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Regulation at complex bacterial promoters: how bacteria use different promoter organizations to produce different regulatory outcomes

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Most bacterial promoters are regulated by several signals. This is reflected in the complexity of their organization, with multiple binding sites for different transcription factors. Studies of a small number of complex promoters have revealed different distinct mechanisms that integrate the effects of multiple transcription factors.

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Abbreviations
CRP cyclic AMP receptor protein
Fis factor for inversion stimulation
IHF integration host factor
Lrp leucine-responsive regulatory protein
Pap pyelonephritis-associated pili
RNAP RNA polymerase
UPEC uropathogenic *E. coli*

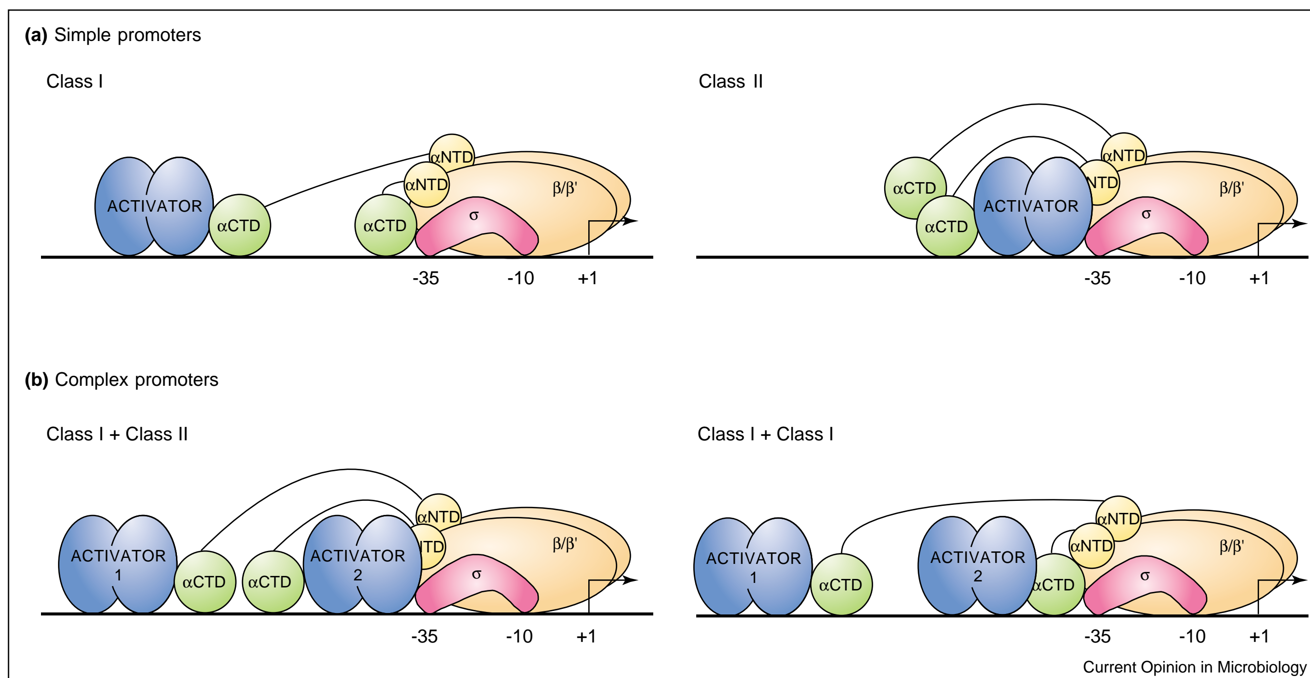
Introduction

Most mechanistic studies on bacterial transcription activation have focused on simple situations where a single transcription factor is sufficient to activate a particular promoter. These studies have taught us that there are two principal mechanisms by which proteins can activate the initiation of transcript formation at a promoter [1]. The majority of activator proteins appear to function by making direct interactions with the multi-subunit bacterial RNA polymerase (RNAP) which recruit it to the target promoter. Perhaps the best examples of these activators are the bacteriophage λ cI protein and the *Escherichia coli* cyclic AMP receptor protein (CRP) [2]. A small number of activators, notably members of the MerR family, use the second, alternative mechanism; they function by altering the conformation of promoter DNA such that it is better recognized by RNAP [3,4].

Most transcription activators function by binding upstream of the transcription start site at their target promoters. Thus, activators that recruit RNAP via a direct protein–protein interaction fall into two classes (Figure 1a). Class I activators bind to upstream locations, usually near position −61, −71, −81 or −91, and function by making a direct interaction with the carboxy-terminal domain of the RNAP α subunit (αCTD). This interaction recruits αCTD, and hence the rest of the RNA polymerase, to the promoter [5]. The location of activator binding at promoters subject to Class I activation is variable, apparently because the linker between αCTD and the α amino-terminal domain is sufficiently flexible to permit the binding of αCTD at different positions. By contrast, Class II activators bind to sites that overlap the target promoter −35 region and, in most cases, activate transcription by making a direct interaction with domain 4 of the RNAP σ subunit [6•]. The binding location of Class II activators cannot be varied because of constraints in the location of σ domain 4. In some cases, activators that function by a Class II mechanism can also make productive contacts with αCTD, which often binds upstream of the bound activator [7]. Perhaps the best-studied case is CRP, which can function either by a Class I or by a Class II mechanism [8]. Recent papers concerning simple CRP-dependent promoters have presented a high resolution structure for CRP and αCTD interacting at a DNA target [9••], have shown that activation requires only one CRP-αCTD contact, despite the fact that CRP is a homodimer and RNAP contains two α subunits [10], and have described the use of a targeted inorganic DNA cleavage reagent to determine the location and orientation of the two RNAP αCTDs at different promoters [11].

Although most of our knowledge about the basic mechanisms of transcription activation has come from the study of simple promoters, such as the *E. coli* CRP-dependent *lac* and *gal* promoters, the bacteriophage λ P*rm* promoter, and the MerR-dependent Tn21/Tn501 *merP* promoter, most naturally occurring activator-dependent promoters are much more complex. Indeed many activator-dependent promoters are also co-regulated either by a repressor or by a second activator (or by both). With the arrival of whole genome sequences, we now know of thousands of promoters with binding sites for multiple factors (see the Regulon DB website at http://www.cifn.unam.mx/Computational_Genomics/regulondb/). It is easy to believe that this complexity arose because of the cell's need to couple the expression of individual genes to

Figure 1



Activation by recruitment with one or two transcription factors. **(a)** Simple promoters activated by contacts with one activator, which recruit RNAP. The figure shows the position of the transcription activator and RNAP subunits at typical Class I and Class II promoters, relative to the transcription start site (+1) and the promoter −10 and −35 elements. The RNAP αCTDs are connected to αNTD by flexible linkers, represented by lines, which allow αCTD to bind at different locations depending on the promoter. At simple promoters, only one αCTD is needed for contact with the transcription activator. **(b)** Complex promoters activated by independent contacts with two activators that recruit RNAP. The organization of model 'Class I + Class II' and 'Class I + Class I' promoters carrying tandem binding sites for two transcription activators is shown. Each activator contacts one of the RNAP αCTDs that are depicted as in (a).

different environmental signals, interpreted by different transcription factors. However, the mechanisms used for this are not so clear.

In this review, we focus on a small number of complex promoters, which are dependent on more than one activator. We have picked these particular examples because they each illustrate a distinct mechanism by which multiple activators can bind and interact at a promoter to integrate regulatory stimuli to give an appropriate output. We explain how their organization accounts for the co-dependence, show how these promoters use different basic mechanisms, and suggest that these may be the paradigms for the many promoters now being discovered in the new age of bacterial genomics.

Integration of independent contacts with RNAP at promoters

A single activator-RNAP contact is sufficient for transcription initiation at some promoters, although other promoters require more than one contact, and this requirement can result in co-dependence on two activators. Thus, at the *E. coli proP* P2 promoter, activation depends on Fis (factor for inversion stimulation) and CRP binding to their cognate targets, at positions −41 and −121, respectively, and each making independent contacts with RNAP [12••]. This mechanism was originally proposed following the observation that a Class II CRP-dependent promoter could be further activated by upstream binding of a second CRP molecule [13]. As only one αCTD is needed for activation by one CRP at a Class II promoter [10], the other αCTD is available for interaction with a second activator. Thus, the two bound CRP molecules both function by contacting αCTD, with the upstream-bound CRP functioning as if it was at a Class I promoter, while the downstream CRP plays a Class II role (Figure 1b). Similarly, at the *proP P2* promoter, CRP and Fis function as Class I and Class II activators respectively. Since, in principle, any Class I activator can combine with any Class II activator, it is unsurprising that many promoters use a similar arrangement of activators that activate synergically by making independent contacts with RNAP [8]. An interesting variation has been found at promoters carrying tandem DNA sites for FNR, an activator related to CRP, which can also activate by Class I or Class II mechanisms [14]. At the *E. coli yfiD* promoter, upstream-bound FNR is unable to contact αCTD and thereby enhance activation. Rather,

it appears to make a direct contact with FNR bound near position −41, preventing the downstream FNR from carrying out Class II activation [15]. As the upstream- and downstream-DNA-sites have different affinities for FNR, this fine-tunes the promoter such that it is activated in a very narrow range of conditions [16]. A recent study has revealed that cis-dependent repression of activation is a property of FNR that is lacking in CRP [17].

At most promoters that are co-dependent on independent contacts by two activators, one activator functions by a Class II mechanism while the other functions by a Class I mechanism. However, in principle, it must also be possible for both activators to function by a Class I mechanism (Figure 1b), and this was proven by studies with model synthetic promoters based on the *E. coli lac* [18] and *melR* promoters [19]. Recent dissection of the *E. coli acs*P2 promoter has identified the first naturally occurring case of such a promoter organization [20].

Co-dependence of activation due to co-operative binding of activators

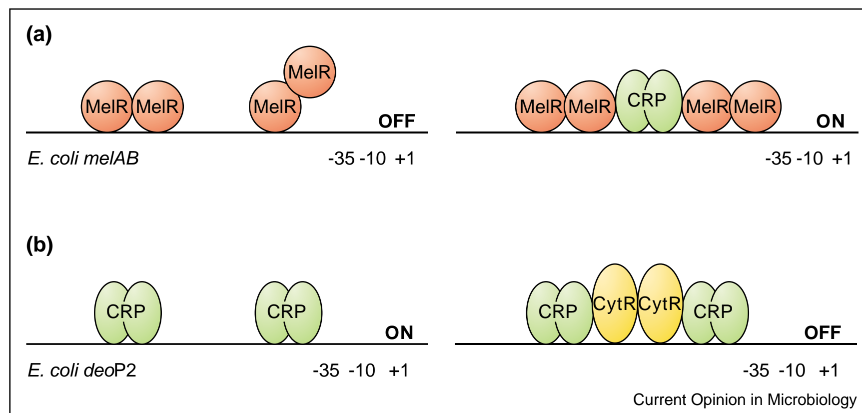
Another way in which co-dependence of promoter activity on two activators can be achieved is by making the binding of one activator contingent on the binding of the other. This is the case at the *E. coli melAB* promoter, which is activated by MelR, binding to four sites organized as two pairs (sites 2 and 2′ and sites 1 and 1′), in combination with CRP, which binds between the two pairs of DNA sites for MelR (Figure 2) [21]. Activation depends on MelR binding to site 2′, such that it can function as a Class II activator that makes a direct contact with domain 4 of the RNAP σ subunit bound at the *melAB* promoter −35 element [22]. The occupation of site 2′ by MelR requires the formation of a complex involving the binding of CRP and four MelR molecules. However, CRP is unable to bind in the absence of MelR. Its binding is contingent on MelR pre-binding to site 1 and site 2 [23]. Curiously, the situation at the *E. coli melAB* promoter, where CRP is recruited as a co-activator by direct protein–protein interactions with tandem-bound MelR, is a mirror image of the situation at some CRP-dependent CytR-repressed promoters [24••], where CytR is recruited as a co-repressor by direct protein–protein interactions with tandem-bound CRP (Figure 2).

Another example of cooperative binding of two activators at a promoter is found with the interactions of AphA and AphB at the *Vibrio cholerae tcpPH* promoter [25]. However, despite the simplicity of this sort of mechanism, examples of cooperative interactions between different transcription factors at bacterial promoters are rare, presumably because the evolution of such interactions would commit the factors to each other (molecular monogamy?) and thereby reduce the possibilities for horizontal evolution.

Co-dependence due to bacterial nucleoid proteins

Transcription initiation at many promoters is modulated by the proteins that shape the bacterial folded chromosome [26]. This is not surprising as most of these proteins are present in large amounts, bind at many target sites and cause big changes in DNA conformation. The expression of some virulence determinants is repressed by these proteins [27,28•,29]. In addition, investigations with the *E. coli nir* operon promoter have shown that the IHF (integration host factor) and Fis proteins confer co-dependence on two activators [30]. The *nir* promoter,

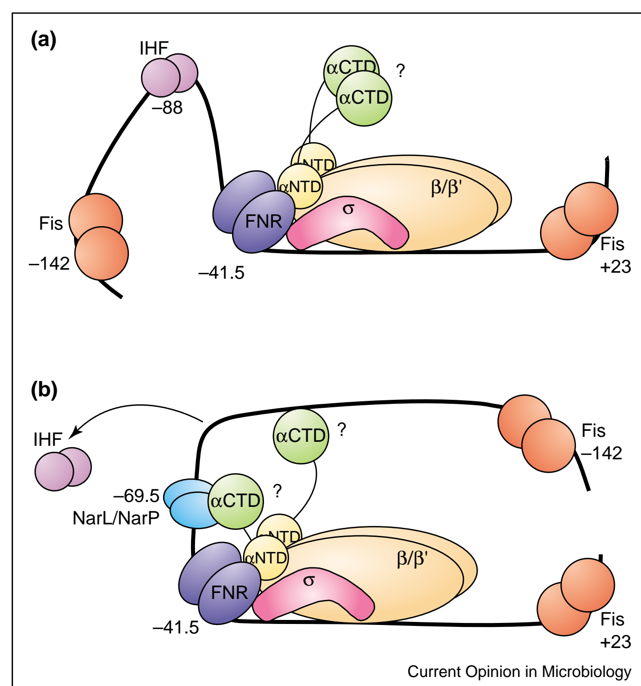
Figure 2



Cooperative interactions can activate or repress a promoter. **(a)** Co-operative binding of CRP to tandem-bound MelR activates transcription at the *E. coli melAB* promoter. The relative locations of MelR and CRP bound to the *melAB* promoter are shown. In the absence of CRP, MelR cannot bind to the promoter proximal site that abuts the −35 element and the *melAB* promoter is inactive (OFF). Co-operative binding of CRP to the promoter permits MelR to occupy the promoter proximal DNA site, and thus activates transcription by a Class II mechanism (ON). **(b)** Co-operative binding of CytR to tandem-bound CRP represses transcription at the *E. coli deo*P2 promoter. CRP bound in tandem activates transcription at the *deo*P2 promoter by a Class II mechanism (ON). Transcription is repressed by CytR binding between the tandem CRP sites (OFF).

which controls expression of an operon that encodes a cytoplasmic NADH-dependent nitrite reductase, is co-dependent on activation by FNR and either NarL or NarP [31]. FNR, the activity of which is triggered by anaerobiosis [14], binds to a single site at position –41.5, and functions as a Class II activator. NarL and NarP, related transcription activators whose activity is triggered by the presence of nitrite or nitrate ions in the growth medium [32], bind to a target site centred at position −69.5. Genetic and biochemical studies have shown that FNR-dependent transcription activation of the *nir* promoter is repressed by IHF binding to a site at position −88 and Fis binding to sites at positions −142 and +23, and that NarL and NarP function by reversing this repression [30,33]. IHF, Fis and FNR bind together to form a repressed complex, but NarL or NarP can displace IHF from its binding site, and this disrupts the repression of FNR-dependent activation. Thus, the co-dependence of the *nir* promoter is the result of a nucleoprotein assembly involving proteins of the bacterial folded chromosome that must be re-modeled by NarL or NarP for the promoter to be activated (Figure 3). The crucial event in remodeling is the displacement of IHF by NarL and NarP. This can be understood in the light of recent structural information about NarL, which shows that NarL binding at the *nir* promoter alters the width of the DNA minor groove such that IHF is less able to bind [33,34•].

Figure 3



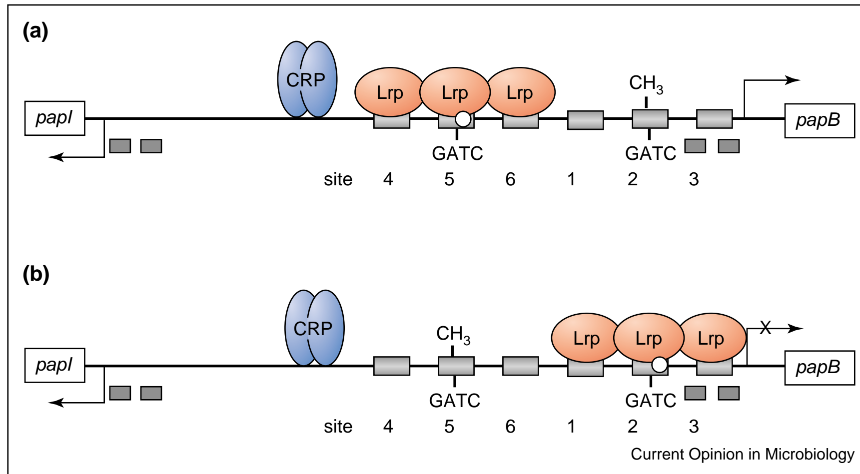
Activation by remodeling a nucleoprotein complex. **(a)** *nir* promoter repressed state. The binding of RNAP, Fis, IHF and FNR relative to the transcription start site of *nir* (+1) is shown. **(b)** *nir* promoter activated state. Binding of NarL or NarP causes dissociation of IHF from its site near position −88, resulting in remodelling of the nucleoprotein assembly into a form that promotes transcription initiation.

Parallel studies with the related *E. coli nrf* operon promoter have revealed many similarities to the *nir* promoter. Expression of the *nrf* promoter is also co-dependent on FNR, which binds at position −41.5, and NarP/NarL, which bind at position −74.5 [31]. FNR-dependent activation of the *nrf* promoter is repressed by IHF, which binds to a target that overlaps the DNA site for NarP/NarL, and Fis, which binds to a site at position −22 [35]. Thus, again, co-dependence on the second activator is due to a nucleoprotein complex involving IHF and Fis that suppresses FNR-dependent activation. For activation, the complex must be remodeled, which NarP or NarL initiate by displacing IHF from its binding site. Presumably there are many other cases where proteins involved in folding the bacterial chromosome confer co-dependence on more than one activator. Interestingly, at the *E. coli nrf* operon regulatory region, bound IHF and bound Fis also play a role in downregulating the divergent *acs* promoters. In particular, IHF and Fis suppress CRP-dependent activation of the *acsP2* promoter, resulting in a growth stage-specific profile of *acsP2* expression [20,36].

Modulation of activator function by an epigenetic mechanism

A very different example of complex activation is found at the *pap* promoter which controls the expression of the pyelonephritis-associated pili (Pap) in uropathogenic *E. coli* (UPEC), that allow UPEC to bind to uroepithelial cells. Expression from this promoter is subject to phase variation so that 'Phase ON' cells are piliated and 'Phase OFF' cells do not have pili. Although the *pap* promoter is dependent on CRP, which binds at position −215 and functions as a Class I activator [37], its expression is controlled by Lrp (leucine-responsive regulatory protein) and PapI, in response to methylation by the *dam* DNA methylase. The crucial observation is that 'Phase ON' and 'Phase OFF' cells exhibit different patterns of DNA methylation at the *pap* promoter and that these methylation patterns toggle Lrp between different target sites such that the promoter is either 'ON' or 'OFF' [38••,39••]. There are six potential binding sites for Lrp at the *pap* promoter (Figure 4). Lrp binds in a mutually exclusive manner to either sites 1–3 or sites 4–6, depending on the phase variation. Sites 2 and 5 contain potential Dam methylation sites, which are protected from the methylase by bound Lrp. In 'Phase OFF' (non-piliated) cells, Lrp binds cooperatively to sites 1–3. Site 3 overlaps the DNA −10 and −35 elements, so Lrp binding prevents recruitment of RNAP to the promoter, thereby repressing *pap* transcription and preventing Dam methylation at the promoter proximal methylation site, located within Lrp binding site 2. Binding of Lrp to sites 1–3 reduces the affinity of Lrp for sites 4–6, which remain unoccupied in 'Phase OFF' cells, thus exposing the distal DNA site for methylation within site 5.

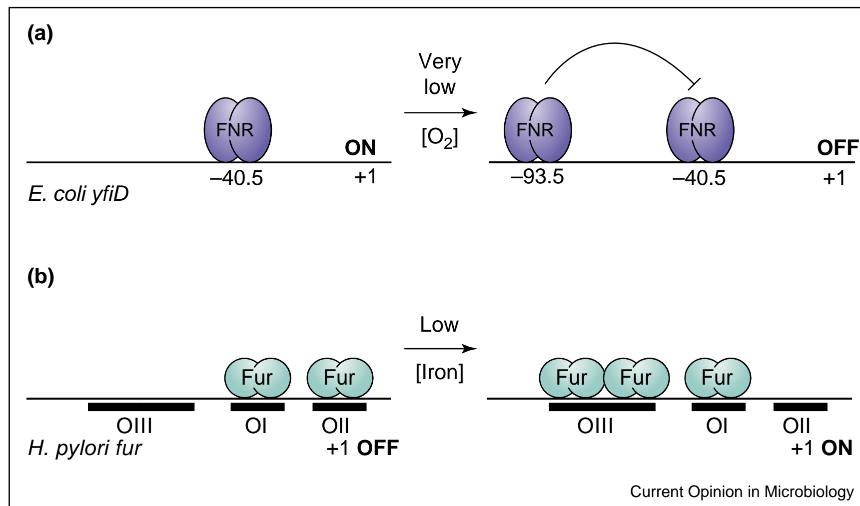
Figure 4



Switching at the *pap* promoter region. Lrp binding sites (numbered 1–6) are shown as gray boxes, and the promoter −10 and −35 elements for the *papB* and *papI* promoters are shown as striped boxes. Transcription start sites are indicated by arrows. Lrp sites 2 and 5 contain GATC sequences that are targets for methylation by Dam. Methylation patterns in **(a)** the the *pap* promoter phase ON (piliated) and **(b)** the *pap* promoter phase OFF (non-piliated) configurations are shown. In the presence of PapI (denoted by a small open circle), Lrp binds co-operatively to non-methylated DNA sites 4, 5 and 6 (phase ON) or sites 1, 2 and 3 (phase OFF). DNA site 3 for Lrp overlaps the *papB* promoter −10 and −35 elements, and, thus, occupation of site 3 in phase OFF promoters leads to inhibition of transcription, indicated by X.

In 'Phase ON' cells, Lrp instead binds cooperatively to sites 4–6, leaving sites 1–3 unoccupied. Thus, Lrp protects the promoter distal methylation site and Dam methylates the promoter proximal site. Because Lrp binding site 3 is unoccupied by Lrp, the −10 and −35 elements are now available for interaction with RNAP. This creates an epigenetic switch that is flipped under the influence of PapB and PapI, triggered by the appearance of hemi-methylated DNA, which appears post-replication. Thus covalent modification of the promoter

Figure 5



Signal-dependent promoter reorganization. **(a)** Signal-dependent repression by an activator at the *E. coli yfiD* promoter. The promoter carries a high affinity DNA site for FNR centered at position −40.5, and a lower affinity site at position −93.5. In microaerobic conditions, FNR preferentially occupies the promoter-proximal higher affinity site at position −40.5 and activates transcription by a Class II mechanism (ON). At lower oxygen levels, FNR additionally occupies the lower affinity site at position −93.5. Simultaneous occupation of both DNA sites by FNR leads to transcription repression (OFF). **(b)** Signal-dependent activation by a repressor at the *H. pylori fur* promoter. When iron levels are high, the Fur repressor protein binds to DNA sites OI and OII. Site OII overlaps the transcription start site (+1) and hence transcription of *fur* is inhibited (OFF). As iron levels fall, Fur protein occupies site OIII in preference to OII. This change in binding by Fur exposes the transcription start site and leads to transcription activation (ON).

target toggles Lrp between functioning as a repressor or a co-activator.

Signal-dependent promoter reorganization

An emerging theme at complex promoters is that ligands can alter the distribution of a transcription factor between different sites at a promoter, thus altering the regulatory outcome. The best-understood example of this mechanism is the action of AraC protein at the *araBAD* promoter [40•]. In the presence of arabinose, AraC binds to two adjacent sites and activates transcription by functioning as a Class II activator. In the absence of arabinose, the activating AraC molecule shifts to bind at a distal site, resulting in loop formation that represses transcription. Another example is the *E. coli yfiD* promoter, described above, where binding of FNR to an upstream site represses activation by FNR bound near position −41 [15,16]. The mirror image of this mechanism is seen at the *Helicobacter pylori fur* promoter, which is autoregulated by the Fur (ferric uptake regulator) protein [41••]. In the presence of free iron, the Fur protein represses the *fur* promoter by binding at two sites that overlap the promoter. At lower iron levels, Fur occupies an upstream site and this relieves repression by downstream-bound Fur [42••]. Thus, at the *E. coli yfiD* promoter, upstream binding of an activator represses expression, whereas at the *H. pylori fur* promoter, upstream binding of a repressor activates expression (Figure 5).

Conclusions

The simple conclusion is that promoters can be complicated. And even greater complexity may already be on our horizon, as illustrated by the following points:

1) Transcription activation by most factors is due to binding to target sites overlapping or upstream of the target promoter −35 hexamer. However, examples have now been described where binding of the factor downstream of the target promoter is needed for activation [43,44•]. Note that this arrangement is extensively used in eukaryotes.
2) It is assumed that transcription factors are free to diffuse in the bacterial cytoplasm, but it seems likely that many will be localized. An indisputable example is the *Vibrio cholerae* ToxT promoter that is co-dependent on two activators, ToxR and TcpP, both of which are anchored to the cell membrane [45,46•].
3) In all cases of co-dependent activation described so far, the primary activator functions by making a direct contact with RNAP [1,47]. An exception may be the *E. coli copA* promoter that is activated by upstream binding of CpxR, and CueR, a MerR homolog that activates by altering promoter conformation [48].
4) Logic modeling suggests many different possibilities for a bacterium to solve its transcription regulatory problems and suggests that there remains considerable space into which evolution can move [49••].

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