THE REGULATION OF BACTERIAL TRANSCRIPTION INITIATION

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Bacteria use their genetic material with great effectiveness to make the right products in the correct amounts at the appropriate time. Studying bacterial transcription initiation in *Escherichia coli* has served as a model for understanding transcriptional control throughout all kingdoms of life. Every step in the pathway between gene and function is exploited to exercise this control, but for reasons of economy, it is plain that the key step to regulate is the initiation of RNA-transcript formation.

CRAB-CLAW STRUCTURE
This is the name given to the structure that is probably common to all multi-subunit RNA polymerases, in which the two largest subunits form a cleft that contains the enzyme active site.

LINKER
In the context of a protein, a linker is a short stretch of amino acids that joins two separately folding domains. Many linkers have a flexible structure that allows the adjacent domains to adopt different juxtapositions with respect to each other.

SIGMA FACTOR
The subunit of RNA polymerase holoenzyme that is required for promoter sequence recognition and ability to initiate transcription.

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The central component in transcriptional regulation in bacteria is the multi-subunit DNA-dependent RNA polymerase, which is responsible for all transcription[1] (FIG. 1). The core enzyme, which is competent for transcription, but not for promoter-directed transcript initiation, has a subunit composition of $\beta\beta'\alpha_2\omega$. High-resolution structural studies on the core enzyme show that it adopts a CRAB-CLAW STRUCTURE, which is similar to the structure that is found in the yeast DNA-dependent RNA polymerase II[2,3]. The active site of the polymerase, which has determinants for the binding of both template DNA and the RNA product during transcription, is formed from the large β and β′ subunits (1,342 and 1,407 residues, respectively, in the *Escherichia coli* core enzyme)[4]. Each of the two identical 329-residue α subunits consist of two independently folded domains that are joined by an ~20-amino-acid flexible LINKER[5]. The larger amino-terminal domain (αNTD; residues 1-235) dimerizes and is responsible for the assembly of the β and β′ subunits. The smaller carboxy-terminal domain (αCTD; residues 250–329) is a DNA-binding module that has an important role at certain promoters[6]. The small 91-residue ω subunit has no direct role in transcription, but seems to function as a chaperone to assist the folding of the β′ subunit[7].

For RNA polymerase to begin transcription at a particular promoter, it must first interact with a σ subunit to form the holoenzyme. The σ subunit has three main functions: to ensure the recognition of specific promoter sequences; to position the RNA polymerase holoenzyme at a target promoter; and to facilitate unwinding of the DNA duplex near the transcript start site[8,9]. Most bacteria contain multiple SIGMA FACTORS that enable the recognition of different sets of promoters. With the important exception of the small σ^{54} family, all σ factors share common features[10]. They are multi-domain proteins that have up to four different domains joined by linkers[11]. Although domains 2, 3 and 4 are known to be involved in promoter recognition[8,12–14], the function of domain 1 is not understood. Indeed, domain 1 is absent from many σ factors.

What happens at promoters

Promoters control the transcription of all genes. Transcription initiation requires the interaction of RNA polymerase with promoter DNA and the formation of an OPEN COMPLEX, in which the duplex DNA around the transcript start-point is unwound[15] (FIG. 2). Synthesis of the DNA template-directed RNA chain then begins, with the formation of the first phosphodiester bond between the initiating and adjacent nucleoside triphosphates. After this initiation phase, RNA polymerase is moved into the elongation complex, which is responsible for RNA-chain extension. The main step in initiation is promoter recognition by RNA polymerase, and the different DNA sequence elements that are responsible for this have been studied intensively[8,16]. Four different sequence elements have been identified. The two principal elements are

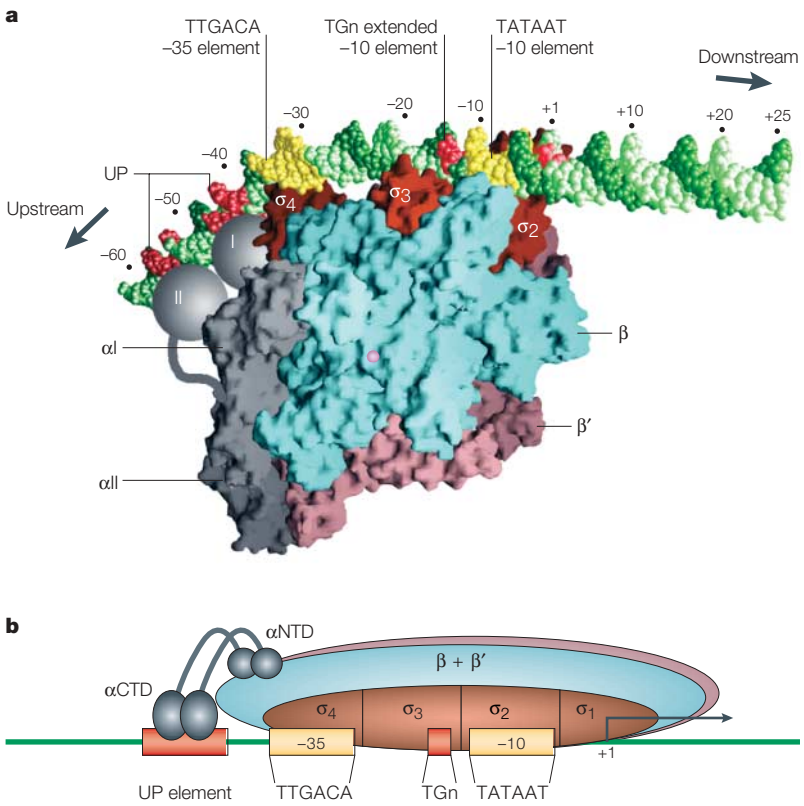

Figure 1 | **RNA polymerase and its interactions at promoters. a** | A model based on crystallographic studies of the initial docking of the RNA polymerase holoenzyme to a promoter. The DNA strands are shown in green, with the –10 and –35 elements highlighted in yellow and the TGn extended –10 and the UP elements highlighted in red. RNA polymerase is shown with the β and β′ subunits coloured light blue and pink, respectively, αNTDs are coloured grey and the different domains of σ are coloured red. Grey spheres labelled I and II, represent the domains of αCTD that bind to the promoter. The RNA polymerase active site is denoted by the Mg^{2+} ion, (magenta). Reproduced with permission from REF. 13 © (2002) American Association for the Advancement of Sciences. **b** | A cartoon illustration of the model shown in part **a**, illustrating the different interactions between promoter elements and the RNA polymerase. The consensus sequences for the -35 (TTGACA), extended –10 (TGn) and -10 (TATAAT) elements are shown.

the –10 hexamer and the –35 hexamer, which are located 10 and 35 base pairs (bp) upstream from the transcript start site, respectively. Promoter –10 elements are recognized by domain 2 of the RNA polymerase σ subunit (specifically, region 2.4). Similarly, promoter –35 elements are recognized by domain 4 of the RNA polymerase σ subunit (specifically, region 4.2). Consensus hexamer sequences for the –10 and –35 elements have been established and crystallographic studies have led to the generation of models that explain how they are recognized by the RNA polymerase[11,13]. The two other important promoter elements are the extended –10 element and the UP ELEMENT. The extended –10 element is a 3–4–bp motif located immediately upstream of the –10 hexamer that is recognized by domain 3 of the RNA polymerase σ subunit[13,17,18], and the UP element is a ~20 bp sequence located upstream of the promoter –35 hexamer that is recognized by the C-terminal domains of the RNA polymerase α subunits[19]. So, together, the –10, –35, extended –10 and UP elements specify the initial binding of RNA polymerase to a promoter (FIG. 1), but the relative contribution of each element differs from promoter to promoter. As the role of these promoter elements seems to be primarily to dock the RNA polymerase such that it is competent for open-complex formation and the succeeding steps of transcription, deficiencies in one element can be compensated by another. Indeed, there is no naturally occurring promoter in which all these elements are perfect — such a promoter would bind RNA polymerase too tightly. After initial binding of RNA polymerase, the DNA strands from approximately position –10 to position +2, just downstream of the transcript start-point, are unwound to form a 'bubble', and to generate the open complex[20,21]. This is due to an ISOMERIZATION, which results in the non-template strand of the 'bubble' being bound by domain 2 of the RNA polymerase σ subunit (specifically, region 2.3). This isomerization is poorly understood, but it must result in movement of the free template strand of the 'bubble' into the active site of the RNA polymerase so that the chemistry of RNA synthesis can begin. So, the pathway to transcript formation involves many steps, all of which could be subject to regulation. For simplicity, most investigators suppose that the main steps that are regulated are the initial binding of RNA polymerase to generate the closed complex, the isomerization to the open complex or the initial steps of RNA-chain synthesis.

The crucial point, when considering microbial gene regulation, is to understand that the RNA polymerase is in short supply. Much of the active RNA polymerase is channelled into copying the genes encoding stable RNAs that are needed for translation. Similarly, some of the RNA polymerase is bound non-productively to the cell's DNA. So, the amount of free RNA polymerase that is available to copy most of the 4,000–5,000 genes in the cell is limited[22]. Additionally, the supply of σ factors is limited, so there is intense competition between different promoters for RNA polymerase holoenzyme[22,23]. This explains how cells can make a lot of one message, but little or none of another.

Five distinct molecular mechanisms seem to ensure the prudent distribution of RNA polymerase between competing promoters. These involve promoter DNA sequences, σ factors, small ligands, transcription factors and the folded bacterial chromosome structure. The different mechanisms are addressed, in turn, below, including how they are exploited to alter profiles of gene expression in response to environmental change. Each mechanism allows variation in the level of expression of genes — known as 'fine tuning'. However, different mechanisms are used in cases where no fine tuning is needed — for example, the control of fimbrial gene expression[24]. The DNA segment carrying the fimbrial gene promoter can be switched from an orientation in which the promoter drives transcription of the fimbrial genes, to the opposite orientation in which the promoter is directed away from them, thereby creating an effective 'on–off' switch.

OPEN COMPLEX
For transcription, the two strands of the DNA duplex must be unwound locally. An open complex is formed when RNA polymerase binds at a promoter, and the duplex around the transcription start is unwound.

UP ELEMENT
This is a DNA sequence element found at some promoters that increases promoter activity by providing a point of contact for the RNA polymerase α subunit C-terminal domains.

ISOMERIZATION
Describes the step in which the DNA segment, in the RNA polymerase-promoter complex, is unwound.

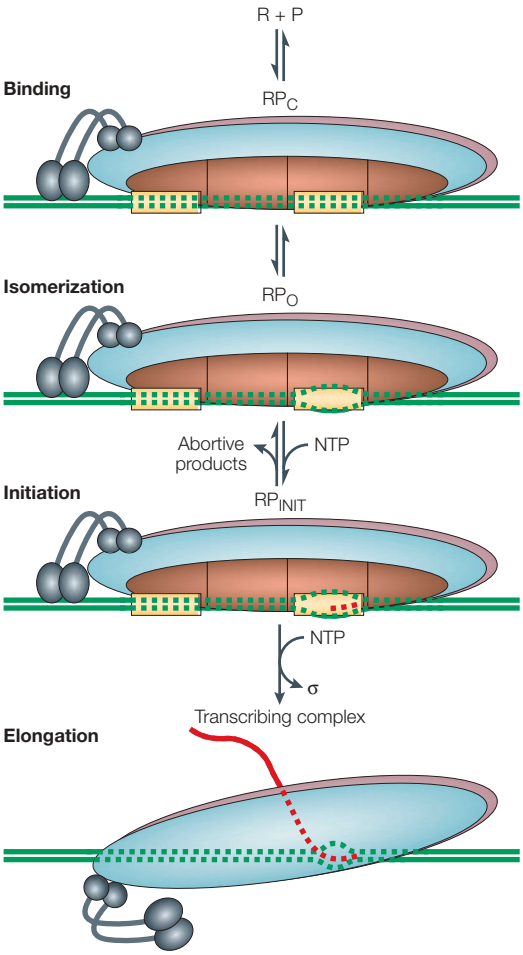

Figure 2 | **The pathway of transcription initiation at bacterial promoters.** The RNA polymerase (R) interacts with promoter DNA (P) to form the closed complex (RP_C). Dashed lines show the promoter DNA that is bound by the RNA polymerase holoenzyme. The duplex DNA around the transcript start site is unwound (represented by a 'bubble' in the DNA that is bound by the RNA polymerase holoenzyme) to form the open complex (RP_O). The initiating complex (RP_{INIT}) is formed and synthesis of the DNA-template-directed RNA chain (shown as a dashed red line) begins with formation of a phosphodiester bond between the initiating and adjacent phosphodiester nucleoside triphosphates (NTPs). Elongation is the final stage, and the RNA chain length increases, shown as a solid red line.

Promoter sequences

In the bacterial cell, RNA polymerase is faced with an array of nearly 2,000 promoter sequences[25], and differences between these sequences act as powerful drivers in the unequal distribution of RNA polymerase between different transcription units. We know that promoters with near-consensus sequence elements function more efficiently. The observation that nearly all promoters possess non-consensus sequences teaches us that the activity of each promoter in the cell is balanced against that of other promoters. Also, it is obvious that promoters that function sub-optimally are amenable to upregulation when the appropriate situation arises. Many of the strongest bacterial promoters have effective UP elements, and these seem to function simply by binding to the RNA polymerase αCTDs[6]. Although differences in promoter sequence elements provide a useful way to control a wide range of promoter activities, these differences provide only static regulation that cannot normally be modulated according to environmental conditions. So, most adaptative regulation is due to modulation by *trans*-acting factors, as discussed below. An exception could arise from the differential distribution of RNA polymerase between promoters, when the free cellular polymerase concentration varies; some promoters might be more affected by changes in RNA polymerase concentration than others.

Sigma factors

E. coli has one main σ factor, σ^{70}, which equips RNA polymerase to recognize most promoters. However, the *E. coli* genome also contains six other σ factors that accumulate in response to specific stresses[22]. As they accumulate, these alternative σ factors compete with σ^{70} for RNA polymerase. They bind a certain number of RNA polymerase molecules and equip these molecules to initiate transcription at promoters carrying particular sequence elements[23]. Specific examples include σ^H and σ^E, which accumulate in response to heat-shock stress in the cytoplasm and periplasm, respectively, and enable the RNA polymerase to recognize promoters that control genes that assist the cell in coping with elevated temperatures[26,27]. Alternative σ factors are widely distributed in bacteria[9], and they all work by binding RNA polymerase molecules so that the holoenzyme that is generated is directed to a specific subset of promoters. Regulation of alternative σ factor activity can be very complicated, involving transcriptional, translational and post-translational control. In many cases, the activity of a σ factor is controlled by an ANTI-SIGMA FACTOR, which sequesters it away from the RNA polymerase[28] (BOX 1).

Small ligands

Small ligands provide an alternative mechanism by which RNA polymerase can respond quickly and efficiently to the environment. The best example is guanosine 3′,5′ bisphosphate (ppGpp), which is synthesized when amino-acid availability is restricted to the extent that translation is also limited[29]. ppGpp works by destabilizing open complexes at promoters that control synthesis of the machinery for translation[30,31]. In fact, although the interaction of ppGpp with RNA polymerase is not promoter-specific, ppGpp-dependent inhibition only occurs at promoters that form unstable open complexes. Such promoters typically have short runs of GC-rich sequences near position +1, and they are found to control many of the genes that encode the products that are needed for translation. Such promoters are also unable to function well at low concentrations of the initiating nucleotide, usually ATP[32,33]. It has been proposed that ppGpp controls expression of the translation machinery in response to sudden starvation, whereas ATP availability controls expression in response to growth rate[34]. Many of these promoters recruit RNA polymerase very effectively and so, potentially,

ANTI-SIGMA FACTORS
A negative transcriptional regulator that acts by binding to a sigma factor and preventing its activity. An anti-anti-sigma factor, in turn, counteracts the action of an anti-sigma factor.

Box 1 | **More about σ factors**

A multiplicity of σ factors

The first bacterial σ factor was discovered in 1969 as the subunit of the *Escherichia coli* RNA polymerase that was essential for promoter selection[80]. The possibility of multiple σ factors in one organism was realized 10 years later, with the finding that several alternative σ factors were essential for sporulation in *Bacillus subtilis*. Shortly after, it was found that certain stress responses (for example, heat shock) in *E. coli* are dependent on alternate σ factors, and whole-genome sequencing has now revealed that multiple σ factors are widespread in bacteria[8]. The number of σ-factor-encoding genes varies, from a single gene in *Mycoplasma genitalium* to 63 genes in *Streptomyces coelicolor*[81]. It seems that, for any bacterium, there is a rough correlation between the number of its genes that encode σ factors and the diversity of environments that it experiences. Usually it is supposed that the binding of σ factors to RNA polymerase is solely a function of relative affinities and concentrations, but this might yet prove to be an oversimplification, and a recent study by Jishage *et al.*[82] has indicated a role for small molecules in σ-factor exchange by RNA polymerase.

Regulation of alternative σ factors

As different σ factors regulate cellular responses to different stresses, it is unsurprising to find that their activities are tightly controlled. Some of this regulation is due to control of σ-factor synthesis, but, in many cases, regulation is effected by anti-sigma factors which modulate the activity of a σ factor independently of its transcription and translation[28]. Many anti-sigma factors sequester their cognate σ factor so that it is not free to combine with RNA polymerase. The activity of the anti-sigma factor is then regulated by ligand binding, covalent modification or proteolysis[83]. In some cases, the anti-sigma factor sequesters its cognate σ factor to the cell membrane, where it is sensitive to extracellular signals. In other cases, the activity of the anti-sigma factor controls proteolytic degradation of its cognate σ factor[84]. The *E. coli* stationary phase σ factor, σ^{38}, binds to the RssB factor which targets σ^{38} to the ClpXP system for proteolysis[85]. Control of this process occurs by phosphorylation of RssB, which regulates delivery of σ^{38} to the ClpXP system.

The σ^{54} family are in a class of their own

Most, but not all, bacteria contain one σ factor that is related to the *E. coli* σ^{54} protein[81]. These σ factors share no sequence similarities with σ^{70}, have a different domain structure, and are unrelated to most of the σ factors[86]. At target promoters, the key elements for RNA polymerase that contains σ^{54} are located near positions –12 and –22. RNA polymerase that contains σ^{54} can recognize these elements, but has an absolute requirement for an activator that must interact with σ^{54} for the RNA polymerase to access the open complex for transcription initiation. Such activators carry specific conserved modules that drive an ATP-dependent remodelling of the RNA polymerase–promoter complex, which results in formation of the open complex. This contrasts with RNA polymerase that contains σ^{70} and that is competent for transcription initiation in the absence of an activator (although at some promoters, an activator is needed to recruit the RNA polymerase).

can initiate transcription at the maximum possible rate. However, to achieve these rates, the open complex must be stabilized and this requires higher ATP and lower ppGpp concentrations.

Transcription factors

The *E. coli* genome contains more than 300 genes that encode proteins that are predicted to bind to promoters, and to either up- or downregulate transcription[35,36]. So far, about half of these have had their functions verified experimentally. Most of these proteins are sequence-specific DNA-binding proteins, and this ensures that their actions are targeted to specific promoters. Some of these proteins control large numbers of genes, whereas others control just one or two genes. It has been estimated that seven transcription factors (CRP, FNR, IHF, Fis, ArcA, NarL and Lrp; see BOX 2) control 50% of all regulated genes, whereas ~60 transcription factors control only a single promoter[37]. Bacterial transcription factors can be grouped into different families on the basis of sequence analysis. So far, a dozen families have been identified[35], the best characterized of these being the LacI, AraC, LysR, CRP and OmpR families.

Transcription factors couple the expression of genes to environmental signals, and they must be regulated — either by controlling their activity or by controlling their expression. Different mechanisms are used to achieve this. First, the DNA-binding affinity of transcription factors can be modulated by small ligands, the concentrations of which fluctuate in response to nutrient availability or stress. The best example of this is the reduction in the DNA-binding affinity of the Lac repressor by the small molecule, allolactose, which signals the presence of lactose in the growth medium[38]. Second, the activity of some transcription factors is modulated by covalent modification. For example, some RESPONSE REGULATORS — such as, NarL — bind to their target DNA only when phosphorylated by their cognate SENSOR KINASE. The sensor kinases are located in the inner bacterial cell membrane, and are regulated by extracellular signals[39]. NarL is controlled by the NarX and NarQ sensor kinases, which are activated by binding to extracellular nitrite or nitrate ions[40]. Third, the concentration of some transcription factors in the cell controls their activity. In these cases, cellular concentration is determined either by regulation of expression of the transcription factor or by proteolysis. For example, one cellular response to oxidative stress is controlled by the concentration of the SoxS protein. The transcription of the gene encoding SoxS is controlled by SoxR, which in turn is regulated directly by interactions with oxidizing ligands[41]. Finally, a less common mechanism for regulating the effective concentration of a transcription factor is sequestration by a regulatory protein to which it binds[42].

When a transcription factor binds to a promoter, it can activate or repress transcription initiation. Some transcription factors function solely as activators or repressors, whereas others can function as either according to the target promoter[35]. Activators improve the performance of a promoter by improving its affinity for RNA polymerase. It is likely that most activators function by binding to target promoters before acting on RNA polymerase. However, an alternative mechanism has recently been proposed for the MarA and SoxS regulators, in which they interact with free RNA polymerase before binding to promoter DNA[43,44]. As the activities of MarA and SoxS are controlled solely by their cellular concentration, this mechanism is reminiscent of that used by σ factors, which 'reserve' a certain number of RNA polymerase molecules by binding to them.

Simple activation. At many promoters, activation of transcription is simple, and involves the action of a single activator. Three general mechanisms are used for 'simple' activation (FIG. 3). In Class I activation (FIG. 3a), the activator binds to a target that is located upstream of the promoter –35 element and recruits RNA polymerase to

RESPONSE REGULATORS
Usually bacterial gene-regulatory proteins that control gene expression in response to external signals. Most response regulators consist of two domains: a DNA-binding domain and a regulatory domain, the activity of which is modulated (indirectly) by the external signal.

SENSOR KINASE
Transmits the external signal to the response regulator.

BOX 2 | **CRP, FNR, IHF, Fis, ArcA, NarL and Lrp**

These are the seven *Escherichia coli* transcription factors that control most of the regulated genes. Like many *E. coli* proteins, their three-letter names derive from acronyms that describe their function. The fourth letter, which is present in some names, denotes a particular gene product amongst several that are involved in the same function. So, CRP is the cyclic AMP receptor protein, a gene regulatory protein that is activated by cyclic AMP. Confusingly, CRP is often also referred to as CAP, the catabolite gene activator protein, which is named after its role in catabolite repression. FNR was named as the factor necessary for induction of fumarate reductase and nitrite reductase, but it is now known to regulate many more genes in response to oxygen starvation. IHF and Fis denote integration host factor and factor for inversion stimulation, respectively. These names refer to functions that are used by certain bacteriophages during infection of *E. coli*, but it is now known that these factors have extensive roles in non-infected cells. Arc and Nar denote anaerobic respiratory control and nitrate regulation, respectively, and ArcA and NarL are the products of particular genes that are involved in each process. Lrp denotes the leucine regulatory protein, which is a little understood regulatory factor that has different roles at many promoters.

the promoter by directly interacting with the RNA polymerase αCTD. The best example of Class I activation is the action of the cyclic AMP receptor protein, CRP, at the *lac* promoter[45]. The linker joining the αCTD and αNTD is flexible, so activators that function using a Class I mechanism can bind at several locations upstream of promoters. In Class II activation (FIG. 3b), the activator binds to a target that overlaps the promoter –35 element and contacts domain 4 of the RNA polymerase σ subunit[46]. This contact also results in recruitment of RNA polymerase to the promoter, but other steps in initiation can also be affected. The best example of this is the activation of the bacteriophage λ PRM promoter by the bacteriophage λ CI protein[47]. Owing to the constraints involved in the binding of domain 4 of the RNA polymerase σ subunit at promoters, there is very little flexibility in the positioning (relative to the transcription start site) of activators that function by a Class II mechanism. At some promoters that are subject to Class II activation, the activator contacts other parts of the RNA polymerase (for example, αNTD) but still binds to a target sequence that overlaps with the promoter –35 element[48].

The third mechanism for simple activation is found in cases where the activator alters the conformation of the target promoter to enable the interaction of RNA polymerase with the promoter –10 and/or –35 elements. This requires the activator to bind at, or very near to, the promoter elements, although one case has been reported of activation at a distance using a relay of conformational changes through the promoter DNA[49,50]. For promoters that are activated by members of the MerR family, the spacing between the –10 and –35 elements at target promoters is not optimal for RNA polymerase binding. MerR-type activators bind to the 'spacer' sequence and twist the DNA to reorientate the -10 and -35 elements so that they can be bound by the RNA polymerase σ subunit[51,52] (FIG. 3c).

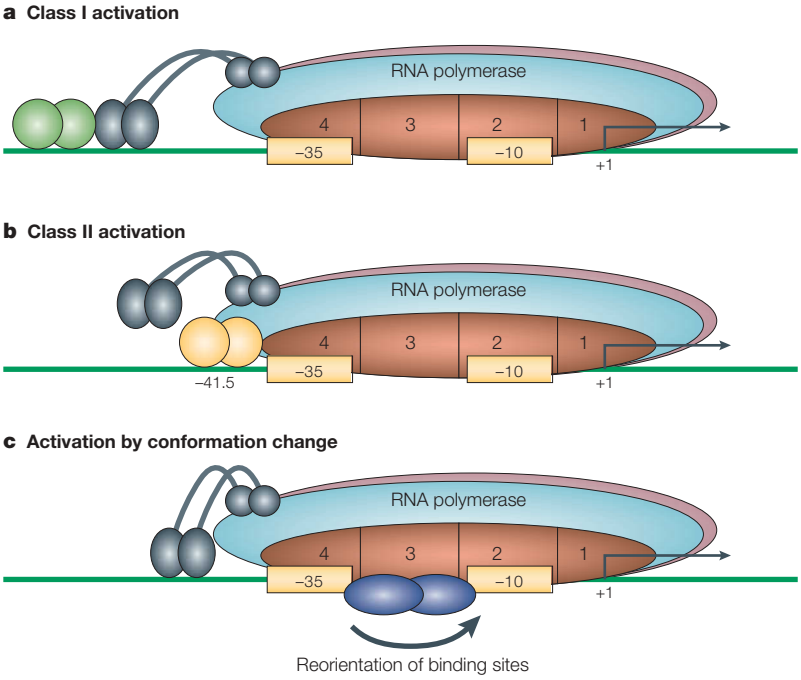

Figure 3 | **Activation at simple promoters.** The figure illustrates the organization of RNA polymerase and activator subunits during activation at simple promoters. Many activators function as dimers, and are shown as dimers here. Interacting proteins are shown adjacent to each other. **a** | Class I activation. The activator is bound to an upstream site and contacts the αCTD of RNA polymerase, thereby recruiting the polymerase to the promoter. **b** | Class II activation. The activator binds to a target that is adjacent to the promoter –35 element, and the bound activator interacts with domain 4 of σ^{70}. In most cases, to contact domain 4 of σ^{70} the class II activator must bind at, or near to, position –41.5. **c** | Activation by conformation changes. The activator (shown in blue) binds at, or near to, the promoter elements and realigns the –10 element and the –35 element so that the RNA polymerase holoenzyme can bind to the promoter.

Simple repression. Repressor proteins reduce transcription initiation at target promoters. At many promoters, repression is simple and involves a single repressor. Three general mechanisms are used (FIG. 4). Steric hindrance of RNA polymerase binding to promoter DNA is probably the simplest mechanism of repression (FIG. 4a). In these instances, the repressor-binding site is located in, or close to, the core promoter elements — for example, the Lac-repressor-binding site at the *lac* promoter[38]. However, in some cases, the repressor might not prevent binding of RNA polymerase to the promoter, but instead might interfere with post-recruitment steps in transcription initiation[53]. At other promoters — for example, the *gal* promoter, which is repressed by GalR[54] — multiple repressor molecules bind to promoter-distal sites, and repression might be caused by DNA looping, which shuts off transcription initiation in the looped domain (FIG. 4b). Finally, complex cases have been found where

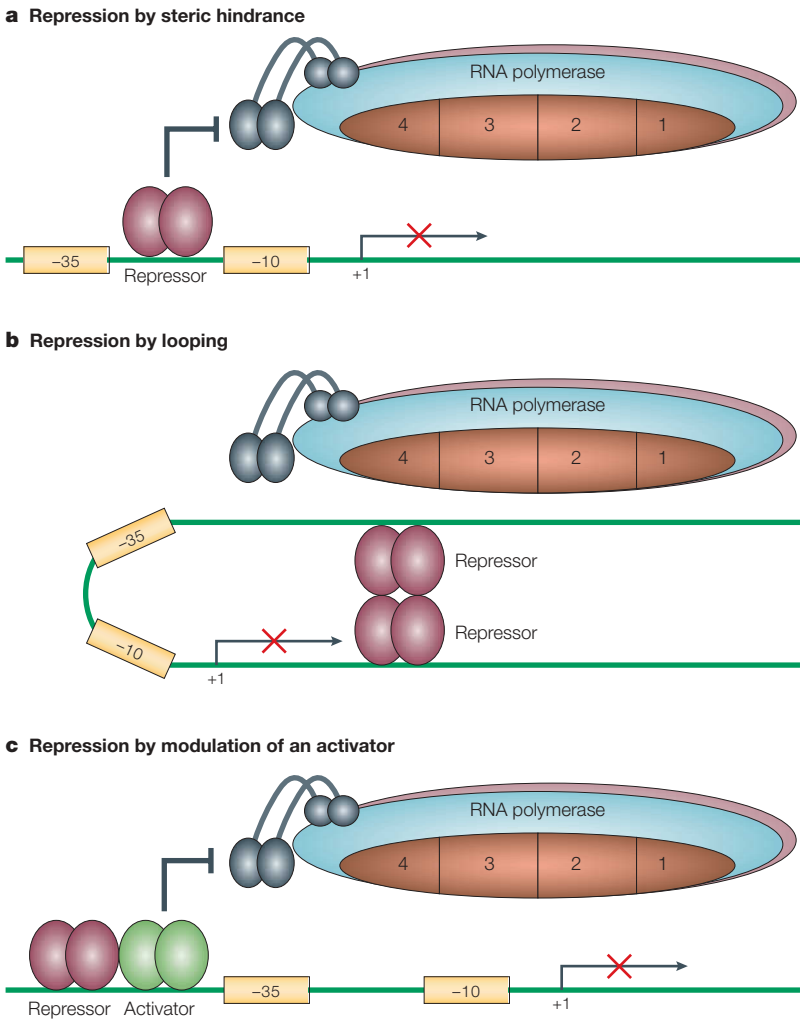

Figure 4 | **Mechanisms of repression. a** | Repression by steric hindrance. The repressor-binding site overlaps core promoter elements and blocks recognition of the promoter by the RNA polymerase holoenzyme. **b** | Repression by looping. Repressors bind to distal sites and interact by looping, repressing the intervening promoter. **c** | Repression by the modulation of an activator protein. The repressor binds to an activator and prevents the activator from functioning by blocking promoter recognition by the RNA polymerase holoenzyme.

the repressor functions as an anti-activator (FIG. 4c). The best examples are at CytR-repressed promoters, which are dependent on activation by CRP. Repression by CytR is through direct interactions between CytR (the repressor) and CRP (the activator) that prevent CRP-dependent activation[55]. At many of these promoters, CytR recognizes tandem-bound CRP molecules, and it is the organization of the tandem-bound CRP which confers specificity for repression by CytR[56].

Folded chromosomes and transcription

Bacterial chromosomes are highly compacted by SUPERCOILING and interactions with proteins and RNA. In *E. coli*, a dozen important proteins are involved in this compaction, including Fis (factor for inversion stimulation), IHF (integration host factor), H-NS and HU (histone-like nucleoid-structuring proteins), StpA (an H-NS homologue) and Dps (DNA-protein from starved cells)[57]. These so-called nucleoid proteins are abundant in the cell, although the concentrations of some, for example, Fis and Dps, fluctuate sharply depending on the growth conditions. Although most of these proteins bind to DNA nonspecifically, some bind with weak specificity so that they occupy specific sites that are distributed throughout the chromosome. The binding of these nucleoid proteins to DNA, and the resulting folding of the bacterial chromosome, must affect the distribution of RNA polymerase between promoters, but as yet, because we are still ignorant as to whether there is a basic unit of bacterial chromatin structure, no general rules have been established. Rather, the effects of these proteins have been unravelled on a case-by-case basis at individual promoters. Perhaps the best-understood case is the H-NS protein, which can completely silence gene expression by forming extended nucleoprotein structures[58–60] — for example, at the *proU* and *bgl* promoters. At other promoters, nucleoid-associated factors work together to influence transcription, causing activation or repression, depending on the context of their binding sites[61]. For example, at the *nir* regulatory region, Fis, IHF and H-NS sequester the DNA into an ordered nucleoprotein complex, which consequently represses transcription[62]. There are also many examples of promoters at which specific nucleoid-associated factors, such as Fis and IHF, have been recruited as activators of transcription initiation[61,63].

Integration of signals

The activity of most bacterial promoters is dependent on multiple environmental cues, rather than just one signal. Many promoters are controlled by two or more transcription factors, with each factor relaying one environmental signal. However, in some cases a regulatory protein can 'integrate' multiple signals. Perhaps the best example is the *Azotobacter vinelandii* NifL–NifA system, which controls the genes that are involved in nitrogen fixation in response to oxygen levels, available carbon and available nitrogen[64]. Regulatory systems of this complexity are quite rare and, in most cases, for a promoter to respond to multiple signals, multiple transcription factors are required. Generally, where two transcription factors are involved, one factor interprets a global metabolic signal, whereas the other responds to a specific metabolic signal. The best illustration of this is the *E. coli lac* promoter[65], which is regulated by CRP — which is dependent on a global signal, glucose starvation — and the Lac repressor — which is controlled by a specific metabolite, allolactose.

There are a few examples of integrated regulation that are solely dependent on repressors, but, in most examples studied so far, complex regulation depends on combinations of repressors and activators, or co-dependence on more than one activator. In most cases in which promoters are controlled by activators and repressors, the different regulators function independently. However, in some cases, such as promoters that are repressed by CytR, the repressor and the activator interact directly[66].

For promoters that are co-dependent on two or more activators, more complicated mechanisms are used. Four general mechanisms have been found at

SUPERCOILING
Describes a state of the DNA in which its conformation deviates from the well-known Watson-Crick double helix, leading to its compaction and favouring local DNA unwinding.

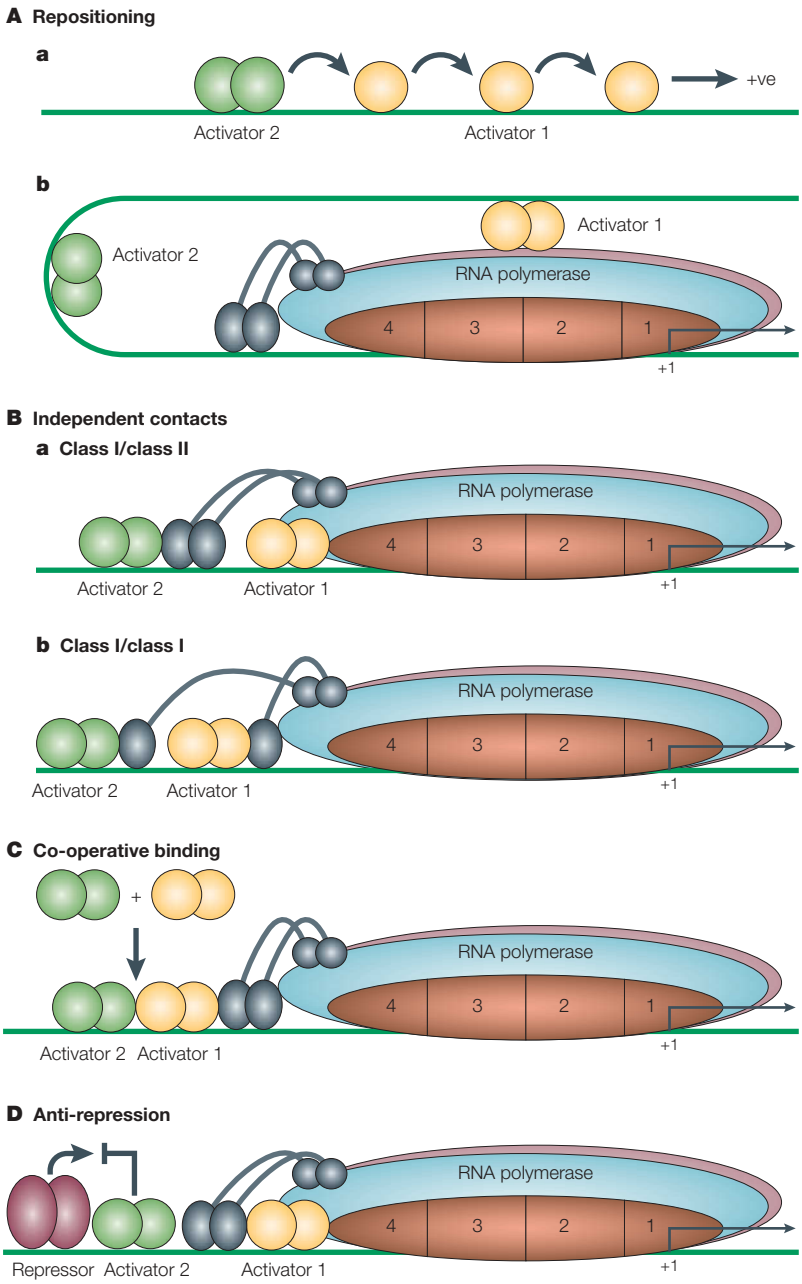

Figure 5 | **Mechanisms of promoter co-dependence on two activator proteins.** **A** | Repositioning of the primary activator by a secondary activator. In (**a**), the secondary activator (green) repositions the primary activator (yellow) from a location where it is unable to activate transcription to a location where it can activate transcription. In (**b**), the secondary activator alters the conformation of the DNA by bending, bringing the primary activator into a position from which it can activate transcription. **B** | Independent contacts by both activators are required for optimal activation. In (**a**), one activator functions by a Class II mechanism, and the second activator functions by a Class I mechanism. In (**b**), both activators function by a Class I mechanism. **C** | Cooperative binding. The binding of one activator is dependent on the binding of the second. **D** | Anti-repression. The binding of the secondary activator is required to counteract the inhibitory effects of a repressor, to allow the primary activator to function.

E. coli promoters, involving activator repositioning, independent activator–RNA-polymerase contacts, cooperative activator binding and anti-repression by an activator (FIG. 5). In repositioning mechanisms, the target promoter could be fully activated by a primary activator, but, in the absence of the secondary activator, the primary activator is mispositioned (FIG. 5A,a). So, the role of the secondary activator is to reposition the primary activator from a location where it is unable to activate transcription, to one where it can activate transcription. This repositioning can involve shifting the primary activator from one DNA site to another — for example, the repositioning of MalT by CRP at the *malK* promoter[67]. Alternatively, the secondary activator can alter the conformation of the DNA — for example, by bending — to allow the primary activator to make interactions with RNA polymerase and thereby activate transcription[68] (FIG. 5A,b).

A different mechanism is found at promoters where both activators bind independently, and where both activators must make independent contacts with RNA polymerase for transcription activation (FIG. 5B). These promoters contrast with simple Class I and Class II activator-dependent promoters, where interactions with a single activator are sufficient for full activation[69,70]. In some cases, complex promoters are dependent on one activator that functions by a Class II mechanism and another that functions by a Class I mechanism — for example, *proP* P2, where Fis and CRP function as Class II and Class I activators, respectively[71,72] (FIG. 5B,a). At other complex promoters, both activators function by a Class I mechanism — for example, *acs* P2, which is dependent on tandem-bound CRP molecules at Class I locations (FIG. 5B,b)[73,74]. The 'independent-contact' mechanism for coupling promoter activity to two activators seems to be ubiquitous. Presumably this is due to many activators being able to combine together because no direct interactions between them are required, so the mechanism presents many evolutionary possibilities.

Experimental evidence indicates that, in most cases, multiple activators bind independently at their target promoters. However, there are a few promoters at which activators bind cooperatively, and this can provide another simple mechanism for ensuring co-dependence, as one activator is unable to bind in the absence of the other (FIG. 5C). The best example of this is the *melAB* promoter, at which CRP is unable to bind unless MelR is already bound[75].

Nucleoid proteins that prevent activation by the primary activator can also confer co-dependence on two activators[76]. The secondary activator is required to reconfigure the nucleoid proteins so that they no longer interfere with activation by the primary activator (FIG. 5D). The best-studied examples of this are the *E. coli nir* and *nrf* promoters, which are co-dependent on FNR — a regulator that is activated by anaerobiosis — and either NarL or NarP — regulators that are activated by nitrite or nitrate ions[62,77]. Both promoters can be fully activated by the primary activator FNR, which interacts with RNA polymerase by a Class II activation mechanism. However, FNR-dependent activation is suppressed by the binding of IHF and Fis to flanking sites. Binding of the secondary activator, NarL or NarP, is needed to counteract the effects of IHF and Fis. Recent studies have shown that NarL or NarP function by displacing IHF from its primary binding site[77].

Conclusions

Bacteria dedicate enormous effort to regulating transcription initiation. So, in a sense, although all genes are equal, some are more equal than others! We surmise that the urge to control RNA synthesis has not been driven simply by bacterial economy, but rather, is the result of millions of rounds of evolution that have been driven by survival. We can but wonder at the solutions that have arisen, which are all simple, but very diverse. The general rule seems to be that there are no general rules! Although it is easy to be seduced by the facile schemes we proffer, they do come with a health warning. The warning is needed because of the vast areas of ignorance that still remain, genomics notwithstanding. We know nothing about the functions of at least 150 putative *E. coli* transcription factors, and many of them cannot even be classified[35]. The actual arrangement of the DNA in the cell is unknown, and it is only recently that we have begun to understand how the rules of gene expression change when the bacterium is outside of the laboratory shake flask[78,79]. However, with the arrival of genomic technologies, and of ever more sophisticated physical biochemistry, we now have the tools to find the answers…and the surprises.

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Competing interests statement

The authors delare that they have no competing financial interests.

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