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Allosteric Transcriptional Regulation *via* changes in the Overall Topology of the Core Promoter

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Abstract

Many transcriptional activators act at a distance from core promoter elements and work by recruiting RNA polymerase through protein-protein interactions. We show here how the prokaryotic regulatory protein CueR both represses and activates transcription by differentially modulating local DNA structure within the promoter. Structural studies reveal that the repressor state slightly bends the promoter DNA, precluding optimal RNA polymerase-promoter recognition. Upon binding a metal ion in the allosteric site, CueR switches into an activator conformation. It maintains all protein-DNA contacts but introduces torsional stresses that kink and undertwist the promoter, stabilizing an A-DNA-like conformation. These factors switch on and off transcription by exerting dynamic control of DNA stereochemistry, reshaping the core promoter and making it a better or worse substrate for polymerase.

While transcription factors can introduce kinks, bends (1), or loops in DNA (2), the mechanistic roles of such distortions are not always clear. Factors in the prokaryotic MerR family alter DNA structure between the core promoter elements where they repress and activate transcription in response to many signals, including metal ion concentration changes (3, 4). The activator conformation of MerR proteins introduces a DNA distortion at the center of the operator (3, 5–7) and structural studies of the activator protein-DNA complexes reveal a kink at this site (8–10). This distortion is thought to stimulate transcription by realigning the suboptimally-spaced –10 and –35 core promoter elements (fig. S1A); however, the mechanisms of allosteric conversion, repression and activation remain unknown. To understand how a protein can switch transcription off and on while bound to a single site, we solved the structures of the DNA complexes of *Escherichia coli* (*E. coli*) CueR in the repressor and activator conformation s.

CueR, a member of the metalloregulatory subfamily of MerR proteins, is a Cu^I and Ag^I-sensing factor that controls expression of metal homeostasis genes *copA* and *cueO* (11–15). The metal-bound state, Ag^I-CueR (i.e. activator), co-crystallized with a 23 base pair (bp) DNA based on *E. coli copA* promoter, P_{copA} (table S1, figs. S1, S2). Given the extreme

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affinity of CueR for copper ($K_d = 2 \times 10^{-21}$ M) (12), crystallization of the metal free (i.e. repressor) complex required mutation of metal-binding residues (C112S, C120S) and deletion of residues disordered in the DNA-free Ag^I-CueR structure (C129-G135) (12). This variant is a repressor in the presence or absence of copper and co-crystallized with a 26 bp *P_{copA}* DNA (table S1, figs. S1, S2). Both structures were solved using molecular replacement (MR) and single-wavelength anomalous diffraction (SAD); the activator and repressor complexes were refined to 2.8 Å and 2.1 Å, with final models that include CueR residues 1–130 and 1–111, respectively (Figs. 1A, B; table S2)

In both structures, the protein is a dimer with each protomer contacting the duplex *via* an N-terminal DNA-binding domain (DBD) composed of four α-helices in a winged helix-turn-helix motif (Fig. 1A). A hinge loop connects the DBD to a long dimerization helix (DH). In the activator structure, the DH is followed by a metal-binding loop (MBL) and a two-turn C-terminal α-helix (CTH) (Figs. 1A, S3A, B). These features, as well as the Ag^I coordination, are similar in the presence and absence of DNA (fig. S3C–F) (12). As discussed below, the MBL and CTH are disordered in the repressor complex.

The most striking difference between these complexes is the DNA conformation. The stereochemistry of the central seven base pairs, which are B-form DNA in the repressor complex, switches in the activator complex to an A-DNA-like structure known as TA-DNA, first described for the TBP/DNA complex (fig. S4A–F, table S3) (16). The two central bp-steps (T₁₂T₁₃ and T₁₃G₁₄) in the repressor complex exhibit elevated roll angles (14° and 10°), consistent with a slight distortion at the center of the DNA. These become highly kinked (33° and 23°) as the minor groove becomes significantly wider than the major groove in the activator complex (Fig. 1C, D) and alters the trajectory of the helical axis by ~36° relative to the repressor complex DNA (Fig. 1E). The repressor undertwists the DNA by ~50° and the activator further undertwists the DNA by ~22°, for a total of ~72° (fig. S5 A). Other MerR-family activator/DNA complexes show similar distortions (table S3, fig. S5B) (8, 10). Molecular dynamics (MD) simulations reveal that the activator duplex structure rapidly relaxes to B-form DNA upon removal of protein constraints, supporting the idea that the DNA distortions are energetically distinct states that are stabilized by two different protein conformations (fig. S6).

Remarkably, protein-DNA contacts in repressor and activator complexes are indistinguishable in the two structures. CueR interacts with phosphate groups at the distal edges of the pseudo-palindrome through three Arg residues from α2 and the loop wing of the DBDs (Arg18, 31 and 37) that serve as clamps that “grip” the DNA backbone (i.e. R-clamps) (figs. S7A, B). Mutagenesis and functional assays reveal that all three residues are required for transcription activation (figs. S7C, D). We conclude that these conserved R-clamp residues (fig. S8) (8–10) play a key role as the activator conformation exerts the torque needed to distort the duplex into the A-DNA-like conformation.

In terms of DNA recognition, Tyr36 inserts into the minor groove and forms H-bonds to N2 of G_{22/23'} and O4' of T_{23/24'}. Lys15 inserts into the major groove and forms H-bonds to N7 and O6 of G_{18/19'} and N7 of G_{17/18'} (fig. S7B) and may confer DNA-binding site specificity (10). These interactions are not conserved among *E. coli* MerR proteins (fig. S8). A

conserved feature among MerR proteins is the van der Waals (VDW) packing of an aromatic residue against a base in the major groove (8–10). In both CueR/DNA structures, the aromatic ring of Phe19 sits perpendicular to and forms VDW contacts with C₁₅ and G_{16'} (fig. S7B). This appears to contribute to the stability of the activator state (10); however these interactions are unchanged in the repressor complex. Thus, the transcriptional switching event is not explained by changes in individual side-chain interactions with DNA.

The allosteric switch starts with metal binding to stabilize the MBL conformation. This triggers displacement of key residues in the hinge between DBD and DH (Fig. 2A). The hinge residue Arg75 provides a direct allosteric link between the MBL and the DBD. The backbone carbonyl oxygen of Ala118' of the MBL forms an H-bond with Arg75 N ϵ , displacing a water and flipping the residue; N η 1 now H-bonds with the backbone carbonyl oxygen atoms of Asp72 and Phe70 on α 4 of the DBD (Fig. 2A). Mutation of Arg75 to alanine decreases transcription activation at P_{copA} *in vivo*, consistent with the loss of the H-bond communication network (Figs. 2C, S9A). The activator conformation is further stabilized when the CTH docks into the opened hydrophobic cavity (Fig. 2B). Hydrophobic residues Ile122, Ile123 and Leu126 project into this cavity, displacing Phe70 but not disrupting the H-bond with Arg75 (Figs. 2A, B). This locks in a new orientation between the two protomers in the activator complex. These changes shift the DHs (α 5 and α 5') in a small “scissors” movement (Fig. 3A) and decrease the distance between the two DBDs by ~6 Å (Fig. 3B). Since the DBDs move as rigid domains that tightly clamp onto the DNA, their rotation (Fig. 3C) and translation (Fig. 3D) readily force the kinking and undertwisting of the intervening DNA.

To test this mechanism, two types of allosteric-control (AC) CueR variants were made: a Cu^I-independent constitutive activator (CueR^{CA}), and a constitutive repressor (CueR^{CR}). Early phenotype screens (17, 18) and studies of A89V/S131L MerR identified variants that activate transcription in the absence of the effector, Hg^{II} (19). We mutated homologous positions in CueR (T84V/N125L) (fig. S9B) and evaluated transcription in a *cueR* knock-out *E. coli* strain (*cueR*) using *in vivo* β -galactosidase reporter assays. This variant exhibits clear Cu^I-independent transcription activation at all added copper concentrations (Fig. 2D), supporting its designation as a constitutive activator. To test whether the CTH communicates the allosteric signal to the DBD *via* docking into the hydrophobic cavity, we evaluated a mutant lacking the CTH (fig. S9C) and found it was a constitutive repressor at all copper concentrations (Fig. 2E), providing additional support for the allosteric mechanism. Inspection of other MerR-family proteins suggests that the repression and activation mechanisms, including docking of the CTH, can be employed by proteins with similar C-termini (e.g. SoxR), and by homologs with larger C-termini (e.g. BmrR). These results reveal how the allosteric transition induces significant stereochemical reorganization of the structure and orientation of the core promoter elements with little or no change in protein-DNA contacts or affinity (20, 21). To understand how these changes lead from repression to activation of transcription, we next considered the promoter geometry from the polymerase point of view.

MerR family proteins are thought to work by adjusting the phase angle and distance between the hexameric polymerase contact sites, namely the –35 and –10 promoter elements (Fig. 4).

In consensus *E. coli* promoters, there are 17 bp between these elements but in MerR-type promoters, there are 19–20 bps (22). Assuming B-form DNA, the –10 and –35 elements of the latter are 72° out of phase with respect to the productive conformation, and have a longer separation between the two polymerase binding sites (Fig. 4A). In the repressor/DNA complex, the promoter is shortened by ~3 Å (Fig. 4B), consistent with hydrodynamic and biophysical studies of apo-MerR-family/DNA complexes (20, 23, 24). The repressor also undertwists the DNA, reducing the phase angle by 50° (to ~130°), a value approaching that of a 17 bp promoter (Fig. 4B, D). Conversion to the activator structure induces further DNA undertwisting and kinking, which shortens the duplex by an additional ~6 Å relative to the repressor complex. This results in phasing and spacing parameters that are close to that of a 17 bp promoter (~108°) (Fig. 4C, D), corroborating studies of other MerR-family/DNA complexes (5, 8–10, 24). While this two-dimensional analysis provides a plausible account for transcription from the activator complex, it also suggests that the repressor, which also decreases the distance and the phase angle between the elements, should stimulate transcription. Clearly, it does not. The question of how the repressor exerts transcriptional silencing is best understood from a three-dimensional perspective.

By examining how these proteins alter the overall shape of the promoter with respect to the polymerase surface, we can account for repression as well as activation. Both structures were modeled onto structurally characterized RNAP/DNA complexes (Figs. S10, 4E) (25). Consistent with results showing that MerR can form stable ternary complexes with the promoter and RNAP (7, 21), we find that CueR and RNAP can bind on opposite faces of DNA without steric penalties (figs. S10A, B). By overlaying CueR/DNA and RNAP complexes at the –35 element where the DNA interacts with the polymerase $\sigma 4$ domain, we find that the repressor-imposed DNA conformation prevents contacts with the –10 element by forcing it ~40 Å away from the $\sigma 2$ domain (figs. S10A, B). This is accomplished by two bends that move the DNA towards the repressor and away from the polymerase (figs. S10C). These bends map to DNaseI hypersensitivity sites in repressor MerR/DNA complexes, indicating that they persist in solution (5). We propose that this undertwisting and shortening of the promoter by the repressor *poises* the conformation of the DNA substrate, but maintains a significant barrier to transcription activation. As it switches from the repressor to activator, CueR introduces a major kink in the central base pairs of the promoter, bringing the –10 element to the $\sigma 2$ domain. The repressor and the activator forms modulate the overall shape of the core promoter in order to place the highly critical –10 element bases (26, 27) away from or near the RNAP $\sigma 2$ domain, respectively (Figs. 4E, S10B). Thus, regulatory factors can reshape a core promoter *via* local changes in DNA stereochemistry and thereby stimulate facile switching between inactive and active transcription states.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Crystal Structures of Repressor and Activator Complexes with DNA
(A) Repressor and activator CueR/DNA structures. **(B)** Sequences of crystallized DNA duplexes. **(C)** DNA groove widths (M=major groove; m=minor groove). **(D)** Kinks at the central bp-steps. The minor grooves are shaded black and kinks are defined by the roll angles (pink lines) between the bp-steps. **(E)** The activator introduces a ~36° angular change in the DNA.

Fig. 2. The Allosteric Signal Is Propagated Through the C-Terminal Loop-Helix Motif to the Hinge

(A) Comparison of repressor (left) and activator (right) CueR depicting the transformations occurring at the CTH and hinge upon Ag^I binding: hinge residues R75, H76 and S77 are displaced (arrows) and form new H-bonds (dashed lines) with CTH residues. (B) The hydrophobic cavity formed by DBD and DH residues (yellow), F70 (orange) and surfaces (gray) are shown in the repressor (left). The CTH' residues and surfaces (pink) dock into the opened hydrophobic cavity in the activator (right). (C, D, E) β-galactosidase activity for allosteric-control (AC) mutants. Error bars (under symbols) correspond to S.D. (C) The *cueR*^{R75A} variant exhibits full repression in the absence of copper and diminished activation upon addition of copper. (D) The *cueR*^{CA} variant exhibits greatly increased activation at all copper concentrations. (E) The *cueR*^{CR} variant exhibits full repression at all copper concentrations.

Fig. 3. The DNA-Binding Domains (DBD) Rotate and Translate Upon Metal Binding
 (A) The slight “scissors” motion of the repressor (top) and activator (bottom) DHs ($\alpha 5$ and $\alpha 5'$) is shown. (B) The repressor DBDs rotate, bringing the wings closer by ~ 6 Å, and (C) decreasing the dihedral angle by $\sim 33^\circ$, resulting in kinked/undertwisted DNA in the activator complex. (D) The complexes from (C) are viewed from the top. The DBDs translate by ~ 7 Å and ~ 10 Å, respectively. The DBDs move as rigid bodies: superposition of the individual repressor and the activator DBDs reveals an RMSD of ~ 0.5 Å for main chain atoms.

Fig. 4. CueR Bends, Undertwists and Kinks DNA for Transcription Control

(A) B-DNA model of *P_{copA}* (19 bp), (B) repressor CueR/DNA complex, (C) activator CueR/DNA complex, and (D) B-DNA model consensus *E. coli* promoter (17 bp). In (B) and (C), the crystallized DNA (tan) is extended with ideal B-DNA (gray) to include promoter elements. The radial plots (left) show relative positions of the two elements looking down the central axis with -35 in front; the corresponding angle between -10 and -35 is given. (E) DNA from the repressor and activator complexes, and the B-DNA model of *P_{copA}* were modeled onto RNAP (see fig. S10). The repressor complex DNA is bent away from $\sigma 2$. The transition from repressor to activator kinks the DNA, positioning the -10 element in close proximity to $\sigma 2$.