Local and global regulation of transcription initiation in bacteria

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Abstract | Gene expression in bacteria relies on promoter recognition by the DNA-dependent RNA polymerase and subsequent transcription initiation. Bacterial cells are able to tune their transcriptional programmes to changing environments, through numerous mechanisms that regulate the activity of RNA polymerase, or change the set of promoters to which the RNA polymerase can bind. In this Review, we outline our current understanding of the different factors that direct the regulation of transcription initiation in bacteria, whether by interacting with promoters, with RNA polymerase or with both, and we discuss the diverse molecular mechanisms that are used by these factors to regulate gene expression.

RNA polymerase core enzyme
The form of bacterial DNA-dependent RNA polymerase that lacks a sigma factor.

Template strand
The strand of the DNA duplex that acts as a template for RNA synthesis.

Open complex
The complex between RNA polymerase and a promoter after DNA duplex unwinding has occurred and the RNA polymerase is ready to start transcription.

Housekeeping sigma factor
The sigma factor in a bacterium that is responsible for the recognition of promoters that control the transcription of most genes.

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In any bacterium, at any moment, expression levels are extremely variable between individual genes. Furthermore, the expression of many genes is subject to variation in response to environmental change. Such regulation of gene expression is crucial for the overall fitness of the bacterial cell. For most genes in most bacteria, the key regulatory step that modulates gene expression seems to be promoter recognition and transcription initiation by RNA polymerase[1]. RNA polymerase core enzyme consists of the large β-subunit and β′-subunit, two α-subunits and the small ω-subunit (FIG. 1a). Each α-subunit consists of independently folded amino-terminal and carboxy-terminal domains that are joined by a flexible linker. The β-subunit and β′-subunit are assembled by binding to the N-terminal domains of the α-subunits, and form a cleft that contains the active site, whereas the ω-subunit is primarily a chaperone for the β′-subunit. However, although competent for DNA-dependent RNA synthesis, the core enzyme is unable to recognize promoters or to initiate transcription without the assistance of one of a set of additional proteins known as sigma factors. The complex that is formed by the binding of a sigma factor to the core enzyme is known as RNA polymerase holoenzyme and is able to orchestrate transcription initiation from specific promoters[2]. Interactions with the promoter position the RNA polymerase holoenzyme such that it unwinds the double-stranded DNA in the region of the transcription start site. Positions +1 and +2 of the unwound template strand (corresponding to the 5′ end of the RNA transcript) are then able to enter the active site to form the transcriptionally competent open complex[3–7]. Subsequent to initiation, the transcription cycle proceeds with escape, elongation and termination steps (FIG. 1a), which have been extensively documented and reviewed elsewhere (see REFS 8–11).

Bacterial promoters contain several discrete sequence motifs, including the –35 element, the extended –10 element, the –10 element and the discriminator region, which are recognized by the sigma factor, and the UP element, which is recognized by the C-terminal domains of the α-subunits[1–3] (BOX 1; FIG. 1b). All bacteria contain one predominant essential sigma factor, known as the housekeeping sigma factor (such as σ^{70} in *Escherichia coli*; also known as RpoD), which is responsible for recognizing most promoters[2]. These housekeeping sigma factors are composed of four structural domains that are connected to one another by flexible linkers. In the RNA polymerase holoenzyme, the sigma factor binds to the subunits of the core enzyme, such that each domain of the sigma factor is positioned to interact with a specific promoter element[2,3]. Contacts involving domain 3 and domain 4 of the sigma factor seem to have the major role in the initial positioning of the RNA polymerase, whereas domain 1 and domain 2 of the sigma factor drive the formation of the open complex[2–7,12] (FIG. 1a). A further function of housekeeping sigma factors, mediated by domain 1, is to ensure that DNA cannot enter the active site until the RNA polymerase is bound to a promoter, which triggers a conformational change that permits DNA access to the active site[13–15].

Transcription initiation can be regulated at the level of the formation of RNA polymerase holoenzyme, promoter recognition by RNA polymerase or RNA polymerase activity, all of which can be mediated by various factors. These regulatory mechanisms can either modulate the function of RNA polymerase itself, or can modulate the accessibility or affinity of promoters for RNA polymerase. In this Review, we discuss the different layers of regulation for each of these targets, focusing on the factors and mechanisms on which they rely.

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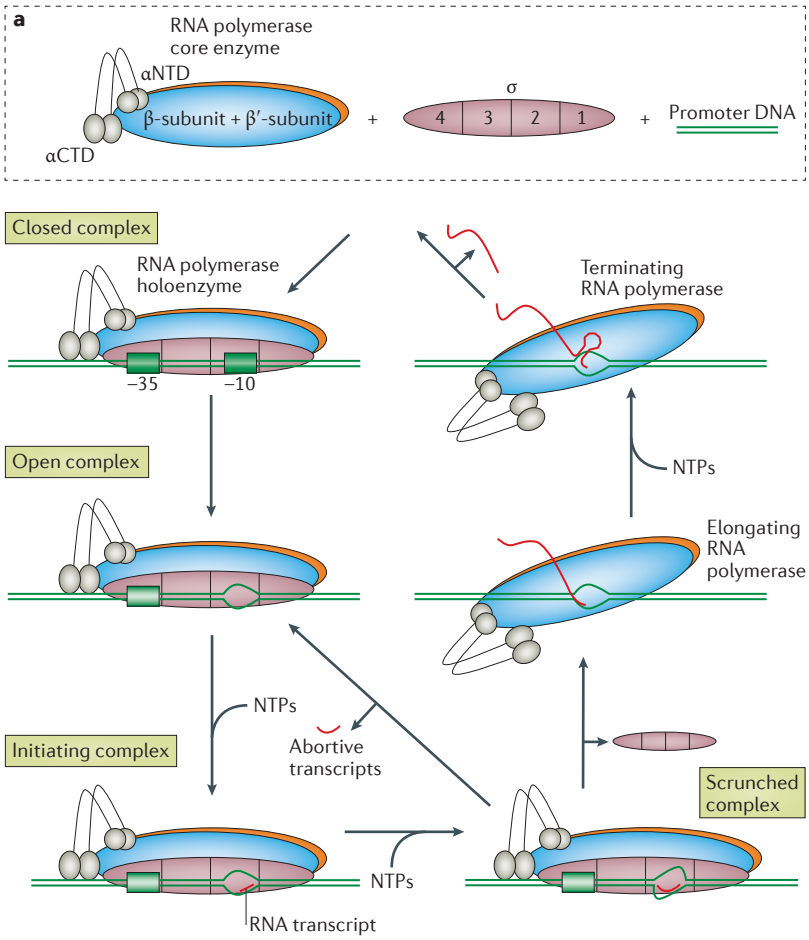

Figure 1 | **Transcription of bacterial genes. a** | The bacterial transcription cycle. RNA polymerase holoenzyme, which comprises the RNA polymerase core enzyme and a σ factor, interacts with promoter DNA to form the closed complex. The closed complex transitions to the open complex by unwinding the DNA duplex in the region of the transcription start site. The addition of nucleoside triphosphates (NTPs) enables a further transition to the initiating complex. Initially, the template strand of the DNA is scrunched in the open complex, which is a process known as 'scrunching'. The scrunched complex can result in transcription at the promoter, which results in cycles of abortive initiation and the release of short RNA fragments. Alternatively, the RNA polymerase can escape from the promoter during the elongation phase, leading to the release of the sigma factor and the formation of the RNA transcript using NTPs and elongation factors (not shown). Elongation proceeds until the RNA polymerase encounters a transcription termination signal, at which point the RNA transcript is released and the polymerase dissociates from the DNA. The polymerase can re-engage with a sigma factor and repeat the cycle. **b** | The promoter sequences that are recognized by RNA polymerase at bacterial promoters. The positions are numbered relative to positions −37 to −58, if the transcriptional start site is denoted +1), the −35 element (positions −35 to −30), the extended −10 element (positions −17 to −14), the −10 element (positions −12 to −7) and the discriminator element (positions −6 to −4). Note that the exact positions of each element can vary for each particular promoter. The regions of the sigma factor (domains 1.1, 2, 3 and 4) and the carboxy-terminal domain of the α-subunit of RNA polymerase (αCTD) that interact with the promoter elements are shown. The image in part **b** is adapted from REF. 161, Nature Publishing Group. αNTD, amino-terminal domain of the α-subunit of RNA polymerase.

Although we mainly describe findings from *E. coli* and other model bacterial species, we also consider regulation in non-model bacterial species, and we suggest that understanding the diversity of these mechanisms may shed light on the parallel evolution of other strategies for gene regulation.

RNA polymerase-centred regulation

Many factors interact directly with bacterial RNA polymerase to influence its activity at different promoters. These factors include sigma factors and various other proteins and ligands that regulate either the formation of RNA polymerase holoenzyme or its activity or promoter preferences.

Sigma factors. The role of s positioning of RNA polyme ers and then to orchestrate the formation of the open complex (FIG. 1a). Thus, the promoter specificity of RNA polymerase depends on its sigma factor[2]. Although the housekeeping sigma factor recruits RNA polymerase to the majority of promoters, nearly all bacteria have one or more alternative sigma factors that guide the RNA polymerase to different promoters, which provides a simple mechanism for reprogramming transcription to a different set of genes[2,16]. The available e that the housekeeping sigma factor and factors bind to the same site on the sur polymerase core enzyme but that, under the housekeeping sigma factor is mor thus able to outcompete alternative sig However, when the abundance of an a factor increases, it can then compete w the housekeeping sigma factor to repro of RNA polymerase molecules[22].

Alternative sigma factors can be grouped into a small number of classes, according to their domain structure, which is usually simpler than that of housekeeping sigma factors[2] (one class has just two domains, corresponding to domain 2 and domain 4 of housekeeping sigma factors[23]). Another difference between alternative sigma factors and housekeeping sigma factors is that most alternative sigma factors bind to a smaller set of promoters, have more stringent sequence specificities and associate with few factors than housekeeping sigma fac results in a narrower range of transcri A possible explanation for these dif many alternative sigma factors have evolved to switch on the concerted expression of sets of genes that have stress-responsive functions, for which fine-tuning is not required (but see REF. 27 for exceptions).

The activity of alternative sigma factors can be controlled by several mechanisms, including covalent modification, sequestration by cognate anti-sigma factors, subcellular localization, the rate of synthesis and proteolytic turnover[16,28]. In some cases, regulation by anti-sigma factors is countered by anti-anti-sigma factors that sequester anti-sigma factors in response to a particular trigger, in a process termed 'partner switching' (REFS 23,28,29). Some anti-anti-sigma factors share

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启动子结构：启动子序列包含-35元件、扩展-10元件、-10元件、鉴别区、UP元件和核心识别元件。

Box 1 | **Recognition of promoters by RNA polymerase**

Optimizing promoter strength

Bacterial promoter sequences contain motifs known as the –35 element, the extended –10 element, the –10 element, the discriminator region, the UP element and the core recognition element. The activity of a promoter is determined by these elements, with stronger promoters often having elements with sequences that better resemble the consensus sequences (FIG. 1b). Bacteria have evolved a 'mix-and-match' approach to setting promoter strength, in which the strengths of individual promoter elements combine to determine the overall activity of the promoter. This modular organization probably has the benefit of enabling promoters to easily evolve to produce different output levels. Most naturally occurring promoters have sequences for each element that are suboptimal for maximum activity[129], although the strength of each element is often not uniform across the promoter, such that different combinations of elements can generate promoters of similar strength[130]. An additional feature that contributes to promoter strength is the initiating nucleotide, which, for example, is important for transcription initiation at rRNA promoters, at which cellular concentrations of initiating nucleotides (either ATP or GTP) influence the formation and stability of the open complex[68,69].

①RNA聚合酶识别的是dsDN，α亚基识别UP元件；σ因子的4结构域识别-35区。-10区发现的都是ssDNA
②核心识别元件由转录起始位点周围的碱基组成，可被RNA聚合酶的β亚基和β'亚基中的残基识别
③RNA聚合酶似乎在-10元件和下游DNA上与dsDNA结合然后DNA被解开产生ssDNA

Double-stranded or single-stranded?

For many promoter elements, recognition by RNA polymerase is specific for double-stranded DNA (dsDNA). For example, the carboxy-terminal domains of the α-subunits recognize the UP element by docking into adjacent minor grooves of the dsDNA[131], the –35 element is recognized specifically as dsDNA by a helix–turn–helix motif in domain 4 of the sigma factor, and the dsDNA of the extended –10 element is bound by a long helix in domain 3 of the sigma factor[2,3]. However, recent structural studies have shown that the –10 element and the discriminator region are both recognized as single-stranded DNA (ssDNA); that is, once the DNA has been unwound during the formation of the transcription bubble[4–7]. To bind to the RNA polymerase, specific bases from the non-template strand of the –10 element (the A and T bases at positions –11 and –7) and the discriminator region (the G at position –6) are flipped out and inserted into pockets in domain 2 and domain 1 of the sigma factor, respectively. The core recognition element, which is formed by bases around the transcription start site, is recognized by residues in the β-subunit and β′-subunit of the RNA polymerase[5–7]. The order in which each element is recognized by RNA polymerase during transcription initiation has been examined using rapid kinetic methods to study the A1 promoter of the T7 phage, which showed that the UP element and the –35 element are bound first, followed by unwinding of the DNA and recognition of the –10 element[132]. Other studies of the *lacUV5* and λP_R promoters showed that RNA polymerase seems to bind to dsDNA at the –10 element and downstream DNA, before the DNA is unwound to produce ssDNA[133,134].

启动子在任何时候都只能被单个RNA聚合酶占据，因此许多启动子上的转录产物形成受到“瓶颈”效应的影响，即开放复合物的形成和转录周期延伸步骤的过渡会被限速

Promoters as 'bottlenecks'

By visualizing fluorescently labelled RNA polymerase in live cells[135] or by measuring transcript levels as a proxy for RNA polymerase activity[136], studies in *Escherichia coli* have shown that a pool of RNA polymerase holoenzyme that scans for promoters seems to be present on bacterial DNA in most conditions. However, as a promoter can only be occupied by a single RNA polymerase at any time, transcript formation at many promoters is subject to a 'bottleneck' effect, in which the formation of the open complex and transition to the elongation step of the transcription cycle become rate-limiting[137–139]. Accordingly, experiments that used chromatin immunoprecipitation (ChIP) to measure promoter occupancy have shown that RNA polymerase is stalled at many promoters, which results in decreased flux of RNA polymerase through specific transcription units[140,141].

使用染色质免疫沉淀(ChIP)测量启动子占用率的实验表明，RNA聚合酶在许多启动子上停滞，导致RNA聚合酶通过特定转录单位的通量下降

structural similarities with the cognate sigma factor, which suggests that they have evolved to be mimics[30,31]. All of these regulatory mechanisms combine to form complex regulatory circuits that adjust the number of RNA polymerase molecules that are programmed by a particular alternative sigma factor according to the environmental conditions.

关于sigma因子交换机制的许多细节仍然不清楚

Despite our knowledge of this circuitry, many details of the mechanisms that mediate sigma factor exchange remain unclear. For example, we do not know how many alternative sigma factors, which have weak binding affinities for the RNA polymerase core enzyme, are able to recruit sufficient RNA polymerase molecules to have a discernible effect on the transcriptional programme[22]. One well-studied case of sigma factor exchange occurs between σ^{38} (also known as RpoS) and the housekeeping sigma factor, σ^{70}, in *E. coli*, in response to certain stresses or at entry into stationary phase[20,21]. Displacement of σ^{70} following an increase in the abundance of σ^{38} is facilitated by Crl[32–34], which interacts with σ^{38}, and by Rsd[35–37], an anti-sigma factor that reduces the level of functional σ^{70}, thereby biasing the competition between the two sigma factors in favour of σ^{38}. In addition, the small guanine nucleotide guanosine tetraphosphate (ppGpp), which undergoes a sharp increase in abundance in response to certain metabolic responses, may have a role in the exchange of some sigma factors[38,39].

举例：大肠杆菌中的σ因子交换（1.σ70的置换是由与σ38相互作用的Crl和降低σ70水平的反σ因子Rsd促进的；2.ppGpp鸟嘌呤核苷酸丰度增加也可发挥作用）

Nearly all alternative sigma factors are evolutionarily related to housekeeping sigma factors, consisting of two, three or four domains that retain common functions in their different contexts[2]. However, most bacteria contain one additional alternative sigma factor that is 'in a class of its own' and belongs to the family of σ^{54} (also known as RpoN) sigma factors, which is evolutionarily unrelated to other sigma factors[40,41]. These sigma factors recognize different elements at target promoters to other sigma factors, with specificity determined by elements at positions –24 and –12, rather than –35 and –10 (REF. 42). Crucially, unlike the complex that is formed by σ^{70}, RNA polymerase ho… is unable to proceed to the … activation by a special class of … see below)[42]. Recent structur… site at which the template str… e formation of the open com… σ^{54} (REF. 19).

大多数细菌都有一个额外的替代σ因子-σ54，它在进化上与其他σ因子无关4。这些σ因子识别目标启动子上的不同元件与其他σ因子，其特异性由-24和-12区的元件决定，而不是-35和-10与由σ70形成的复合物不同，含有σ54的RNA聚合酶全酶不能进入开放复合物，而需要由一种特殊的ATP依赖激活剂激活。

Chromatin immunoprecipitation (ChIP). A method whereby antibodies are used to isolate DNA fragments that have been cross-linked to a specific protein.

Stationary phase
The period when bacteria have stopped growing.

Guanosine tetraphosphate (ppGpp). A small molecule that is synthesized in response to certain stresses. ppGpp is often referred to as 'magic spot',

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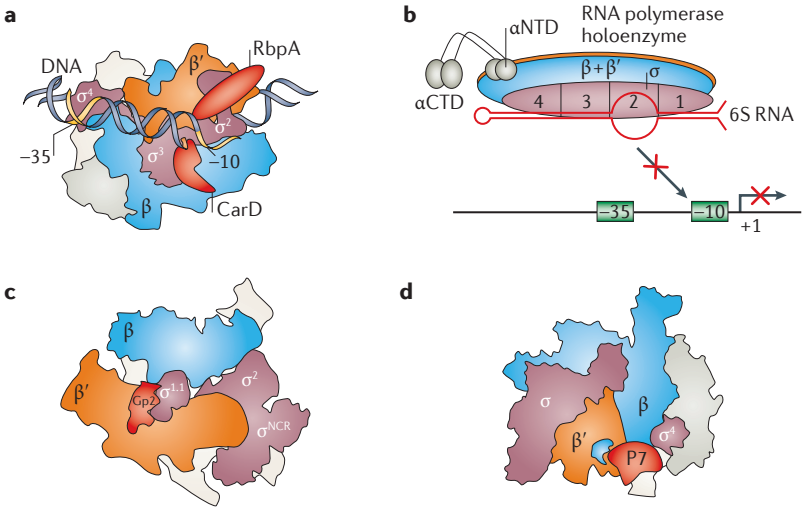

Figure 2 | **Modulation of RNA polymerase activity. a** | Open complex stabilizers. Both RbpA and CarD are global transcription factors that bind directly to RNA polymerase to regulate transcription at promoters. RbpA contacts domain 2 of the housekeeping sigma factor, whereas CarD binds to the β-subunit of RNA polymerase. Both are positioned to stabilize the open complex by interacting with promoter DNA in addition to the RNA polymerase. **b** | Regulation by a promoter mimic. The structure of the 6S RNA mimics that of a promoter in an open complex, which enables the 6S RNA to sequester RNA polymerase holoenzyme that contains the housekeeping sigma factor and thus downregulate global transcription. **c** | Sequestration of a sigma factor domain. On infection of *Escherichia coli* by phage T7, the phage Gp2 protein binds to two regions of the *E. coli* RNA polymerase holoenzyme: the channel that contains the active site and domain 1 of the sigma factor. These interactions inhibit transcription initiation by preventing DNA from entering the active site and by stopping the movement of domain 1 of the sigma factor, respectively. **d** | Sigma factor displacement. On infection of *Xanthomonas oryzae* by phage Xp10, the phage protein P7 binds to the β-subunit and β′-subunit of the bacterial RNA polymerase, which inhibits transcription by displacing domain 4 of the sigma factor from the holoenzyme when the RNA polymerase is associated with promoter DNA. The image in part **a** is adapted with permission from REF. 44, National Academy of Sciences. The image in part **c** is a adapted with permission from REF. 14, National Academy of Sciences. The image in part **d** is adapted from Liu, B. *et al.* A bacteriophage transcription regulator inhibits bacterial transcription initiation by sigma-factor displacement. *Nucleic Acids Res.* (2014), **42**, 7, 4294–4305, by permission of Oxford University Press. αCTD, carboxy-terminal domain of the α-subunit of RNA polymerase. σNCR, sigma factor non-conserved region. αNTD, amino-terminal domain of the α-subunit of RNA polymerase.

Interestingly, different activators are required at different target promoters and, at least in *E. coli*, levels of σ^{54} seem to be unregulated[21]. Together, these features of σ^{54} suggest that, in keeping with being in a class of its own, σ^{54} uses a different regulatory strategy to other sigma factors, in that transcription initiation is regulated by the presence of an activator rather than the abundance of the sigma factor.

Regulators of holoenzyme activity. The simplicity and ubiquity of regulation by sigma factors have detracted attention from numerous other regulatory factors that interact with RNA polymerase (FIG. 2). Although many of these regulatory factors act at the level of transcript elongation and termination (such as N-utilization substance (Nus) factors)[9], and are, as such, beyond the scope of this Review article, a small number are, similarly to sigma factors, regulators of transcription initiation. Some of these regulator
to particular groups of bacteria, suc
CarD, which bind to and stabilize op
Actinomycetes[43] (FIG. 2a). Recent studies
RbpA binds directly to domain 2 of the housekeeping sigma factor and is positioned to make direct stabilizing contacts with the upstream end of unwound DNA in open complexes[44]. CarD has a C-terminal domain that makes stabilizing contacts that are complementary to those made by RbpA, which suggests that RbpA and CarD function coordinately, whereas the N-terminal domain of CarD interacts with the RNA polymerase β-subunit[45,46].

Although *E. coli* and related members of the Enterobacteriaceae lack RbpA and CarD, these bacteria use other factors to modulate the stability of open complexes. For example, the extended coiled-coil protein motif in DksA inserts into a narrow cha
the secondary channel) that leads from t
RNA polymerase to the active site[47–50].
DksA, in cooperation with ppGpp, selec
or destabilizes open RNA polymerase–
plexes, depending on the promoter, w
a sigma factor-free mechanism to reprogramme the transcriptome in response to increased levels of ppGpp, which can result from certain metabolic stresses[51,52]. The mechanism of regulation by DksA awaits elucidation but probably involves a lowering of the energy barrier between the RNA polymerase–promoter closed complex and open complex[47–52]. The structure of DksA and its mode of binding to the polymerase resemble those of the GreA and GreB transcription elongation factors[47,53], and recent studies suggest that DksA, GreA and GreB all function as 'inspectors' that continually probe the enzyme active site by making rapid transient 'visits' to the secondary channel[54–56].

Other factors decrease the number of RNA polymerase molecules that are available for transcription by sequestering the holoenzyme. One example is 6S RNA, an approximately 180-nucleotide non-coding RNA that is synthesized in response to slow growth and forms a 1/1 complex with the RNA polymerase holoenzyme[57] (FIG. 2b). In *E. coli*, 6S RNA is a mimetic for the DNA of promoters that are targets for the housekeeping RNA polymerase holoenzyme (that is, holoenzyme that contains σ^{70}). Consequently, th
functional (that is, non-sequestered) R
holoenzymes that contain σ^{38} increase
transcription potential of the cell is redu
sequesters the housekeeping RNA po
enzyme[57]. 6S RNA seems to work coope
Rsd anti-sigma factor (see above) to cou
sion to growth, as the levels of both fac
cell growth slows[37].

Finally, some phages have factors that inhibit the activity of bacterial RNA polymerases to favour the activity of their own bespoke RNA polymerases. For example, during infection of *E. coli* cells, the Gp2 protein of phage T7 induces a conformational change in a part of domain 1 of σ^{70} that blocks the access of template DNA to the active site[14] (FIG. 2c). By contrast, the P7 protein

Actinomycetes
A class of soil bacteria with a

Coiled-coil
An extended motif found in proteins.

Closed complex
The complex between RNA polymerase and a promoter before DNA duplex unwinding has occurred.

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一些噬菌体具有抑制细菌RNA聚合酶活性的因子；在感染大肠杆菌细胞时，T7噬菌体的Gp2蛋白在σ70的1域的一部分引起构象改变，从而阻止模板DNA进入活性位点(图2c)以抑制转录过程

of the related phage Xp10, which infects *Xanthomonas oryzae*, inhibits the activity of RNA polymerase by displacing the sigma factor from the holoenzyme; the association of P7 with RNA polymerase is stable, which prevents the recruitment of a replacement sigma factor to reform the holoenzyme[58] (FIG. 2d).

调节RNA聚合酶全酶的其他机制

Other factors that regulate RNA polymerase holoenzyme. Some regulators of RNA polymerase holoenzyme, known as 'appropriators', remodel parts of the polymerase to alter promoter preferences (FIG. 3), and thus have a similar function to alternative sigma factors. The most well-studied example of a phage appropriator is the AsiA protein of phage T4, which infects *E. coli*. As phage T4 does not encode its own RNA polymerase, its invasion strategy is to appropriate, rather than to silence, the host RNA polymerase. AsiA is encoded by a gene that is expressed during the early stage of the T4 life cycle, and remodels and repositions domain 4 of σ^{70} so that it is unable to recognize –35 elements[59,60] and instead becomes a target for the T4 MotA transcription activator. MotA, which is encoded by early-stage genes, is the essential activator for transcription of genes that are expressed during the middle stage of the T4 life cycle[61] (FIG. 3a). Thus, AsiA and MotA redirect the housekeeping RNA polymerase holoenzyme from the transcription of host genes to the transcription of phage genes. Interestingly, the T4 proteins Alt and ModA also alter the promoter preferences of the host RNA polymerase, although the mechanism by which they do so is to ADP-ribosylate residue R265 in the C terminus of either one or both RNA polymerase α-subunits. This modification prevents the RNA polymerase from recognizing UP elements and so helps divert the polymerase from transcribing host genes to transcribing T4 genes[61] (FIG. 3b).

蛋白调控

重塑聚合酶的部分构象以改变启动子的偏好，具有与替代σ因子相似的功能；研究最充分的是感染大肠杆菌的T4噬菌体的AsiA蛋白、MotA蛋白和Alt蛋白；
①AsiA和MotA蛋白：AsiA蛋白是由T4噬菌体生命周期早期基因编码，能对σ70的4域进行重构，使其不能识别启动子的-35区，而成为T4 MotA转录激活子的靶点，MotA由早期基因编码，是T4生命周期中期基因转录的必要激活因子。在感染过程中，T4噬菌体的AsiA蛋白重塑了大肠杆菌管家σ因子的4域结构。重组的σ因子与另一种噬菌体蛋白MotA直接相互作用，MotA是一种转录激活子，与T4感染周期中期基因的启动子结合。重组的σ因子和MotA之间的相互作用引导RNA聚合酶来激活这些基因的转录。即将管家RNA聚合酶全酶从对宿主基因的转录转向对噬菌体基因的转录
②AMotA蛋白ModA蛋白：机制是修饰NA聚合酶α亚基的一个或两个C末端的ADP-核糖基残基R265。这种修饰阻止RNA聚合酶识别UP元件，从而帮助聚合酶从转录宿主基因转移到转录T4基因

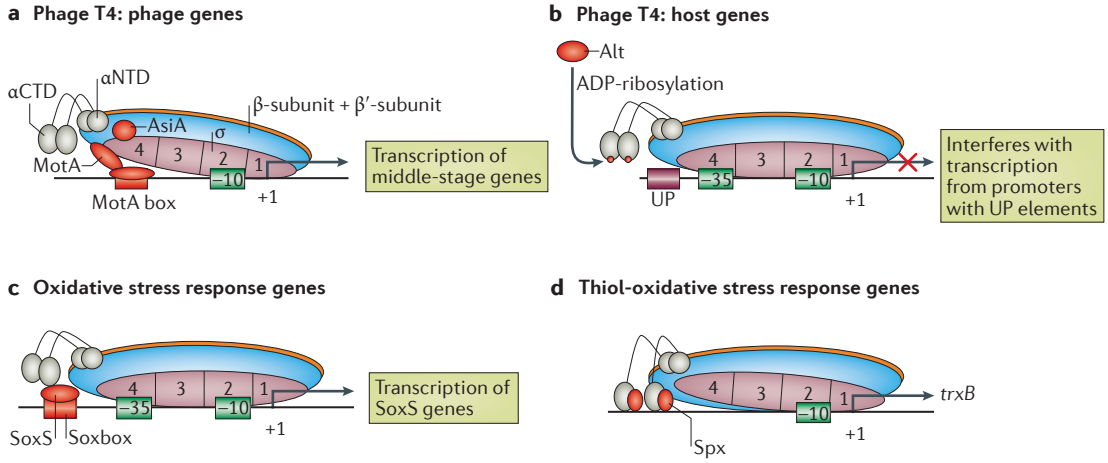

Figure 3 | **Appropriation of RNA polymerase for specific transcriptional programmes. a** | During infection, the AsiA protein of phage T4 remodels the structure of domain 4 of the *Escherichia coli* housekeeping sigma factor. The remodelled sigma factor interacts directly with another phage protein, MotA, which is a transcription activator that binds to promoters of middle-stage genes of the T4 infection cycle. The interaction between the remodelled sigma factor and MotA recruits RNA polymerase to activate the transcription of these genes. **b** | Early during infection, the phage T4 protein Alt ADP-ribosylates amino acid residue R265 on the carboxy-terminal domains of the α-subunits of *E. coli* RNA polymerase (αCTD). This modification modulates the recognition of UP element sequences in bacterial promoters, thereby increasing the availability of RNA polymerase for transcription of phage genes. **c** | In *E. coli*, SoxS is expressed in response to oxidative stress. By binding to the C-terminal domains of the α-subunits of RNA polymerase, SoxS directs RNA polymerase to promoters that contain 'Sox-box' SoxS-recognition sites in upstream regions, which leads to the expression of genes that are controlled by these promoters. **d** | In *Bacillus subtilis*, the Spx protein is activated in response to thiol-oxidative stress. Activated Spx is able to associate with the C-terminal domains of the α-subunits of RNA polymerase, which modulates the binding preferences of RNA polymerase such that the expression of genes such as *trxB* is induced to combat thiol-oxidative stress. αNTD, the amino-terminal domains of the α-subunits of RNA polymerase.

③大肠杆菌中的SoxS：SoxS在氧化应激反应中表达，SoxS通过与RNA聚合酶α亚基的c端结构域结合将RNA聚合酶全酶靶向到具有上游Sox-box序列的启动子上，也就是说，在SoxS - RNA聚合酶全酶复合物与DNA结合之前，SoxS接触每个RNA聚合酶α亚基C端结构域的DNA识别位点。SoxS-RNA聚合酶全酶复合物不能识别UP元件，但可以识别Sox-boxes，从而导致由这些启动子控制的基因表达。
④枯草芽孢杆菌中的Spx：Spx蛋白在硫醇氧化应激反应中被激活。激活的Spx能够与RNA聚合酶α亚基的C端结构域结合，从而调节RNA聚合酶的结合偏好，从而诱导trxB等基因的表达，以对抗硫醇氧化应激。

α NTD: RNA聚合酶α亚基的氨基末端结构域。

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Michaelis constant
The concentration of a substrate at which the reaction catalysed by an enzyme proceeds at half of its

除了蛋白质调控外，RNA聚合酶全酶的活性还可以通过其四种核苷三磷酸底物水平的波动来调控：
①大肠杆菌中，rRNA转录起始的NTP是ATP，rRNA启动子的转录起始对细胞内ATP浓度敏感，随着ATP浓度增加细胞内大量合成rRNA合成。
②枯草芽孢杆菌中，rRNA转录起始的NTP是GTP，当它被ppGpp的合成所消耗时，其数量会减少，因此，枯草芽孢杆菌中ppGpp水平通过相应的GTP水平变化，间接将代谢与RNA聚合酶活性结合起来

rms after a
he is
compacted inside a bacterium.

Superhelical density
The measure of the degree to which the winding of one DNA strand around the other differs from the periodicity of the Watson–Crick structure.

Finally, in addition to regulation by proteins, the activity of RNA polymerase holoenzyme can be regulated by fluctuations in the levels of its four nucleoside triphosphate (NTP) substrates. Note that the Michaelis constant for the initiating nucleotide is higher than that for subsequently added NTPs[67]; that is, for a given rate of transcription, the required concentration of the initiating NTP is higher than that of subsequent NTPs, which means that the concentration of the initiating NTP is most crucial to the activity of the RNA polymerase. As the initiating NTP for rRNA transcripts in *E. coli* is ATP, transcription initiation at rRNA promoters is expected to be sensitive to the cellular concentration of ATP, which increases as cells leave stationary phase with a concomitant burst of rRNA synthesis[68,69]. In *B. subtilis*, the initiating NTP for rRNA is GTP, which decreases in abundance when it is consumed by the synthesis of ppGpp. Therefore, ppGpp levels in *B. subtilis* indirectly couple metabolism to RNA polymerase activity through corresponding changes to GTP levels[70,71]. This contrasts with *E. coli*, in which the cooperative action of ppGpp and DksA directly regulates the stability of RNA polymerase holoenzyme open complexes[51] (see above).

More complex effects of NTP levels are observed during transcription initiation at *E. coli* promoters that control genes that are involved in pyrimidine biosynthesis. At many of these promoters, fluctuations in the levels of UTP or CTP affect transcript stability and translation efficiency. This can occur either by changing the location of the transcript start site or by non-templated insertion of extra bases into the nascent transcript[72]. As UTP and CTP are both pyrimidine-based nucleotides, this form of regulation produces a feedback mechanism in which pyrimidine biosynthesis can be adjusted in response to changes in the levels of these nucleotides[72].

以启动子为中心的调控

Promoter-centred regulation

Regulation by factors that bind directly to the RNA polymerase is complemented by factors that directly target the promoter DNA (FIG. 4). Such regulation can be mediated by many different mechanisms, which range from supercoiling to transcription factors that simply block access to the promoter. In this scenario, first imagined by François Jacob and collaborators (BOX 2), transcription factors have structural motifs that bind to promoters that contain cognate 'operators', which are specific sequences of base pairs. The operators for most bacterial DNA-binding structural motifs, such as the helix–turn–helix motif, have 4–5 base pairs. As any given 5-base pair sequence will arise, on average, once every 4^5 base pairs, further specificity is required to target transcription factors only to the desired promoters. Such specificity can be achieved using various mechanisms, including homodimerization (or higher order multimerization) of the transcription factor, association of the transcription factor with another DNA-binding factor, or the incorporation of several DNA-binding structural motifs into a single transcription factor. Of these mechanisms, dimerization or further multimerization is the most common, and therefore most operators contain direct or inverted repeats of a 4–5 base pair sequence.

直接与RNA聚合酶结合的调控因子与直接以启动子DNA为靶点的调控因子相辅相成，这种调控可以通过许多不同的机制介导。

（不用做材料）
转录因子具有结构基因，结合到包含同源“操作符”的启动子上，这是特定的碱基对序列。大多数细菌DNA结合结构基因的操作符，如螺旋-旋-螺旋基序，有4-5个碱基对。由于任何给定的5个碱基对序列平均每45个碱基对出现一次，因此需要进一步的特异性，使转录因子只针对所需的启动子。这种特异性可以通过多种机制实现，包括转录因子的同源二聚(或更高阶多聚)，转录因子与另一个DNA结合因子的结合，或将几个DNA结合结构基因合并到一个转录因子中。在这些机制中，二聚或进一步多聚是最常见的，因此大多数操作符包含4-5碱基对序列的直接重复或反向重复。

The key role of transcription factors is to couple promoter activity to environmental cues, so their activity has to be regulated in response to these cues. In many transcription factors, regulatory modules mediate DNA binding in response to the cues
by the binding of a small ligand or prot
modification. The activities of other tr
tors depend on abundance and availab
be regulated by synthesis, turnover o
Although some transcription factors re
gle promoter, most transcription factor
promoters. In addition, at least in *E. col*
ers are regulated by more than one facto
many transcription factors are expresse
ers that are themselves regulated by oth
factors. Promoter regulation by trans
therefore generates a complex regulat
which the concerted activities of spec
master regulators orchestrate the distr
polymerase to the various transcriptio
present in the genome[73–76].

（不用做材料）
在许多转录因子中，调节模块介导DNA结合，以响应小配体或蛋白质的结合或共价修饰所感知的信号。其他转录因子的活性取决于丰度和可用性，可通过合成、周转或隔离来调节。虽然有些转录因子只调控单个启动子，但大多数转录因子调控多个启动子。此外，至少在大肠杆菌中，大多数启动子受不止一个因子的调控。此外，许多转录因子是由启动子表达的，启动子本身受其他转录因子的调控。因此，转录因子对启动子的调控产生了一个复杂的调控网络。

转录起始发生在细菌的类核环境中，而在类核形成过程中发生的细菌染色体的压缩被认为对启动子的活性有负面影响。因此，通过调节类核的局部结构来改变压缩程度可能导致转录的改变。压缩主要是由于超螺旋和扭曲，这是由核苷酸相关蛋白(NAP)诱导的。如反转刺激因子(Fis)和整合宿主因子(IHF)，显然具有序列特异性，这使得它们能够介导启动子对局部DNA结构的特异性作用，因此可以认为它们是转录因子

反转刺激因子(Fis)抑制了控制主要固定相DNA结合蛋白Dps表达的启动子的活性，使启动子在染色体压缩状态下活跃，而在染色体松弛快速生长的细胞中不活跃。超螺旋结构的变化，可能是由ATP水平的波动介导的，导致许多启动子的上调和下调;在某些情况下，这会导致局部超螺旋密度的改变，从而调节特定靶启动子的转录活性

It is important to appreciate that tra
ation occurs in the context of the bacte
that the compaction of the bacterial ch
occurs during the formation of the nuc
to have an overall negative effect on p
ity. Therefore, modulation of the local structure of the nucleoid to change the level of compaction may result in changes in transcription. Compaction is primarily due to supercoiling and to the contortions that are induced in the DNA by nucleoid-associated proteins (NAPs), which 'sculpt' the bacterial chromosome by bending, wrapping, looping and twisting DNA such that it fits inside the bacterial cell[77] (BOX 3). Many NAPs are abundant and bind to numerous target sites using a relaxed sequence specificity[78–80]. By contrast, some NAPs, such as factor for inversion stimulation (Fis) and integration host factor (IHF), clearly do have sequence specificity, which enables them to mediate promoter-specific effects on the local DNA structure, such that they can be regarded as transcription factors[81]. For example, Fis represses the activity of the promoter that controls the expression of the major stationary phase DNA-binding protein, Dps, so that the promoter is active in stationary phase but inactive in stress-free rapidly growing cells[82]. In parallel, global alterations in supercoiling, which are probably mediated by fluctuations in the levels of ATP, lead to upregulation and downregulation at scores of promoters; in some cases, this results in local superhelical density changes that modulate the transcription activity at specific target promoters[83–88].

Recent observations that the activities of certain promoters vary according to their precise location in the folded bacterial chromosome may be best explained by the suggestion that the availability of free RNA polymerase holoenzyme, or at least the degree to which the enzyme can access promoter regions, is not the same at every location[89]. Thus, the long-standing assumption that promoter activity in bacteria is determined only

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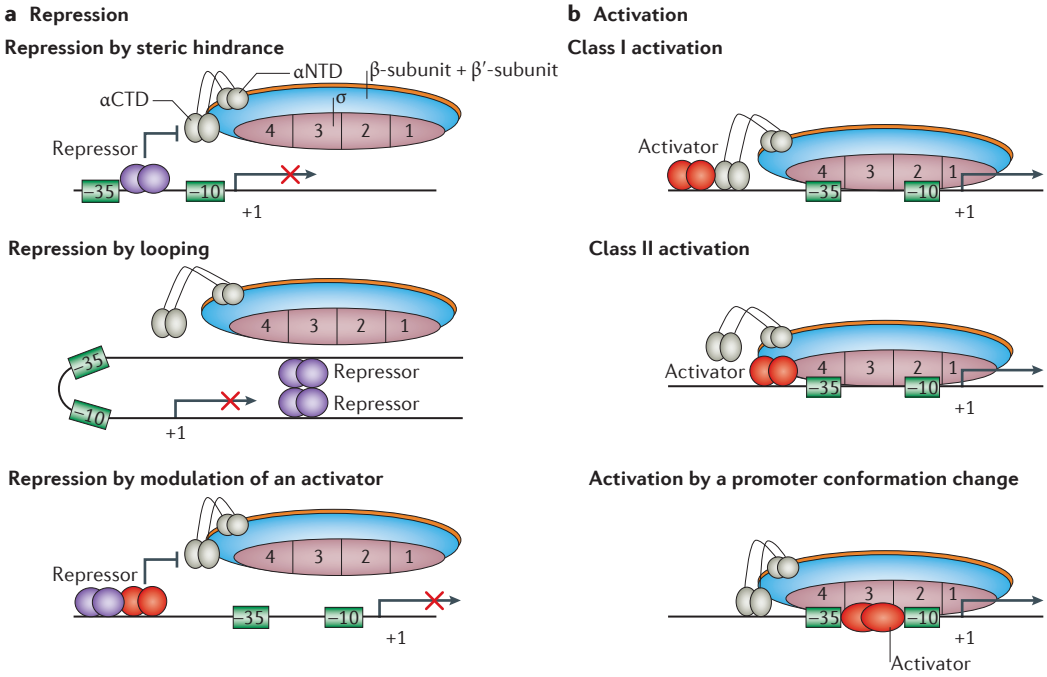

转录因子对启动子的抑制或激活

Figure 4 | **Repression or activation at promoters by transcription factors.** Transcription factors known as repressors and activators use one of several mechanisms to repress and activate transcription initiation, respectively. The principal mechanisms are shown here, using the example of the RNA polymerase holoenzyme that contains σ^{70} in *Escherichia coli*. **a** | In repression by steric hindrance, the repressor binds to a site that overlaps the core elements of the promoter that is recognized by RNA polymerase and thus blocks the binding of RNA polymerase to the promoter. In repression by looping, protein–protein interactions are formed between repressors that bind to sites upstream and downstream of the promoter, inducing looping of the DNA between the two repressor binding sites. DNA looping prevents the recognition of promoter elements by RNA polymerase, and thus transcription is repressed. For those genes that require activators for transcription, repressors can modulate activators to prevent recruitment of RNA polymerase. Although these repressors indirectly modulate RNA polymerase activity by targeting activators, in contrast to repressors that directly modulate RNA polymerase activity by masking promoter elements, the ultimate effect remains to prevent recognition of the promoter and thus repress transcription. **b** | In class I activation, the activator binds to a site upstream of the promoter and recruits RNA polymerase to the promoter by contacting the carboxy-terminal domain of the α-subunit (αCTD). In class II activation, the activator binds to a site in the promoter adjacent to (or overlapping with) the −35 element, where it recruits RNA polymerase through direct interactions with domain 4 of the sigma factor. Rather than directly recruit RNA polymerase, some activators induce a conformational change in the promoter DNA to activate transcription. These activators bind at, or near to, the core RNA polymerase recognition elements of the promoter and often realign the −10 and −35 elements so that they can be recognized by RNA polymerase, thereby enabling the recruitment of RNA polymerase to the promoter and activation of transcription. αNTD, the amino-terminal domains of the α-subunits of RNA polymerase.

这里以大肠杆菌中含有σ70的RNA聚合酶全酶为例，展示了其主要机制

①在链状DNA转录抑制中，抑制因子与RNA聚合酶识别的启动子的核心元件结合从而阻止RNA聚合酶与启动子的结合。

②在环状DNA转录抑制中，抑制因子与启动子的上游和下游结合，诱导DNA在两个抑制因子结合位点之间形成环状。DNA环化阻止了RNA聚合酶对动子元件的识别，从而抑制了转录

③对于那些需要激活因子才能转录的基因，抑制因子可以通过调节激活因子来抑制RNA聚合酶的引导。虽然这些阻遏物通过靶向激活物间接调节RNA聚合酶活性，但与通过沉默启动子元件，直接调节RNA聚合酶活性的阻遏物不同，其最终效果仍然是阻止启动子的识别从而抑制转录。

①在I类激活中，激活子与启动子上游的一个位点结合，通过与α-亚基的羧基端结构域(α CTD)接触，将RN聚合酶引导到启动子上。

②在II类激活中，激活因子结合到启动子中与-35元件相邻(或重叠)的一个位点，在此通过与σ因子的4域直接相互作用引导RNA聚合酶。

③有些激活剂不是直接引导RNA聚合酶到启动子上的，而是诱导启动子DNA的构象改变来激活转录。这些激活子结合在启动子的核心RNA聚合酶识别元件处或附近，并对-10和-35元件进行重组，使其能够被RNA聚合酶识别，从而使RNA聚合酶能够被引导到启动子上并激活转录。

α NTD: RNA聚合酶α亚基的氨基末端结构域。

by local transcription factors and by how sequences of promoter elements correspond to consensus sequences (BOX 1) is an oversimplification.

抑制转录因子，称为阻遏子，其抑制转录起始的机制（如上所述）举例子-乳糖操纵子

Repressive transcription factors. Several mechanisms have been described for the repression of transcription initiation by repressive transcription factors (known as 'repressors'). At many promoters, repression of transcription initiation occurs simply by steric hindrance, in which repressors bind to operators that overlap the −10 or −35 elements of the promoter and thus block access of RNA polymerase to the promoter[1] (FIG. 4a). Many promoters contain arrays of operators that are organized adjacently to each other. For these promoters, a larger number of bound repressors usually increases the strength of repression. At other promoters, the simultaneous binding of a repressor to operators that are located distally to one another gives rise to a loop in the local DNA structure that prevents binding by ase[90] (FIG. 4a). The best-documented case DNA looping is mediated by the GalR galactose operon in *E. coli*. The operat ognized by GalR are located upstream a of the different promoter elements of the do not overlap with these elements[90,91]. S can be bound (usually independently) different repressors, which can lead to v sion, such as at promoters that contro of colicin[92,93].

（不用做材料）

在许多启动子中，转录起始的抑制仅仅通过链状抑制发生，抑制子与启动子的-10或-35元素重叠的操作子结合

从而阻断RNA聚合酶对启动子的识别与结合(图4a)。许多启动子包含相互邻接的操作符数组。对于这些启动子，大量的结合抑制子通常会增加抑制的强度。在其他启动子上，阻遏因子与彼此相距较远的操作子同时结合

在局部DNA结构中形成环状结构，阻止RNA聚合酶的结合(图4a)。在大肠杆菌中，DNA环转录抑制是由半乳糖操纵子的GalR抑制子介导的。被GalR识别的操作子位于gal操纵子的不同启动子元件的上游和下游，但不与这些元件重叠。

At some promoters, repression is more complicated and is mediated by 'anti-activator' repressors (FIG. 4a), which counter the function of activating transcription factors ('activators'; see below). For example, the CytR repressor is as an anti-activator that simultaneously interacts with its operator and an adjacent activator, the cyclic AMP (cAMP) receptor protein (CRP; also known as

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Box 2 | **50 years of studying gene regulatory mechanisms**

In 1965, François Jacob, André Lwoff and Jacques Monod received a Nobel Prize for 'discoveries concerning the genetic regulation of enzyme and virus syntheses'. Together, they had discovered the mechanism by which λ phages that infect *Escherichia coli* are silenced as lysogens until exposure to UV light, and the mechanism by which β-galactosidase activity is 'silenced' until *E. coli* is grown in a medium containing lactose. In both cases, the mechanism centred on a transcriptional repressor. As the fundamentals of molecular biology had only just been established, the regulation of transcription had not been considered as a possible mechanism when the work was started. Indeed, the idea that the solution was a transcriptional repressor was resisted by Monod[142] until he was persuaded by the clarity of genetic experiments[143]. The discovery that these functions are regulated at the level of transcription led to the development of a research area that covered promoters, transcription start sites and operators, together with all of the protein factors and other components that mediate the induction and repression of gene expression. From these studies emerged the concept that what matters is not the genes one has, but how one expresses them, and it is no exaggeration to state that all work in this area has its origins in the discovery of transcriptional repressors. However, although his work represented fundamental contributions to biological research, two shortcomings are notable in the writings of Monod. First, he assumed that all regulation would be explained by the activity of repressors and, unlike Jacob, was never persuaded that transcriptional regulation might also rely on activators[144]. Second, the notion popularized by Monod that 'anything found to be true of *E. coli* must also be true of elephants' gave undue prominence to *E. coli* and its *lac* operon as a model for molecular biology, the limitations of which were appreciated by Jacob[121] and, more recently, articulated by Victor de Lorenzo as the black cat–white cat principle[145].

catabolite activator protein (CAP)). At some promoters, CytR binding requires a combination of CytR–CRP and CytR–DNA interactions to prevent the binding of RNA polymerase[94]. Another complex mechanism of repression can occur when the repressor interacts directly with bound RNA polymerase. For example, the p4 protein encoded by phage φ29, which infects *B. subtilis*, simultaneously binds to the C-terminal domain of the α-subunit of RNA polymerase and to the DNA upstream of the polymerase, thereby preventing promoter clearance[95].

激活转录因子(称为“激活因子”)通过以下三种机制之一，通常从较低的基础水平增加启动子的活性；:I类激活、II类激活或构象改变激活

Activating transcription factors. Activating transcription factors (known as 'activators') increase the activity of promoters, often from low basal levels, by one of three mechanisms: class I activation, class II activation or activation by a conformational change[96] (FIG. 4b). In class I activation, the activator binds to an operator that is located upstream of the promoter elements and then recruits RNA polymerase to the promoter through interactions formed between a small surface-exposed patch on the activator (known as an activating region) and the C-terminal domain of the RNA polymerase α-subunit[96]. Structural analysis of activation by CRP shows that this interaction occurs without any structural change in either partner, and, as such, is often referred to as 'velcro' (REF. 97). Furthermore, class I activation occurs at promoters that require activators to recruit RNA polymerase because one or more of the promoter elements has a sequence that is suboptimal for the binding of RNA polymerase. Thus, for those promoters that are dependent on class I activation, RNA polymerase is recruited by protein–protein interactions rather than protein–DNA interactions. Optimal class I activation occurs when the activator binds to the same face of the DNA helix as the RNA polymerase α-subunit[98], which facilitates the interaction between the two proteins such that activation is reduced if the operator is displaced by 1–5 base pairs (corresponding to up to half a turn of the helix) but is restored if the displacement is approximately 10 base pairs (corresponding to a full turn of the helix). In some cases, decreased activation due to suboptimal placement of an activator can be reversed if the bases of one or more of the promoter elements are modified to enhance the binding of RNA polymerase[99].

（不用做材料）

I类激活发生在启动子上，启动子需要激活子来引导RNA聚合酶到启动子上，因为一个或多个启动子元件的序列不适合RNA聚合酶的结合。因此，对于那些依赖于I类激活的启动子，RNA聚合酶是通过蛋白质-蛋白质相互作用而不是蛋白质- DNA相互作用引导的。当激活物与RNA聚合酶 α -亚单位98结合在DNA螺旋的同一面时，最佳的I类激活就会发生，这促进了两个蛋白质之间的相互作用。

在II类激活中，激活子与启动子的-35区重叠的操纵序列结合。一旦与操作分子结合，激活剂中的离散激活区域通过与σ因子的结构域4、 α 亚基的N端结构域或RNA聚合酶的其他组分形成直接相互作用来引导RNA聚合酶到启动子上(图4b)。

第三种机制是单个激活子可以驱动启动子上的转录起始，这涉及到激活子与位于启动子-35和-10元件之间的操作子的结合(图4b)。因此被调控的启动子通常在两个元件之间有非最佳间距，目前的观点是，激活涉及DNA的扭曲，以便启动子的-35和-10元件更好地定位于RNA聚合酶结合。现有的结构证据表明，这种扭曲在-35和-10元素之间不均匀分布。

In class II activation, the activator binds to an operator that overlaps the –35 element of the promoter. Once bound to the operator, discrete activating regions in the activator recruit the RNA polymerase by forming direct interactions with domain 4 of the sigma factor, with the N-terminal domain of the α-subunit or with other components of the RNA polymerase[96] (FIG. 4b). At some promoters, the interactions between the activator and the RNA polymerase are more favourable in the open complex, which facilitates the transition from the closed to the open complex[100–103]. One of the consequences of class II activation is that the C-terminal domains of the RNA polymerase α-subunits are unable to bind to their preferred binding site immediately upstream of the –35 promoter element and instead bind immediately upstream of the activator[96]. This permits class II activators to function together with class I activators, and this combination is responsible for synergy at many bacterial promoters in which activity is co-dependent on two signals[1].

A third mechanism by which a single activator can drive transcription initiation at a promoter involves the binding of the activator to an operator that is located between the –35 and –10 elements of the promoter (FIG. 4b). Promoters that are thus regulated usually have non-optimal spacing between the two elements, and the current view is that activation involves distortion of the DNA so that the –35 and –10 elements of the promoter are better positioned for RNA polymerase binding[104,105]. The available structural evidence indicates that this distortion is unevenly spread across the DNA between the –35 and –10 elements[105,106].

In the above examples of 'direct' activation, the activator alters the target promoter to make it more 'attractive' to the RNA polymerase. However, activation can also occur indirectly, usually by removing a repressor, and in these cases the activator can be thought of as an anti-repressor[1]. For example, in enterohaemorrhagic *E. coli*, the expression of one of the principal virulence determinants is repressed by histone-like nucleoid-structuring protein (H-NS), but the expression of Ler, an H-NS homologue, disrupts the repression and triggers virulence[107,108]. A more complex example occurs at the *nir* promoter, which controls the expression of a nitrite reductase in *E. coli*. Class II activation, repression by NAPs and indirect activation by anti-repressors all compete to regulate the activity of this promoter: class II activation of *nir* is mediated by an activator known as Fnr (fumarate and nitrate reduction regulatory protein) in response to oxygen deprivation, but is suppressed by the NAPs IHF and Fis, which bind to specific target sites that are located just upstream of the DNA binding site for Fnr[109,110]. However, this suppression is disrupted by either NarL or

Enterohaemorrhagic *E. coli*
A virulent strain of *Escherichia coli* that causes bloody diarrhoea.

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Box 3 | Nucleoid-associated proteins

Although not enclosed by a membrane in a nucleus, bacterial chromosomes are compacted and highly folded, forming a structure known as the nucleoid. This compaction enables large bacterial chromosomes to fit inside cells and is achieved by the combined effects of DNA supercoiling, molecular crowding, and the presence of RNA and nucleoid-associated proteins (NAPs). Initially, it was thought that NAPs would have similar structures to eukaryotic histones, but it is now clear that NAPs are instead a diverse group of proteins that recognize DNA target sites using many different structural motifs[77,146]. *Escherichia coli* has at least 12 different NAPs, including factor for inversion stimulation (Fis), integration host factor (IHF), Histone-like protein originally isolated from an *E. coli* strain labelled U93 (HU), histone-like nucleoid-structuring protein (H-NS)) and leucine-responsive protein (LRP), all of which have been studied extensively[77,79]. The most studied example is probably H-NS, which forms filaments along AT-rich segments of DNA; these filaments then form bridges between different segments to repress the activity of any associated promoter[147]. How the activity of H-NS is modulated is not fully understood, but one mechanism that is used to relieve repression is the expression of H-NS homologues, such as Ler, that are incorporated into the filaments but are unable to form the repressive bridges[148].

Much of what we understand about NAPs c… …m studies of *E. coli*…owever, the set of NAPs that are present in a bacterium va… …or example, Fis is restricted to members of the Gammapro… …und in almost all bacteria and is the principal NAP in … …essential protein[77,149,150]. Some species in the Actinomyc… …an amino-terminal domain that is similar to HU, b… …that resembles eukaryotic histone H1 (REF. 151). Ac… …vels of some NAPs change in response to growth con… …ch means that the composition of the nucleoid of a cell … …mple, rapidly growing *E. coli* cells at early exponential phase… …lecules of Fis, whereas the number of Fis molecules in ce… … zero[78,152].

Characterization of the binding preferences of individual NAPs has shown that some of these proteins, such as IHF and Fis, are sequence-specific DNA-binding proteins, whereas other NAPs, such as H-NS and HU, bind to DNA nonspecifically, often to distorted DNA structures[79]. Biochemical, biophysical and single-molecule experiments have shown that NAPs package DNA by several different mechanisms. For example, IHF and Fis package DNA by bending the target site, whereas, as mentioned above, H-NS forms bridges between DNA molecules… …precipitation (ChIP) studies have determined the ger… …ome NAPs, which have shown that many NAPs bind to hu… …ny of these target sites are located in intergenic regulator… …erent NAPs may bind[156]. Thus, it is not surprising that ma… …ed in which NAPs regulate gene expression by directly m… …or example, the formation of extended filaments of H-N… …oters[147], and even prevents the unwanted expression of h… …nes, which often encode virulence factors[159]. By contrast… …tion initiation through various mechanisms and can a… …tly by interacting with RNA polymerase holoenzyme usin… …her NAPs can prevent transcription across a much lar… …omosome and a genome-wide survey of the *E. coli* chro… …00 loci at which transcription seemed to be silenced[160]. … …transcription suggests that evolution has blurred the … …transcription factors so as to ensure both nucleoid in… …egulation[124].

调节细菌启动子活性的其他机制包括特定碱基的修饰或碱基序列的改变。这些修饰既可以改变转录因子的亲和性，也可以调节RNA聚合酶的结合或亲和性。

最常见的化学修饰是DNA甲基化，它可以导致特定转录因子与操纵基因结合亲和力的变化，从而可能导致转录程序的全局变化。

调控区域碱基序列变化的最极端的例子是DNA片段的反转，即启动子对信号的反应方向发生逆转。这就产生了“开启”或“关闭”的启动子，这取决于它们是朝向还是远离它们控制的基因，这与直接由转录因子控制的启动子形成了对比，后者的输出可以与输入信号成比例。

DNA腺嘌呤甲基化酶(Dam)对大肠杆菌的两个操作基因pap和agn43进行甲基化修饰，导致抑制因子结合的缺失，从而诱导基因表达。抑制在子细胞中重新建立，因为复制过程中合成的DNA是未甲基化的。

DNA反转调控的一个例子是启动子，它控制了大肠杆菌中编码1型菌毛的膜操纵子的表达(图5b)。正如预期的那样，在这个操纵子上催化DNA转化的FimB和FimE酶本身受到严格的调控。启动子的局部序列变异提供了一种更复杂的DNA反转策略。这通常是由重复单个核苷酸(或二核苷酸)的可变长度束驱动的，通常在启动子的-35元件附近(图5c)。例如，流感嗜血杆菌hifA和hifB的启动子具有可变数量的TA重复，这调节了启动子的活性。群体中的单个细菌细胞在启动子的可变区域有不同数量的重复，每个通道长度对应不同的转录活性水平。因此，在任何时候，在给定的条件下，一部分细胞在启动子处都具有最优的转录活性水平

NarP, which are anti-repressors that have activities that are regulated by levels of extracellular nitrate and nitrite ions[111]. Thus, expression from Fnr-regulated promoters integrates two different environmental signals, oxygen and nitrate (or nitrite).

The primary role of many activation mechanisms is simply to facilitate the recruitment of RNA polymerase to the promoter, rather than to promote the transition of the RNA polymerase closed complex to the open complex, which, in any case, does not require an activator for most RNA polymerase holoenzymes. However RNA polymerase holoenzymes that contain σ^{54}-family sigma factors are unable to make this transition without the assistance of activators known as enhancer binding proteins (EBPs), which use ATP hydrolysis to induce a conformational change that drives the formation of the open complex and enables transcriptionally competent complexes to be formed[112]. As with activators that mediate class I activation, EBPs bind to DNA upstream of promoter regions; however, as EBPs form interactions with the sigma factor — rather than the more proximal α-subunits — of the RNA polymerase holoenzyme, looping of upstream DNA has to occur to 'deliver' the EBP to the sigma factor, and this is often assisted by DNA-bending proteins such as IHF[112,113].

Promoter modifications. Other mechanisms by which the activity of bacterial promoters can be regulated involve the modification of specific bases or changes to the base sequence. These modifications can either alter the affinity of an operator for a transcription factor or modulate the binding or affinity of RNA polymerase. The most common chemical modification is DNA methylation, which can result in changes in the binding affinities of specific transcription factors to operators that can potentially lead to global changes in the transcriptional programme[114–118]. For example, methylation of the operators of two *E. coli* genes, *pap* and *agn43*, by the DNA adenine methylase (Dam) results in the loss of repressor binding, which leads to the induction of gene expression. Repression is re-established in daughter cells, as the DNA that is synthesized during replication is unmethylated (FIG. 5a).

The most extreme examples of base sequence changes at regulatory regions are inversions of DNA segments that reverse the orientation of the promoter in response to a signal. This creates promoters that are either 'on' or 'off', depending on whether they are oriented towards or away from the genes that they control, which contrasts with promoters that are directly controlled by transcription factors, for which output can be proportional to the input signal[117,118]. An example of regulation by DNA inversion is the promoter that controls the expression of the *fim* operon, which encodes type 1 fimbriae in *E. coli* (FIG. 5b). As might be expected, the FimB and FimE enzymes that catalyse DNA inversion at this operon are themselves tightly regulated.

Local sequence variation at promoters provides a more sophisticated alternative strategy to DNA inversion. This is usually driven by tracts of variable length that repeat a single nucleotide (or dinucleotide), often in the vicinity of the −35 element of the promoter (FIG. 5c). For example, the promoters of *hifA* and *hifB* in *Haemophilus influenzae* have variable numbers of TA repeats, which modulates the activity of the promoters. Individual bacterial cells in a population have a different number of repeats in the variable region of the promoter, with each tract length corresponding to a different level of transcriptional activity. Thus, at any moment, a subset of cells will have the optimal level of transcriptional activity at the promoter for the given conditions[117–120]. This regulation 'by lottery', which is driven by repetitive sequences that differ from one generation to another,

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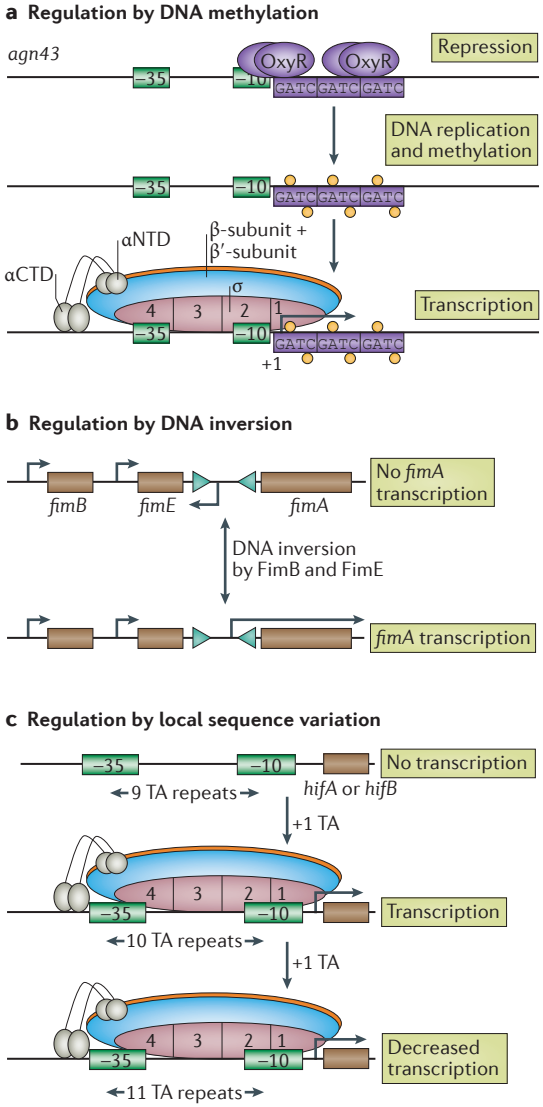
通过DNA甲基化调控；

为了抑制agn43的转录，转录因子OxyR与agn43位点的启动子区域中含有未甲基化的GATC序列的位点结合。使转录受到抑制，因为OxyR阻断了RNA聚合酶对启动子的识别与结合。DNA复制后，DNA腺嘌呤甲基化酶(Dam)可以在一条或两条链上发生GATC序列的腺嘌呤的甲基化，从而阻止OxyR结合。一旦GATC位点不再被OxyR占据，RNA聚合酶就能够结合到启动子上并启动转录。

通过DNA反转调控；

膜操纵子的fimA和其他基因的表达是通过启动子区DNA元件的反转来调控的。这种反转是由重组酶FimB和FimE介导的，并在启动子的开关状态之间切换方向。在“关闭”状态下，启动子的方向远离fimA，导致非编码转录本的产生，而在“开启”状态下，启动子的方向使fimA转录本的产生成为可能。

3、局部序列变异调控
在流感嗜血杆菌中，hifA和hifB的启动子编码菌毛成分，具有可变数量的ta-二核苷酸重复，这改变了-10和-35元件之间的间距。由于这些元件之间的间距是RNA聚合酶全酶结合的决定因素，因此启动子的强度，重复次数的变化产生基因表达的变化，从而表达可以完全关闭或调整到不同的水平。



◀ Figure 5 | **Regulation by promoter DNA modification.** **a** | Regulation by DNA methylation. To repress transcription of *agn43*, the transcription factor OxyR binds to sites in the promoter region of the *agn43* locus that contain unmethylated GATC sequences. This results in transcriptional repression because OxyR blocks the access of RNA polymerase to the promoter. After DNA replication, the DNA adenine methylase (Dam) can methylate the adenines of these GATC sequences on one or both strands, which prevents OxyR binding. Once the GATC sites are no longer occupied by OxyR, RNA polymerase is able to bind to the promoter and initiate transcription. **b** | Regulation by DNA inversion. The expression of *fimA* and other genes of the *fim* operon is regulated by inversion of a DNA element in the promoter region. The inversion is mediated by the recombinases FimB and FimE and switches the orientation of the promoter between off and on states. In the 'off' state, the promoter is oriented away from *fimA*, which results in the production of non-coding transcripts, whereas, in the 'on' state, the orientation of the promoter enables the production of *fimA* transcripts. The *fim* locus is not drawn to scale. **c** | Regulation by local sequence variation. In *Haemophilus influenzae*, the promoters of *hifA* and *hifB*, which encode fimbrial components, have a variable number of TA-dinucleotide repeats, which alter the spacing between the −10 and −35 elements. As the spacing between these elements is a determinant of RNA polymerase holoenzyme binding, and thus promoter strength, variation of the number of repeats produces changes in gene expression, such that expression can be switched off entirely or adjusted to different levels. αCTD, carboxy-terminal domain of the α-subunit of RNA polymerase. αNTD, the amino-terminal domains of the α-subunits of RNA polymerase.

contrasts sharply with regulation by transcription factors, which drive specific responses to specific environmental cues. Therefore, transcription factors may not always be essential for bacterial survival, as other strategies can be used by bacteria to adapt their transcriptional programmes to changing environments.

Evolutionary origins

François Jacob stated that 'evolution was a tinkerer' and nowhere is this famous statement more evident than in the regulation of transcription initiation in bacteria[121]. From the perspective of evolutionary history, we can assume that transcription was present in the first cells and it is easy to imagine that this transcription might have been much less specific and much less regulated than in modern-day cells. Indeed, it has been suggested that the widely observed, unregulated, pervasive transcription that is seen in many bacteria is an evolutionary relic from the time before control was exerted[122]. Furthermore, the RNA polymerase core enzyme, which is common to all three domains of life, can make transcripts, but lacks the ability to select specific start sites or efficiently initiate transcription[123]. In bacteria, these functions were acquired through the evolution of sigma factors, whereas different mechanisms evolved to solve this problem in archaea and eukaryotes[123].

Following the acquisition of sigma factors, the bacterial RNA polymerase had to overcome the compacted DNA structure of the nucleoid to access the many potential promoters that were probably blocked by NAPs (BOX 3). In fact, NAPs may have been important in the evolution of transcription regulation, which has been argued to have involved the co-option of NAPs to regulate the repression or activation of specific genes[124]. Therefore, the reversal of NAP-mediated repression (such as that mediated by Ler, NarL or NarP) may well reflect the oldest form of activation, with the acquisition of activating regions that recruit RNA polymerase occurring later in evolution. Evidence for a common origin for transcription factors and NAPs comes from findings that DNA binding by transcription factors does not always produce a measurable effect on transcription[125,126], and that many NAPs can function as regulators of transcription by using activating regions to recruit RNA polymerase[127,128].

When considering the various regulatory mechanisms of sigma factors, transcription factors, appropriators and other factors, one can conclude that the complexity of the regulation of transcription initiation in bacteria has probably evolved through the sequential addition of layers of regulation. Furthermore, the evolution of these regulatory layers continues today, as can be

Pervasive transcription
The synthesis of transcripts that seem not to correspond to any functional genetic unit.

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seen in the different extents to which different bacteria use sigma factors and transcription factors, which underscores the danger of making generalized conclusions based solely on studies using *E. coli* as a model.

Outlook

Since 2004, when we last reviewed this topic[1], enormous progress has been made in understanding the molecular details of transcription initiation, together with mechanisms that regulate its activation and repression. Fundamental to this progress has been an improved understanding of the structure and dynamics of the bacterial RNA polymerase, which has enabled further studies to elucidate the mechanisms of many of the factors that interact with the polymerase. In parallel, the availability of omics data made possible by the development of next-generation sequencing and other high-throughput methods has alerted us to the diversity of factors that regulate RNA polymerase in different bacteria and to the breadth of the regulatory repertoire. However, despite these advances, we are still unable to predict transcriptomes and regulatory patterns from first principles. Therefore, we believe that an important aspiration for the years ahead is to develop our understanding of regulatory mechanisms such that the many layers of regulation can be modelled to accurately predict transcriptional outcomes from a bacterial genome sequence.

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Competing interests statement
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