The majority of bacterial promoters are regulated by at least two transcription factors. Many of these promoters are regulated by two activators, with expression being co-dependent on the activity of both activators [1]. At most of these promoters, one of the activators responds to a ‘global’ regulatory signal whilst the other responds to a ‘specific’ signal. For example, transcription initiation at the \textit{Escherichia coli} narG promoter is induced by oxygen starvation (a global signal, interpreted by the global regulator, FNR) and nitrate ions (a specific metabolic signal, interpreted by NarL). The \textit{E. coli} narG promoter is co-dependent on FNR and NarL. We are interested in understanding the mechanisms by which expression from a promoter can be co-dependent on two activators.

In recent studies with promoters regulated by single transcription activators, we have demonstrated that direct protein–protein interaction between the activator and RNA polymerase (RNAP) is essential for transcription activation. A number of studies have shown that RNAP carries two separate contact sites for such activators: one is located in the C-terminal domain of the \(\sigma\)-subunit (\(\sigma\)CTD) whilst the other is near the C-terminus of the \(\sigma\)-subunit (reviewed in [2,3]). Most activators that make contact with \(\sigma\) bind just upstream of the –35 region, whilst activators that contact \(\sigma\)CTD generally bind further upstream. This suggests a simple model to explain co-dependence on two activators, illustrated in Figure 1, in which one of the activators makes contact with the RNAP \(\sigma\)-subunit and the second activator makes contact with \(\sigma\)CTD. Interestingly, in almost all documented cases of promoters co-dependent on two activators, one of the activators binds just upstream of the –35 region and is well placed to contact \(\sigma\) (recall that the C-terminus of the \(\sigma\)-subunit of \textit{E. coli} RNA polymerase makes direct contact with the –35 hexamer at promoters). In these cases it is likely that \(\sigma\)CTD is displaced upstream of this activator (see [4] for an example). In the ‘simultaneous touching’ model shown in Figure 1, we propose that the second activator functions synergically with the first by making simultaneous contact with the displaced \(\sigma\)CTD.

Evidence for the ‘simultaneous touching’ model in Figure 1 has been obtained from experiments using a set of semisynthetic promoters dependent on the \textit{E. coli} cyclic AMP receptor protein (CRP) [5].

**Figure 1**

Simultaneous touching at a co-dependent promoter

The Figure shows the organization of the different subunits of RNAP, \(\sigma\) and CRP, and two dimeric transcription activators, A and B, at a ‘typical’ promoter. The RNAP \(\sigma\)-subunit consists of two domains, \(\sigma\)CTD and \(\sigma\)NTD, tethered by a linker. The RNAP \(\sigma\)-subunit recognizes the –10 and –35 promoter elements located upstream of the transcription start at +1. Activator A binds just upstream of the –35 sequence and, in most cases, makes contact with the RNAP \(\sigma\)-subunit. This results in displacement of \(\sigma\)CTD which contacts activator B, bound further upstream.

Abbreviations used: \(\sigma\)CTD, \(\sigma\)NTD, C- or N-terminal domain of the \(\sigma\)-subunit; CRP, cyclic AMP receptor protein; RNAP, RNA polymerase.
receptor protein (CRP), a 'global' transcription activator that contains surface-exposed activating regions that can interact with both $\sigma$ and zCTD [5]. The starting point of this work was the promoter CC(−41.5), which carries a single consensus 22 bp sequence for the CRP dimer centred between bp 41 and 42 (−41.5) upstream of the E. coli melR transcription start region (Figure 2). This promoter was cloned into a lac expression vector and promoter activity was measured in a number of genetic backgrounds and growth conditions. Our studies showed that the activity of CC(−41.5) was totally dependent on CRP. When a binding site for either a second dimer of CRP (CC) or for FNR (FF) was inserted upstream of the first CRP-binding site (the new site being centred at −90.5), promoter activity was increased 3–5-fold (Figure 2). In control experiments, the insertion of the related LL binding site, which is unable to bind either wild-type CRP or FNR, caused no increase in promoter activity. Further control experiments established that, when bound at −90.5, neither CRP nor FNR was able to activate the promoter in the absence of CRP bound at −41.5. We conclude that, at these promoters, either two CRP dimers or CRP and FNR are acting synergically in transcription activation [6].

In previous work with CRP-dependent promoters at which the CRP dimer binds to a site centred at −41.5, we characterized the direct contacts made between CRP and RNAP that are essential for promoter activation [5]. Figure 3 illustrates an experiment to investigate the possibility of direct contacts between the upstream bound CRP or FNR and RNAP at the two promoters carrying tandemly bound activators. We have exploited the observation that the single amino acid substitutions H159L in CRP and S73F in FNR interfere with contacts with zCTD [3, 4]. Figure 3(4) shows an experiment to investigate the promoter with tandem bound CRP dimers at −41.5 and −90.5. The upstream CRP-binding site was altered to the control LL sequence, a derivative of CC recognized by CRP carrying the E181V substitution but not by wild-type CRP [6]. CRP carrying the E181V substitution is able to bind to the LL sequence [6], and is able to interact synergically with wild-type CRP bound at −41.5. Figure 3(4) shows that CRP carrying the E181V and H159L substitutions is unable to give synergic activation. Since the H159L substitution specifically interferes with the activating region of CRP that makes contact with zCTD [3, 4], we conclude that CRP bound upstream must be making direct contact with zCTD, and that this contact is responsible for synergic activation of transcription initiation (see Figure 1).

A similar rationale, illustrated in Figure 3(B), was used to investigate the promoter dependent on FNR bound at −90.5 and CRP at −41.5. Wild-type FNR bound upstream of CRP results in synergic activation but this synergy is suppressed by the S73F substitution in FNR.

**Figure 2**

Promoters activated by a single or tandem bound transcription factors

The Figure shows sketches of the organization of the different promoters discussed in the text. Each promoter carries DNA-binding sites for CRP (CC: CRP is shown as open circles) or FNR (FF: FNR is shown as open squares) upstream of the melR−10 region (−10) and transcript start site (shown by an arrow). One promoter carries the LL sequence, a derivative of CC recognized by CRP carrying the E181V substitution but not by wild-type CRP [6]. The location of the centre of the CC-, FF- and LL-binding sites with respect to the transcript start site is shown in each case. Each of the promoters was cloned on an EcoRI-HindIII fragment into the lac expression vector pRW50 (described fully in [6]). The recombinants were transformed in E. coli M182 crp′ fnr′ cells, and β-galactosidase expression was determined as a measure of the activity of that promoter. The activity of each promoter (in arbitrary units) is shown above the arrow indicating the transcript start. In each case activity was reduced to ≥30 units in M182Δcrp cells.
Since this substitution specifically interferes with the FNR activating region that makes contact with αCTD [7], we conclude that upstream bound FNR must be making a direct contact with αCTD and that this contact is required for synergic activation of transcription initiation (cf. Figure 1).

In summary, we have presented clear evidence in favour of the 'simultaneous touching'

**Figure 3**

**Upstream bound activator must make a direct contact with αCTD**

The Figure shows experiments with two different promoter derivatives, illustrated as described in the legend to Figure 2. Each derivative was cloned in pRW50, and β-galactosidase expression was determined in different host backgrounds. Activities in each case are shown above the arrow indicating the transcript start. (A) The promoter carries a CC site centred at -41.5 and an LL site centred at -90.5. CRP dimers (open circles) can occupy the CC site but not the LL site in M182 crp' cells. CRP E181V (filled circles; encoded by a second plasmid introduced into M182) can occupy the LL site and increase transcription activation. CRP E181V H159L (shown by filled circles with crosses to denote mutation of the activating region that contacts αCTD) can occupy the LL site but is unable to increase transcription activation. (B) The promoter carries a CC site centred at -41.5 and an FF site centred at -90.5. CRP dimers (open circles) can occupy the CC site but not the FF site in JRG1728 crp'Δfnr cells. FNR (open squares; encoded by a second plasmid introduced into JRG1728) can occupy the FF site and increase transcription activation. However, FNR S73F (shown by open squares with crosses to denote mutation of the activating region that contacts αCTD) can occupy the FF site but is unable to increase transcription activation.

A model for co-dependent transcription activation at a number of semisynthetic promoters carrying combinations of DNA sites for CRP and FNR. A recent study by Scott and co-workers [8] strongly suggests that this model is applicable to the *E. coli* and *Salmonella typhimurium ansB* promoters, which are also regulated by CRP and FNR. It remains to be seen whether this model is applicable to other co-activated promoters. An attractive feature of the model derives from the fact that αCTD and αNTD are joined by a linker which can allow considerable flexibility in the location of the upstream activator [3,7,9]: thus promoters co-dependent on two activators that touch RNAP simultaneously will have access to a diverse range of permissible architectures.

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