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Transcription activation in *Escherichia coli* and *Salmonella*

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SUMMARY

Promoter-specific activation of transcript initiation provides an important regulatory device in *Escherichia coli* and *Salmonella*. Here, we describe the different mechanisms that operate, focussing on how they have evolved to manage the 'housekeeping' bacterial transcription machinery. Some mechanisms involve assisting the bacterial DNA-dependent RNA polymerase, or replacing or remodelling one of its subunits. Others are directed to chromosomal DNA, improving promoter function or relieving repression. We discuss how different activators work together at promoters, and how the present complex network of transcription factors evolved.

Introduction

Changes in the expression of individual genes are essential for bacteria to cope with fluctuating environments, and *Escherichia coli* and *Salmonella* are 'virtuosos' in gene regulation. In fact, gene expression is regulated at many different levels, but here we focus solely on the activation of gene transcription at promoters. Promoters are defined as segments of genomic DNA that direct transcript initiation at a defined location. Each promoter will contain one or more key sequence elements that are recognised by the DNA-dependent RNA polymerase (RNAP) (Fig. 1). Many bacterial promoters are subject to regulation by activation, and several strategies are used, with regulatory factors primarily interacting either with specific promoter DNA elements (promoter-centric regulation) or with the DNA-dependent RNA polymerase (RNAP-centric regulation) (1-3). Mechanisms to activate the expression of specific transcripts have evolved to exploit the 'hardware' that assures transcription, so, firstly, here, we briefly outline the molecular biology of bacterial transcript initiation and its context, before describing different regulatory mechanisms.

Transcript initiation: the key role of sigma (σ)

Transcription of DNA into RNA in all bacteria is done by a highly-conserved multi-subunit DNA-dependent RNA polymerase enzyme (RNAP), which consists of two large subunits, denoted β and β', two α subunits and the small ω subunit (4). Each α subunit comprises two domains; the larger N-terminal domain (αNTD) dimerises to provide the socle for the assembly of the other subunits. The enzyme active site, located in a cleft between the β and β' subunits, is organised to carry single-stranded DNA that acts as a template for incoming nucleoside triphosphates (NTPs). During transcript elongation, RNAP catalyses the formation of a phosphodiester bond between the 3'-OH of the 3'-end nucleotide of the nascent RNA chain in the enzyme product site and the incoming NTP in the acceptor site. This requires local unwinding of 12-15 base pairs of the duplex DNA double helix, in order for the single stranded template-strand DNA to be routed into the active site. This transcription 'bubble' and the β-β'-α_2-ω form of RNAP (known as the core enzyme) translocate efficiently along the DNA during the elongation phase of transcription, powered by the incoming NTPs (and helped by various elongation factors). RNAP core enzyme can also catalyse the initiation of a transcript by attaching an incoming NTP in the acceptor site to the 3'-OH of an NTP or a short oligonucleotide, bound in the product site, with

both initiating entities specified by base-pairing to the DNA template strand. However, transcript initiation by core RNAP is very inefficient, simply because it is not equipped to create the local DNA unwinding that is essential for a single-stranded DNA template to access the RNAP active site and, in addition, it is unable to select locations for transcription to begin (4-6).

Comparison of multisubunit RNAPs from the three kingdoms of life reveals a common enzymatic mechanism for DNA-templated RNA synthesis, and eukaryotic and archaeal RNAPs contain subunits that are highly similar to the bacterial β, β', α, and ω subunits (7). In contrast, different strategies are adopted by RNAP in each of the three kingdoms to facilitate transcript initiation: in bacteria, an additional RNAP subunit, σ, assures this function by recognising specific sequence elements at promoters and then opening the DNA duplex to create the transcription bubble, so that the single stranded DNA template is guided into the RNAP active site (Fig. 1) (8). Core RNAP carries determinants on the β and β'subunits that provide the docking site for just one σ subunit (9, 10): bacterial RNAP associated with a σ factor is known as holo enzyme, and is competent for transcript initiation at locations specified by its σ factor.

Escherichia coli and *Salmonella* chromosomes carry genes for seven different σ factors (denoted *rpoD*, *rpoS*, *rpoH*, *rpoE*, *rpoF*, *fecI* and *rpoN* from studies with *E. coli* lab strains). In most growth conditions, σ^{70}, encoded by *rpoD*, is the predominant factor, known as the housekeeping σ factor, which orchestrates transcript initiation at the majority of promoters. Housekeeping σ factors always contain four independently folding domains (known as Domains 1, 2, 3 and 4), each of which has a discrete function (Fig. 1) (11). Domain 2, found in all σ^{70}-family σ factors, is directly responsible for the formation of the transcription bubble, and Domain 1 acts as a gatekeeper, preventing entry of DNA into the active site cleft (6). Domains 4 and 3 are essentially promoter binding modules that recognise specific sequence elements in promoter DNA, thereby positioning holo RNAP for transcript initiation. Key bases in the promoter -35 element and promoter extended -10 element are recognised by σ Domains 4 and 3 respectively, and this positions Domain 2 for interaction with the upstream end of the -10 hexamer element (consensus 5'-TATAAT-3') (Fig. 1).

The engagement of holo RNAP with a promoter starts a series of isomerisations leading to the formation of a transcriptionally-competent RNAP-promoter complex, known as the 'open' complex, as the DNA around the transcript start point (usually referred to as position +1) is unwound (Fig. 1) (12, 13). A stepwise process then creates the transcription bubble by opening 12-15 base pairs, displacing σ Domain 1 from the active site cleft, and facilitating the entry of the template strand into the enzyme active site. DNA unwinding by σ Domain 2 starts precisely at the A:T base pair at position 2 of the -10 element, but appears to be initiated by transient distortion of the T:A base pair at position 1 (13). A major driver of stepwise DNA unwinding is the deployment of several conserved tryptophan residues in σ Domain 2 that trap the unwound non-template strand A at position 2 in a flipped conformation in a binding pocket, and also provide a wedge to trap the upstream end of the bubble. Other σ Domain 2 determinants interact with non-template bases at positions 3-6 of the -10 element, as well as several bases immediately downstream, known as the

discriminator element (Fig. 1) (5, 13-15). The consequence of this is that the non-template strand is held firmly, whilst the template strand is free to enter the RNAP active site. Usually, the template bases 7 and 8 bases downstream from the promoter -10 hexamer element guide the choice of nucleotides, bound in the product and acceptor sites, for transcript initiation, but there is some flexibility (16, 17).

In most conditions, the number of potential binding targets for RNAP on the *E. coli* chromosome is far greater than the number of available RNAP holoenzyme molecules. As well as bona fide promoters, these targets include thousands of other sites where RNAP binds transiently without the formation of a transcriptionally-competent open complex (18-20). Efficient duplex opening by RNAP carrying the σ^{70} housekeeping factor at any target promoter depends on precise positioning of σ Domain 2 with respect to the -10 element, and this is assured by Domains 3 and 4 of σ binding to the extended -10 element and the -35 element, respectively (Fig. 1). Additionally, at some promoters, the C-terminal domain (αCTD) of one or both of the RNAP α subunits contributes to RNAP binding. These independently-folded αCTDs are linked to the corresponding αNTD by a flexible linker and they can interact with the DNA via the so-called α 265-determinant, which contains residue R265 that interacts with the DNA minor groove, with a preference for AT-tracts, known as UP-elements (Fig. 1) (21). These are found at some promoters just upstream of the -35 element, and they contribute to RNAP recruitment. Additionally, at some promoters the promoter-proximal UP-element is positioned so that the bound αCTD is immediately adjacent to σ Domain 4 (22). This results in a productive interaction involving a surface of αCTD known as the α 261-determinant (including α residue E261) and a surface of σ Domain 4 known as the 593-604 determinant (because of the σ^{70} residues involved). Hence, transcript initiation at any location will depend on the efficiency of 'capture' of RNAP, guided by the different promoter-RNAP interactions, and subsequent formation of a transcription bubble driven by engagement of σ Domain 2 (23). Note that efficient capture of RNAP at a promoter does not require input from every possible promoter element, and, as long as a certain number of RNAP-promoter interactions are assured, transcript initiation will proceed (23-25). For each promoter element that interacts with a determinant in RNAP during transcript initiation at a promoter, there is a consensus 'best binding' sequence, and promoters located in regulatory regions adjacent to functional genes appear to have evolved different combinations of promoter elements to assure appropriate levels of gene expression. However, deep sequencing of transcripts has now shown low levels of transcript initiation (known as spurious or pervasive transcription) driven by thousands of -10 elements that were, hitherto, thought to have no function (26, 27). Moreover, at many loci, the intrinsic symmetry of the -10 element leads to bidirectional transcription (28).

The *E. coli* housekeeping σ factor, σ^{70}, is capable of orchestrating transcript initiation by RNAP, as described above. However, in some bacterial clades, the housekeeping σ is insufficient, and other protein factors support its function. For example, some factors act as tethers, promoting σ binding to core RNAP, and, in some cases, directly interacting with the promoter DNA. Thus, though *E. coli* and *Salmonella* use a single protein subunit (σ) to 'solve' the problems of transcript initiation, other

bacteria adopt more complex solutions (29). This underscores that evolution does not always adopt what, to us, seems the simplest solution. Similarly, the majority of alternative σ factors appear to have evolved from the same ancestor as the housekeeping σ, all containing the crucial Domain 2, but, sometimes, lacking one or two of the other domains (11). These alternative σ factors can be assigned to different groups, depending on their domain structure. Hence members of one group carry just Domains 2 and 4, whilst members of other groups carry Domains 2, 3 and 4, or even all four domains. The DNA-binding domains of alternative σ factors carry different DNA-recognition determinants, thereby guiding RNAP to different promoter sequences. In each case, σ appears to have evolved to steer Domain 2 to the -10 element at target promoters, where it initiates local DNA unwinding and transcription bubble formation, holding the non-template strand, thereby moving the DNA template strand into the RNAP active site. The observed variation in σ domain structure shows that there are several different ways to manoeuvre Domain 2 to its target. However, most alternative σ factors employ a Domain 4, and the ubiquity of Domains 2 and 4 in different σ factors probably reflects that they contact the major RNAP core determinants for σ binding. Hence σ Domain 2 contacts a determinant in β'subunit, whilst σ Domain 4 contacts a determinant in β subunit. Note that all the above applies to the alternative sigma factors in *E. coli* and *Salmonella*, with the single exception of σ^{54}, encoded by *rpoN*, that differs in domain structure and competence to orchestrate formation of transcriptionally competent open complexes (30) (see below).

The current textbook view is that the strength of any bacterial promoter depends on a combination of different promoter element sequences that, together, direct RNAP to initiate transcription. However, this is an oversimplification because it assumes that, *in vivo*, all promoters are equally available to RNAP holoenzyme. The biggest driver of inequality is likely to be the ~1000-fold compaction required to fit 1 mm of DNA into a cell that is 1 μm in length. This compaction is managed by DNA supercoiling, and, also, a set of proteins known as nucleoid associated proteins (NAPs), that include H-NS, Fis, HU and IHF (integration host factor), whose primary task is to sculpture the bacterial chromosome by bending, wrapping and looping bacterial DNA. Many of these proteins are abundant with widespread binding across the genome, and this likely explains the >20-fold variation in transcriptional propensity across the *E. coli* genome with positions of high and low transcription (31-34). In addition, the activity of many promoters is affected by supercoiling and again, the local supercoiling regime depends on location. Note that DNA supercoiling and the abundance of NAPs vary according to conditions and, also, the binding of NAPs and local DNA supercoiling are connected, and hence variation of either can easily cause changes in patterns of gene expression (35-37). Other global factors that can influence bacterial promoter activities are fluctuations in the levels of nucleotides, variations in the levels of signalling molecules such as ppGpp (38), as well as epigenetic markers such as DNA methylation (39).

Overview of activation

The rationale for the evolution of bacterial transcription activatory circuits is to couple gene expression to environmental change (40). Hence the activity of the protein

factors involved is tightly regulated (Fig. 2). Mechanisms of regulation include the reversible binding of small ligands and covalent modification that can be either inhibitory or activatory, and often involves an independently folding regulatory domain. Alternatively, activatory factors can be sequestered by binding to other proteins, or by restriction to a particular location such as the inner membrane of the cell. In some cases, regulation is mediated by setting the cellular level of transcription activatory factors, and this can be driven either at the level of their biosynthesis or their degradation which, in turn, can be regulated by environmental factors (1, 23). Another key issue for DNA-binding activators is the number of competing binding sites on the bacterial chromosome compared to functional targets (41). Experimental approaches such as chromatin immunoprecipitation have shown that, for many factors, binding preferences for specific operator sequences are not as strong as previously supposed (42). Hence the effective functional concentration for any activator protein is highly buffered by competing sites and, in some cases, this competition is exploited to control activation.

E. coli holo RNAP containing housekeeping σ^{70} is fully competent for sequence-specific transcript initiation, with the potential activity of any promoter being set by the precise sequence of each promoter element, together with local factors such as supercoiling and NAP binding, as described in the previous section. Regulation can be mediated either by repressors or activator proteins that are targeted to certain promoters by DNA-binding modules that have evolved to recognise specific base sequences. However, whilst all promoters are susceptible to regulation by repression, regulation by activation can only be effective if the promoter has the potential to work better. Thus, activator-dependent promoters must be defective in some way, either due to some promoter elements being sub-optimal, or due to repression, either by specific repressors or by NAPs. There are many ways by which transcript initiation at specific promoters can be activated, and evolution has exploited these to manage beneficial changes in gene expression. We stress that activation mechanisms depend on the 'problem' faced by the RNAP at specific promoters. Hence, for RNAP σ^{70} holoenzyme at weaker promoters, the main problem is competition with other promoters, and so activators work by recruiting more RNAP. Some activators simply assist RNAP to engage, whilst others remodel or even replace RNAP domains or subunits (Fig. 2).

More complicated mechanisms are required at promoters where the problem is the formation or the stability of the open complex and these invariably require RNAP remodelling. Hence, for RNAP σ^{54} holoenzyme, because the pathway to open complex formation is blocked (see below), the principal action of activators is to remodel σ^{54} (30). In contrast, at promoters where the problem is repression, activators often simply act as anti-repressors (Fig. 2). Below we discuss each of the main types of activation found in *E. coli* and *Salmonella*, but we also highlight novel mechanisms found in other bacterial clades. Several themes are recurring, with activators targeting promoter DNA and/or RNAP and functioning by mechanisms involving assistance, remodelling, repositioning or replacement. We also consider how different activators can work together at target promoters, thereby coupling gene expression to two or more independent signals.

Activation by σ subunit replacement

Since the specificity of holo RNAP for promoters is mainly determined by its σ factor, alternative σ factors provide an easy route to channel RNAP to particular promoters (Fig. 2). For *E. coli*, in many nutrient-rich conditions, the activity of each of the five alternative σ^{70}-family members is kept low, either by regulating transcription of the cognate gene, inefficient translation, sequestration, or proteolysis (43). However, these circuits are wired so that the activity of specific alternative σ factors increases in response to particular signals, and this results in a number of core RNAP molecules being captured by the alternative σ (6, 19, 29, 44). These alternative σ factors carry Domain 2 and Domain 4 determinants that target different promoter sequences, and hence some promoters can only be served by a specific RNAP holoenzyme. High resolution structural analysis suggests that the initiation pathway orchestrated by most (if not all) σ^{70} -family factors is similar (45-47). Although originally, it was thought that each bacterial promoter was highly specific for a particular σ factor, it is now clear that, for many cases, there is substantial overlap (48, 49).

Efficient activation by an alternative σ requires displacement of the resident housekeeping σ in a sub-population of RNAP molecules. Since both housekeeping and alternative σ factors bind to the same core RNAP determinants, each RNAP can carry only one σ factor and so relative binding affinities are important (44). In some cases, the activity of an alternative σ factor is dependent on helpers such as *E. coli* Crl that tethers σ^{38}, encoded by *rpoS*, to RNAP (50). In parallel, Rsd binding to σ^{70}, reduces the activity of the competing housekeeping σ (51). Hence transcription activation mediated by an alternative σ may not only require a signal-dependent increase in the activity of that factor but also the induction of factors to facilitate σ exchange (52).

Activators that recruit RNAP via αCTD

Many activators bind to a specific DNA operator at target promoters and display a surface (known as an Activating Region) that interacts directly with αCTD, thereby assisting the recruitment of RNAP (Figs. 2 & 3). Such promoters are usually defective for one or more promoter elements, and the activator-αCTD contact compensates for the missing RNAP-promoter element interactions to recruit RNAP so that the σ subunit is positioned to open the transcription bubble (2). In many cases, the activator binds to an αCTD surface that is distinct from the DNA-interacting 265-determinant and this supports αCTD-promoter interactions (Fig. 3A) (53). In other cases, the activator binds to an αCTD surface that includes the 265-determinant, thereby ‘docking’ that αCTD and effectively changing its DNA-binding specificity (Fig. 3B) (54-56).

A well-understood example is the *E. coli* homodimeric cyclic AMP receptor protein (CRP, also known as CAP) that activates transcription by binding upstream from scores of different promoters. The activating surface of CRP (known as AR1) in the downstream subunit of the CRP dimer interacts directly with a surface of αCTD

(known as the 287-determinant) and this promotes interaction between the 265-determinant and promoter DNA, thereby supporting recruitment of RNAP (Fig. 3C) (57). Here, CRP and αCTD bind adjacent to each other on the same face of the promoter DNA, but for this interaction to be productive, the recruited RNAP must be correctly juxtaposed with respect to the different downstream promoter elements. Hence, activation is dependent on precise positioning of CRP, though the flexibility of the linker that connects αCTD to αNTD permits different architectures (53). The best studied case is the *E. coli lac* operon promoter where high resolution structural analysis shows AR1 of CRP interacting with the 287-determinant of a single αCTD that simultaneously interacts with promoter DNA via its 265-determinant and also with σ Domain 4 via its 261-determinant (Fig. 3C) (58, 59). A similar situation is seen with 'phage λ cII activator protein that also positions αCTD so that it can interact both with promoter DNA and with σ Domain 4 (Fig. 3D). However, in this case, two cII dimers bind on the opposite face of target promoter DNA from RNAP, but side chains in the upstream subunit make contact with a determinant in αCTD that includes α residue K271 (60). In all these cases, the activator-αCTD interaction assists RNAP, positioning it so that it can engage with the 'normal' transcript initiation pathway at the target promoter.

A different situation is found with other activators that bind to the αCTD surface that includes α R265. Hence *E. coli* SoxS and MarA, and *Salmonella* RamA, that can function as monomers, 're-educate' one of the RNAP αCTDs to a different DNA binding specificity, and this results in recruitment of RNAP to promoters carrying the operator sequence for each activator (54, 55, 61, 62). Here, essentially, the activator replaces one of the RNAP promoter binding modules and, apparently because of the α subunit inter-domain linker, many different organisations are possible (Figs. 3B & 3E). Hence, with SoxS, the activator can be oriented in either direction according to how its operator sequence (known as the Sox box) is positioned, and, whilst one αCTD is bound to SoxS, off the promoter DNA, the other αCTD can bind promoter DNA immediately upstream of the -35 region and interact with σ Domain 4 via its 261-determinant (61). It has been noted that, for SoxS, and similar activators that hold αCTD off the promoter DNA, the interacting surface is more extensive than for activators, like CRP, that hold αCTD on the promoter DNA. Hence activator binding to free RNAP is possible, underscoring that these activators work by altering the binding preferences of RNAP (56, 61). This mode of activation is known as 'prerecuitment', where a DNA-binding transcription factor binds first to RNAP holoenzyme, in contrast to 'recruitment', where the activator binds first to its operator at the target promoter (63, 64).

Activators that target RNAP σ Domain 4

For some activators, their primary target is σ Domain 4, and so their binding site abuts or overlaps a promoter -35 region. Some of these just assist σ Domain 4 binding to their target promoter -35 element, whilst others reposition or remodel the domain (Fig. 4A & 4B)(65). The simplest example is the 'phage λ cI regulator protein which activates the 'phage λ P_{RM} promoter after binding to a target immediately adjacent to the promoter -35 element. This facilitates an interaction between a surface-exposed cI activating region and a target in the σ Domain 4 593-604

determinant that assists promoter binding by σ Domain 4, and hence recruitment of RNAP and subsequent transcript initiation. Many bacterial transcription factors appear to work by this simple mechanism, whereby the activator occupies the position that would normally be taken by the promoter proximal αCTD (2). The consequence of this is that αCTD is displaced and, due to the flexible α subunit interdomain linker, has the possibility of making upstream interactions either with promoter DNA or with other transcription factors (as discussed later).

For some activators, their contact with σ Domain 4 results in its occlusion from the promoter -35 element, effectively replacing the binding specificity of σ Domain 4 with that of the operator for the activator (61, 66). An extreme case is that of 'phage T4 AsiA protein that binds and remodels σ^{70} Domain 4 such that it becomes susceptible to interactions with the T4 MotA transcription factor that is responsible for activating middle order transcription during the 'phage T4 infection cycle (Fig. 4C) (67, 68).

Surface-exposed activating regions are a key feature of transcription factors that activate by contacting specific RNAP determinants (53). Some transcription factors with a σ Domain 4-directed activating region also carry an activating region that can interact with αCTD. Such activators are referred to as 'ambidextrous' and can therefore activate by exploiting either RNAP target, depending on the promoter architecture (69, 70). This is the case for *E. coli* SoxS, MarA and Rob, and *Salmonella* RamA, and, when they bind close to target promoter -35 regions and interact with σ Domain 4, the 265-determinant in one of the RNAP αCTDs binds to the αCTD-directed activating region, and this contributes to RNAP recruitment (Fig. 4D) (61, 62, 66). Similarly, for some homodimeric members of the CRP family of transcription factors, activation is primarily driven by an activating region in the downstream subunit of the promoter-bound dimer interacting with the σ Domain 4 593-604 determinant, but AR1 in the upstream subunit interacts with the displaced αCTD that docks to the first available upstream minor groove on the same face of the promoter DNA (Fig. 4E) (71, 72). Interestingly, for CRP itself, when bound to DNA sites that overlap promoter -35 regions, the major contact made by the downstream subunit is with αNTD rather than σ Domain 4 (73). This underscores that interactions which recruit RNAP can be made with any convenient surface determinant, the key criterion being that RNAP is positioned so that σ Domain 2 can orchestrate transcript bubble opening and subsequent transcript initiation. Note that these simple adhesive contacts can sometimes stabilise RNAP-promoter intermediates that form after initial binding (74) (see Fig. 4E).

Activators that target promoter structure

Some transcription activator proteins alter promoter structure. This is the case for the action of MerR-family activators at target promoters where the spacing between the -10 and -35 elements is longer than the optimal 17 base pairs (Fig. 2) (75). Activators such as MerR, SoxR and CueR bind to operators located between the -10 and -35 elements, inducing a distortion that repositions the key promoter elements. Several high resolution structures show that this involves DNA bending triggered by distortion at specific base pairs that reduces the distance between the -10 and -35 elements, so that σ Domain 2 is then correctly positioned (76-78). In another

scenario, the activator binds upstream and alters the conformation of downstream DNA to promote transcript initiation, likely, this involves constraining negative supercoiling in promoter DNA. This was first reported for the *E. coli ilvG* promoter that is activated by upstream binding of IHF: the proposed working model is that IHF binding facilitates downstream promoter opening due to local increased negative supercoiling (79). Other promoters may be similarly induced by mechanisms involving alternative DNA structures such as G quadruplex formation (80, 81).

Activation by factors that interact with RNAP but not DNA

The secondary channel is an important feature in RNAP. It provides a direct pathway to the enzyme active site, thereby facilitating the supply of nucleoside triphosphate (NTP) precursors and access for certain elongation factors (82). In open complexes, the secondary channel is the target for a factor dubbed DksA and small nucleotides, collectively known as ppGpp (derived from GDP/GTP and ATP) that accumulate during growth arrest. Together, ppGpp-DksA destabilises open complexes at certain promoters, but promotes open complex formation at others (38, 83, 84). Both effects are thought to result from changes in the kinetics of different steps in the sequential process of transcription bubble opening and displacement of σ Domain 1 from the RNAP active site cleft. Whether the changes result in activation or repression is determined by the starting parameters at the target promoter (83). Remarkably, many bacteriophage and plasmids encode DksA paralogues that work in a similar way, but without the requirement for ppGpp (85, 86).

Activation by anti-repression

Initiation at many bacterial promoters is repressed by promoter-specific transcription factors (87). The paradigm is the *E. coli* lactose (*lac*) operon promoter where the *lac* operon repressor binds with high affinity to specific operator sequences at the promoter region, thereby hindering RNAP access. Activation follows ligand-dependent reduction in *lac* repressor binding: essentially, the ligand effector, which is often a metabolite, is the inducer of expression from the target promoter. However, the action of repressors can also be countered by activator proteins that behave as anti-repressors, simply by displacing the repressor (Fig. 2) (88).

In many cases, unlike with the *lac* operon repressor, repression is due to nucleoid associated proteins (33, 34, 89). A prime example is H-NS, that forms filaments that occlude promoters (90). Repression can be reversed by the targeted binding of specific transcription factors, usually in response to a signal, that disrupts the repression. For example, PhoP can function as a ‘disruptive counter-silencer’, though, at some target promoters, it activates directly by interacting with RNAP (91). Here activation is simply due to the unmasking of promoters, and this can be done with any targeted DNA binding protein, even the *lac* operon repressor (92). Alternatively, repression by H-NS filaments can be disrupted by the insertion of full-length or partial H-NS paralogues, forming heterodimers with H-NS (93).

Activation at promoters dependent on σ^{54}

The vast majority of bacterial σ factors share common features with σ^{70}. However, a small number are unrelated, and belong to the σ^{54} family, named after the *E. coli*

rpoN gene product, σ^{54}, that is structurally unrelated to σ^{70}. *E. coli* σ^{54} contains three functional regions (RI, RII and RIII) (30). Moreover, promoters recognised by holo RNAP carrying a σ^{54}-family σ subunit have a different organisation, with the key promoter elements located 12 and 24 base pairs upstream from the transcript starts (94). The -24 element at such promoters is recognised by a highly conserved domain known as the RpoN box that is located at the C-terminal end of RIII (Fig. 5Ai). In σ^{54}, the RpoN box is preceded by a structure known as the ELH-HTH, which is a 55 Å extra-long-helix running into a helix-turn-helix, and the HTH is responsible for recognition of the promoter -12 element and the initiation of DNA melting (95, 96). RNAP σ^{54}-family holoenzyme is competent for promoter recognition, but, unlike RNAP holoenzyme carrying a σ^{70}-family σ factor, is unable to drive formation of the fully open transcription bubble. Structural studies show that this is because σ^{54} Region I obstructs the ELH-HTH and, thus, DNA duplex opening (95, 96). Hence a supplementary factor is required and this is provided by the specialised AAA+ domain, found in a family of activatory transcription factors, known as enhancer-binding proteins (EBPs). AAA+ domains (ATPases Associated with a variety of cellular Activities) contribute to a variety of protein functions across all kingdoms of life and can couple ATP hydrolysis to protein remodelling (97). In EBPs, AAA+ domains have been recruited to implement ATP-driven reorganisation of RI of σ^{54}-family σ factors, so that a transcriptionally-competent RNAP-promoter complex with local DNA unwinding can form (98, 99). To do this, the active surface of the AAA+ domain, which contains protruding loops that interact with the σ^{54}-family σ factor, is delivered to the face of the holo RNAP that is engaging the promoter -12 element. To facilitate this, most EBPs bind as multimers to a target upstream of the promoter and the intervening DNA must be bent to facilitate the activator-RNAP interaction (100). At some promoters, the bending requires the intervention of one or more nucleoid-associated proteins, such as IHF (Fig. 5Ai) (101). Note that the fundamental mechanism of action of σ^{54}-family members differs from that of σ^{70}-family members, likely because it evolved independently to 'solve' the problems of transcript initiation. A consequence of this is that distinctive activation mechanisms are found at promoters served by RNAP with a σ^{54}-family σ subunit (94).

Integration of activatory signals at promoters

The variety of different activatory mechanisms has been exploited by evolution so that activation of a particular promoter can be coupled to two signals (102, 103). In some cases, activation can be assured by either one factor or another, but in many cases, activation is co-dependent on two factors, both of which are essential for full promoter activity. Hence, the promoter essentially acts as an integrator to couple different inputs to the output of transcript initiation.

Sometimes, σ factors are involved in signal integration, for example, at certain target promoters served by RNAP σ^{28} holoenzyme, CRP is required for optimal activity (104). At σ^{54}-dependent promoters, whilst the EBP facilitates open complex formation in response to one signal, sometimes a supplementary factor, often triggered by a second effector, is also required (Fig. 5Ai). Dependent on the promoter, this second factor may bend upstream DNA to facilitate 'delivery' of the

EBP to the RNAP σ^{54} holoenzyme, or interact with αCTD to promote the initial recruitment of σ^{54} holoenzyme (98).

In *E. coli*, the most common co-activation scenario is when the promoter is dependent on two activators that each contact a separate RNAP target surface independently (Fig. 5B). Because of the flexibility of the linker connecting αNTD and αCTD, in most of these cases, at least one of the activators makes a recruiting contact with αCTD, and, often, the other contacts σ Domain 4. Clearly, for co-dependence, the promoter must be organised so that either activator on its own is unable to do the job, and this is often due to non-optimal spacing of individual promoter elements or non-optimal binding sites for either of the factors (70, 102).

At most promoters that are co-dependent on two activators, the primary activator factor has a shortcoming. In some case, the primary factor binds at a location where it is unable to activate transcription, and the role of the second activator is to reposition it to a location where it can activate (Fig. 5Aii) (105). In other cases, the primary activator binds at the correct place, but its action is suppressed by repressor proteins, such as NAPs, so the role of the second activator is to relieve this repression (Fig. 5C) (106). Finally, at a small number of promoters, the binding of the primary activator at the target promoter requires direct interaction with the second activator (Fig. 5D) (107).

Transcription activation: the big picture

When RNAP σ^{70} holoenzyme encounters a bacterial promoter, the pathway to transcript initiation is certainly not simple, but very similar processes occur at other promoters, and it is easy to grasp the logic of the different transactions. The same can be said for promoter escape, transcript elongation and transcript termination, but, when it comes to regulation, we are faced with a bewildering array of different mechanisms, and it is not clear why, for any promoter, one is used rather than another. One way to appreciate this complexity is to consider an evolutionary pathway that begins with the simple stark fact that managing DNA and transcribing DNA are both absolutely essential for cellular life, whereas regulation is an optional extra, albeit a useful and desirable one, that appeared later. Hence, chromosomal DNA was compacted by a combination of NAPs and supercoiling, and core RNAP assured transcription, albeit inefficiently with little or no specificity. In this context, it is then easy to imagine how σ Domain 2 evolved to improve the efficiency of transcription bubble opening, how Domain 1 evolved to be the gatekeeper for template DNA entry into the active site, and how Domains 3 and 4 evolved to specify transcript starts to intergenic regions. The benefits of being able to initiate transcripts at specific locations are twofold. First it ensures that transcripts encode complete proteins rather than just fragments. Second, it opens the gate for regulation, and, given the importance of NAPs and the link between DNA compaction and transcription repression, it is plausible that the first bacterial attempts at regulation involved NAPs and relief of NAP-mediated repression at specific locations. This may explain why NAPs play such an important role in the current regulatory hierarchy (108, 109), and it has been argued that many bacterial transcription factors evolved from NAPs by increasing their DNA-binding selectivity and by the addition of

regulatory domains (110-112). The subsequent evolution of activating regions gave some of these proteins the ability to interact with and recruit RNAP to promoter DNA, resulting in the finely tuned activation that we see now at many promoters in *E. coli* and *Salmonella.* However, because each promoter is different, and each regulatory feature evolved separately as an add-on, there is no single model for activation. Key evidence for this is that remnants of the initial regime, such as pervasive transcription (26, 27), bidirectional transcription (28), and non-functional binding of transcription factors, remain, and, possibly, have been retained to provide 'fodder' for future evolution (113).

In this context, it is worth considering σ^{54} as different to other alternative RNAP σ factors, since its activity appears not to be subject to environment-sensitive modulation (18). Rather, transcript initiation at each σ^{54}- dependent promoter is activated by an EBP, most of which carry a regulatory domain, controlled either by ligand binding, covalent modification, or interaction with a protein partner, that modulates DNA binding and AAA-domain activity (98). Hence, we might regard σ^{54} as the product of a parallel attempt by evolution to facilitate transcription bubble opening at specific locations on bacterial chromosomes, and although it never succeeded to become a housekeeping σ, it has been retained, likely because it confers strong activator-dependence when incorporated into RNAP holoenzyme (114).

Transcription activation: what we can learn from other bacteria

Jacques Monod, the founding father of this research field, famously stated that what is true for *Escherichia coli* is true for elephants (115), but, given the view that regulation in general, and activation in particular, are add-ons to gene expression, restricting consideration to *E. coli* and *Salmonella* is short-sighted. Hence studies of transcription regulation in other bacterial clades have already suggested alternative activatory mechanisms that may or may not be operative in *E. coli* and *Salmonella* (29). A good example is to be found with transcription activators that interact with RNAP σ domain 2 (116). In Actinobacteria, the housekeeping σ factor is defective at many promoters and the CarD and RbpA proteins support Domain 2 function by tethering it to core RNAP, and by making complementary interactions with promoter DNA (117). Here, CarD and RbpA are more akin to being part of the RNAP holoenzyme than response-driven activators. In contrast, *Caulobacter* GcrA, which consists of a DNA binding domain flexibly linked to a σ-interacting domain, binds to a specific operator either upstream or downstream of target promoter -10 elements, such that the σ-interacting domain binds to and co-operates with the housekeeping σ factor Domain 2 to stabilise the open complex, making several direct interactions with certain bases (118). Here, as with CarD and RbpA, the activator can be considered as a helper for σ Domain 2. Similarly, outside of *E. coli* and *Salmonella*, clade-specific factors that cooperate with the RNAP α subunit are readily found (119-122). Monod's colleague, Francois Jacob, remarked that the process of evolution resembles the work of a tinkerer who "works with no specific end in mind, collecting any materials at his disposal, and rearranges them into a workable object" (123). Half a century after this comment, continuing studies of the different mechanisms by which transcript initiation can be activated reenforce this comment, and suggest that

there will be more to come, as we explore more genes and more genomes. Hence, for example, a recent study with the *Rhodobacter* GafA transcription factor showed that it targets the small ω subunit of RNAP (124). This underscores the significance of a previous report in which the *E. coli rpoZ* gene (encoding ω) was engineered so as to interact with an engineered 'phage λ cI regulator protein, creating an artificial 'tinkered' system, where transcription at a target promoter was activated by an 'arbitrary' protein-protein interaction (125).

Transcription activation: its biological role

A century ago, before the advent of molecular biology, it was known that the activity of many microbial enzymes changed substantially as microbial growth conditions altered. The biological significance of this was clear to all at the time, but the mechanism was not, and the working hypothesis was that the enzymes themselves adapted to their hosts' growth conditions (111). The great achievement of Jacob and Monod was to deduce that many adaptation processes are due to the regulation of transcription by promoter-targeted repressors (115), and, shortly afterwards, Ellis Englesberg showed that, for the *E. coli* arabinose operon genes, changes in expression were due to an activator, AraC (126). Following this, hundreds of transcription activators and repressors have been discovered in *E. coli* (127), with most of the research driven by the notion that the activity of each individual factor is triggered by a distinct environmental signal via the action of an effector (40). Hence, activators contribute to the ability of *E. coli* cells to adapt in order optimally to benefit from their environment. This comforting view dominates the literature, and yet it may not be the whole story since we know that, within bacterial populations, especially during infections, there is cell-to-cell variation in the expression of certain gene products, and this variation may ensure the survival of some in really hostile environments, such as a mammalian host. Whilst there are several possible sources of such variation (128, 129), one involves transcription activators, whose activity may not be coupled to any effector, but, rather, is subject to random cell-to-cell variation. For activators whose transcription depends on the activator itself via a feed-forward loop, this variation is accentuated, and can be a major source of cell-to-cell phenotypic variation (130, 131). In such cases, the bacterial community overall benefits from variation rather than uniformity. A good example can be found with AggR, a transcription activator protein that is directly responsible for activation of dozens of virulence genes in the enteroaggregative *E. coli* pathotype (132). Current models for its regulation suggest that its activity is controlled by a feed-forward loop, with dampening, such that, in any bacterial population, only a small proportion of cells are actually virulent (133), and this may well explain why many strains of this pathotype are harmless for many individuals (134). Such regulation 'by lottery' may be widespread for certain key bacterial activators, and adds a new dimension to our understanding of bacterial adaptation and the role of transcription activation.

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Figure Legends

Figure 1: Interactions between holo RNA polymerase and promoters leading to transcript initiation. Panel (A) shows the key promoter sequence elements: each element is denoted by a coloured rectangle positioned to indicate its location relative to position +1, the transcript start point. The sequence below each box denotes the consensus for *E. coli* σ^{70} holoenzyme. The labels -35, Ext, -10, Dis, and CRE, denote the promoter -35 hexamer element, the extended -10 element, the -10 hexamer element, the discriminator element, and the core recognition element, respectively. Panel (B) illustrates the interaction of parts of the holo RNAP with different promoter elements in the closed complex. RNAP is drawn as a brown oval with the α subunit N- and C-terminal domains shown as blue circles. The 4 independently folding domains of the housekeeping σ subunit are shown as purple-shaded ovals marked σ^1, σ^2, σ^3, and σ^4, located to indicate the interactions described in the text. Panel (C) illustrates the interaction of parts of the holo RNAP with different promoter elements in the open complex. Using the same convention as in (B), the figure shows the transcription bubble with the template strand (orange) held by the CRE, and the non-template strand (blue) held by σ Domain 2. The figure has been adapted from (111).

Figure 2: Regulation and roles of activatory transcription factors in bacteria. The left side of the figure notes that the function of an activatory factor (denoted by a yellow oval) can be regulated by (i) its level (set by its synthesis and/or its degradation), (ii) interaction with a protein partner, (iii) interaction with a ligand, (iv) sequestration to a location (*e.g.*, the cytoplasmic face of the bacterial inner membrane), or (v) covalent modification. The right side of the figure illustrates how the active form of the factor may function by (i) replacing an RNAP subunit (e.g. the housekeeping σ subunit is replaced by an alternative σ), (ii) assisting RNAP to follow the 'normal' pathway to transcript initiation, as in Fig. 1 (e.g. by promoting the interaction of αCTD with promoter DNA and σ), (iii) remodelling part of RNAP (e.g. by binding to the 265-determinant of αCTD, thereby changing its base sequence preferences), (iv) remodelling the promoter (e.g. by altering the juxtaposition between the promoter -10 and -35 elements), or (v) by removing a repressor (shown as a red oval bound at a target promoter). In each case, specificity is determined by recognition of target promoter sequence elements by the activatory factor. The figure is adapted from (102) and, for simplicity, just one αCTD is shown.

Figure 3: Activation by RNAP recruitment via αCTD. The upper line of the figure illustrates the two major strategies used by activators to recruit RNAP to target promoters via αCTD. The figure uses drawing conventions from Figs. 1 and 2, with functional interactions denoted by coloured dots, listed in the inset box. Panel (A) illustrates assistance in which an activator-αCTD interaction promotes αCTD binding to promoter DNA and contact with σ Domain 4. Panel (B) illustrates remodelling, in which the activator contacts the DNA-binding 265-determinant of αCTD, thereby altering its binding specificity. The lower line illustrates three specific examples: in

each case specificity is determined by an operator sequence that is targeted by the activator. Panel (C) illustrates activation of the *E. coli lac* operon promoter by CRP. An activating region (AR1) in the downstream subunit of the CRP dimer interacts with a determinant in αCTD (the 287-determinant), thereby promoting the interaction of the 265- and 261-determinants of αCTD with promoter DNA and σ Domain 4 respectively (58, 59). Here, for a productive interaction, CRP and αCTD must be bound to the same face of the DNA helix, and this can facilitate activation at other promoters by CRP bound at locations further upstream (73). Note that this type of activation is sometimes referred to as Class I activation (2). Panel (D) illustrates activation of the 'phage λ_{pRE} promoter by cII protein. An activating region in the upstream subunit of the cII tetramer interacts with the 271-determinant in αCTD, thereby promoting the interaction of the 265- and 261-determinants of αCTD with promoter DNA and σ Domain 4 respectively (60). Here, for a productive interaction, cII and αCTD must be bound to opposite faces of the DNA helix, and a similar arrangement is found at some promoters that are activated by members of the response-regulator family (135). Panel (E) illustrates activation of the *E. coli zwf* promoter by SoxS that makes interactions with the DNA-binding 265-determinant of one αCTD. A second activating region contacts the other αCTD, thereby promoting its interaction with promoter DNA and σ Domain 4 (61). Note that other arrangements can be found at different SoxS-activated promoters (61, 136).

Figure 4: Activation by targeting Domain 4 of the RNAP σ subunit. The upper line of the figure illustrates the two major strategies used by activators that target RNAP σ^4. Panel (A) illustrates assistance, in which an activator-σ^4 interaction promotes σ Domain 4 binding to the promoter -35 element, and, thereby open complex formation and transcript initiation: the activator binds to an operator sequence that abuts the promoter -35 element. Panel (B) illustrates remodelling, in which the activator contacts and repositions RNAP σ Domain 4. Essentially, the DNA binding specificity of σ Domain 4 is complemented by, or replaced with, that of the activator protein. The figure uses the same drawing style as Fig. 3 and functional interactions are denoted by coloured dots, listed in the inset box. The lower line illustrates three specific examples. Panel (C) illustrates activation at 'phage T4 middle promoters by the early phage-encoded AsiA and MotA proteins. Essentially, AsiA remodels host RNAP σ Domain 4 so that it becomes susceptible to activation by MotA (67, 68, 137, 138). Panel (D) illustrates activation of the *E. coli micF* promoter by SoxS that makes interactions with both the DNA-binding 265-determinant of one αCTD and with σ Domain 4 (61). The interactions, together, alter the binding preferences for RNAP in and upstream of the -35 region. Panel (E) illustrates activation of the *E. coli gal* operon P1 promoter by CRP: note that *gal*P1 is typical of the many *E. coli* promoters where CRP binds to a target that abuts the promoter -35 region (139). Here, αCTD is displaced and binds upstream, making a productive interaction with an activating region (AR1) in the upstream subunit of the CRP dimer. A second activating region (AR2) in the downstream CRP subunit interacts with a determinant in αNTD, whilst a third activating region (AR3) in the downstream CRP can contact σ Domain 4 (73, 140). Note that this type of activation

is sometimes referred to as Class II activation (2). During Class II CRP-dependent activation, AR2 is the predominant activating region whilst, for other CRP family members, such as FNR, AR3 is predominant (71-73), and different adhesive activator-RNAP interactions can stabilise different intermediates along the pathway to transcript initiation (72, 74).

Figure 5: Mechanisms of promoter co-dependence on two activatory factors. The figure shows illustrations of each of the known mechanisms whereby the activity of a bacterial promoter can be dependent on two activators, shown as rust-coloured and yellow ovals, denoted Activator 1 and Activator 2. The figure uses the same drawing style as Figs. 3 and 4, with some functional interactions denoted by coloured dots, listed in the inset box. Panel (A) illustrates mechanisms in which Activator 2 is needed to position Activator 1 at a location where it is functional for activation. (i) illustrates activation with RNAP σ^{54} holoenzyme. The atypical σ^{54} is illustrated as a series of tangerine-shaded ovals, labelled according to (96). RNAP σ^{54} holo enzyme contacts promoter -24 and -12 elements (pink rectangles) but is unable to proceed from the closed to open complex, as the activity of determinants in the ELH-HTH region is occluded by the RI domain. The ATP-driven action of an Enhancer Binding Protein (Activator 1) is required to relieve this blockage so that the transcription bubble can open (facilitated by ELH-HTH, see text). At some σ^{54}-dependent promoters, Activator 2 is required to assure the correct positioning of Activator 1, by bending the upstream DNA. Note that, in some cases, Activator 2 also assists with initial recruitment of RNAP σ^{54} holoenzyme by interacting with αCTD (141, 142). (ii) illustrates the original report of repositioning (105), where the binding of Activator 2 repositions Activator 1 from a location where it is unable to activate transcription to a location where it is able to activate transcription (in this case, Activator 2 is CRP and Activator 1 is MalT). Panel (B) illustrates co-dependence in which Activator 1 and Activator 2 bind independently to their targets and make independent but complementary contacts with different parts of RNAP σ^{70} holoenzyme. In the majority of such cases, as illustrated here, one activator contacts σ Domain 4, whilst the other contacts one of the displaced αCTDs, but there are some promoters where both activators bind further upstream and only contact αCTD (143). Panel (C) illustrates co-dependence in which a repressor blocks the function of Activator 1, and the role of Activator 2 is to stop the action of the repressor. This mechanism was discovered at the *E. coli nir* operon promoter, where FNR-dependent activation is suppressed by the repressive action of two NAPs, IHF and Fis, but repression is countered by the binding of NarL (106, 144). Panel (D) illustrates co-dependence in which the binding of one factor requires binding of the other and vice-versa. The scenario requires direct interaction between Activator 1 and Activator 2. Whilst infrequent in *E. coli* and *Salmonella*, direct interactions between different transcription factors are being discovered in other bacterial clades (145-147). The figure is adapted and redrawn from (102).

Biographies

Steve Busby (right) is currently Professor of Biochemistry at the University of Birmingham, U.K. His interest in bacterial promoters began whilst he was a postdoctoral research fellow at the Pasteur Institute in Paris and, since moving the Birmingham in 1983, he has focussed his research on both fundamental and applied aspects of bacterial gene regulation.

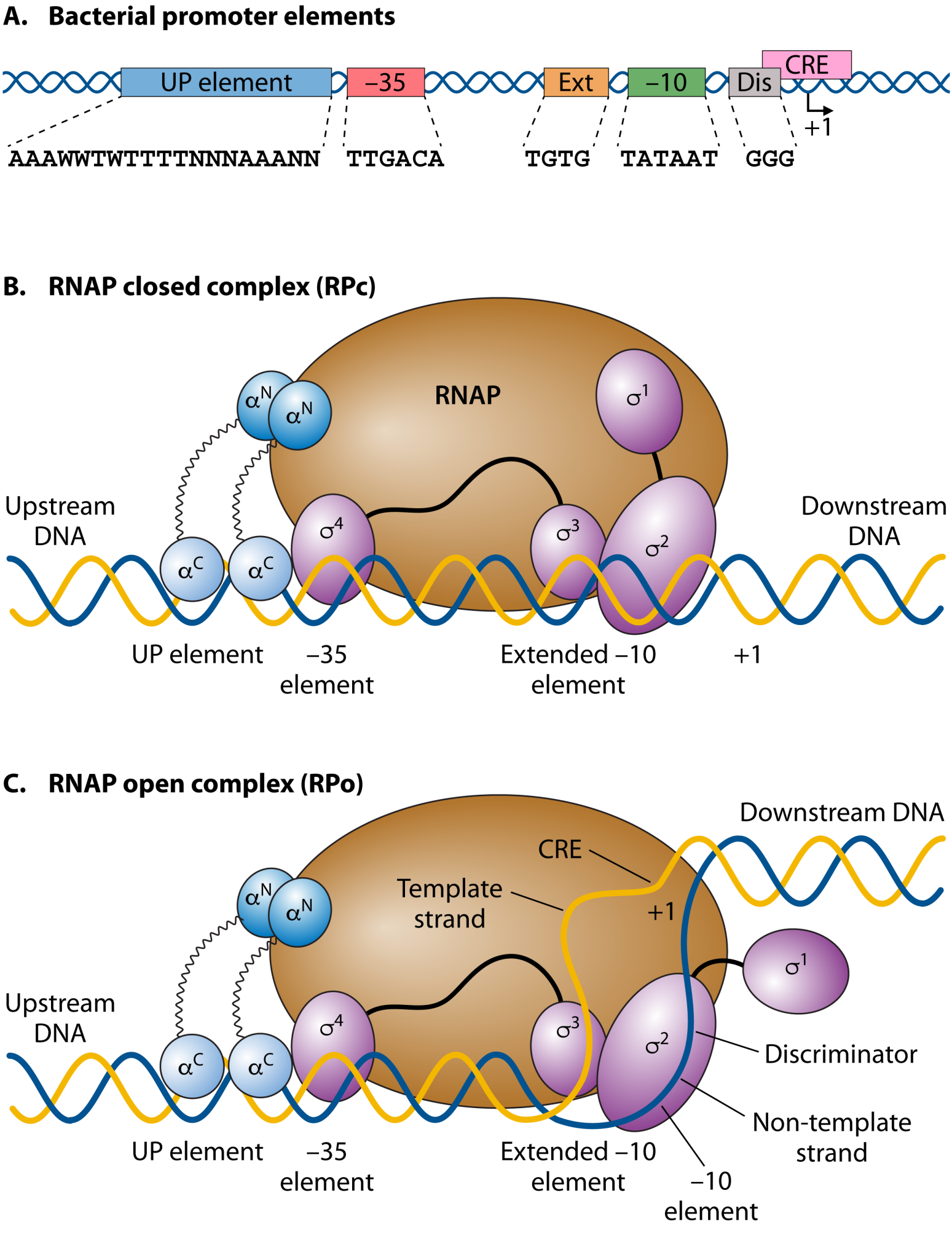
Doug Browning (left) is currently a Lecturer in Biosciences at Aston University in Birmingham, U.K. Doug's current research interests are in bacterial genomics, gene regulation, and outer membrane assembly, but, for many years, he worked with Steve Busby on how signals conveyed by different effectors are integrated at bacterial promoters.

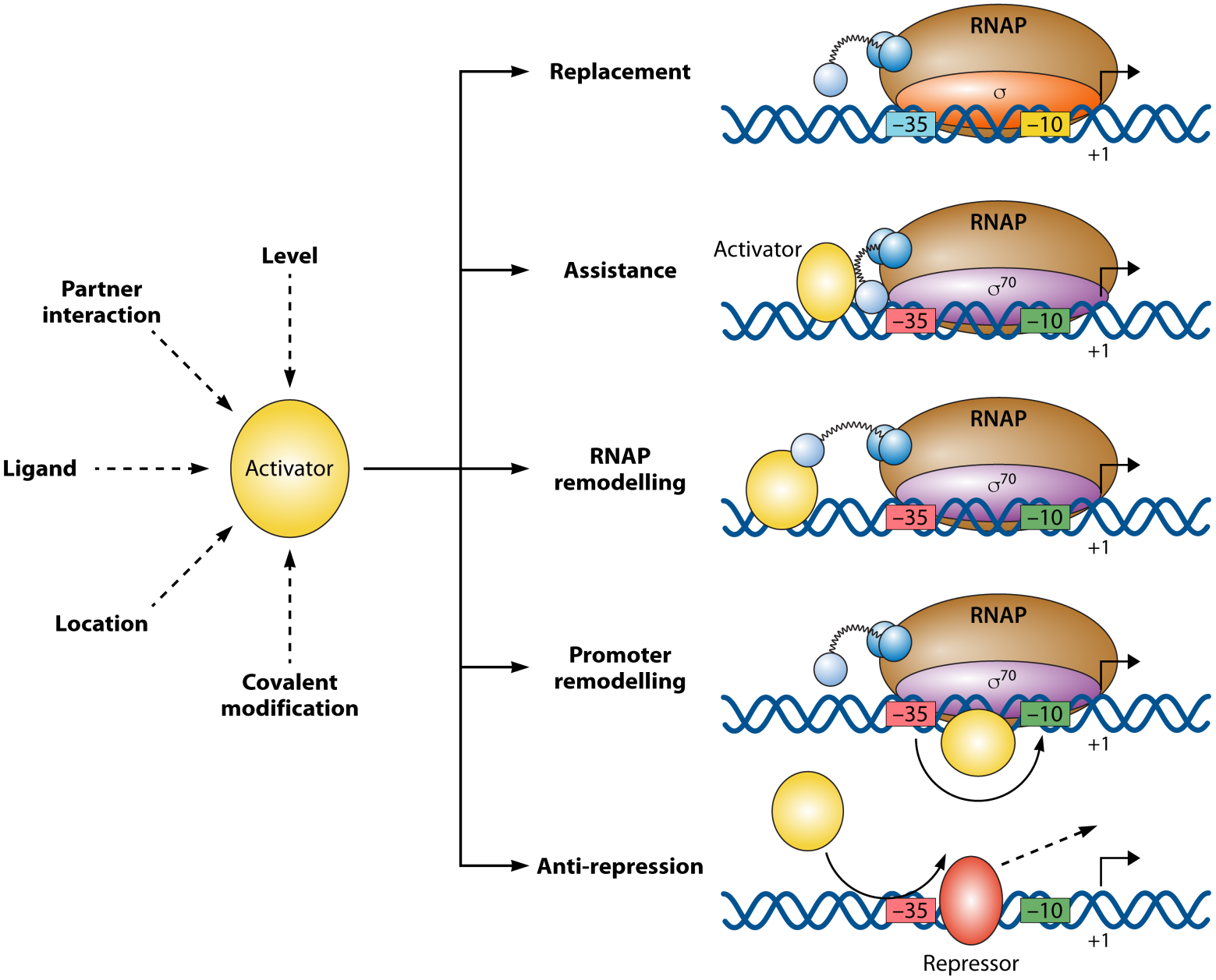
Appendix: list of relevant *E. coli* genes and EcoCyc accession numbers

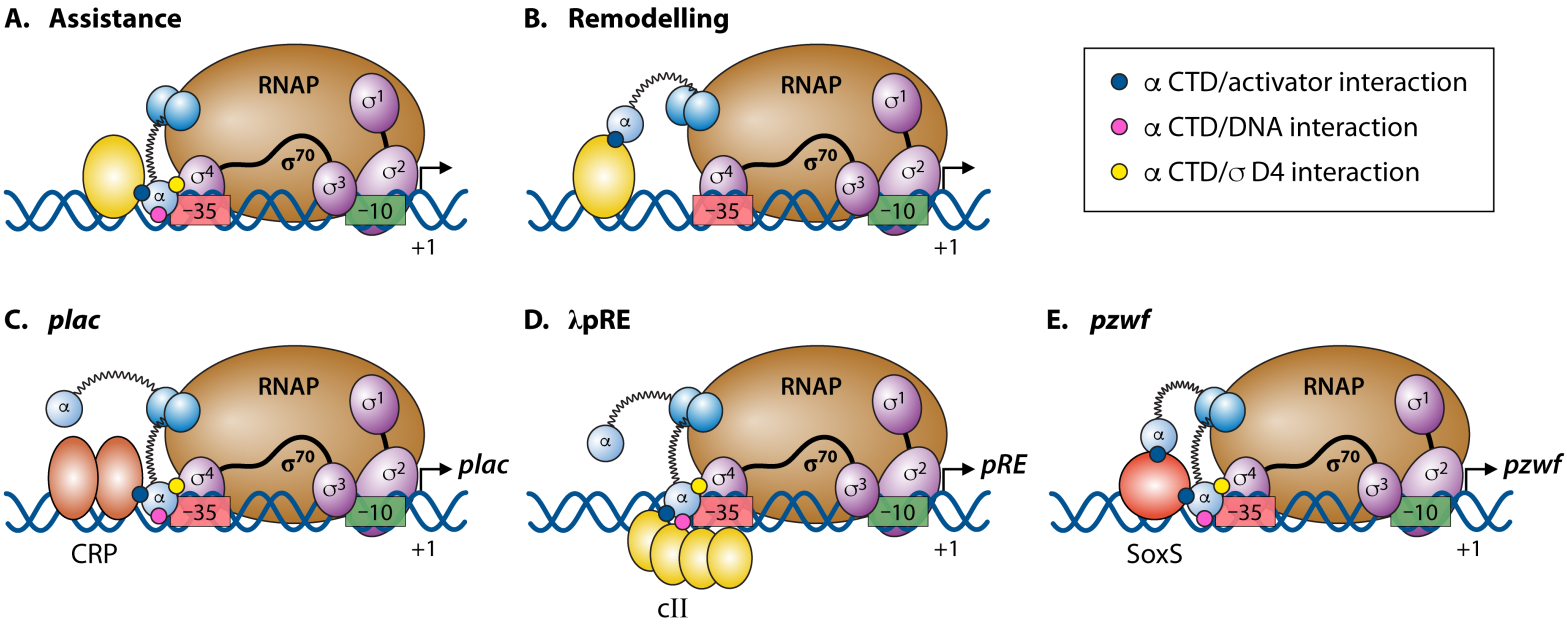
gene name	product	EcoCyc accession ID
RNA polymerase core subunits		
rpoA	DNA-dependent RNA polymerase α subunit	EG10893
rpoB	DNA-dependent RNA polymerase β subunit	EG10894
rpoC	DNA-dependent RNA polymerase β' subunit	EG10895
rpoZ	DNA-dependent RNA polymerase ω subunit	EG10899
RNA polymerase σ subunits		
rpoD	Major ('housekeeping') σ^{70} subunit	EG10896
rpoS	General stress/stationary phase σ^{38} subunit	EG10510
rpoH	Alternative σ^{32} subunit involved in heat shock	EG10897
rpoE	Alternative σ^{24} subunit involved in periplasmic stress	EG11897
rpoF	Alternative σ^{28} subunit involved in motility	EG11355
fecI	Alternative σ^{19} 'iron starvation' σ	EG10291
rpoN	Alternative non-canonical σ^{54} subunit	EG10898
RNA polymerase accessory proteins		
dksA	DksA RNAP-binding transcript initiation modulator	EG10230
crl	Promotes the activity of σ^{38} by acting as a tether	EG11092
rsd	Reduces the activity of 'housekeeping' σ^{70}	EG11738

Nucleoid-associated proteins		
fis	Nucleoid-associated DNA-bending protein	EG10317
hns	Nucleoid-structuring nucleoid-associated protein	EG10457
hupA & *hupB*	Subunits of HU, nucleoid-organising protein	EG10466 & EG10467
ihfA & *ihfB*	Integration host factors α & β subunits	EG10440 & EG10441
Transcription Factors		
lacI	Lactose utilisation operon repressor	EG10525
malT	Activator of maltose utilisation operons	EG10562
araC	Activator of arabinose utilisation operons	EG10054
phoP	Global response regulator	EG10731
narL	Global response regulator triggered by nitrate/nitrite	EG10643
crp	Cyclic AMP receptor protein: a global regulator	EG10164
fnr	Global transcription factor controlled by oxygen	EG10325
soxS	Regulator that controls oxidative stress responses	EG10958
marA	Regulator involved in diverse stress responses	EG11434
rob	Regulator with similar targets to SoxS and MarA	EG11366
soxR	Redox-regulated transcription factor	EG10957
cueR	Copper-triggered transcription regulator	G6263
Genes adjacent to exemplar regulatory targets		
lacZ	β -galactosidase enzyme	EG10527
galE	UDP glucose 4-epimerase	EG10362
zwf	NADP-coupled glucose-6-phosphate dehydrogenase	EG11221
micF	Small regulatory RNA	EG30063
ilvG	Enzyme in branched chain aminoacid biosynthesis	G8221

1125


A. Bacterial promoter elements
UP element
–35
Ext
–10
Dis
CRE
+1
AAAWWTWTTTTNNNAAANN
TTGACA
TGTG
TATAAT
GGG
B. RNAP closed complex (RPc)
RNAP
Upstream DNA
Downstream DNA
UP element
–35 element
Extended –10 element
+1
C. RNAP open complex (RPo)
Downstream DNA
CRE
Template strand
+1
Upstream DNA
Discriminator
Non-template strand
UP element
–35 element
Extended –10 element
–10 element


Level
Partner interaction
Ligand
Activator
Location
Covalent modification
Replacement
Assistance
RNAP remodelling
Promoter remodelling
Anti-repression
RNAP
σ
−35
−10
+1
Activator
σ70
Repressor


A. Assistance
RNAP
σ1
σ70
σ4
σ3
σ2
α
−35
−10
+1
B. Remodelling
RNAP
σ1
σ70
σ4
σ3
σ2
α
−35
−10
+1
α CTD/activator interaction
α CTD/DNA interaction
α CTD/σ D4 interaction
C. plac
RNAP
σ1
σ70
σ4
σ3
σ2
α
−35
−10
plac
CRP
+1
D. λpRE
RNAP
σ1
σ70
σ4
σ3
σ2
α
−35
−10
pRE
cII
+1
E. pzwf
RNAP
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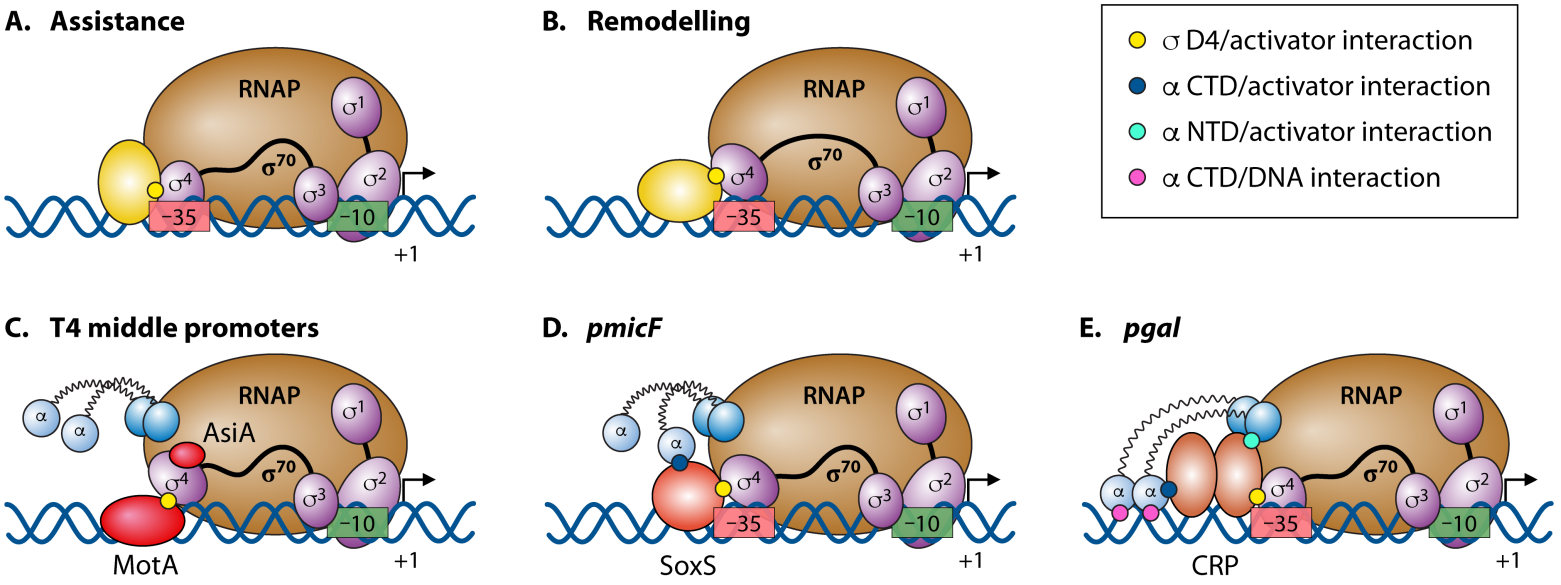
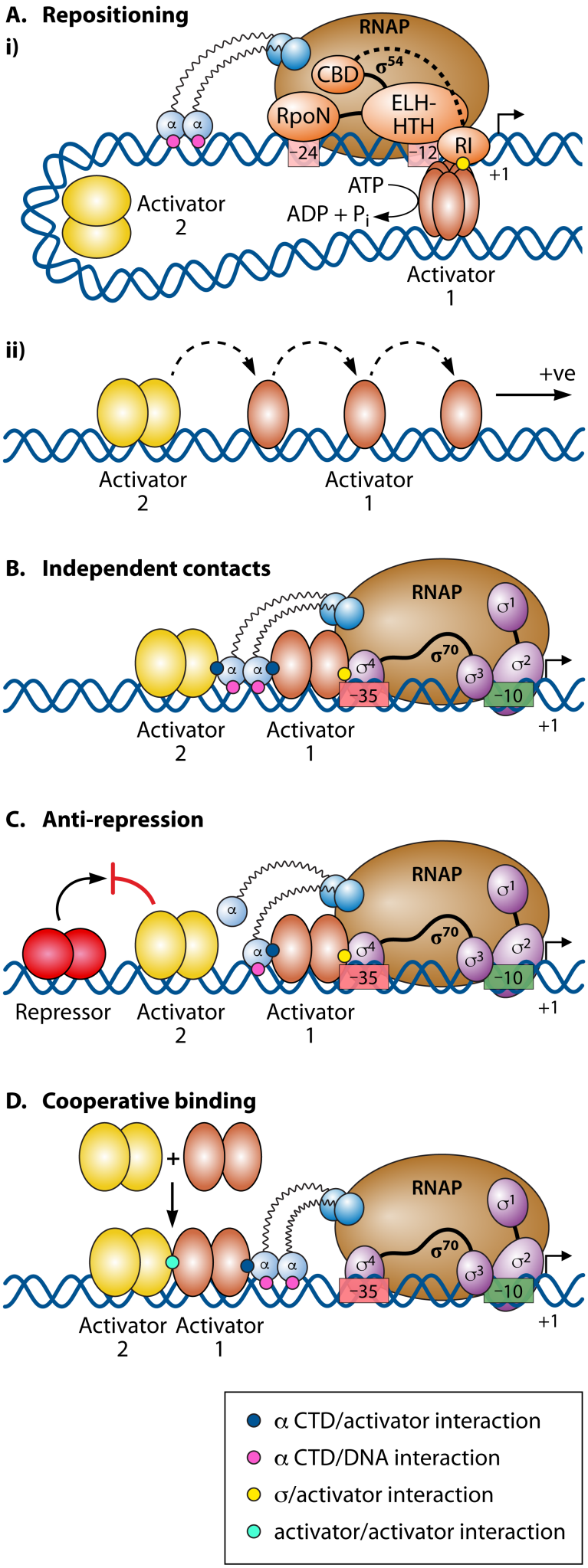
pzwf
SoxS
+1


A. Assistance
RNAP
σ1
σ70
σ4
σ3
σ2
−35
−10
+1
B. Remodelling
RNAP
σ1
σ70
σ4
σ3
σ2
−35
−10
+1
σ D4/activator interaction
α CTD/activator interaction
α NTD/activator interaction
α CTD/DNA interaction
C. T4 middle promoters
α
α
RNAP
AsiA
σ1
σ70
σ4
σ3
σ2
−10
MotA
+1
D. pmicF
α
α
RNAP
σ1
σ70
σ4
σ3
σ2
−35
−10
SoxS
+1
E. pgal
RNAP
σ1
α
α
σ70
σ4
σ3
σ2
−35
−10
CRP
+1


A. Repositioning
i)
RNAP
CBD
σ54
RpoN
ELH-HTH
RI
α
α
−24
−12
+1
ATP
ADP + Pi
Activator 2
Activator 1
ii)
+ve
Activator 2
Activator 1
B. Independent contacts
RNAP
σ1
σ70
σ4
σ3
σ2
−35
−10
+1
Activator 2
Activator 1
C. Anti-repression
RNAP
Repressor
Activator 2
Activator 1
D. Cooperative binding
RNAP
Activator 2
Activator 1
α CTD/activator interaction
α CTD/DNA interaction
σ/activator interaction
activator/activator interaction