79, 120). Although these interactions contribute to activation at most Class II promoters, they are not indispensable (21). Hence, holo-RNA polymerase reconstituted with α subunits lacking the C-terminal domain is competent for Class II activation (58). For CRP-dependent Class II promoters, the major activatory determinant is a second activating region, AR2, that is a small, positively charged surface, distinct from AR1, that interacts with a target on the surface of the αNTD (100). Kinetic analysis shows that, while the role of the AR1-αCTD interaction promotes RNA polymerase recruitment, the AR2-αNTD interaction promotes transition to the open complex (100, 110, 134).

Wild-type CRP appears not to make any direct activatory interaction with σ at Class II promoters, but substitution of CRP residue K52 unmasks a third activating region, AR3, that interacts with σ Domain 4 (108, 109). Hence, CRP can easily be converted from activating by interaction with one part of RNA polymerase (αNTD) to activating by interaction with another part (σ Domain 4). Experiments with oriented heterodimers of CRP have shown that AR2 and AR3
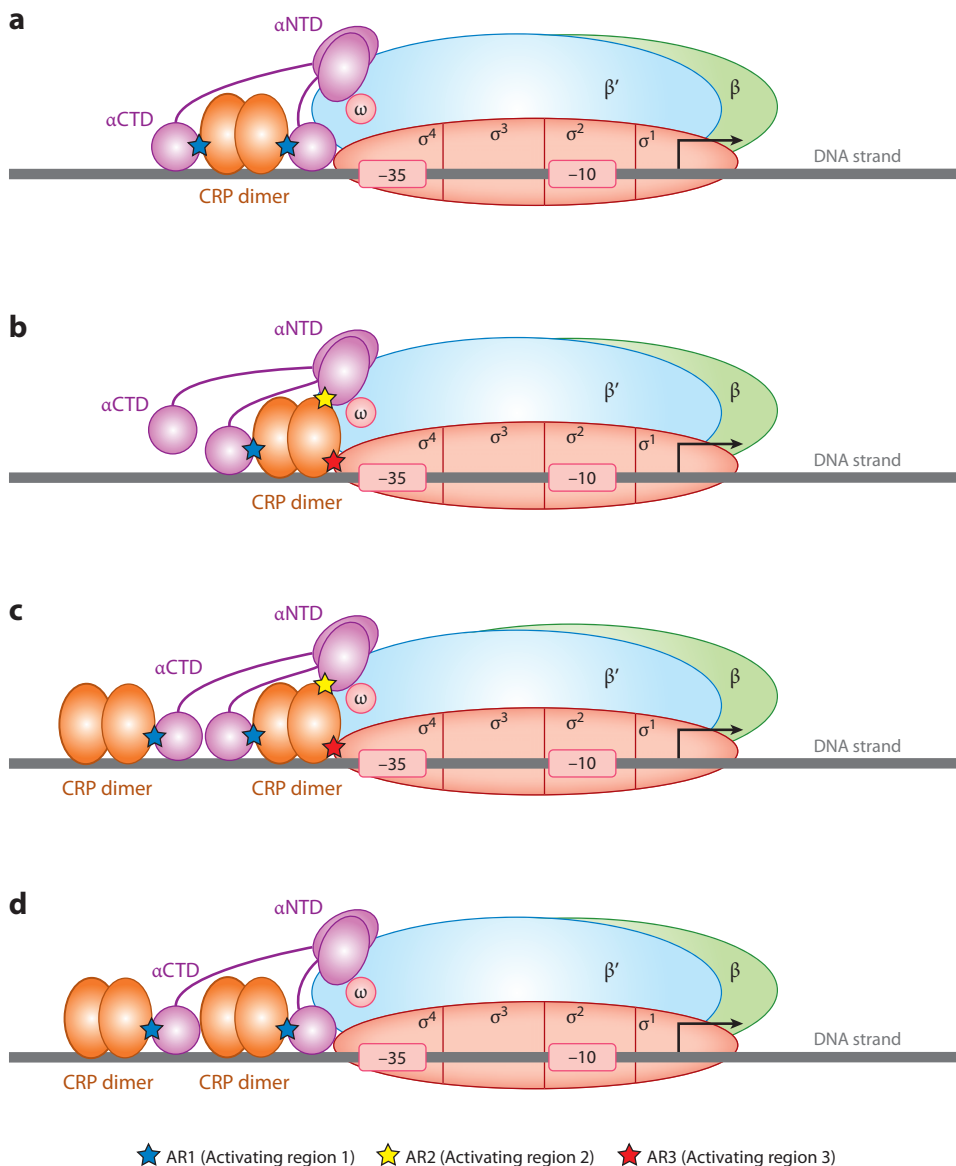
Figure 4

Activation of transcription by the cyclic AMP receptor protein (CRP). (*a*) The CRP dimer binds upstream of
RNA polymerase and contacts one or both α subunit C-terminal domains (CTDs) via AR1 (activating
region 1) (*blue star*). (*b*) CRP binds adjacent to RNA polymerase and interacts with different polymerase
subunits: AR1 interacts with αCTD, AR2 interacts with an α subunit N-terminal domain (NTD), and AR3
interacts with σ^4. (*c*) Activation by tandem-bound CRP dimers where the downstream CRP is in a Class II
position (as in panel *b*). (*d*) Activation by tandem-bound CRP dimers where the downstream CRP is in a
Class I position (as in panel *a*).

are functional in the downstream subunit of the homodimer (100, 143). Hence, at Class II promoters, both subunits of the CRP dimer make activatory interactions with RNA polymerase. For FNR-dependent Class II promoters, the major activatory determinant in FNR corresponds to AR3 of CRP and AR2 plays a much smaller role (80). Thus, for members of the CRP-FNR family, different combinations of functional activating regions must have evolved, thereby enabling activation at promoters with differing architectures. It is easy to imagine how similar evolution has occurred in other transcription factor families.

Promoters with Tandem DNA Sites for CRP or FNR

Figure 4 illustrates some of the diverse organizations found at CRP-dependent promoters. Many CRP-regulated promoters carry two or more DNA sites for CRP (129, 149). At some promoters, full activation can be achieved with just one bound CRP and thus the other target sites are redundant. In other cases, two bound CRPs are essential for optimal activation, and at many of these promoters, the two sites have different binding affinities for CRP. This can result in a nonlinear output from the promoter during growth transitions when CRP is activated or deactivated. It is presumed that this complexity has evolved to tailor the output of certain promoters during such transitions (23, 73).

At promoters that are dependent on CRP binding to tandem targets, each bound CRP makes an independent activatory contact with one of the two RNA polymerase αCTDs (6, 69, 95). This mechanism works because, at simple CRP-dependent promoters, there is always a spare αCTD available for interaction with a correctly positioned second CRP (83). The most commonly found organization is for one CRP site to overlap the promoter −35 element and to activate by the Class II mechanism described above (**Figure 4c**). Further activation is observed when a second CRP binds upstream near positions −74, −82, −93, or −102, and extensive biochemical studies have shown that AR1 in the upstream-bound CRP recruits the spare αCTD to the promoter (6, 95). At this type of promoter, often referred to as a Class III promoter, the two bound CRPs together exploit the two RNA polymerase αCTDs to generate enough velcro for optimal RNA polymerase recruitment. Hence, one-armed RNA polymerase reconstituted with one full-length α subunit and one α subunit lacking αCTD, though competent for CRP-mediated activation at a promoter dependent on one bound CRP, is unable to activate at a promoter dependent on tandem-bound CRP (83). Similar synergy between tandem-bound CRPs can be generated when both CRPs bind upstream of the promoter −35 element (**Figure 4d**) (4, 69, 135). The simplest situation is when the promoter-proximal CRP, located at position −61.5, as at the *lac* promoter, activates together with upstream-bound CRP near position −92 or −103. Again, both αCTDs are essential for such activation.

Because FNR is so similar to CRP, it was expected that some promoters would be codependent on tandem-bound FNR for optimal activation. However, surprisingly, FNR-dependent promoters cannot be substantially further activated by upstream-bound FNR, and indeed, when located at certain positions, upstream-bound FNR is inhibitory (3). The clearest example of this is the *E. coli yfiD* promoter, which is dependent on FNR binding to a single target centered at position −40.5 (87). Binding of FNR to a second weaker upstream site at position −93.5 causes a repression of *yfiD* promoter activity, and this appears to be due to a direct interaction involving AR1 of FNR. This complexity provides a mechanism for microaerobic induction of gene expression. Thus, when FNR becomes activated in response to oxygen depletion, it occupies the site at position −40.5 and activates *yfiD* expression. Subsequently, as FNR becomes fully activated, the upstream site at −93.5 is filled, resulting in downregulation of expression. Hence, the interplay of FNR

binding at two targets with different regulatory outcomes results in transient promoter activation when the environment switches from aerobic to fully anaerobic conditions.

PROMOTERS DEPENDENT ON σ^{54}: THE SECOND ACTIVATION PARADIGM

Unlike holo-RNA polymerase containing σ^{70} (and most alternative σ factors), polymerase containing σ^{54} is unable to serve target promoters independently of a transcription activator. Genes encoding σ^{54}-like proteins are found in $\sim$60% of bacteria but the origins of σ^{54} are unclear because it is structurally distinct from σ factors related to σ^{70} and does not share their characteristic domains (91).

RNA polymerase containing σ^{54} serves a variety of target promoters, and the key sequence elements at these promoters are located near positions -12 and -24. RNA polymerase can recognize these promoters, but it is unable to form transcriptionally competent open complexes without the intervention of a specialized transcription activator that contains an AAA+ activator domain that interacts directly with σ^{54} (43). These activators, often known as enhancer-binding proteins, typically contain three domains, a regulatory domain responsive to a particular metabolic signal, the AAA+ activator domain, and a DNA-binding domain, and they self-assemble into hexamers that bind to DNA targets at σ^{54}-dependent promoters. These targets are mostly found 100–150 base pairs upstream of the transcript start, but they can function up to 1,000 base pairs upstream and a DNA loop has to form in order for the activator domain to contact σ^{54}. DNA-bending proteins such as IHF can help in the formation of these loops (**Figure 5**), and a careful study of the requirements for positioning IHF sites led to the conclusion that the activator domain was presented to RNA polymerase from the opposite side of the target DNA (57). Cryo-electron microscopy of complexes between the PspF activator domain and RNA polymerase σ^{54} holoenzyme showed this directly, and it was possible to visualize protrusions from the activator domain that correspond to surface-exposed loops that make direct contact with σ^{54} in the holoenzyme (10).

AAA+ activator domains couple ATP hydrolysis to movement in a wide variety of systems in all kingdoms of life. Thus, the current model for σ^{54}-dependent activation is that ATP hydrolysis causes motion of the surface-exposed loops in the activator domain so that they interact with σ^{54} to overcome the blockage between the closed and open complexes (67). This has been referred to as the second paradigm for transcription activation because the activator domain is targeted directly to push an essential button needed to remove the obstacle to open complex formation
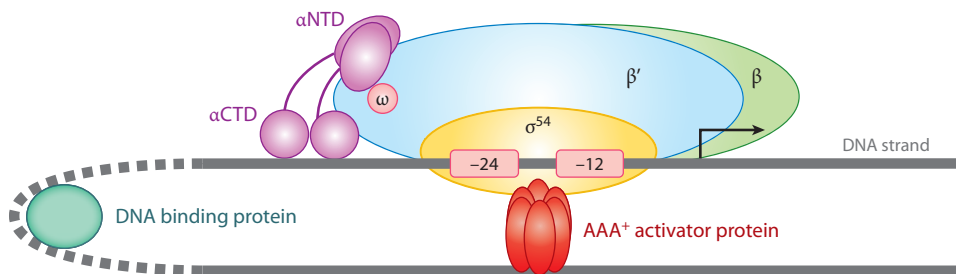

Figure 5

Transcription activation at a σ^{54}-dependent promoter. RNA polymerase containing σ^{54} binds to the -12 and -24 promoter elements. Interaction with the AAA+ activator protein is often facilitated by DNA looping caused by a DNA-bending protein. Abbreviations: CTD, C-terminal domain; NTD, N-terminal domain.

(15). This is in contrast to most of the mechanisms discussed above, where the activator's strategy is to recruit or guide RNA polymerase holoenzyme, but then let it get on with transcript formation as an activator-independent promoter.

At least in the cases that have been studied thus far, there are no big fluctuations in σ^{54} levels during bacterial growth or adaptation (66). Thus, regulation is imposed by the different AAA+ activator proteins whose activities are modulated by diverse mechanisms in response to different metabolic or environmental conditions. This is in sharp contrast with most other alternative σ factors whose activity is regulated either by their level or their availability, without the intervention of transcription factors (see below).

RNA POLYMERASE APPROPRIATION

Alternative σ Factors

RNA polymerase–centric activation mechanisms involve proteins that reprogram the promoter preferences of the polymerase holoenzyme (as opposed to improving the attractiveness of promoters for RNA polymerase); some of these mechanisms are illustrated in **Figure 6**. The best example is when, in response to specific signals, the housekeeping factor is replaced with an alternative σ factor, thereby changing the promoter preferences of a proportion of the cell's polymerase (50). In some cases, this reprogramming mediates global responses to a general stress, while in others, the alternative σ factor participates in driving a developmental pathway such as sporulation. The activity of most alternative σ factors is controlled by their availability in the cell, and therefore, target promoters tend not to require transcription activators and contain recognition elements that resemble the consensus (74). However, there are some exceptions. For example, the σ^{28}-dependent *E. coli aer* promoter is activated by CRP (54). The consequence of this is that, in certain conditions, when σ^{28} levels are rising or falling, the *aer* promoter has an advantage over other σ^{28}-dependent promoters. Although it was previously thought that regulons controlled by different σ factors contain distinct sets of genes, genome-wide studies have now shown that many genes can be served by RNA polymerases carrying different σ factors (140).

Appropriation and Pre-Recruitment

It can be argued that mechanisms that focus on RNA polymerase rather than promoters are the regulatory method of choice when bacteria need to respond efficiently to ensure their survival. Hence, a sudden heat shock demands instant action to avoid death, and this can be done by σ factors appropriating RNA polymerase, whereas the choice to metabolize arabinose or lactose is not so pressing and is resolved by transcription factors that change the competition between different promoters (50). Examples of this include the *E. coli* SoxS and MarA transcription activators, each of which induces expression of large stress regulons by binding to a common DNA target known as the Mar box (88). Both factors are AraC family members that contain just the DNA binding/activation domain and their activity is controlled by their level. They were thought to bind to Mar boxes at target promoters and activate by the Class I or Class II mechanisms outlined above. However, there is now some evidence that SoxS and MarA bind directly to RNA polymerase away from target promoters, and this has been dubbed pre-recruitment (47, 48, 89). Genetic and biochemical analyses argue that the binding target in RNA polymerase is the determinant in αCTD that interacts with UP elements (29, 126). Hence, upon binding to RNA polymerase, SoxS and MarA switch the αCTD DNA-binding determinant from recognizing UP elements to recognizing Mar boxes. Because UP elements play a big role in directing large amounts of
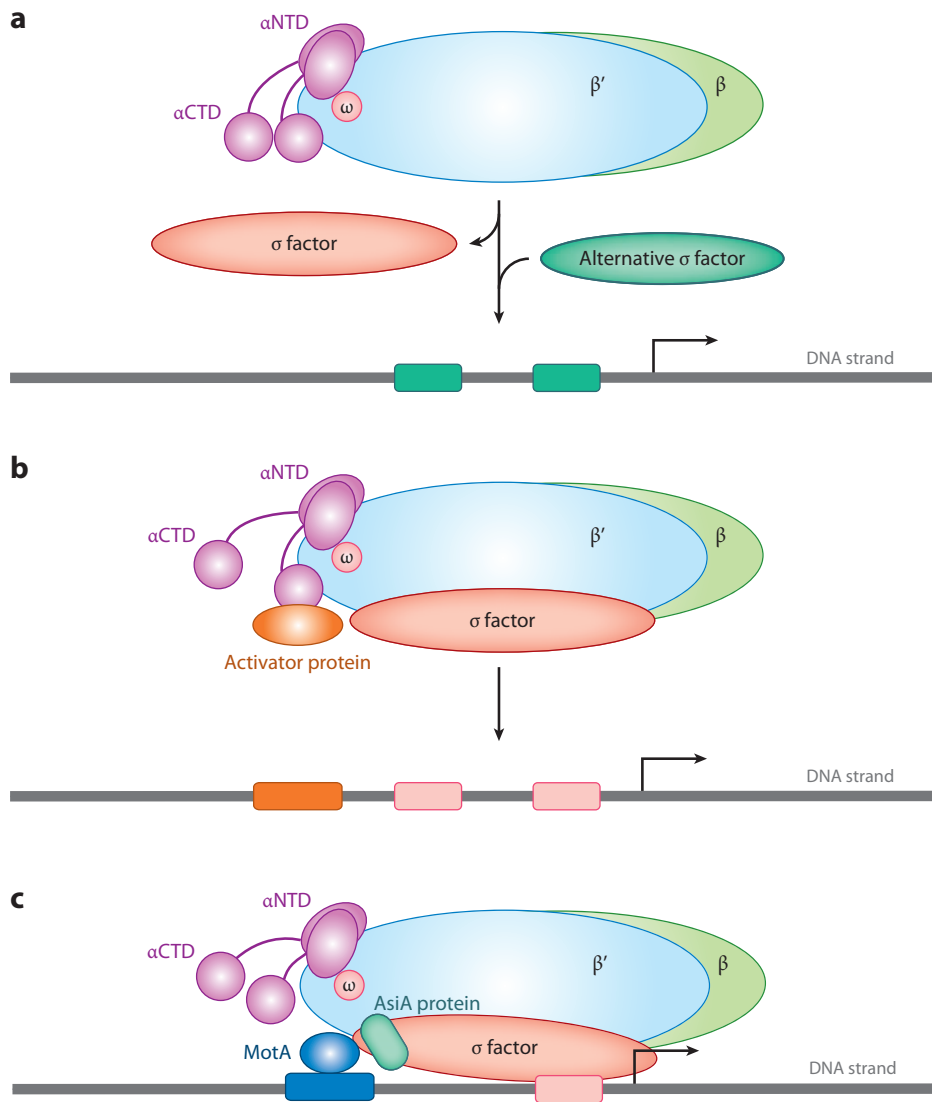
Figure 6

Activation by appropriation and pre-recruitment. (*a*) σ factor appropriation. RNA polymerase is
reprogrammed by an alternative σ factor, thus altering the promoter preference. (*b*) Pre-recruitment by an
activator protein directs RNA polymerase to a specific set of promoters. (*c*) Appropriation of RNA
polymerase by the T4 phage AsiA protein. AsiA remodels σ^{70}, enabling MotA to interact and direct
transcription from middle phase T4 promoters. Abbreviations: CTD, C-terminal domain;
NTD, N-terminal domain.

RNA polymerase to transcription units involved in growth, it is easy to see how the SoxS/MarA
pre-recruitment can result in a population of RNA polymerase molecules tasked to deal with an
urgent stress rather than with growth (141, 146).

　　As expected, bacterial viruses are the masters of appropriation. The best example is perhaps
the AsiA gene product of bacteriophage T4, which is dependent on the host multisubunit RNA

polymerase for its development because its genome does not encode its own bespoke polymerase. AsiA protein is expressed early during infection, binds tightly to σ^{70}, and was originally thought to be just an anti sigma, thereby shutting down host transcription (28). However, it was subsequently shown that AsiA binds to and remodels Domain 4 of σ^{70} in the host RNA polymerase holoenzyme, and that this remodeling is essential for the function of the T4-encoded MotA transcription activator that is required for the middle phase of T4 transcription (24, 52, 75). Thus, AsiA can be thought of as an appropriator that remodels part of σ^{70} to prevent recognition of host -35 elements while facilitating MotA action and the development of T4 infection.

INDIRECT ACTIVATION

Alongside the direct transcription activation mechanisms discussed above, bacteria use other ingenious indirect ways to control transcription positively. For example, upregulation of gene expression can be due to inversion switching of a DNA segment carrying a promoter, or it can result from the insertion of a transposon that carries a full promoter or a promoter element (118). Alternatively, in some bacteria, clonal variation of promoter sequences is responsible for up- or downregulation of gene expression (94).

The most frequently found mechanism for indirect activation of transcription in bacteria is the relief of repression. In one scenario *dam*-mediated DNA methylation prevents repressor binding, and at some promoters this is an important mechanism for toggling promoters between on and off states (85). However, in most cases, relief of repression is due to the binding of a protein factor that then disrupts repression caused either by a specific repressor (41) or by nucleoid-associated proteins (32). Such protein factors, which appear to function as activators, are perhaps more aptly termed antirepressors. Good examples of antirepressors are found in many pathogenic bacteria where the expression of genes involved in virulence is repressed by the H-NS nucleoid-associated protein. Hence, Ler protein in enteropathogenic *E. coli* and VirB in *Shigella flexneri* are unable to activate transcription directly at promoters, but they trigger the expression of virulence genes by disrupting repression mediated by H-NS (132). Such repression can be disrupted in many different ways, including by a bona fide repressor. Thus, in a clever piece of synthetic biology, Caramel & Schnetz (25) showed that the Lac repressor could break H-NS-dependent repression of the *E. coli bgl* promoter, thereby demonstrating that the best-known transcription repressor could also become involved in transcription activation.

INTEGRATION OF REGULATORY SIGNALS AT PROMOTERS

Bacterial transcription factors have evolved to couple environmental signals to transcription output; thus, each factor's activity is directly or indirectly controlled by a specific signal. Because bacteria have to sense different combinations of signals, it is unsurprising to find that most promoters are regulated by multiple transcription factors and that the role of the promoter is to integrate the different signals into one output. Many promoters are codependent on two or more transcription activators, and the most common scenario is for each of the activators to bind independently and make independent contacts with RNA polymerase. Thus, just as some CRP-dependent promoters depend on CRP binding at two upstream sites, a promoter can be dependent on two different upstream-bound activators. The first reported cases of this were an artificial promoter dependent on activation by CRP and phage λ cI protein (68) and the *E. coli ansB* promoter (125), but subsequently, scores of examples have been found and they appear to fall into two groups (71). In the first group, the promoter-proximal activator overlaps the target promoter -35 element and functions as a Class II activator (**Figure 7a①**). In the second group, the promoter-proximal activator binds
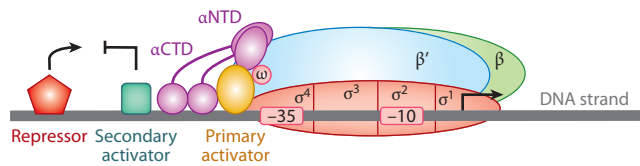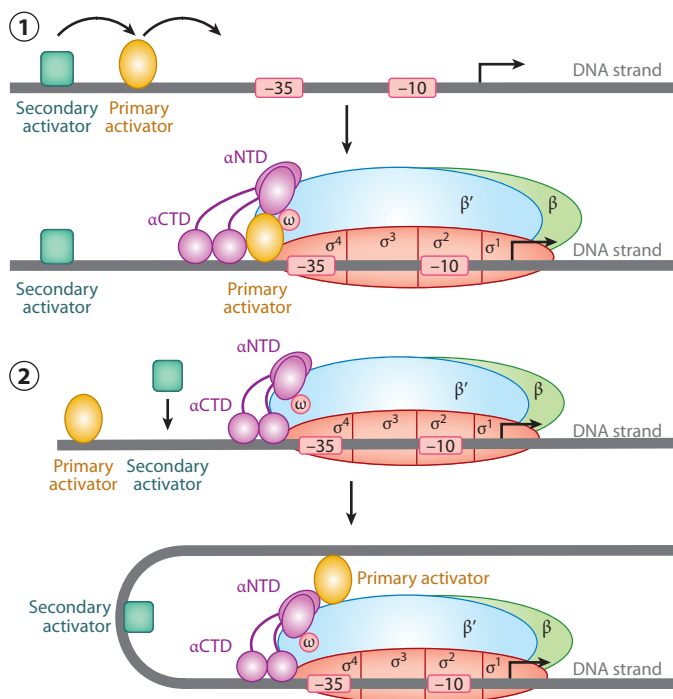
a Independent contacts
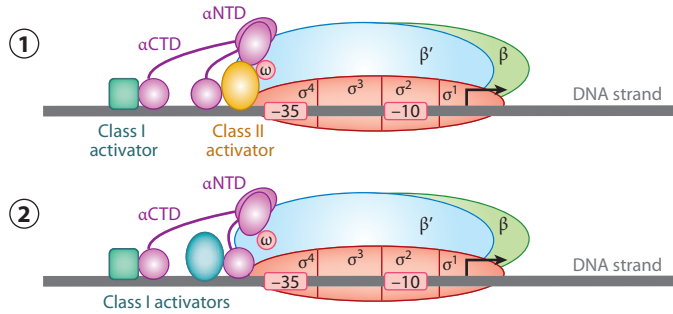
b Cooperative binding
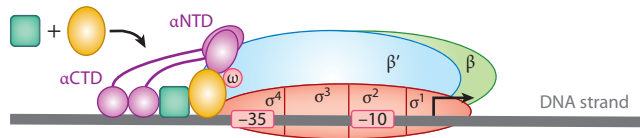
c Antirepression

d Repositioning

upstream of the −35 element and functions as a Class I activator (**Figure 7a②**). In both groups, the promoter-distal activator contacts the spare αCTD. At such promoters, the flexible linker connecting the α subunit domains allows considerable variability in the way the activator targets can be organized. Note too that for a promoter to be codependent on two activators, it must evolve such that each individual activator is unable to do the job by itself. This is often fixed by weaker binding sites, suboptimal −35 elements or UP elements, or suboptimal spacing. For example, in the pathogenic enteroaggregative *E. coli* strain 042, the promoter-controlling expression of the plasmid-encoded toxin depends on both upstream-bound Fis and CRP binding to a target that overlaps the −35 element. CRP appears unable to activate the promoter alone because its DNA site is too close to the −10 element (114). Hence, insertion of a single base between the promoter −10 and −35 elements relieves the dependence on Fis.

At most bacterial promoters where more than one different activator is required, these bind independently. A likely explanation for this is that bacteria rely on rapid evolution for their survival, and one element of this involves mixing and matching transcription activators to activate synergically at promoters. This is facilitated if direct interactions between the different factors are not required. However, there are a few examples in which different activators bind cooperatively (**Figure 7b**), such as MelR and CRP at the *E. coli melAB* promoter (139); possibly ToxR and TcpP at the *Vibrio cholerae* promoter (93); and GadE and RcsB at the *E. coli gadA* promoter, where a GadE/RcsB heterodimer forms (26).

At some bacterial promoters that are codependent on two activators, the primary activator has the potential to fully activate the target promoter, but its activity is suppressed by repressors (**Figure 7c**). An example is the *E. coli nir* promoter, which can be fully activated by FNR, but activation is suppressed by upstream binding of two nucleoid-associated proteins, IHF and Fis (13). This suppression can be released by the binding of either of the nitrate-activated factors, NarL and NarP, that displace IHF from one of its targets.

At other codependent promoters, the primary activator is unable to make an activatory contact with RNA polymerase without being repositioned by a secondary activator (**Figure 7d**). For example, at the *E. coli malE* promoter, CRP repositions MalT from a location where it is unable to activate transcription to one where it can activate (111). Other examples are found at promoters where an essential activator binds at a far-upstream target and requires a DNA-bending protein such as IHF to bring it closer to the core promoter elements. This appears to be the case for the *E. coli narG* promoter (122), and this is also often found at σ^{54}-dependent promoters where IHF is needed to position the enhancer-binding protein so that it can interact with σ^{54} in RNA polymerase holoenzyme (138). This is done by inducing a sharp bend that brings the enhancer binding protein close to the promoter −12 region.

←——

Figure 7

Integration of multiple regulatory signals. (*a*) Independent contacts by different activator proteins. In ①, transcription is dependent on independent contacts from a Class II activator and a Class I activator. In ②, transcription is dependent on independent contacts from two Class I activators. (*b*) Cooperative binding. The binding of the first activator requires binding of the second activator. (*c*) Antirepression. Maximum activation by the primary activator is facilitated by the second activator, which overcomes the inhibitory action of the repressor (*red*). (*d*) Repositioning. In ①, the secondary activator repositions the primary activator, enabling interactions with RNA polymerase at the promoter. In ②, the secondary activator alters the DNA conformation, thereby presenting the primary activator for interaction with RNA polymerase. Abbreviations: CTD, C-terminal domain; NTD, N-terminal domain.

CONCLUDING COMMENTS

Although transcription activation at bacterial promoters has been studied directly for nearly 40 years, the number of cases for which the details are fully understood is still small. Fortunately, the models that have been developed over this period appear to be applicable to most newly sequenced genomes, but it is unthinkable that new paradigms won't emerge from some of these genomes over the next few years. One big area of current weakness is a quantitative understanding of transcription initiation, and the recent discovery of cases in which promoter escape is limiting and regulated adds an extra dimension of complexity. Another area in need of progress is our understanding of how the folding of bacterial chromosomes affects transcription. It has recently been suggested that location within these structures may be important (117). Perhaps the most pressing need is to find a simple method by which to monitor directly the distribution of RNA polymerase throughout an operon in a time-resolved manner. Methods based on chromatin immunoprecipitation have potential but single-cell approaches are needed. Finally, the challenge is to exploit our newfound knowledge and devise antibacterial therapies that target signaling pathways and transcription factors.

DISCLOSURE STATEMENT

ACKNOWLEDGMENTS

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