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Advances in bacterial promoter recognition and its control by factors that do not bind DNA

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Abstract

Early work identified two promoter regions, the –10 and –35 elements, that interact sequence specifically with bacterial RNA polymerase (RNAP). However, we now know that several additional promoter elements contact RNAP and influence transcription initiation. Furthermore, our picture of promoter control has evolved beyond one in which regulation results solely from activators and repressors that bind to DNA sequences near the RNAP binding site: many important transcription factors bind directly to RNAP without binding to DNA. These factors can target promoters by affecting specific kinetic steps on the pathway to open complex formation, thereby regulating RNA output from specific promoters.

Because many, and perhaps most, gene products are conserved among different species of bacteria, the extraordinary variety of bacterial life must result from differences in the relative amounts of these products and in the timing of their expression. Regulation can occur at every step on the pathway to gene expression, but transcription initiation is probably the most frequently regulated step. Differences in intrinsic promoter strength and in the action of transcription factors that regulate promoters are sufficient to account for much of the variation in the levels of RNA that are produced by different operons^{1–3}.

In contrast to archaea and eukaryotes, bacteria contain only one form of RNA polymerase (RNAP) core enzyme (E), which is a complex of five subunits ($\alpha_2\beta\beta'\omega$). However, in most bacterial species, there are multiple forms of the σ -factor specificity subunit, and thus multiple forms of RNAP holoenzyme (referred to as $E\sigma$). Some differences in promoter use and activity result from changes in the availability of different RNAP holoenzymes under different nutritional or environmental conditions. However, the strengths of different promoters that are recognized by the same form of holoenzyme, for example $E\sigma^{70}$ (the main holoenzyme in *Escherichia coli*), can vary over many orders of magnitude, with some promoters producing less than one RNA copy per cell generation and other promoters producing tens of thousands of RNA copies per cell generation^{1–3}.

Alignments of *E. coli* promoter sequences from as long ago as 1975 identified the –10 and –35 hexamers, the primary determinants of basal promoter strength^{1,2}. Furthermore, several additional promoter elements that affect strength and regulation have been identified since that time^{4–6} (FIG. 1). Sequence variation within individual promoter elements affects specific interactions of the promoter with two RNAP subunits, σ and α , and results in the vast range of basal promoter strengths that are found in nature^{1–4}. At many or most promoters, basal promoter strength is increased or decreased by activator or repressor proteins that bind to specific DNA sequences near to, or overlapping, the RNAP binding

site, thereby restricting the effects of these regulators to only those promoters that contain the transcription-factor binding sites³.

In this Review, we first provide a brief overview of RNAP structure and the sequence of events that occur during bacterial transcription initiation. We then review recent advances in our understanding of the individual interactions between RNAP and DNA that participate in promoter recognition. Finally, we discuss the regulation of transcription initiation, in which we emphasize mechanisms other than those of the classical activators and repressors, which have been reviewed elsewhere (for a recent review, see REF. 3). The unconventional regulators described in this Review include small proteins, RNAs, nucleotides, nucleotide analogues and ions. The amounts and/or activities of these regulators change according to nutritional and environmental conditions. Similar to the conventional transcription factors, the effects of these factors are promoter specific, but they differ from conventional transcription factors in that promoter specificity is not achieved by binding to DNA⁷.

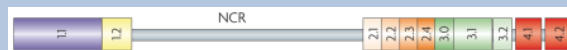
Transcription initiation

RNAP structure and the kinetics of the reaction

The six subunits of the holoenzyme $\alpha_2\beta\beta'\omega\sigma'$ are highly conserved in bacteria, and all except σ have homologues in archaea and eukaryotes. The α dimer forms the scaffold on which β and β' , the catalytic subunits, assemble, and the ω subunit assists β' binding to the α_2 sub-assembly^{1,8}. The overall structure of the bacterial core enzyme⁹ is similar to that of the archaeal RNAP¹⁰ and the eukaryotic RNAPs¹¹, and the only major differences are on the surface of the complex^{9–11}. In both the core and holoenzyme structures of bacterial RNAP, the overall architecture resembles that of a crab claw, in which β forms one pincer and β' the other^{12–15} (FIG. 2a). In the holoenzyme–DNA complex, σ is oriented with the carboxy (C)-terminal domain upstream and the amino (N)-terminal domain downstream with respect to the promoter¹⁴. The conformation of σ is extended, with the domains spread primarily along one face of RNAP (BOX 1; FIG. 2b, c). The interface between σ and core RNAP is extensive, comprising more than 8,200 Å² of surface¹².

Box 1

σ factors



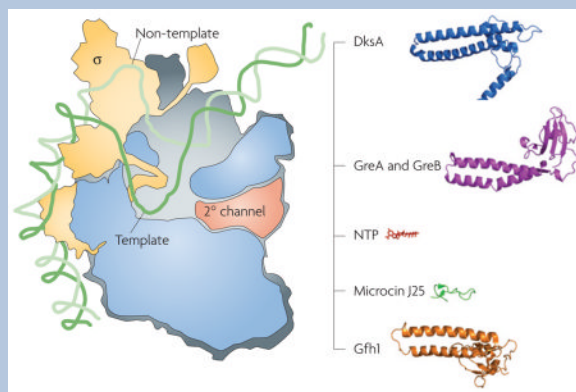
Most bacteria encode multiple σ factors, and, in some species, there are >60 (REF. 3). Each of the seven different σ factors in *Escherichia coli*, which range from 173 to 613 amino acids in length, recognizes its own cognate consensus promoter sequence. The most abundant σ factor in the bacterial cell, referred to as σ^{70} in *E. coli* (σ^A in most other species), is responsible for recognizing most of the cell's promoters. σ^{70} contains four regions of conserved amino acid sequences, $\sigma 1$ – $\sigma 4$, that are further divided into subregions¹ (see the figure). In addition to region 1.1 and the nonconserved region (NCR; a segment of σ^{70} that, even in all members of the σ^{70} class, is not always present and the sequence and length of which varies widely⁷⁹), these regions of sequence conservation are also present in the alternative σ factors, but not in the σ^{54} class (the most divergent of the σ factors^{1,84}). In the main text, we refer primarily to the *E. coli* $E\sigma^{70}$ holoenzyme for brevity. However, the mechanisms of the alternative holoenzymes are also probably similar to that of $E\sigma^{70}$, with the exception of $E\sigma^{54}$. Core RNA polymerase (RNAP) is limiting, so the different affinities of the σ factors for core RNAP, as well as dramatic variation in the amounts of the different σ factors under different nutritional and

environmental conditions, contribute to the regulation of promoter utilization by the different holoenzymes.

There are two major channels in RNAP, which are bifurcated by a long, evolutionarily conserved feature of the enzyme, the bridge helix (FIG. 2a). These channels provide solvent access to the active site in the absence of DNA. However, promoter DNA fills the main channel that is formed by the cleft between the β and β' pincers. Therefore, it is thought that the much narrower secondary channel provides the usual route whereby nucleoside 5'-triphosphates (NTPs) access the active site¹⁶ (BOX 2).

Box 2

Factors that bind in the secondary channel of RNAP



The secondary channel of RNA polymerase (RNAP) is too narrow to accommodate double-stranded DNA (see the figure). However, it provides access for solvent and nucleoside 5'-triphosphates (NTPs) to the enzyme's active site and is wide enough to accommodate back-tracked RNA (which occurs when the RNA 3' end is no longer aligned with the active site)¹⁷. In addition, the secondary channel has emerged in recent years as a route by which small molecules and small proteins can access the RNAP active site and exert a wide range of effects on transcription^{7,16,106,107}.

The structural similarity between DksA and the Gre factors^{107,119,120} raised the possibility that Gre factors also affect ribosomal RNA (rRNA) transcription initiation. However, GreA does not mimic the effects of DksA *in vitro* nor compensate for the absence of DksA *in vivo*, even when produced at concentrations that are sufficient to compensate for its lower concentration and its apparently weaker affinity for RNAP than DksA¹²². Thus, binding in the secondary channel of RNAP is insufficient to fully explain the effect of DksA on transcription initiation. By contrast, GreB and DksA have similar effects on rRNA promoter complexes *in vitro*, but the GreB concentration is much lower than that of DksA, which explains the absence of an effect of *greB* mutants on rRNA transcription *in vivo*¹²². When GreA was overexpressed in an otherwise wild-type strain, however, it competed with DksA and prevented DksA from modulating rRNA transcription¹⁴⁶.

There is no DksA homologue in *Thermus thermophilus*, but it has been proposed that another Gre factor homologue, Gfh1, acts as an anti-Gre factor by binding in the RNAP secondary channel in this bacterium¹⁴⁷. The secondary channel is also the target of antibiotics, such as microcin J25 (REF. 148). Figure modified, with permission, from REF. 15 © (2003) Elsevier Science and REF. 149 © (2004) Elsevier Science.

The X-ray crystal structure of the promoter–*Thermus aquaticus* RNAP complex, known as the fork–junction complex¹⁴, provided valuable insights about the placement of DNA within RNAP. Although the 6.5 Å resolution of the fork–junction complex was insufficient to discern details of the protein–protein and protein–DNA interactions in the promoter complex, the availability of data from biochemical and genetic studies^{1,2,17}, higher resolution structures of individual components of the promoter complex (reviewed in REFS 9,12) and a high resolution structure of the elongation complex¹⁸ make it possible to construct high-quality models of the open complex from the low-resolution fork–junction structure^{14,15,19}.

Nevertheless, it should be emphasized that the structure is a snapshot of only one step in a multistep process and components that are not present in this structure have important roles in transcription initiation. For example, the ~85-residue C-terminal domains of the two α subunits²⁰ and the highly acidic ~90-amino-acid N-terminal domain of σ (region 1.1)²¹, although modelled in some panels of FIGS 2,3 are not resolved in the structure, probably because they are mobile. The complex captured in the crystal also lacks DNA upstream of the –35 hexamer, downstream of the –10 hexamer on the non-template strand and downstream of position –12 on the template strand. Therefore, the picture of transcription initiation that was afforded by the fork–junction complex is incomplete. It should be noted that the lengths of the DNA spacers that separate different promoter elements vary from promoter to promoter, which can lead to ambiguity in nomenclature. For consistency, we use numbering that corresponds to the most frequent situation, in which the transcription start site (+1) is the seventh position downstream from the –10 hexamer, and there is a 17 bp spacer between the –10 and the –35 hexamers.

Because formation of a complex that is capable of initiating transcription is a multistep process, we need to correlate structural descriptions of complexes with specific kinetic intermediates^{1,2,22}. It is generally assumed that there is a common mechanism that is shared by all promoters¹. However, there are difficulties inherent in the detection of rapidly forming, transient complexes. Furthermore, the correlation of specific structures with specific steps in the mechanism has been complicated by variation in the precise upstream and downstream end-points of promoter complexes that have been assigned by footprinting and other biochemical assays^{1,2,4,22–29}. Some variation results from differences in the structural details of complexes that are formed by different promoters, but effects of differences in template topology (for example, DNA supercoiling), ionic conditions and temperature have also contributed to the difficulty in correlating specific structures with experimentally derived kinetically significant intermediates (for a review, see REF. 1).

For simplicity, we refer to three intermediate complexes of RNAP with promoter DNA (RP), RP closed (RP_C), RP intermediate (RP_I) and RP open (RP_O) (FIG. 3), but we recognize that there is some ambiguity in these terms. The energetics of this process have been described as “bind, nucleate, melt” (REFS 1,2,22). The movements of the transcriptional machine that occur on the pathway to initiation are driven not by the hydrolysis of ATP, but by binding free energy — the establishment of interfaces and conformations in earlier intermediates that trigger subsequent rearrangements to form later intermediates²². Ultimately, these successive rearrangements result in DNA opening and alignment of the start-site base of the template strand with the catalytic site of the enzyme. The largest of the conformational changes in the enzyme (in terms of the burial of polar amide surface area) that occurs on the pathway to formation of the transcriptionally competent complex appears to take place after DNA-strand opening and could correspond to the clamping of the enzyme’s jaw (or jaws) onto the downstream DNA²⁷.

In the first step in the general scheme (FIG. 3a), RNAP binds to promoter DNA to form the closed complex RP_C (FIG. 3b). In this complex, RNAP protects DNA in footprints from approximately -55 to approximately $+1$ relative to the transcription start site, and the DNA is completely double stranded^{1,2,22-24}. Although RNAP protects both the -35 hexamer and the -10 hexamer in these footprints, the DNA has not yet entered the main channel^{22,24,27}. The complex then undergoes large changes that are referred to as isomerizations, in which the conformations of both RNAP and DNA change^{1,2,22,27} (discussed below). The downstream boundary of the intermediate complex RP_I extends beyond the transcription start site, perhaps as far as $+12$ (FIG. 3c). In this complex, the DNA strands are still base paired^{1,2,15,22,24,30}, although there could be a few transiently opened base pairs within the -10 hexamer. Ultimately, in the open complex RP_O , the DNA strands are separated from approximately -11 to approximately $+3$ and the footprint extends downstream to approximately $+20$, leaving a base on the template strand (designated $+1$) that is able to pair with the first NTP (initiating NTP (iNTP))^{1,2} (FIG. 3d).

As discussed below, although DNA downstream of the -10 hexamer is not in the crystallized fork-junction complex, crosslinking experiments indicate that the non-template strand immediately downstream of the -10 hexamer is held in a groove that is formed between β and σ (REFS 17,26), where it can interact with a highly conserved module in σ region 1.2 (REFS 6,31,32) (BOX 1; FIG. 2b). The template strand threads through RNAP to the active site, and the DNA strands re-anneal at approximately $+4$ (REFS 2,33). Modelling based on biochemical data and on the structure of the elongation complex suggests that the double-stranded DNA downstream to approximately $+12$ is housed in a protein tunnel that is formed by β and β' (REFS 14,17-19,26).

The N-terminal domain of σ region 1.1 is disordered in all the X-ray structures, but biophysical approaches have shown that σ region 1.1 is in the main DNA channel of the holoenzyme (FIG. 3b) and is displaced by double-stranded DNA during open complex formation²¹ (FIG. 3d). The molecular details of this process remain to be determined. However, the end result of the conformational changes that contribute to melting of the -10 hexamer is an approximately 90° change in the trajectory of the DNA^{15,30} (FIG. 3d).

Once the open complex forms, NTP incorporation drives the transcription reaction forward. However, at most promoters, RNAP synthesizes short, abortive products before transitioning to the elongation complex. During this time, the leading edge and the active site of the enzyme move forward, but the contacts between the trailing edge of RNAP and the -35 hexamer remain intact. It appears that both DNA strands in the vicinity of the -10 hexamer are extruded from the main DNA channel onto the surface of the enzyme during this transition in a process that is called scrunching^{34,35} (FIG. 3e). It is proposed that the energy stored in this scrunched intermediate is used to break the interactions between RNAP and the promoter, thereby allowing RNAP to begin the transition to the elongation phase of transcription (FIG. 3f). Ultimately, formation of the elongation complex involves staged disruption of a series of contacts between σ and core RNAP^{33,36} (discussed below).

In the discussion that follows, we describe interactions between individual promoter DNA elements and modules of RNAP in more detail, moving from upstream to downstream with respect to the transcription start site (FIG. 1).

α CTD-DNA interactions and promoter recognition

Although the two α subunits are identical in amino acid sequence, they are not functionally equivalent, as the core enzyme is asymmetric: one α subunit interacts primarily with β and one interacts primarily with β' (REF. 9) (FIG. 2c). Each α subunit is comprised of two domains that are tethered by a flexible linker of ~ 15 amino acid residues²⁰. The α N-

terminal domain (α NTD) provides the dimerization interface as well as the scaffold for core-enzyme assembly⁹, whereas the α C-terminal domain (α CTD) binds to DNA⁴. DNA sequences that result in specific binding by the α CTDs upstream of the -35 element are referred to as UP elements^{4,37}. UP elements are widely distributed in bacterial, plasmid and phage promoters. Extensive biochemical and genetic analyses of the α CTD–DNA interaction and a high resolution X-ray structure provide a cohesive picture of the complex, in which α CTD interacts in and across the DNA minor groove using a helix–hairpin–helix motif^{37–40}.

The two α CTDs increase promoter activity most when bound to the two DNA minor grooves just upstream of the -35 hexamer^{4,37} (FIGS 1,2c), but the flexible tether that links the two domains of α , coupled with bending of the DNA, can allow the α CTDs to interact with DNA that is as much as 5–6 minor grooves upstream of the -35 hexamer²⁶. DNA binding by a single α CTD to half of an UP element (an UP element subsite) can also stimulate transcription, but to a lesser extent than binding to a full UP element³⁷.

α CTD can also interact with DNA sequences in a base-nonspecific manner (that is, with the DNA backbone)⁴¹. The kinetic consequences of sequence-specific and nonspecific α CTD binding to DNA for transcription initiation have been investigated. Unexpectedly, not all of the effects are attributable to initial recruitment of RNAP to the promoter^{41–43}. Elimination of α CTD (or truncation of DNA upstream of the -35 hexamer) not only decreases K_B , the equilibrium binding constant for the first kinetically significant complex, but it also slows down k_i , the composite rate of isomerization, by at least fourfold. Exactly how α CTD affects k_i remains unclear. One model is that α CTD allosterically affects a part of RNAP that contributes to the isomerization step⁴¹. An alternative model is that DNA upstream of the α CTD binding site (or sites) directly contacts a part of RNAP that is involved in the isomerization step (or steps), and that this is facilitated by the α CTD–DNA interaction^{28,43}.

σ region 4 interactions with the -35 element and with the α CTD

Extensive genetic, biochemical and structural information has provided a detailed picture of recognition of the -35 element by a helix–turn–helix motif in σ region 4.2 (REFS 1,2,44,45) (FIG. 2a). The helix–turn–helix contacts backbone positions and bases on both the template and non-template strands, which are among the first sequence-specific interactions made between RNAP and the promoter^{29,46,47}. When bound to the proximal UP-element subsite that is centred at approximately -41 , α CTD can interact directly with σ ⁷⁰ region 4.2, which is bound to the -35 element^{48,49} (FIG. 2c). The α CTD– σ region 4.2 interface appears to differ slightly depending on whether CRP (cyclic AMP receptor protein), which activates transcription through contact with the α CTD, is bound to a DNA site that is centred at -61.5 (REFS 48,49). This suggests that transcription factors remodel the α CTD– σ region 4.2 interaction.

Extended -10 element interactions with σ region 3.0

Surface-exposed residues in σ region 3.0, an α helix that is perpendicular to region 2.4, bind in the major groove of the extended -10 element, which is separated from the -10 element by one base^{5,12–14} (FIGS 1,2b, c). This interaction is crucial for transcription from a subset of promoters with poor matches to the -35 or -10 consensus hexamers^{5,50–52}. Although only a subset of *E. coli* promoters have consensus-extended -10 elements, these are more frequent in *Bacillus subtilis*^{53–54}. The kinetic effects of the extended -10 interaction with σ region 3.0 have not been characterized extensively, but the interaction seems to stimulate transcription, both by increasing the rate of association and by stabilizing the promoter complex^{6,53,54}. The identity of promoter position -13 , the position between the extended

–10 and –10 elements, also contributes to specific recognition by at least some RNAP holoenzymes, particularly $E\sigma^S$ (REF. 55).

The interaction between the –10 hexamer and σ region 2

The literature on the –10 hexamer– σ region 2 interaction is too detailed and extensive to be covered here in depth (see, for example, REFS 1,2). To summarize, the interactions between σ region 2, the most highly conserved region in σ , and the –10 hexamer are complex, as this element is recognized by RNAP, first, in the double-stranded DNA form and, after melting, as single-stranded DNA^{56–60} (FIG. 2b). Sections of σ regions 2.3 and 2.4 form a continuous α helix that interacts with non-template strand bases in the –10 hexamer, and aromatic side chains in these sections of σ probably stack on bases in the –10 element during DNA-strand opening^{14,24,56–61}. The non-template adenine at –11 seems to be the most crucial base for the nucleation of strand separation. Localized melting may involve base flipping at –11, and stacking of an aromatic residue in region 2.3 on the base at –12 is proposed to prevent DNA unwinding further upstream^{14,60–66}. Unsurprisingly, because the –10 hexamer is contacted in both the double-stranded and single-stranded DNA forms, mutations in the –10 hexamer affect both K_B and k_i (REF. 67).

Discriminator interactions with σ region 1.2

The term discriminator was first used by Andrew Travers⁶⁸ to describe a G+C-rich region between the –10 hexamer and the transcription start site in ribosomal RNA (rRNA) and transfer RNA (tRNA) promoters. Like the UP element and the extended –10 element, the base sequence in this region is not highly conserved in *E. coli* promoters and was therefore not originally recognized as a sequence-specific recognition element for RNAP. However, recent work has shown that σ region 1.2 contacts the non-template strand base two positions downstream from the –10 hexamer (–5 at most promoters)^{6,31} (FIG. 2a). Promoter sequences that contain a guanine at –5 are the most favourable for the interaction with *E. coli* σ^{70} (REFS 6,31). The guanine at –5 crosslinks to the N-terminal residues of two perpendicular α helices in *E. coli* σ^{70} region 1.2, probably with residues Y101 and/or M102 (REF. 31). The discriminator– σ region 1.2 interaction affects the rate of dissociation of RP_O to RP_C (REF. 6), which is a major determinant of the susceptibility of rRNA and other promoters to negative regulation by ppGpp and DksA (discussed below). Consistent with a contact between σ region 1.2 in *E. coli* RNAP and a guanine in the discriminator, the major σ factor in *T. aquaticus* binds best to a DNA sequence that contains three guanines immediately downstream of the –10 hexamer³².

Promoter escape and σ release

The σ cycle paradigm, in which σ is released upon promoter clearance and is then recycled for use by other core RNAP enzymes⁶⁹, has been questioned in recent years^{70,71}. Although σ release is not obligatory for promoter clearance⁷², it now seems clear that σ is usually released in a stochastic manner within the first few hundred base pairs of the start site^{73–74} (as proposed more than 20 years ago⁷⁵).

Because the interaction of σ with core RNAP to form the holoenzyme is a multistep process^{76–78}, it is not surprising that dissociation of σ from core RNAP also takes place in stages (reviewed in REF. 72). As discussed above, displacement of σ region 1.1 from the main channel of the enzyme occurs on the pathway to RP_O formation to allow entry of double-stranded DNA^{14,15,21,30} (FIG. 3b–d). As the nascent transcript grows to ~6 nucleotides, interactions of the $\sigma 3$ – $\sigma 4$ linker within the RNA exit channel are displaced (FIG. 3e), and σ region 4 contacts are disrupted from the β flap at a length of ~16–17 nucleotides (that is, when the transcript reaches the end of the RNA exit channel)³⁶. The interaction of σ region 2 with the coiled-coil region of β' is sufficient for the association of

σ with core RNAP⁷⁸. Furthermore, the interaction of σ region 2.2 with the coiled-coil itself could be the last contact to be disrupted to release σ from core RNAP. It also may be sufficient for the retention (or reassociation) of σ in some proportion of the elongation complexes⁷². The non-conserved region (σ NCR) (BOX 1) that is located between σ region 1.2 and σ region 2.1 (and/or RNAP-associated transcription factors) could have roles in disruption of the σ region 2.2 interaction with the β' coiled coil^{74,79}.

Classical regulators of transcription

Classical repressors (for example, the Lac and λ repressors) usually prevent binding of RNAP by occluding the promoter in the vicinity of the -10 and -35 region³, but a repressor has also recently been identified that inhibits transcription solely by competing for an UP element⁸⁰. Repression mechanisms have also been reported in which proteins do not prevent RNAP binding, but instead block formation of intermediates in the initiation pathway^{81–83}. Classical activators typically bind near RNAP, contacting α CTD or σ region 4.2 (REF. 3) and thereby recruiting RNAP to the promoter or facilitating a conformational change later on the pathway to initiation. Several different activators and co-activators often function together on the same promoter³.

These conventional activators and repressors share an important property: they restrict their effects to specific promoters by binding to specific DNA sequences that are near to or overlap the promoter. Below, we focus on regulators of transcription initiation that bind to RNAP and achieve specificity without binding to DNA. Some other transcription factors (reviewed in REF. 84), including many activators of $E\sigma^{54}$ -dependent promoters, as well as the activator of $E\sigma^{70}$, bacteriophage N4 single-stranded DNA-binding protein, bind to DNA, but differ in their mechanisms of action from either conventional regulators or the regulators that are described below.

Regulators that do not bind DNA

Anti- σ and anti- α factors

Anti- σ factors are proteins that regulate transcription by sequestering one or more of the σ surfaces that bind to core RNAP or to promoter DNA⁸⁵. Anti- σ factors are σ specific (for example, anti- σ^F , anti- σ^E or anti- σ^{32}), and are widespread in the bacterial kingdom. The mechanisms of action of several members of this class have been defined at the atomic level⁸⁶.

The phage T4-encoded regulator AsiA uses a slightly different strategy. It targets specific promoter subsets by binding to σ rather than to DNA, and it functions by interfering with -35 element recognition, both by preventing the interaction of the β flap with σ region 4 (REF. 87) and by deforming the DNA-binding surface of σ region 4 (REF. 88). In conjunction with the T4-encoded transcription factor MotA, AsiA mediates a global change in gene expression from T4 early genes to T4 middle genes.

Whereas the anti- σ factors target promoters that use a specific σ factor, the *B. subtilis* protein Spx modulates the function of a different RNAP subunit — α . Spx functions as an anti- α factor by sequestering activator and σ -region-4 binding surfaces on α CTD, and it can activate or inhibit transcription at specific promoters during oxidative or disulphide stress conditions^{89,90}.

A factor that stimulates holoenzyme assembly?

Crl, a 15.8 kDa protein first identified in *E. coli* and found in many gammaproteobacteria, is required for efficient expression of some $E\sigma^S$ -dependent promoters^{91–93}. Crl interacts

directly with σ^S (and perhaps other σ factors) and increases transcription from a subset of promoters *in vivo* and *in vitro*^{91–96}. Because stimulation of transcription by Crl was much greater when it was added to σ before assembly of σ with core RNAP, and Crl stimulated transcription only when the σ concentration was low, it was proposed that Crl aids binding of σ to core RNAP⁹³. This model is consistent with analyses of $E\sigma^S$ formation in extracts from wild-type and *crI* mutant strains⁹². One attractive model is that Crl serves as a chaperone that helps fold or unfold σ to facilitate its binding to the core⁹³.

As indicated above, Crl does not increase transcription *in vivo* from every promoter that is recognized by $E\sigma^S$, and it does not bind to DNA. Because Crl seems to increase the effective concentration of RNAP, it was suggested that its promoter specificity results from differences in the intrinsic binding constants of different promoters for $E\sigma^S$ (REF. 93). Further studies are required to determine whether Crl-activated promoters are simply those that bind $E\sigma^S$ most weakly and are therefore most sensitive to alterations in $E\sigma^S$ concentrations.

Global negative control by ppGpp and DksA

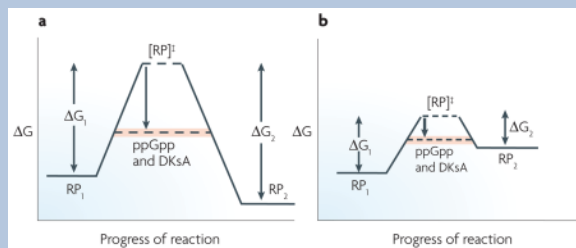
The unusual nucleotides guanosine tetraphosphate and pentaphosphate (collectively referred to here as ppGpp) were identified approximately 40 years ago (reviewed in REFS 7,97). ppGpp is distributed widely in bacteria and is also found in chloroplasts, which evolved from bacteria^{97,98}. Control of transcription by ppGpp has been described in many bacterial species⁹⁷, and its effects on transcription have been characterized on a genome-wide scale in both *E. coli*^{99,100} and *B. subtilis*¹⁰¹. ppGpp directly and specifically inhibits promoters for rRNAs and most tRNAs^{7,97,102,103}, as well as some promoters for mRNAs (for example, *pyrBI*¹⁰⁴ and *fts*¹⁰⁵). It was recently discovered that ppGpp has a cofactor in *E. coli* and many other bacterial species: the 151 amino acid protein DksA¹⁰⁶, although DksA is not as widely distributed as ppGpp^{7,107}. In addition to inhibiting some promoters, ppGpp and DksA activate a number of other promoters directly and/or indirectly^{7,108–111} (discussed below).

The discovery that DksA mediates the effect of ppGpp on transcription in *E. coli* resolved the discrepancy between the 2- to 3-fold inhibition of rRNA promoters by ppGpp that has been observed *in vitro* in reactions that contain only RNAP and promoter DNA, and the ~20-fold inhibition that has been observed in cells^{102,103,106,112}. Inactivation of the *dksA* gene virtually abolishes inhibition of rRNA promoters by ppGpp *in vivo*, thereby preventing the regulation of ribosome synthesis¹⁰⁶. Elimination or overexpression of *dksA* has been reported to affect not only ribosome synthesis, but also several other functions, such as amino acid biosynthesis, chaperone function, cell division, quorum sensing, phage sensitivity, responses to envelope stress and virulence in a range of bacterial species (reviewed in REF. 7).

There is a strong correlation between the short lifetime of the competitor-resistant promoter complex and negative regulation by ppGpp and DksA^{6,7} — the competitors for RNAP binding that are used in such studies are typically either the polyanion heparin or double-stranded promoter DNA. rRNA promoter complexes are extremely short-lived, with the equilibrium between competitor-resistant complexes (for example, RP_O) and competitor-sensitive intermediates (for example, RP_C) becoming shifted in the dissociation direction even in the absence of ppGpp and DksA (BOX 3b; FIG. 3a). Although ppGpp and DksA dramatically reduce the lifetimes of all promoter complexes that have been examined, they only inhibit transcription initiation from those promoters that form short-lived complexes with RNAP^{6,7,102,106}. Presumably, ppGpp and DksA fail to inhibit output from promoters that make long-lived complexes because RNAP escapes to the elongation phase before promoter occupancy declines significantly.

Box 3

ppGpp and DksA inhibit some promoters and activate others



A kinetic framework for explaining both the direct negative and positive effects observed for ppGpp and DksA on transcription is shown in the figure. It is proposed that the binding of these factors to RNA polymerase (RNAP) lowers the free energy (ΔG) of an intermediate and/or transition state ($[RP]^\ddagger$) (dashed lines) between intermediates (labelled here as RP_1 and RP_2 on the pathway to open-complex formation)¹⁰⁸. The consequence for transcription would depend on the intrinsic kinetic constants that are ultimately derived from the DNA sequence of an individual promoter. For example, at positively regulated amino acid promoters (see the figure, parta), the RP_2 complex might be stable (have a lower free energy than RP_1)¹⁰⁸, which would make the promoter insensitive to the inhibitory effects of ppGpp and DksA. If there were also a high free-energy barrier between the two intermediates, ppGpp and DksA could lower this energy barrier, thereby decreasing the activation energy and increasing transcription initiation.

By contrast, at ribosomal RNA promoters (see the figure, partb), the activation energy for movement between the two free-energy states might be low and might not require ppGpp and DksA. However, if RP_2 had a higher free energy than RP_1 , as for promoters that do not make stable open complexes at equilibrium, such as *rnmBP1*^{102,144}, then the destabilizing effects of ppGpp and DksA would lower the barrier between the two states in the dissociation direction, thereby discouraging RP_2 formation and inhibiting transcription. Thus, depending on the intrinsic kinetics of different promoters, ppGpp and DksA could regulate transcription on a global scale. The main kinetic properties that would define whether ppGpp and DksA have positive or negative effects on transcription would be the initial binding constant for RNAP and the dissociation rate of the competitor-resistant complex. The promoter sequences that define these kinetic properties are complex, which precludes identification of regulated promoters by sequence analysis alone⁶. Figure reproduced, with permission, from REF. 108 (2005) National

The features that contribute to the short half-life of the rRNA promoter complex with RNAP include a suboptimal -35 element, suboptimal spacing between the -10 and -35 hexamers, a suboptimal extended -10 element and a suboptimal discriminator sequence⁶. The UP element interaction with α CTD that recruits RNAP to the promoter does not contribute to the stability of the competitor-resistant complex. Therefore, the UP element has no effect on regulation by ppGpp and DksA^{6,7}. Conversely, ppGpp and DksA probably have no effect on initial recruitment of RNAP to the rRNA promoter^{6,7,108}. Separation of recruitment from the regulation functions of the promoter allows for finely tuned rates of ribosome synthesis even under conditions of high promoter occupancy.

High-resolution structures of ppGpp and DksA complexed with RNAP would greatly facilitate our understanding of their mechanisms of action. An X-ray crystal structure of

ppGpp bound to RNAP holoenzyme from *Thermus thermophilus*¹¹³ revealed that ppGpp bound to a site that overlapped the so-called entry site for NTPs (the E site), as defined in the yeast RNA polymerase II elongation complex¹¹⁴. However, extensive biochemical and genetic analysis of the analogous site in *E. coli* RNAP, and the finding that ppGpp does not inhibit *T. thermophilus* RNAP *in vitro* or *in vivo* strongly suggest that this site is not responsible for the biologically significant effects of ppGpp on transcription initiation^{115,116}.

RNAP preparations that lack the ω subunit are unable to respond to ppGpp *in vitro*^{117,118}. The basis for the observed ω requirement in ppGpp function is not yet understood, but ω is not located near the E site of RNAP.

An X-ray crystal structure of *E. coli* DksA has also been solved¹⁰⁷, although there is no RNAP–DksA co-crystal. The structure of DksA is reminiscent of that of GreA and GreB, bacterial transcription elongation factors that prevent transcription-elongation arrest and facilitate promoter escape^{119–121}. Similar to the Gre factors, DksA has a globular domain and a coiled-coil domain with an acidic tip and binds in the RNAP secondary channel^{107,122}.

Exactly how DksA binding to RNAP leads to its effects on the lifetime of the promoter complex and on transcription initiation remains unclear. The proposal that the acidic residues at the tip of the DksA coiled-coil coordinate an Mg^{2+} ion bound to ppGpp¹⁰⁷ requires re-evaluation if ppGpp functions elsewhere on RNAP¹¹⁵. Furthermore, this model does not address the mechanism that is responsible for the effects of DksA that are observed in the absence of ppGpp^{105,106,123,124} (discussed below).

Positive control of transcription by ppGpp and DksA

ppGpp and DksA work together to increase transcription from many promoters in response to nutrient limitation or other stresses. Some of these promoters are probably activated indirectly — that is, by the increase in RNAP concentration that results from the liberation of RNAP from rRNA operons (rRNA transcription represents the bulk of cellular transcription at high cellular growth rates)^{125,126}. Consistent with the indirect (or passive) model, positively regulated promoters that have been found to be activated indirectly have low affinity for RNAP and require high concentrations of RNAP for transcription *in vitro*¹²⁶. Furthermore, they make long-lived complexes with RNAP, which accounts for their insensitivity to direct inhibition by ppGpp and DksA^{102,126}. ppGpp and DksA also seem to indirectly regulate some promoters that are transcribed by other RNAP holoenzymes: for example, $E\sigma^{54}$ and $E\sigma^S$ (REFS 127,128). To explain the stimulatory effect of ppGpp on transcription from certain promoters that are dependent on alternative σ factors, it has also been suggested that ppGpp might directly facilitate the competition of alternative σ factors for core RNAP. However, to our knowledge, there is no evidence that ppGpp directly decreases the affinity of σ^{70} for core RNAP or directly increases the affinities of alternative σ factors for core RNAP (discussed in REF. 129).

Various promoters are also activated directly by ppGpp and DksA (BOX 3). These include the promoters for several amino acid biosynthesis operons¹⁰⁸, promoters found in LEE (locus of enterocyte effacement) pathogenesis islands in pathogenic *E. coli* isolates¹⁰⁹, some $E\sigma^E$ -dependent promoters¹¹⁰ and the promoter for the global regulator Hfq¹¹¹. Unsurprisingly, mutations in *relA* and *spoT* (which code for the enzymes that synthesize ppGpp) and mutations in *dksA* are pleiotropic.

NTP concentration and the control of transcription initiation

All promoters require higher concentrations of the iNTP than the subsequent NTPs, but certain promoters — for example, rRNA, some tRNA promoters^{7,103,130} and the *fis* promoter¹³¹ — require even higher concentrations of the iNTP than promoters in general. In

extended stationary phase, depletion of NTPs therefore preferentially inhibits these promoters even though little or no ppGpp is present^{103,131}. The rapid increase in NTP concentration is responsible for the increase in rRNA transcription that occurs when cells emerge from stationary phase¹⁰³.

The mechanism by which changes in the iNTP concentration selectively affect rRNA synthesis has been debated. The transient stabilization of the intrinsically short-lived open complex by pairing of the first NTP (and subsequent NTPs) with the template strand may be sufficient to stimulate the initiation reaction by mass action^{7,102,130}. Alternatively, it was proposed that binding of the first two NTPs results in a conformational change in RNAP and that this conformational change promotes transcript initiation independently of its role in stabilization of promoter–RNAP interactions¹³².

DksA amplifies the effects of low iNTP concentration on promoters that make intrinsically short-lived complexes with RNAP by increasing the probability of DNA-strand collapse before incorporation of the initial NTPs^{7,106}. ppGpp-independent effects of DksA that have been observed on promoters *in vivo*^{123,124} might be attributed to the effects of promoter-complex lifetime on the iNTP concentration requirement. In addition, high DksA concentrations could increase the iNTP requirement by partially occluding the secondary channel, thereby reducing the rate of NTP diffusion to the active site^{7,105,106}. It is also possible that there are times when regulation by DksA *in vivo* works independently of changes in ppGpp or NTP concentrations^{123,124}.

The absence of a requirement for promoter sequence-specific DNA-binding proteins in regulation by ppGpp and iNTP concentrations suggests that the regulatory purposes of these factors evolved early in the history of life and therefore might occur in bacterial species that are evolutionarily distant from *E. coli*. Consistent with this hypothesis, changes in ppGpp and iNTP concentrations regulate rRNA promoters in *B. subtilis*¹³³. However, ppGpp does not inhibit *B. subtilis* rRNA promoters directly. Rather, ppGpp reduces GTP levels and, because all *B. subtilis* rRNA promoters initiate with GTP, this reduction in GTP levels decreases rRNA promoter activity. Also consistent with this model is the fact that changing the identity of the base at position +1 in *B. subtilis* rRNA promoters results in a loss of regulation by ppGpp¹³³. By contrast, *E. coli* rRNA transcription can begin with ATP, GTP or cytosine 5'-triphosphate (CTP) and still be regulated by ppGpp^{7,103,130}. ppGpp probably reduces *B. subtilis* GTP pools in two ways: GTP is consumed in making ppGpp and ppGpp directly inhibits an enzyme in the GTP biosynthetic pathway^{97,133}. Thus, *E. coli* and *B. subtilis* use the same signalling molecules to solve the same regulatory problem, but use different mechanisms.

Elegant systems also exist for the control of nucleotide biosynthesis operons in which NTP concentrations regulate gene expression. However, most of these mechanisms differ from those that control rRNA promoter activity. For example, in the *E. coli* *pyrBI* and *carAB* operons, an increase in the concentration of uridine 5'-triphosphate (UTP) results in reiterative transcription (which occurs when the initial transcript slips on the DNA template and non-coded nucleotides are added to the 3' end of the RNA). High UTP levels lead to reiterative transcription and transcript release, which results in feedback inhibition of promoter activity^{134,135}. *pyrBI* is also regulated at the level of transcription initiation by ppGpp and at the level of transcription elongation by UTP concentration-dependent attenuation^{104,134}.

UTP concentrations also control transcription at the *codBA* and *upp* promoters. When UTP concentrations are high, transcripts initiate with ATP and form weak RNA–DNA hybrids that are more likely to undergo reiterative transcription, which leads to transcript release and

decreased expression. When UTP concentrations are low, transcripts initiate with GTP and form stronger RNA–DNA hybrids that are less likely to undergo reiterative transcription^{136,137}.

At the *pyrC* promoter, high CTP concentrations lead to initiation at a cytosine residue seven nucleotides from the end of the –10 hexamer, whereas low CTP concentration leads to initiation with GTP two nucleotides further downstream. Only the CTP-initiated transcript forms a hairpin that occludes ribosome binding¹³⁸.

Changes in NTP concentration also seem to be crucial for regulation by the ~180-nucleotide 6S non-coding RNA¹³⁹. Small RNAs regulate gene expression in all three biological kingdoms, but usually after the transcription-initiation step. However, 6S RNA is a promoter-specific inhibitor of transcription initiation that is found in a wide range of bacteria. 6S RNA binds stably to $E\sigma^{70}$ without binding to DNA, thereby competing with promoters for RNAP by mimicking a transcription bubble. Furthermore, $E\sigma^{70}$ can initiate RNA synthesis using 6S RNA as a template when the NTP concentration is high¹⁴⁰. Because bound RNAP is released upon RNA synthesis, it has been proposed that regulation by 6S is achieved through changes in NTP concentration: when NTP concentrations are low (for example, during extended stationary phase), 6S traps RNAP, but when NTP concentrations rise during outgrowth from stationary phase, RNAP is released and is free to bind to promoters^{103,139,140}.

Exactly how 6S RNA inhibits only some promoters is not fully understood¹⁴¹. Presumably, specific promoters have different affinities compared with 6S RNA for parts of σ^{70} (including region 4.2 (REF. 141)), and different rates of initiation of RNA synthesis from specific promoters and 6S RNA determine the magnitude of inhibition of specific promoters by 6S RNA. In any case, as in the examples described above, the intrinsic kinetic properties of the core promoter, as determined by its DNA sequence, determine its susceptibility to regulation by 6S RNA.

Osmoregulation

High external osmolarity increases cytoplasmic potassium glutamate and other organic anion concentrations by reducing the amount of cytoplasmic water. Bacteria have evolved elaborate strategies to deal with the high salt concentrations that can occur in their natural environments. At most promoters, the stability of open complexes, coupled with the accumulation of osmoprotectants and increased macromolecular crowding, buffer the RNAP–promoter interaction against the potential inhibitory effects of high external osmolarity (reviewed in REF. 142). However, rRNA promoters are dramatically inhibited when the salt concentration in *E. coli* cultures is increased¹⁴³. The mechanism that is responsible has not been elucidated, but ppGpp does not seem to have a role in this response¹⁴³. Because rRNA promoters are also especially sensitive to increased salt concentration *in vitro*^{144,145}, it seems likely that the short lifetime and low level of RP_O occupancy of the rRNA promoter complex results in its inhibition by high osmolarity *in vivo*. This inhibition of rRNA promoters by high osmolarity seems to be another situation in which the intrinsic kinetic characteristics of the rRNA promoter complex result in its specific regulation by environmental or nutritional signals.

Conclusions and future perspectives

The textbook description, in which bacterial promoter recognition resulted entirely from interactions between the –10 and –35 hexamers and the σ subunit of RNAP, has evolved in recent years with the discoveries of additional promoter elements and their interacting partners in RNAP. Likewise, the traditional view that the regulation of transcription

initiation results primarily from activators and repressors that bind to specific DNA sequences has also been amended. We now know that regulators of transcription initiation can achieve specificity, in some cases, by binding directly to RNAP without binding to DNA and by exploiting the kinetic variation that ultimately results from differences in promoter sequences. We are only now beginning to develop an appreciation for the complexity of basal promoter activity and its role in transcription regulation in bacteria. The role of regulation of basal promoter activity should be a fertile area for research on the control of archaeal and eukaryotic gene expression in the future.

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Glossary

Template strand	The strand of DNA that enters the active site of RNA polymerase and is used as a guide for RNA synthesis. +1 is defined as the position where the template strand pairs with the nucleoside 5'-triphosphate that forms the 5' end of the transcript. The RNA transcript is the reverse complement of the template strand and has the same sequence as the non-template strand
Footprinting	A biochemical assay for detecting protein binding sites on DNA. A protein is allowed to bind to end-labelled DNA, the DNA is subjected to limited enzymatic or chemical nuclease cleavage and DNA fragments are separated by polyacrylamide electrophoresis under conditions that allow single-nucleotide resolution
Crosslinking	A biochemical technique for identifying interactions between macromolecules. Typically, covalent bonds between macromolecules are induced by ultraviolet or chemical exposure
Promoter clearance	The step in transcription in which RNAP breaks its interactions with the promoter and begins productive RNA synthesis

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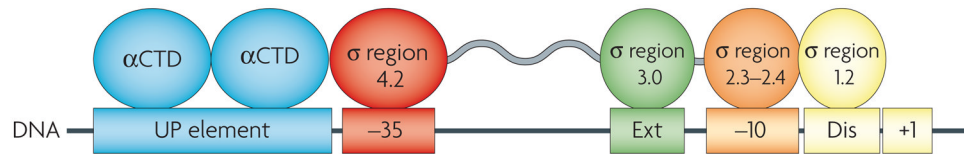


Figure 1. DNA elements and RNAP modules that contribute to promoter recognition by $E\sigma^{70}$

The optimal UP element (α binding site) is an alternating series of A and T tracts (-57 to -38; 5'-AAAWWTWTTTTNNNAAANN-3'; W = A or T; and N = any base)³⁷. -35 refers to the -35 element, consensus sequence 5'-TTGACA-3' from -35 to -30 (REF. 1). Ext refers to the extended -10 element, consensus sequence 5'-TGTG-3' from -17 to -14 (REF. 50). -10 refers to the -10 element, consensus sequence 5'-TATAAT-3' from -12 to -7 (REFS 1,2). Dis refers to the discriminator region, optimal sequence 5'-GGG-3' from -6 to -4 (REFS 6,32,68). +1 is the transcription start site. The segments of the σ and α subunits of RNA polymerase (RNAP) that interact with these elements are described in the main text and REFS 1,2,6,37. α CTD, α carboxy-terminal domain.

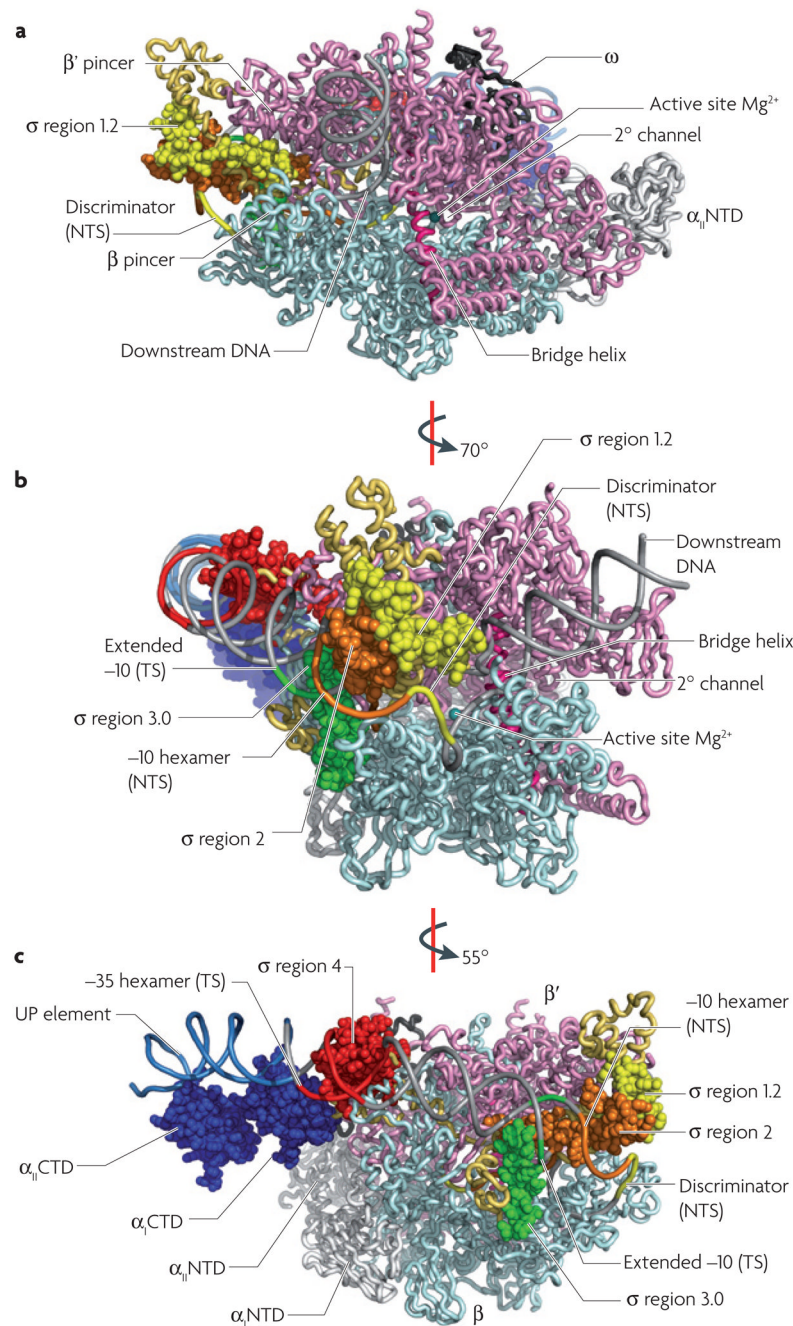


Figure 2. Models of the open complex

a, b | Promoter elements and the regions of $E\sigma^{70}$ RNA polymerase (RNAP) that recognize promoter elements, the secondary channel and some other features of the enzyme are labelled. Segments of RNAP that bind to promoters are shown in space-fill, and ribbon representations of the DNA sections that are bound by these parts of RNAP are represented by matching colours (β , blue; β' , pink; α amino-terminal domain (α NTD), light grey; and ω , dark grey). The structures are adapted from the model of the open complex¹⁹, which is based on the crystal structure of the fork-junction complex¹⁴. The paths of the individual strands downstream from the -10 hexamer are based on modelling and biochemical studies^{17,19}, as DNA downstream of the -10 element is not present in the fork-junction

complex. ϵ | α carboxy-terminal domains (α CTDs) and upstream DNA are included. The α CTD structure is from REF. 40. The positions of the α CTDs and the path of the DNA upstream of the -35 hexamer are based on modelling and biochemical studies^{19,30}. Region 1.1 of σ was not resolved in the structure, but would be outside the main channel in the open complex²¹. NTS, non-transcribed strand; TS, transcribed strand. Coordinates for the model from REF. 19 were courtesy of R. Ebright and C. Lawson, Rutgers University, New Jersey, USA.

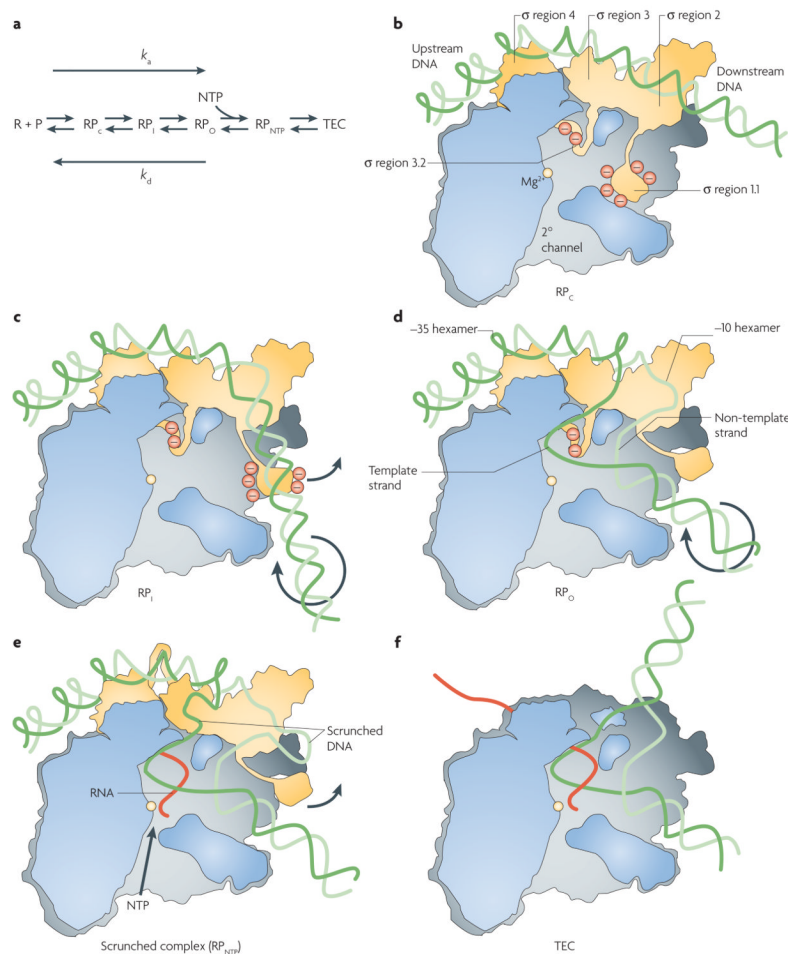


Figure 3. Steps in transcription initiation

In **a**, RP_C refers to the earliest promoter complex with RNA polymerase (RNAP), although this complex is not always kinetically detectable at all promoters. RP_O refers to the final complex before nucleoside 5'-triphosphate occupancy of RNAP. RP_I is used as an abbreviation for all the intermediates between RP_C and RP_O . The structures of these intermediates remain to be defined and could even differ at different promoters. k_a is the composite association-rate constant for RP_O formation. k_d is the composite dissociation constant. Individual steps are shown in **b–f** (REFS 15,30), and are described in more detail in the main text. The template DNA strand is in dark green; the non-template DNA strand is in light green; and the RNA transcript that emerges from the RNA channel is in red. The catalytic Mg^{2+} ion in the active site is indicated by a yellow sphere. Red circles represent acidic residues in regions 1.1 and 3.2, which are mobile modules in σ^{70} . In RP_C (**b**), the DNA is double stranded and downstream DNA has not moved into the main channel. In RP_I (**c**), σ region 1.1 begins to move out of, and downstream DNA begins to move into, the main channel. This probably occurs concurrent with DNA-strand separation^{22,27}, but the intermediates (RP_I) remain to be determined. In RP_O (**d**), the DNA strands have separated, with the template strand moving into position for base pairing with the first nucleoside 5'-triphosphate (NTP). In the 'scrunched complex' (REFS 34,35) (**e**), RNA synthesis has begun, and the DNA strands in or downstream of the -10 hexamer are temporarily 'extruded' out of their respective channels before promoter clearance. An NTP is shown entering the enzyme through the secondary channel. In the transcription elongation complex

(TEC) (**f**), σ has dissociated from core RNAP. Figure adapted, with permission, from REF. 15 (2003) Elsevier Science.