How bacterial genes get turned on

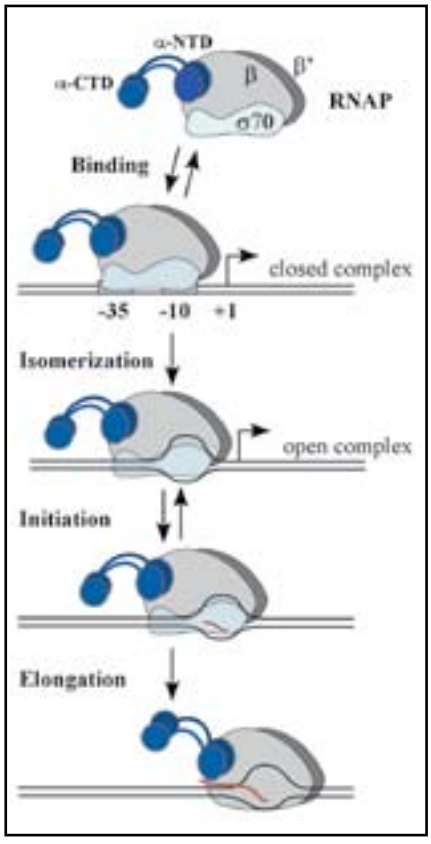
Rachel A Schubert and Keith E Shearwin

Discipline of Biochemistry
School of Molecular and Biomedical Sciences
University of Adelaide
Adelaide SA 5005
Tel: (08) 8303 5361
Fax: (08) 8303 4362
E-mail: keith.shearwin@adelaide.edu.au
E-mail: rachel.schubert@adelaide.edu.au

DNA-dependent RNA polymerase (RNAP) from prokaryotic to eukaryotic cells is conserved in sequence and structure [1] due to the universality of DNA as a store of information and the need to decode this information into protein expression through copying into messenger RNA.

Regulation of protein production is fundamental to allowing cells to maintain a phenotypic state and to respond efficiently to environmental signals and, not surprisingly, many regulators of transcription have been described which enhance the action of RNAP.

The recent solution of prokaryotic RNAP structures at different stages of transcription [2, 3], the elucidation of activator structures bound to DNA [4-6] and in complex with RNAP subunits [7, 8], and the sophisticated modelling of larger protein complexes [9, 10], has augmented biochemical and genetic analyses and greatly enhanced our understanding of the molecular mechanisms of transcriptional activation in bacteria.



General characteristics of transcriptional activation

From the large number of studies on prokaryotic activators, several general observations can be made. Firstly, potentially, any step of transcription by the multisubunit RNAP (Figure 1) can be enhanced by a regulatory protein, depending upon which process (or processes) are rate-limiting at a given promoter. Thus, an activator may target binding of the RNAP to the promoter to form a closed complex; the isomerisation that occurs upon unwinding of the –10 region of the DNA to form the open complex; the initial polymerisation of nucleotides to form the nascent RNA; clearance of promoter DNA by RNAP; or successful production of full length mRNA by the elongating RNA polymerase. However, most activators work at the level of initiation of transcription to enhance RNAP binding or isomerisation of the closed RNAP-promoter complex.

Secondly, most transcriptional activators recognise and bind to particular DNA sequences in the vicinity of promoters to give specificity to the action of RNAP. Increased transcription may result from a direct interaction between the regulator and RNAP at the promoter or, in some cases, in changes in the DNA conformation caused by activator binding.

Thirdly, most activators work as multimers or in combination with other regulatory proteins. These cooperative interactions can produce a large increase in promoter activity over a small change in activator concentration and can allow combinatorial control of transcription.

Some examples are given below to illustrate a number of different mechanisms of transcriptional activation. Many of these activators have homologues in other bacteria which are expected to exhibit similar mechanisms of activation.

Activation by DNA-bound proteins through contact with RNAP

The majority of bacterial activators work by binding specific DNA recognition sites upstream of promoter sequences and making protein-protein contacts with one or more subunits of RNAP to enhance transcription. The steps of transcription targeted by the activator are dependent upon the position of the DNA-bound activator relative to the promoter (which determines the RNAP subunit contacted) and on the nature of the protein-protein interface [11].

Figure 1. The steps of transcription in prokaryotes.

Escherichia coli **RNA polymerase (RNAP) is made up of two α subunits (each with N- and C- terminal domains, α-NTD and α-CTD, connected by a flexible linker); β and β' subunits and a sigma subunit (usually σ^{70} – which recognises specific promoter sequences).**

Transcription occurs through a number of intermediate steps [2]. Firstly, RNAP recognises and binds, via the σ^{70} subunit, specific DNA sequences (indicated by boxes) centred at approximately –10 and –35 of the promoter relative to the start site of transcription (+1, shown by an arrow), to form a closed complex. The CTD of an α subunit may also contact the upstream DNA.

Isomerisation of the RNAP-promoter complex results in localised unwinding of the DNA helix at the –10 region (represented by a 'bubble') to form the open complex. Transcription initiates with the polymerisation of nucleotides at the active site, and the growing nascent RNA emerges from a secondary channel in the RNAP structure as elongation proceeds. It remains unclear whether the sigma subunit is lost from the RNAP complex during elongation.

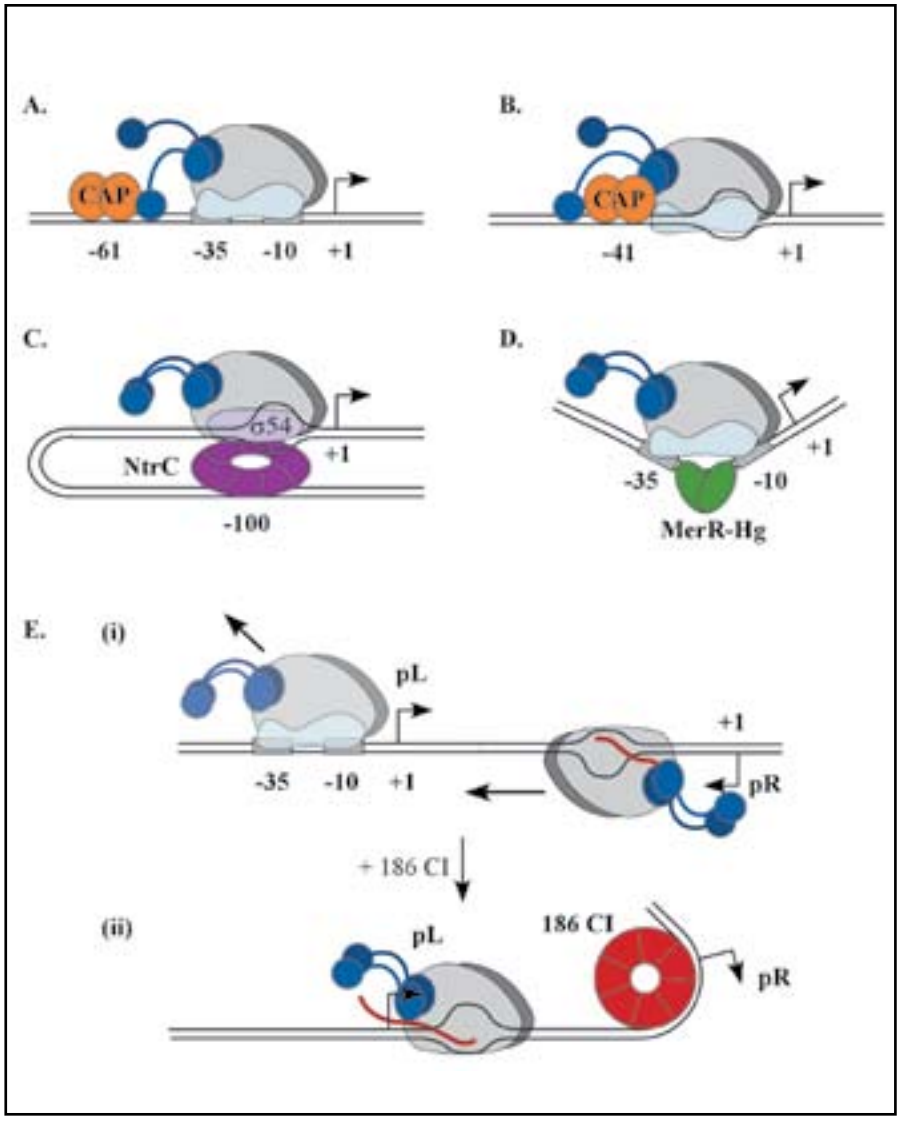


Figure 2. Some examples of transcriptional activators (RNAP and sequences in the promoter DNA are represented as for Figure 1).

A. Activation by a CAP dimer bound upstream of the RNAP closed complex. The promoter proximal CAP subunit interacts with the C-terminal domain of an RNAP α subunit to stabilise DNA binding by the α-CTD.

B. Activation by a CAP dimer bound adjacent to the RNAP closed complex. The promoter distal CAP interacts with an α-CTD to facilitate DNA binding by RNAP, and the promoter-proximal CAP contacts an α-NTD and σ^{70} to enhance isomerisation.

C. Activation of transcription by NtrC. DNA-looping allows a stable RNAP-σ^{54} closed complex at the target promoter to interact with an NtrC hexamer bound approximately 100 nt upstream. ATP hydrolysis by NtrC stimulates conformational changes in σ^{54}, which facilitates open complex formation.

D. Activation by members of the MerR protein family. Binding of a mercuric ion to a MerR dimer bound between –10 and –35 of the target promoter causes the protein to distort and bend the DNA; thereby shortening the distance between these promoter elements and allowing proper binding of the σ^{70} subunit of RNAP.

E. Activation by the 186 CI protein. (i) Frequent binding and firing of RNA polymerases from the strong pR promoter of coliphage 186 causes open or closed complexes at the face-to-face promoter pL to be knocked off the DNA. (ii) When present, the 186 CI protein forms a wheel-like 14mer (a 7mer of dimers) which binds over the pR promoter sequence to prevent RNAP access to pR. Polymerases at pL are then able to initiate transcription in the absence of interfering transcription from pR.

The paradigm for this type of activation is the dimeric catabolite activator protein (CAP) of *Escherichia coli*, which increases transcription at a number of promoters [9, 12]. At promoters where CAP binds at –61 or further upstream and on the same face of the DNA helix as RNAP, the activator contacts the C-terminal domain of an RNAP alpha subunit (α-CTD) to enhance its binding to the adjacent DNA sequence, thereby stabilising RNAP binding to the promoter (Figure 2A). In fact, provided that formation of the RNAP-promoter closed complex is a rate-limiting step at the promoter, any protein contact between a DNA-bound protein and RNAP can foster polymerase binding and enhance initiation of transcription [13].

At a second group of promoters, CAP binds to a site overlapping the –35 sequence and forms specific contacts with the N-terminal domain of an alpha subunit and the sigma70 (σ^{70}) subunit of RNAP, as well as the α-CTD (Figure 2B). These interactions assist the α-CTD to bind the DNA (upstream of CAP) as before, but also act to stimulate isomerisation, presumably by selectively interacting with and stabilising the RNAP open complex [9, 12].

In contrast, the CI protein from the *E. coli* virus (coliphage) lambda, which similarly binds to a recognition site abutting the –35 sequence of the phage pRM promoter, contacts the RNAP σ^{70} subunit alone to enhance the rate of isomerisation [7, 12]. Interestingly, a single amino acid mutation in the region of σ^{70} that interacts with λ CI results in improved polymerase binding but not isomerisation at the promoter – presumably due to λ CI now interacting with residues only exposed in the closed complex.

These examples demonstrate that the nature and strength of the activator-RNAP protein interface determines the effect of that contact on the level of transcription [7].

A variation on the theme of activation by RNAP contact is employed by the nitrogen regulation protein of *E. coli*, NtrC, and related family members [14, 15]. Under various stress conditions, RNAP subunits form a complex with an alternative sigma subunit, σ^{54}, which recognises different promoter sequences to σ^{70}, and forms a very stable closed complex that is naturally deficient at isomerisation.

Activated NtrC binds as a hexameric ring to enhancer elements ~100 nt upstream of the promoter and looping of the intervening DNA allows the NtrC multimer to also interact with the promoter on the opposite face of the helix to RNAP-σ^{54} [10] (Figure 2C). Conformational changes in the NtrC hexamer due to ATP hydrolysis appear to be transmitted to σ^{54} to destabilise RNAP interactions with the promoter and allow opening of the DNA.

Transcription activation by DNA conformational changes

Positive regulators of transcription have also been characterised which bind to specific promoters but do not appear to directly interact with the RNAP complex. An example of this is the MerR protein family, whose members activate transcription in response to metal ions or some antibiotics [5, 16]. In the absence of their ligands, these proteins bind as dimers to recognition sequences between the –10 and –35 sites of target promoters and on the opposite side of the DNA helix to RNAP.

The –35 sequence is bound by the σ^{70} subunit of the RNAP, but a larger than usual spacing between the –10 and –35 sequences (19-20 nt compared to 16-18 nt) prevents further interaction with the DNA.

When MerR is bound by mercuric ions (its ligand), it undergoes a conformational change which results in distortion of the promoter DNA – the DNA is unwound, bent at an angle of 50 degrees away from the MerR dimer and is squashed together at the site of the bend. This distortion of the DNA realigns the –10 and –35 sites to their usual spacing to allow proper binding of the σ^{70} subunit and transcription (Figure 2D).

Activation by relief of repression

Repression of transcription is a widely used regulatory mechanism to control gene expression, and removal of this inhibition in response to a specific signal provides another way to enhance production of a particular gene product.

A recently elucidated example of this type of regulator is the QacR repressor, which is involved in antibiotic resistance in *Staphylococcus aureus* [4-6]. A pair of QacR dimers attach to opposite sides of the promoter DNA by recognition of sequences centred at +8 of the promoter. The dimers bind cooperatively without direct contact but through unwinding and twisting of the DNA, and are thought to block escape of RNAP bound at the promoter. Binding of a small ligand (including drugs such as antibiotics) to a QacR dimer disrupts its interaction with the DNA and ultimately allows the expression of drug exporter proteins.

An unusual example of relief of repression involves the CI protein of coliphage 186 [17]. The 186 CI protein indirectly stimulates its own production from the weak pL promoter by preventing interfering transcription from a strong convergent promoter, pR [18]. In the absence of 186 CI, polymerases which bind to pL but are slow to fire ('sitting ducks'), are knocked off the DNA by the barrage of RNAP molecules coming from pR [19, 20] (Figure 2E(i)).

A wheel-like complex of 14 CI proteins is thought to wrap around sites over the interfering promoter to prevent polymerase access to pR [17]. This removes the interference of pR on pL and allows polymerases at pL time to commence elongation (Figure 2E(ii)). The 186 CI-DNA complex downstream of pL does not appear to present a barrier to RNAP elongating from pL, perhaps due to tethering of the regulator wheel by cooperative binding to additional operators ~300 nt away [17, 18].

Conclusions

The mechanism of activation employed by a transcriptional regulator at a particular promoter results from a combination of factors relating to both the promoter sequence and to properties of the activator protein. These factors overall determine which step/s of transcription are rate-limiting, whether the activator is positioned favourably to interact with RNAP and has a conformation able to do so, and whether activator binding will stimulate conformational changes in the promoter structure. The characterisation of increasing numbers of regulators of *E. coli* and other bacteria will no doubt reveal further novel and interesting mechanisms of transcriptional regulation.

Acknowledgements

Thanks to Professor Barry Egan for valuable suggestions and discussions.

References

1. Ebright RH. RNA polymerase: structural similarities between bacterial RNA polymerase and eukaryotic RNA polymerase II. *J Mol Biol* 2000; 304:687-698.
2. Murakami KS & Darst SA. Bacterial RNA polymerases: the whole story. *Curr Opin Struct Biol* 2003; 13:31-39.
3. Murakami KS, Masuda S, Campbell EA, Muzzin O & Darst SA. Structural basis of transcription initiation: an RNA polymerase holoenzyme-DNA complex. *Science* 2002; 296:1285-1290.
4. Grkovic S, Brown MH & Skurray RA. Regulation of bacterial drug export systems. *Microbiol Mol Biol Rev* 2002; 66:671-701.
5. Schumacher MA & Brennan RG. Structural mechanisms of multidrug recognition and regulation by bacterial multidrug transcription factors. *Mol Microbiol* 2002; 45:885-893.
6. Ramos JL, Martinez-Bueno M, Molina-Henares AJ *et al.* The TetR family of transcriptional repressors. *Microbiol Mol Biol Rev* 2005; 69:326-356.
7. Jain D, Nickels BE, Sun L, Hochschild A & Darst SA. Structure of a ternary transcription activation complex. *Mol Cell* 2004; 13:45-53.
8. Benoff B, Yang H, Lawson CL *et al.* Structural basis of transcription activation: the CAP-alpha CTD-DNA complex. *Science* 2002; 297:1562-1566.
9. Lawson CL, Swigon D, Murakami KS, Darst SA, Berman HM & Ebright RH. Catabolite activator protein: DNA binding and transcription activation. *Curr Opin Struct Biol* 2004; 14:10-20.
10. Huo YX, Tian ZX, Rappas M *et al.* Protein-induced DNA bending clarifies the architectural organization of the sigma54-dependent glnAp2 promoter. *Mol Microbiol* 2006; 59:168-180.
11. Browning DF & Busby SJ. The regulation of bacterial transcription initiation. *Nat Rev Microbiol* 2004; 2:57-65.
12. Dove SL, Darst SA & Hochschild A. Region 4 of sigma as a target for transcription regulation. *Mol Microbiol* 2003; 48:863-874.
13. Hochschild A & Dove SL. Protein-protein contacts that activate and repress prokaryotic transcription. *Cell* 1998; 92:597-600.
14. Zhang X, Chaney M, Wigneshweraraj SR *et al.* Mechanochemical ATPases and transcriptional activation. *Mol Microbiol* 2002; 45:895-903.
15. Gralla JD. Signaling through sigma. *Nat Struct Biol* 2000; 7:530-532.
16. Brown NL, Stoyanov JV, Kidd SP & Hobman JL. The MerR family of transcriptional regulators. *FEMS Microbiol Rev* 2003; 27:145-163.
17. Pinkett HW, Shearwin KE, Stayrook S *et al.* The structural basis of cooperative regulation at an alternate genetic switch. *Mol Cell* 2006; 21:605-615.
18. Dodd IB & Egan JB. Action at a distance in CI repressor regulation of the bacteriophage 186 genetic switch. *Mol Microbiol* 2002; 45:697-710.
19. Callen BP, Shearwin KE & Egan JB. Transcriptional interference between convergent promoters caused by elongation over the promoter. *Mol Cell* 2004; 14:647-656.
20. Shearwin KE, Callen BP & Egan JB. Transcriptional interference: a crash course. *Trends Genet* 2005; 21:339-345.