Enhancer-like long-range transcriptional activation by λ CI-mediated DNA looping

Lun Cui¹, Iain Murchland¹, Keith E. Shearwin, and Ian B. Dodd²

School of Molecular and Biomedical Science (Biochemistry), University of Adelaide, Adelaide, SA 5005, Australia

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How distant enhancer elements regulate the assembly of a transcription complex at a promoter remains poorly understood. Here, we use long-range gene regulation by the bacteriophage λ CI protein as a powerful system to examine this process in vivo. A 2.3-kb DNA loop, formed by CI bridging its binding sites at *OR* and *OL*, is known already to enhance repression at the lysogenic promoter *PRM*, located at *OR*. Here, we show that CI looping also activates *PRM* by allowing the C-terminal domain of the α subunit of the RNA polymerase bound at *PRM* to contact a DNA site adjacent to the distal CI sites at *OL*. Our results establish *OL* as a multifaceted enhancer element, able to activate transcription from long distances independently of orientation and position. We develop a physicochemical model of our in vivo data and use it to show that the observed activation is consistent with a simple recruitment mechanism, where the α -C-terminal domain to DNA contact need only provide ~ 2.7 kcal/mol of additional binding energy for RNA polymerase. Structural modeling of this complete enhancer–promoter complex reveals how the contact is achieved and regulated, and suggests that distal enhancer elements, once appropriately positioned at the promoter, can function in essentially the same way as proximal promoter elements.

In eukaryotes, initiation of transcription is often regulated by enhancers, DNA sequences that can be located many kilobases away from the promoter. Enhancers are usually complex, being composed of multiple DNA elements that bind a variety of proteins (1, 2). The enhancer and the promoter are brought into close contact, looping the intervening DNA (3, 4), thus placing these binding elements close to the promoter. However, due to the lack of detailed understanding of enhancer–promoter complexes, how enhancers efficiently and specifically regulate initiation remains obscure (5).

The action of DNA elements at and around a promoter is easier to understand. These sequences can be envisaged as a local scaffold for the assembly of transcription-favoring or transcription-inhibiting protein complexes. The promoter elements bind the transcription machinery, and promoter-proximal DNA elements position other proteins so that they can interact, favorably or unfavorably, with this machinery (2, 6–9). In prokaryotes, these promoter complexes are relatively simple. Repression of transcription is usually achieved by proteins positioned to compete with binding of RNA polymerase (RNAP), whereas transcription can be activated by proteins positioned adjacent to RNAP that contact it to stabilize initiation intermediates (8, 10, 11).

The CI repressor of bacteriophage λ has been seminal in understanding how promoter-proximal elements are used in transcriptional regulation. Two CI binding sites, *ORI* and *OR2*, overlap the lytic *PR* promoter and cooperative binding of two CI dimers to these sites competes with RNAP binding and represses the promoter. Extensive study of this complex has culminated in crystal structures that reveal how CI binds to the DNA and how the dimers interact with each other (12–14). The presence of a CI dimer at *OR2* simultaneously activates the divergent lysogenic promoter *PRM* (Fig. 1A). Extensive genetic, biochemical, and structural studies have shown that amino acids in the N-terminal domain (NTD) of CI at *OR2* make favorable contacts with amino acids in the σ subunit of the RNAP open complex (11).

λ CI also provides a powerful model for regulation of transcription by distant sites because CI binding at *OR* is affected by the *OL*

site, located 2.3 kb away. A CI tetramer bound to *ORI*/*OR2* interacts with another CI tetramer bound to *OL*, forming a CI octamer and a DNA loop (13, 15). This long-range cooperative interaction improves CI repression of *PR* (15–17) and is also necessary for CI repression of *PRM* because it allows a CI dimer bound to a third operator at *OL* to assist CI binding to the very weak *OR3* site overlapping *PRM* (16–20) (Fig. 1C).

In addition to this long-range stimulation of repression, the *OL* site has been shown to increase CI activation of *PRM* both in vivo (21, 22) and in vitro (20). Two models have been proposed for this long-range activation (21): (i) a “looped octamer activation model” in which octamer formation somehow alters the interaction between the CI dimer at *OR2* and RNAP at *PRM*, and (ii) a “UP contact model” in which *OL*–*OR* looping brings an UP element for the *PL* promoter, located adjacent to *OL3* (23, 24) sufficiently close to *PRM* that it can be contacted by the C-terminal domain (CTD) of the α subunit of an RNAP bound at *PRM* (Fig. 1B). The CTDs of the two α subunits are attached to RNAP by flexible linkers, allowing these domains to make activatory contacts with proteins positioned up to a few tens of base pairs upstream of the promoter (25, 26). The α CTDs can also bind directly to DNA sequences termed UP elements (27), which have been shown to stimulate transcription if located just upstream of the promoter (28). In fact, the α CTD is also involved in CI activation of *PRM*, contacting the DNA between CI dimers at *ORI* and *OR2* (29).

Using in vivo reporters, we confirm the UP contact model, showing that activation of *PRM* by *OL* requires the UP element, an intact α CTD, and CI-mediated DNA looping. Taking advantage of existing structural data on CI and RNAP, we build structural models of the *OL*–CI–*OR*–RNAP complex that show that contact between the α CTD and the UP element is structurally feasible. However, the DNA bending that is needed for this contact is unlikely to be possible in complexes in which CI is bound to *OL3*. Indeed, we show that forcing CI to bind to *OL3* abolishes activation by *OL*. The structurally informed UP contact model is shown to be consistent with an updated statistical–mechanical model of *PRM* regulation, which provides estimates of the binding free energies involved in this enhancer-like interaction.

Results

Activation Requires the UP Element Proximal to *OL*. To understand how the distant *OL* site can activate *PRM*, we first distinguished between the looped octamer activation and UP contact models using chromosomal *PRM*–*lacZ* fusions (Fig. 2A) exposed to a range of fixed CI concentrations (*Materials and Methods*) (Figs. S1–S3).

To maintain the native 2.3-kbp spacing between *OL* and *OR*, *OL* was placed upstream of *PRM*, rather than in the natural downstream position. *OL* was oriented so that the simple U-shaped *OL*–*OR* loop gives antiparallel alignment of *OL* and *OR*,

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¹L.C. and I.M. contributed equally to this work.

²To whom correspondence should be addressed. E-mail: ian.dodd@adelaide.edu.au.

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The UP element did not affect *PRM* in the absence of CI, presumably because it requires CI-mediated DNA looping to bring it close to the promoter. Accordingly, disrupting DNA looping by mutating all *OL* operators prevented UP element stimulation of *PRM* in the presence of CI (UPonly; Fig. 2C).

Activation Requires the α Subunit of RNAP. As expected, if the α subunit contacts the UP element at *OL*, the stimulatory effect of the *OL3* proximal sequence was inhibited by overexpression of an α subunit bearing the R265A mutation known to block UP recognition (27) (Fig. 2D). As a further test of α -subunit involvement, we replaced the UP element with a binding site for the α -contacting activator CAP (10). A CAP site adjacent to *OL3* had no effect on *PRM* in the absence of CI but could increase CI activation of *PRM* ~2.5-fold over that seen without *OL* (CAP; Fig. 2C).

OL was able to activate *PRM* when its orientation was reversed (REV; Fig. 2C), a classical property of enhancers. We also extended the 2.3-kb spacer between *OL* and *OR* to 10 kb. The effect of the UP element became undetectable at this distance; however, stimulation of *PRM* activation by a CAP site at *OL* was still substantial at 10 kb (Fig. 2C). Thus, if appropriately positioned, CAP can function over very large DNA distances.

Structural Modeling of UP Activation. There are eight basic configurations in which a CI octamer can link *OL* and *OR* and leave *PRM* free for RNAP binding (Fig. 3). As proposed by Anderson and Yang (21), each of these configurations is likely to have a different propensity for UP contact. To better understand how, and in which loop configurations, the α CTD can reach from the RNAP at *PRM* to the UP element at *OL*, we used existing crystal structures to assemble structural models of various *OL*–CI–*OR*–RNAP complexes.

Linker flexibility is likely to be needed for CI cooperativity. The first step was to make a model of a CI tetramer bound to DNA containing two adjacent operators by combining the structures of a full-length CI dimer bound to DNA (14) [Protein Data Bank (PDB) ID 3BDN] with the octamer structure of the CI CTD (13) (PDB ID 1KCA). Although the residue 93–131 segment of CI has traditionally been considered a flexible interdomain linker, the crystal structure shows most of this region folded against the CTD (14). Stayrook et al. (14) present a model of dimers bound to adjacent operators and formation of the tetramer without linker flexibility, but they did not consider the spacer DNA between the operators. However, we found that using average B-DNA parameters for the DNA between the operators and then docking CI dimers onto the *OR1* and *OR2* operators by alignment with the PDB 3BDN crystal structure resulted in a separation of their CTDs that was inconsistent with tetramerization.

We thus tested whether bending and twisting or untwisting of the interoperator spacer would allow alignment of the CTDs. However, of 22,680 tested structural variants of the spacer DNA (*Materials and Methods*), none allowed a CI tetramer docked at *OR2* to come within 10 Å of its ideal position at *OR1*. We also observed that a model of the CI–DNA–RNAP ternary complex created by alignment of the RNAP open complex model (30) and the CI dimer (PDB 3BDN) to the σ –CI–NTD crystal structure (11) results in significant clashes between RNAP and CI. Thus, we concluded that

flexibility of the CI interdomain linker is needed to yield a plausible model of the DNA-bound tetramer.

To introduce conformational flexibility of the CI dimer, we used a torsion angle dynamics simulated annealing protocol to vary the torsions of residues 93–101 (the “short linker”). The lowest energy dimer structures with intact CTD and NTD structures were then used to generate *OR2*-anchored CI tetramers, and the one aligning most closely with *OR1* was refined by multibody docking. This structure is shown in Fig. 4A and Fig. S5.

The *OL1.2* tetramer has the same operator spacing as that of *OR1.2*, so was assumed to adopt the same structural configuration. The *OL2.3*-bound tetramer was successfully modeled by the same protocol; due to the shorter interoperator spacer, this yielded a distinct tetramer model.

Our short linker model of dimer flexibility thus provides structural models of the DNA-bound CI tetramer that are consistent with the experimental observations that CI cooperativity is relatively independent of operator spacing (31), that deletion of the interdomain linker makes cooperativity highly dependent on operator spacing (32), and that a CI octamer cannot bind to three adjacent operators (14).

CI octamer-mediated DNA looping and RNAP at *PRM*. Models of DNA-bound CI octamers were then constructed by alignment of the CI CTDs of two DNA-bound tetramers with the CI CTD octamer structure (PDB 1KCA). For a given loop class (Fig. 3), there are two ways of positioning the asymmetric CI tetramer on the operators at *OL* and similarly two orientations at *OR*. Combined with the asymmetry of the CI CTD octamer, this gives rise to eight possible octamer–DNA conformations, each equally plausible, for each of the eight loop classes. A single representative of such ensembles has been chosen for depiction in Fig. 4.

RNAP at *PRM* was added to these *OL*–CI₈–*OR* structures by first aligning the common σ and DNA portions of the crystal structure of the complex of DNA, the CI NTD and the $\sigma 4$ domain of Taq RNAP (11) with a model of the full Taq RNAP open complex (30). This CI NTD–RNAP_o–*PRM* structure was then joined to the CI octamer–DNA structures by aligning their common CI NTD and DNA portions (Fig. 4B). Importantly, this could be achieved without major steric clashes for all of the *OL*–CI₈–*OR* structures tested, indicating that the model of the CI-looped complex is compatible with active RNAP at *PRM*.

Modeling the contact between RNAP α CTD and UP. We were then in a position to test the structural feasibility of the α CTD–UP contact in the *OL*–CI₈–*OR*–RNAP complex. Because of the flexibility of the α linkers, we generated an ensemble of structures for this 20-aa polypeptide segment (*Materials and Methods*). This showed that the DNA-contacting R265 residues of the two α CTDs circumscribe a hemispherical zone behind RNAP (Fig. 4C). As expected, this zone extends to the α CTD contact at the –54 position of *PRM* (29). However, the α CTD is not able to reach the UP element at *OL* in any loop conformation without bending of the DNA between UP and *OL2*.

We therefore allowed the DNA between UP and *OL2* to flex by random natural B-DNA variation. These DNA ensembles showed that the flexibility of this DNA could permit the UP element to reach the α CTD zone in certain cases (Fig. 4C). Fig. 4D and E shows one such contact for an antiparallel *OL1.2*–*OR1.2* case (loop 1). Significantly, we found α CTD–UP overlap only in specific *OL1.2*–*OR1.2* linked structures of the loop 1 and 5 classes. When the octamer is bound to *OL2.3* (loops 2, 3, 6, and 7), DNA flexure is confined to the region between *OL3* and UP, restricting DNA bending and preventing α CTD–UP overlap (Fig. 4F). We note that the idea that the *OR1.2*–*OL2.3* octamer is less favorable for *PRM* activity than the *OR1.2*–*OL1.2* octamer was previously suggested from an analysis of CI activation of *PRM* in vitro (20).

We found that binding of a CI dimer to *OL3* in the *OL1.2*–*OR1.2* octamer structures (loops 4 and 8) is also predicted to inhibit α CTD–UP contact. The CI dimer at *OL3* restricts UP element movement, but less severely than in the *OL2.3* octamer because the *OL2*–*OL3* spacer is able to flex. However, in the models, CI binding bends the *OL3* operator such that UP is

Fig. 3. The basic *OL*–*OR* loop configurations in which *PRM* is free. Numbering is from ref. 21. The UP element is predicted to be contactable only in certain loop 1 and loop 5 structures.

Fig. 4. Structural modeling of the looped CI-DNA-RNAP complex. (A) Model of the CI tetramer bound to *OR1* and *OR2*. (B) Model of the open complex RNAP at *PRM* and a CI octamer bound to operators *OR1*, *OR2*, *OL1*, and *OL2*. *OL* DNA is shown in two overlapping orientations (parallel and antiparallel, loops 1 and 5 in Fig. 3), resulting in two different positions for the UP element. (C) Flexibility in the RNAP α subunit linker and DNA allow the α CTD and UP element to contact in some configurations but not in others. The blue spheres represent the positions of base pairs 14 and 20 of the UP element (numbered from the *OL3* end) from 5,000 randomly sampled conformations of the *OL3*-proximal DNA. The green spheres represent the positions of the centroid of the α CTD DNA binding residue R265 from 10,000 randomly sampled conformations of each of the α -subunit linkers. (D) Space-filling model of a structure from C in which the α CTD (green) can contact the minor groove of the UP element in a native-like manner. (E) Detail of D, also showing a second possible α CTD-UP contact. (F) CI octamer binding to *OL2.3* restricts DNA flexibility, and inhibits UP element contact with the α CTD in all loop configurations examined (representative example shown). (G) CI dimer binding to *OL3* restricts DNA flexibility and also induces DNA curvature that moves the UP element away from RNAP, preventing UP element contact with the α CTD in all loop configurations examined (representative example shown). Figures were generated using Chimera.

moved further away from RNAP (Fig. 4G). These effects of dimer occupation of *OL3* are true for all *OL1.2-OR1.2* loop configurations in which we have found UP- α CTD contacts to be plausible.

Although we have not systematically examined all 64 of the looped structures for UP- α CTD contact, it was evident from the analysis of many of these, and from knowledge of the structural variation allowed by the CI asymmetries, that none of the structures involving CI binding at *OL3*, either as a dimer or part of an octamer, would allow UP contact.

Thus, the structural modeling indicates that contact between the α CTD at *PRM* and UP at *OL* is feasible as long as CI is not bound to *OL3*.

Physicochemical Modeling of UP Activation. The wealth of physicochemical data available for λ CI makes it possible to test the feasibility of the UP contact model by mathematical modeling. We thus extended our previous statistical-mechanical model of *PRM* regulation by CI-mediated DNA looping (16) (*SI Materials and Methods*; Figs. S6 and S7). First, following Anderson and Yang (21), we distinguished the eight different basic loop orientations in which *PRM* can be active (Fig. 3). Second, we specifically modeled RNAP binding at *PRM*. Basal RNAP binding to *PRM* (in the absence of CI) was defined by a free energy term. When CI is present at *OR2* and not part of an octamer, an additional free energy term was added for RNAP binding. A further free energy was added if the CI at *OR2* is part of a looped octamer; this parameter allows for activation or inhibition by looping itself. Another free energy was added for species in which the UP element is engaged. These parameters allow CI, DNA looping, and the UP element to regulate *PRM* by stabilizing or inhibiting RNAP binding at the promoter. These and other parameters were fitted to the 70 data points of Fig. 2B (*PRM*) and Fig. S4 (*PR*).

We found that a model that implements the insights from the structural modeling, that is, one that allows UP contact only in loops 1 and 5 (applying the same UP-contact energy for both loops), was able to reproduce the data well (Fig. 2B and Fig. S4). In contrast, if

we allowed equal UP contact in all eight looped configurations, the fit of the model to the data were considerably worse. In particular, this “all-loops” model (i) does not reproduce the magnitude of the drop in *PRM* activity at high [CI] in the longOL.*OL3*⁺*OR3*[−] reporter; and (ii) slightly underestimates the contrast between activated and repressed *PRM* activity in the wild-type longOL reporter—the steepness of repression at high [CI] (Fig. 5A). We found that the model can accurately reproduce these two features (and the rest of the data) as long as UP is allowed to be contacted in at least one of the loops containing four CI dimers (loops 1, 2, 5, and 7) and in none of the loops containing five CI dimers (loops 3, 4, 7, and 8). In such UP contact schemes, the UP element can be used at lower CI concentrations but its stimulatory effect is

Fig. 5. Statistical-mechanical modeling of UP contact. (A) Data for the longOL reporters (as Fig. 2B). The solid curves show the optimal fits from the statistical-mechanical model with UP contact allowed only for loops 1 and 5. The dashed curves show optimal fits if all loops have equal UP contact energies. (B) The fitted values for the net free energy changes due to RNAP occupation in the four activation states of *PRM*. The UP contact improves binding by 2.7 kcal/mol.

removed at higher CI concentrations, reducing *PRM* activity. The simple scheme indicated by the structural modeling—*OL3* occupation inhibits UP contact—fulfills this requirement and is thus consistent with the reporter data, although it is not the only scheme to be so.

The parameter fitting converged on values that are biochemically reasonable, supporting the feasibility of the UP contact model (Fig. S7). The values for the DNA looping parameters, $\Delta G_{\text{oct}} = -0.5$ and $\Delta G_{\text{tet}} = -2.4$, are close to those estimated previously (16). The fitted free energy values for RNAP binding and UP contact are moderate: basal binding of RNAP to *PRM* is slightly unfavorable, CI at *OR2* improves RNAP binding by 1.4 kcal/mol, DNA looping inhibits RNAP binding by 0.4 kcal/mol, and UP contact provides a 2.7 kcal/mol improvement in RNAP binding (Fig. 5B).

The slight inhibition of *PRM* activation by DNA looping itself is needed to produce the small drop in maximal *PRM* activity in the shortOL reporters relative to the noOL reporters (Fig. 2B). The CTD of the CI dimer that is bound to the *OL* operator opposite *OR2* is quite close to RNAP in some of the octamer structures, possibly resulting in mildly unfavorable contacts.

The reporter data are unable to provide a test of the proposal that the CI dimer at the third *OL* operator opposite *PRM* in loops 4 and 7 directly blocks RNAP binding at *PRM* (21), because very similar fits were obtained by setting either no inhibition or complete inhibition of *PRM* in these species. However, the structural modeling suggests that there are no significant clashes between RNAP and CI in these cases.

Testing the Inhibition of UP Contact by CI at *OL3*. Thus, the reporter data and the structural and statistical-mechanical modeling indicate that the stimulatory effect of *OL* on *PRM* is due to an α CTD-UP contact that is mediated by an *OL1.2-OR1.2* loop, and that CI binding to *OL3* removes this activation by preventing the DNA bending needed for this contact.

We reasoned that mutation of *OL1* should provide a strong test of the prediction that CI binding to *OL3* prevents UP contact because it should eliminate all looped species in which *OL1* is occupied (loops 1, 3, 4, 5, 7, and 8), including all of the loops in which UP contact is expected to be possible, leaving only loops 2 and 6. Eliminating CI binding to *OL1* in the statistical-mechanical model confirmed this expectation, showing that the *PRM* activity of the longOL.*OL3*⁺*OR3*⁺ reporter should be reduced to that of the shortOL.*OL3*⁺*OR3*⁺ reporter (Fig. 6). As predicted, when we mutated *OL1* in these reporters, UP activation was lost (Fig. 6). Also, the repression of the *OL1*⁺ longOL.*OL3*⁺*OR3*⁺ reporter, due to filling of *OL3* at high [CI], was also lost, because in the absence of *OL1*, a CI tetramer at *OL2.OL3* forms at lower CI concentrations.

Fig. 6. Mutation of *OL1* confirms that *OL2.3* looping does not allow UP activation. Observed (points; error bars are SD; $n = 4$) and predicted (curves) *PRM* activity for longOL.*OL1*⁺*OR3*⁺ and shortOL.*OL1*⁺*OR3*⁺ reporters (green), plotted with *OL1*⁺*OR3*⁺ data and predictions from Fig. 5A (blue).

Discussion

Role of the UP Element in λ . The UP element is located at the -80 to -102 region of the λ lytic *PL* promoter (23, 24). This position is normally too far away to affect the promoter (28); however, the DNA-bending integration host factor (IHF) binds to a region overlapping the UP element (23, 33). It was proposed that DNA bending by IHF positions the UP element so that it can be contacted by the α CTD of the RNAP at *PL*, allowing the IHF and UP sites to stimulate *PL* some twofold to threefold in vivo (24). Thus, when CI is absent, the UP element is used to stimulate lytic transcription from the nearby *PL* promoter, but when CI is present and *PL* is repressed, the UP element is commandeered to stimulate lysogenic transcription from the distant *PRM* promoter.

The primary effect of activation of *PRM* by the UP element is to increase maximal CI-stimulated *PRM* activity approximately two-fold, making it almost as strong a promoter as *PR* (Fig. S4), without increasing basal activity. We expect that the higher *PRM* activity and thus higher lysogenic CI concentration in a λ lysogen due to UP activation would tend to stabilize the lysogenic state. λ lysogens are destabilized by expression of certain activation-defective α subunits, a result interpreted as due to defective α binding to the CI *ORI-OR2* complex (29). However, at least some of this effect may be due to a loss of *PRM* activation by the *OL* UP element. Testing this role of the UP element in the phage is complex because of its effect on *PL*. A secondary effect of the UP activation mechanism is to sharpen CI regulation of *PRM*, because UP activation occurs at low CI concentrations but is removed at higher CI concentrations as *OL3* becomes occupied by a CI dimer. This steeper repression of *PRM* as CI concentration increases beyond 0.5 lysogenic units should buffer the CI concentration in the lysogen.

A question arises whether the IHF site has any role in UP element stimulation of *PRM*. The UPcon1 and UPcon2 constructs (Fig. 2C) show that substitution of the IHF recognition sequence between *OL3* and the UP element (23) does not prevent UP activation of *PRM*.

***OL* as a Complex Enhancer.** The *OL* site has the defining properties of an enhancer, regulating transcription from long distances independently of orientation and position. *OL* was known to increase the repression of *PRM* (and *PR*) from as far as 3.8 kb downstream of the promoter (16), and here we have shown that repression is also improved when *OL* is 2.3 kb upstream of *PRM*. *OL* was known to stimulate activation of *PRM* when located 2.3 kb downstream (21), and we show here that activation works with *OL* placed 2.3 kb upstream of *PRM* and in either orientation. With the stronger activation provided by a CAP binding site, *OL* can stimulate *PRM* activity from 10 kb upstream.

OL is also a complex enhancer, containing multiple DNA sequences that both activate and repress the distant promoter in a variety of ways. Our analysis shows in unprecedented detail how these distal DNA elements work together with the proximal elements to regulate transcription. *OL1* and *OL2* target the enhancer to the promoter, binding a CI tetramer that interacts strongly with the *ORI.2* tetramer to overcome the entropic cost of forming a specific, stable long-range DNA loop. Formation of the CI octamer assists location of CI at *ORI.2* to help repress *PR* and activate *PRM* at low CI concentrations but also positions the distal *OL3* and the UP sequences so that they can function like proximal elements. The UP element then behaves essentially as a core promoter element, able to be directly contacted by the RNAP at the distant promoter via the α CTD. The *OL3* site has dual repressive roles. CI binding at *OL3* acts architecturally, preventing the DNA bending needed for this UP contact and thus indirectly competing with RNAP binding. In addition, a CI dimer at *OL3* is positioned to interact with a CI dimer at *OR3* to effect repression of *PRM*. In all, *PRM* is thus regulated by three core promoter elements (-10 , -35 , and *UP*) and six sites for the CI transcription factor. These five proximal and four distal sites assemble 8–12 CI monomers and 5 RNAP subunits into different enhancer-promoter complexes that determine whether transcription occurs.

λ OL thus provides an alternative prokaryotic enhancer model to the example of σ 54-dependent long-range promoter activation. Although OL works noncatalytically, demonstrating enhancement by simple recruitment mechanisms, activation via σ 54 is catalytic. The activators interact with the bound σ 54 RNAP by DNA looping and remodel the polymerase in an ATP-dependent reaction needed for open complex formation (34). Thus, these two systems illustrate how these fundamentally different regulatory mechanisms (6, 10) can operate from long distances. Activation by the catalytic mechanism requires only transient enhancer–promoter contact and thus could be suited to situations where a single enhancer must activate multiple promoters. The stable enhancer–promoter contact of the recruitment mechanism would likely be suited to enhancers that provide highly specific regulation of one promoter at a time.

Materials and Methods

Reporter and CI Expression Constructs. The *lacZ* reporter constructs (Figs. S1 and S2) (*SI Materials and Methods*) were integrated into the λ *attB* site in MG1655 *rph⁺ ΔlacIZYA*. A λ *att80* prophage and various *PRM.cI.OL* CI expression constructs inserted at ϕ 186 *attB* (Fig. S1) were used to supply different fixed CI levels, measured by Western blotting (*SI Materials and Methods*) (Fig. S3). The RNAP α subunits (Fig. 2D) were expressed from Plac

on multicopy plasmids. LacZ assays were by a modified microtiter plate method (16) (*SI Materials and Methods*).

Statistical–Mechanical Modeling. Full details of the modeling are given in *SI Materials and Methods*. Fig. S6 lists the species and assignments; Fig. S7 lists the model parameters.

Structural Modeling. DNA flexibility modeling was based on observed base pair step parameters (35). The simulated annealing protocol was that implemented in CNS (36). Conformations of the α linker were generated by randomly sampling ϕ and ψ backbone torsions in a manner proportionate to their residue-specific Ramachandran probabilities, as implemented by the Rosetta modeling program (37). Full details of the structural modeling are given in *SI Materials and Methods*.

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