

Structural Basis of Transcription Activation: The CAP- α CTD-DNA Complex

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The *Escherichia coli* catabolite activator protein (CAP) activates transcription at P_{lac} , P_{gal} , and other promoters through interactions with the RNA polymerase α subunit carboxyl-terminal domain (α CTD). We determined the crystal structure of the CAP- α CTD-DNA complex at a resolution of 3.1 angstroms. CAP makes direct protein-protein interactions with α CTD, and α CTD makes direct protein-DNA interactions with the DNA segment adjacent to the DNA site for CAP. There are no large-scale conformational changes in CAP and α CTD, and the interface between CAP and α CTD is small. These findings are consistent with the proposal that activation involves a simple "recruitment" mechanism.

The catabolite activator protein (CAP) [also referred to as the cyclic adenosine monophosphate (cAMP) receptor protein] activates transcription by binding to a DNA site located in or upstream of the core promoter and interacting with the RNA polymerase (RNAP) α subunit COOH-terminal domain (α CTD) [reviewed in (1)], an 85-amino acid, independently folded domain that is flexibly tethered to the remainder of RNAP [reviewed in (2)]. Interaction of CAP with α CTD facilitates binding of α CTD (and, through it, the remainder of RNAP) to promoter DNA, thereby stimulating transcription initiation (1). At class I CAP-dependent promoters, such as the P_{lac} promoter, CAP- α CTD interaction is the sole basis of activation (1). At class II CAP-dependent promoters, such as the P_{gal} promoter, interaction of CAP with α CTD is one of multiple interactions involved in activation (1, 3).

CAP binds to DNA as a dimer of two identical subunits and recognizes a 22-base pair (bp), two-fold symmetric consensus DNA site (1, 4–6). Transcription activation by CAP requires a determinant within CAP termed "activating region 1" (AR1) (residues 156 to 164) (1, 7–11), which is functionally presented by one of the two subunits of the CAP dimer (1, 12, 13). Transcription activa-

tion also requires the COOH-terminal residue of CAP (residue 209) (14), which, in the structure of the CAP-DNA complex, is located adjacent to, and is in contact with, AR1 (6).

Transcription activation by CAP requires three distinct determinants within α CTD (1, 15–19): (i) the "287 determinant" (residues 285 to 290, 315, 317, and 318), proposed to mediate protein-protein interaction with AR1 of CAP; (ii) the "265 determinant" (residues 265, 294, 296, 298, 299, and 302), proposed to mediate protein-DNA interaction with the DNA segment adjacent to the DNA site for CAP; and (iii) the "261 determinant" (residues 257, 258, 259, and 261), proposed to mediate protein-protein interaction with σ^{70} at a subset of class I CAP-dependent promoters, including P_{lac} .

Transcription activation by CAP also requires the structural integrity of the DNA segment adjacent to the DNA site for CAP (1, 20). In the ternary complex of CAP, RNAP, and promoter, α CTD interacts with the DNA segment adjacent to the DNA site for CAP, contacting the DNA minor groove centered 18 or 19 bp from the center of the DNA site for CAP (1, 21, 22). At most CAP-dependent promoters, including P_{lac} , α CTD interacts nonspecifically with the DNA segment adjacent to the DNA site for CAP, contacting arbitrary, nonspecific DNA sequences (1, 23, 24). However, replacement of these nonspecific DNA sequences by high-affinity, specific DNA sites for α CTD (e.g., 5'-AAAAAA-3') (25) facilitates formation of the ternary complex of CAP, RNAP, and promoter (1, 24, 26–28).

In previous work, the CAP-DNA complex was crystallized using a 30-bp two-fold symmetric DNA fragment containing the 22-bp two-fold symmetric consensus DNA site for CAP and 4 bp of flanking DNA on each side

(Fig. 1A, top) [(6); see also (4, 5)]. In our current work, we crystallized the CAP- α CTD-DNA complex using an analogous 44-bp two-fold symmetric DNA fragment containing the 22-bp two-fold symmetric consensus DNA site for CAP and 11 bp of flanking DNA—with an optimally positioned, high-affinity, specific DNA site for α CTD (i.e., 5'-AAAAAA-3') (25, 27, 28)—on each side (Fig. 1B, top) (29). It was anticipated that this DNA fragment would yield a two-fold symmetric complex consisting of a central CAP dimer flanked on each side by α CTD (Fig. 1B).

The structure was solved by molecular replacement using the crystal structure of the CAP-DNA complex as the initial model and iterative cycles of Fourier refinement and model building to place the rest of the structure. The final model R and R_{free} values are, respectively, 21.1 and 24.4% against 3.1 Å diffraction data (Fig. 1C) (30).

As anticipated, the two-fold symmetric crystallization DNA fragment yielded a two-fold symmetric structure (Fig. 1B). Each half of the two-fold symmetric structure contains one subunit of CAP, one-half of the crystallization DNA fragment, one molecule of α CTD that interacts with CAP and DNA (α CTD^{CAP,DNA}), and one molecule of α CTD that interacts exclusively with DNA, interacting with an A/T-rich DNA-minor-groove segment that, fortuitously, is accessible in the crystal lattice (α CTD^{DNA}). We suggest that the structure defines two distinct sets of biologically relevant interactions: (i) CAP- α CTD-DNA interactions at a class I or class II CAP-dependent promoter (Fig. 2; CAP- α CTD^{CAP,DNA}-DNA) and (ii) α CTD-DNA interactions with an A/T-rich DNA minor groove at an UP element subsite-dependent promoter [Fig. 3; α CTD^{DNA}-DNA (2, 25)].

The structures of the CAP-DNA complex (Fig. 1A) (6) and of CAP-DNA within the present complex (Fig. 1B) are superimposable [root mean square deviation of backbone atoms ($\text{RMSD}_{\text{backbone}}$) = 1.0 Å]. We conclude that the conformations of CAP and the DNA segment in contact with CAP do not change substantially upon interaction with α CTD. Similarly, the structures of free α CTD (31), α CTD^{CAP,DNA}, and α CTD^{DNA} are superimposable ($\text{RMSD}_{\text{backbone}}$ = 0.7 to 2.1 Å). We conclude that the conformation of α CTD does not change substantially upon interaction with CAP and/or with DNA. The structure of the DNA segment between CAP and α CTD^{CAP,DNA} is moderately distorted (roll of 15° and twist deficit of 10° centered 13 bp from the center of the DNA site for CAP, resulting in compression by ~2.5 Å of the DNA major groove centered 13 bp from the center of the DNA site for CAP, and reduction by ~7.5 Å of the distance between CAP and α CTD^{CAP,DNA}) (32, 33). The struc-

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tures of the DNA segments in contact with α CTD^{CAP,DNA} and α CTD^{DNA} are nearly canonical B-form DNA, with only very slight distortion (compression by ~ 1.5 Å of the DNA minor groove).

The interaction between CAP and α CTD^{CAP,DNA} involves AR1 and the COOH-terminal residue of CAP and the 287 determinant of α CTD^{CAP,DNA} (Fig. 2, A through C). The interaction involves a strikingly small interface (six residues each of CAP and α CTD; 630 Å² of buried surface area). Residues 157 to 160 and 164 of AR1 contact residues 285 to 288 of the 287 determinant, with the Thr¹⁵⁸ side-chain hydroxyl making two hydrogen bonds (to residues 285 and 286), the Thr¹⁵⁸ backbone carbonyl making two hydrogen bonds (to residues 285 and 287), the Thr¹⁵⁸ side-chain methyl making van der Waals interactions (to residue 286), and residues 157, 159, 160, and 164 making additional van der Waals interactions (Fig. 2B). The COOH-terminal residue of CAP (Arg²⁰⁹) contacts residues 315 and 317 of the 287 determinant of α CTD, with the COOH-terminal residue side chain making a hydrogen bond to residue 315 and the COOH-terminal backbone carboxylate making a salt bridge with residue 317 (Fig. 2C). The COOH-terminal residue side chain also makes two buttressing hydrogen bonds to AR1 (to backbone carbonyl oxygens of resi-

dues 159 and 160). The observed contacts provide a structural rationalization for genetic results indicating that Thr¹⁵⁸ is the critical residue in AR1 (the only residue in AR1 for which side-chain atoms beyond C β are required for activation) (10) and indicating involvement of residues 157 to 160, 164, and the COOH-terminus of CAP, and residues 285 to 288, 315, and 317 of α CTD (1, 7–11, 14, 18, 19). The structure also provides a rationalization for genetic results indicating involvement of residues 162 and 163 of CAP and residues 289 and 290 of α CTD (1, 8–11, 19); in the structure, these residues underlie (and make hydrogen bonds and van der Waals interactions with) residues of AR1 and the 287 determinant of α CTD and, as such, are likely to be critical determinants of the conformations of AR1 and the 287 determinant. The observed small size of the interface between CAP and α CTD^{CAP,DNA} is in agreement with genetic results ruling out involvement of other residues (10, 18, 19) and with biochemical results indicating a modest magnitude of transcriptional activation (a factor of about 10 at P_{lac}) (34) and a modest magnitude of CAP-RNAP interaction free energy (about -1 to -2 kcal/mol at P_{lac}) (35–37).

The interaction between α CTD^{CAP,DNA} and DNA involves the 265 determinant of α CTD^{CAP,DNA} and the DNA backbone and minor groove of the 6-bp DNA segment cen-

tered 19 bp from the center of the DNA site for CAP (5'-AAAAAG-3') (Fig. 2, A, D, and E). Residues 264, 265, 268, 294, 296, 298, and 299 of the 265 determinant make direct contacts with the DNA backbone (Fig. 2, D and E). No residue makes direct contacts with DNA base-pair edges (Fig. 2, D and E). However, the Arg²⁶⁵ side chain penetrates into the DNA minor groove and makes at least two, and possibly four, water-mediated hydrogen bonds with DNA base-pair edges (cyan in Fig. 2, D and E). The Arg²⁶⁵ side-chain guanidinium makes two water-mediated hydrogen bonds (to the thymine O2 atom of the A:T base pair 18.5 bp from the center of the DNA site for CAP, and to the adenine N3 atom of the A:T base pair 19.5 bp from the center of the DNA site for CAP) through interaction with a water molecule positioned as in the "minor-groove spine of water" characteristic of A/T-rich DNA (38–41) (cyan in Fig. 2, D and E). In addition, the Arg²⁶⁵ side-chain guanidinium is positioned so as potentially to make two additional water-mediated hydrogen bonds (to the thymine O2 atom of the A:T base pair 19.5 bp from the center of the DNA site for CAP, and to the adenine N3 atom of the A:T base pair 20.5 bp from the center of the DNA site for CAP) through interaction with a putative second water molecule positioned as in the minor-groove spine of water (a water molecule not

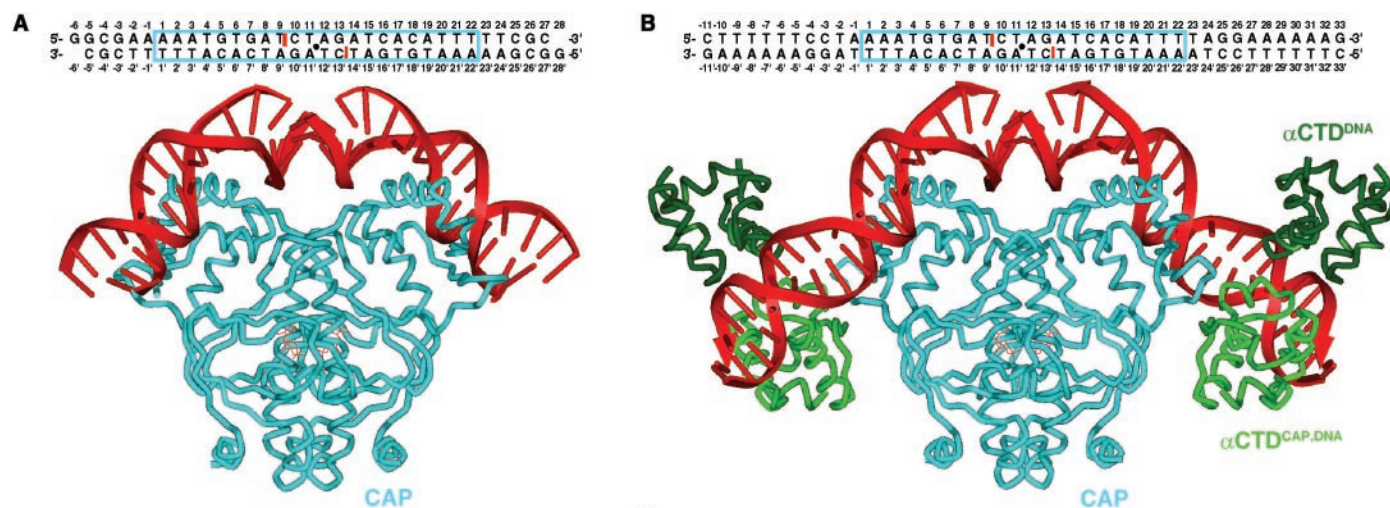


Fig. 1. Structure determination. (A) Top, 30-bp DNA fragment used to crystallize the CAP-DNA complex in (6) (box, DNA site for CAP; solid circle, two-fold symmetry axis of DNA site for CAP; solid bars, single-phosphate gaps). Bottom, structure of the CAP-DNA complex in (6). CAP is in cyan; DNA and cAMP are in red. (B) Top, 44-bp DNA fragment used to crystallize the CAP- α CTD-DNA complex in this work (box, DNA site for CAP; solid circle, two-fold symmetry axis of DNA site for CAP; solid bars, single-phosphate gaps). Bottom, structure of the CAP- α CTD-DNA complex of this work. CAP is in cyan, α CTD^{CAP,DNA} is in light green, α CTD^{DNA} is in dark green, and DNA and cAMP are in red. (C) Crystallographic data collection and refinement statistics. *I*, intensity.

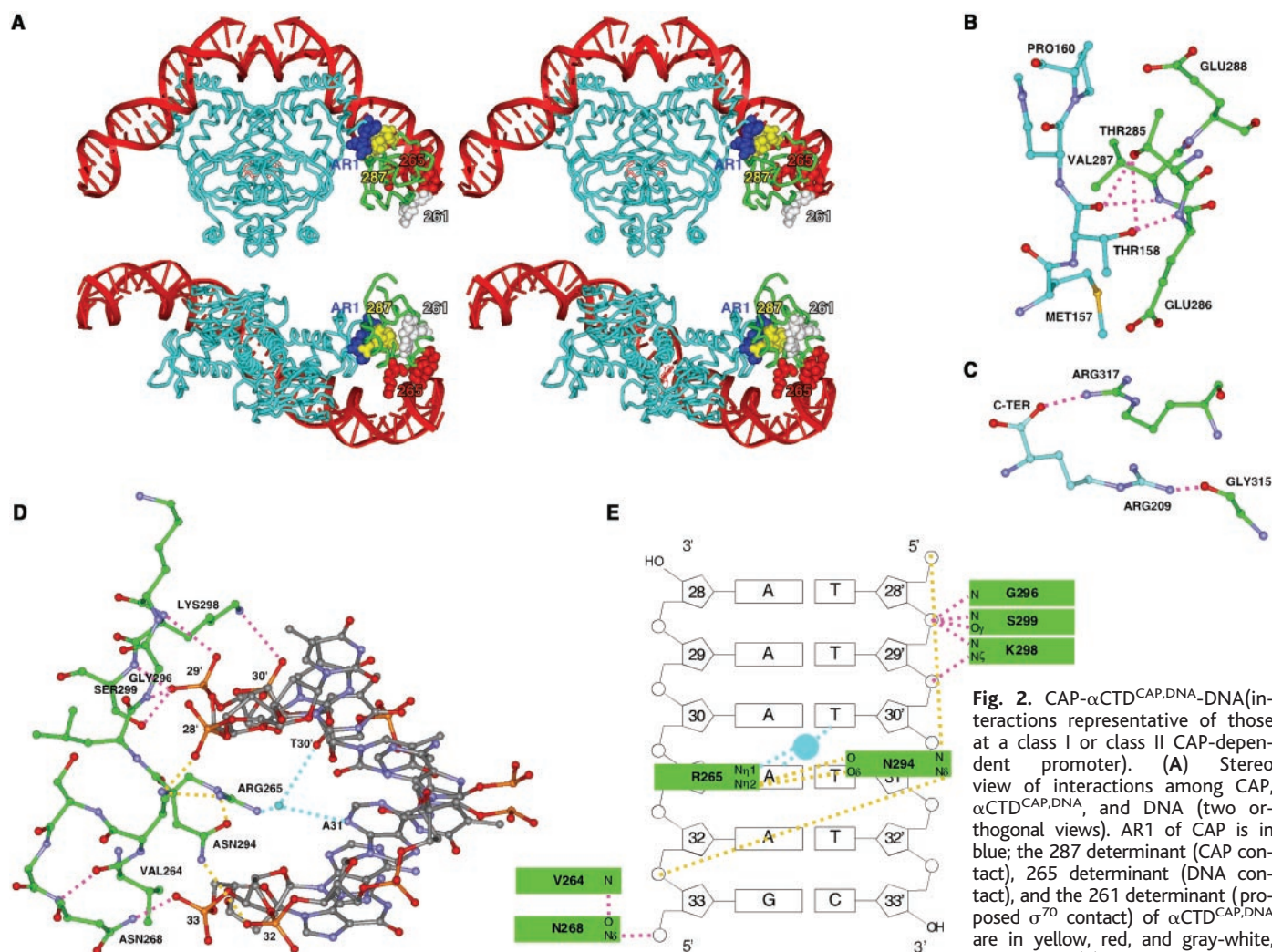


Fig. 2. CAP- α CTD^{CAP,DNA}-DNA (interactions representative of those at a class I or class II CAP-dependent promoter). (A) Stereo view of interactions among CAP, α CTD^{CAP,DNA}, and DNA (two orthogonal views). AR1 of CAP is in blue; the 287 determinant (CAP contact), 265 determinant (DNA contact), and the 261 determinant (proposed σ^{70} contact) of α CTD^{CAP,DNA} are in yellow, red, and gray-white, respectively. (B) Interactions between AR1 of CAP and residues 285 to 288

of the 287 determinant of α CTD^{CAP,DNA}. Hydrogen bonds are in magenta. (C) Interactions between the COOH-terminal residue of CAP (Arg²⁰⁹) and residues 315 and 317 of the 287 determinant of α CTD^{CAP,DNA}. Hydrogen bonds are in magenta. C-TER, COOH-terminus. (D) Interactions between α CTD^{CAP,DNA} and DNA (view along DNA minor-groove axis). Water-mediated hydrogen bonds involving the Arg²⁶⁵ side-chain guanidinium, DNA bases, and an experimentally defined water molecule (sphere near center) are in cyan. The network of hydrogen bonds buttressing the Arg²⁶⁵ side-chain guanidinium relative to the phosphate backbones of the two DNA strands is in yellow. Other hydrogen bonds are in magenta. (E) Summary of interactions between α CTD^{CAP,DNA} and DNA. Colors are as in (D). G, Gly; K, Lys; N, Asn; R, Arg; S, Ser; and V, Val.

observed in this structure at 3.1 Å (39). The Arg²⁶⁵ side-chain guanidinium is held in a precise orientation relative to the DNA minor groove and bound water through buttressing van der Waals interactions with DNA-backbone sugars of the top and bottom DNA strands (Fig. 2D) and by a cross-helix network of buttressing hydrogen bonds, involving two hydrogen bonds between the Arg²⁶⁵ side-chain guanidinium and Asn²⁹⁴, one hydrogen bond between Asn²⁹⁴ and a DNA-backbone phosphate of the top DNA strand, and one hydrogen bond between Asn²⁹⁴ and a DNA-backbone phosphate of the bottom DNA strand (yellow in Fig. 2, D and E). The observed contacts provide a structural rationalization for genetic and nuclear magnetic resonance (NMR)-spectroscopic results indicating involvement of residues 264, 265, 268, 294, 296, 298, and 299 (with Arg²⁶⁵ as the

most critical residue) in CAP- α CTD-DNA and α CTD-DNA interactions (1, 15–19, 25, 42, 43), for biochemical and NMR-spectroscopic results indicating involvement of the DNA minor groove in CAP- α CTD-DNA and α CTD-DNA interactions (22, 43, 44), and for interference-footprinting results indicating involvement of the adenine N3 atom in α CTD-DNA interactions (44). The observed contacts also provide two structural rationalizations for the specificity of α CTD for A/T-rich DNA: (i) Arg²⁶⁵ makes water-mediated hydrogen bonds through interaction with the minor-groove spine of water, a structural feature present in A/T-rich DNA (40, 41), and (ii) Arg²⁶⁵ is buttressed by a network of interactions spanning a compressed, narrowed minor groove, a structural feature present in A/T-rich DNA (45, 46).

The 261 determinant of α CTD^{CAP,DNA} is

located on the face of α CTD^{CAP,DNA} opposite from CAP, ~23 bp from the center of the DNA site for CAP, and is prominently exposed (gray-white in Fig. 2A). The position and prominent exposure of the 261 determinant are consistent with the proposal that the 261 determinant participates in α CTD- σ^{70} interactions at the subset of class I CAP-dependent promoters, including P_{lac}, where CAP binds in the –60 region, with ~23 bp between the center of the DNA site for CAP and the upstream edge of the DNA segment contacted by σ^{70} (1, 19, 47).

As noted above, the crystal structure contains a second molecule of α CTD that interacts exclusively with DNA, interacting with an UP element subsite-like DNA segment (5'-GAAAAA-3') [compare with (25)] that, fortuitously, is accessible in the crystal lattice: α CTD^{DNA} (dark green in Fig. 1B; Fig.

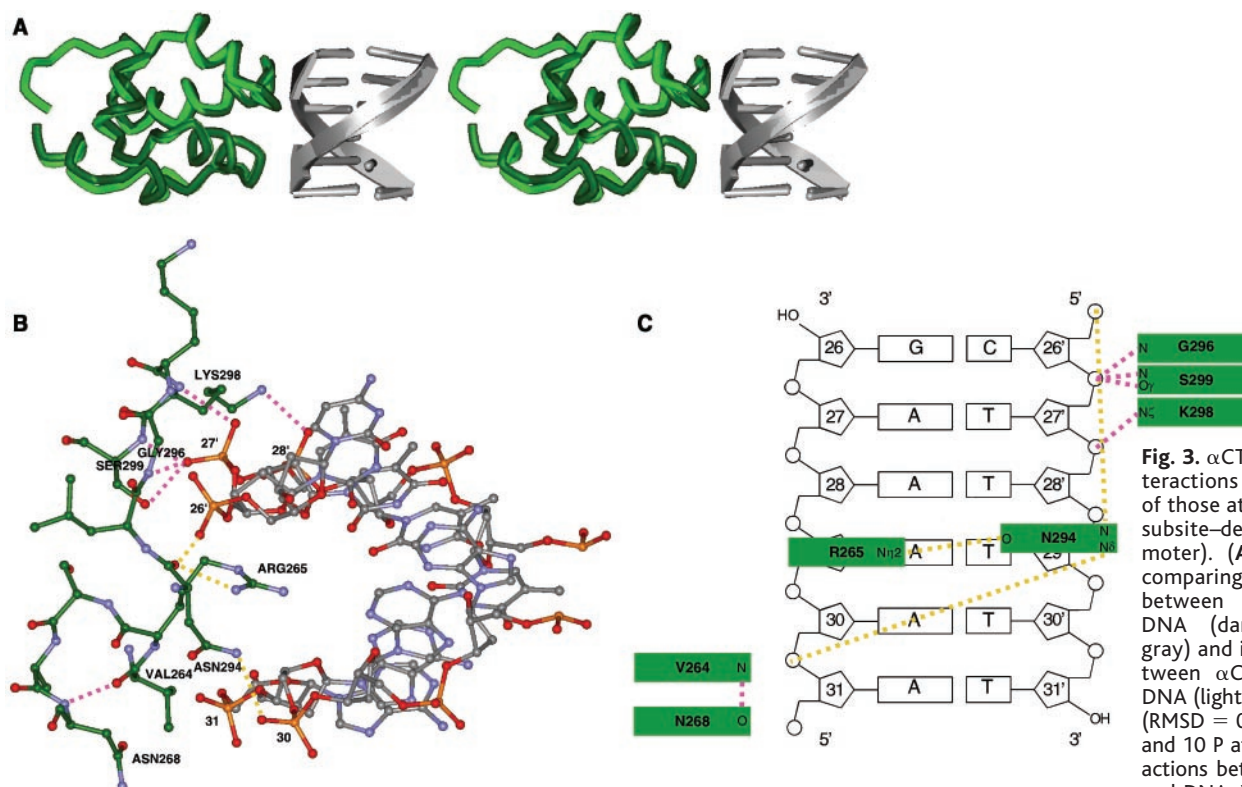


Fig. 3. α CTD^{DNA}-DNA (interactions representative of those at an UP element subsite-dependent promoter). (A) Stereo view comparing interactions between α CTD^{DNA} and DNA (dark green and gray) and interactions between α CTD^{CAP-DNA} and DNA (light green and gray) (RMSD = 0.74 Å for 72 C α and 10 P atoms). (B) Interactions between α CTD^{DNA} and DNA. View and colors are as in Fig. 2, D and E.

are as in Fig. 2D. No water molecules were observed in the α CTD^{DNA}-DNA interface in this structure at 3.1 Å. However, the positions of the Arg²⁶⁵ side-chain guanidinium and DNA bases are compatible with the establishment of water-mediated hydrogen bonds identical to those at the α CTD^{CAP-DNA}-DNA interface (Fig. 2D) (39). (C) Summary of interactions between α CTD^{DNA} and DNA. Colors are as in Fig. 2, D and E.

3). α CTD^{DNA}-DNA and α CTD^{CAP-DNA}-DNA interactions are nearly identical, both in overall organization and in detail (Fig. 3). We infer that similar α CTD-DNA interactions are made in UP element subsite-dependent transcription and in CAP-dependent transcription, consistent with similar requirements for the 265 determinant (1, 15–19, 25, 42, 43) and similar preferences for A/T-rich DNA (24–28).

The results in this report provide a high-resolution structural description of interactions between a transcriptional activator and its target within the general transcription machinery. Two striking findings are that transcriptional activation can occur without conformational change in activator and target and that transcriptional activation can involve a small interface between activator and target. These findings support the proposal that transcriptional activation can involve a simple “recruitment” mechanism—that is, simple “adhesive” interactions between activator and target that facilitate and/or stabilize interaction of the general transcription machinery with promoter DNA (1, 2, 48–50). Activation by recruitment does not require conformational signaling within or through the target; does not require extensive, high-information-content interactions between activator and target; and entails modest net interaction energies between activator and target (interac-

tion energies comparable to the magnitude of activation) (48–50). We suggest that the results provide a structural paradigm for understanding other examples of transcriptional activation, both in bacteria (where most activators are thought to function through recruitment and to contact the same target, α CTD) (1, 2, 48–50) and in eukaryotes (where most activators are thought to function through recruitment) (49, 50).

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29. Crystals were grown by vapor diffusion at 20°C as hanging drops in solutions containing 0.1 to 0.2 mM CAP (5), 0.2 to 0.4 mM α CTD (α residues 245 to 329) (42), 0.1 to 0.3 mM DNA fragment [prepared as in (5)], and 0.8 mM cAMP in buffer A [50 mM sodium acetate (pH 4.5), 250 mM NaCl], equilibrated against a reservoir containing 2 \times buffer A. Crystals were stabilized by successive transfers (2 min per transfer) to 2 \times buffer A containing 5, 10, 15, 20, 25, and 30% ethylene glycol and were mounted on loops and flash frozen at -165°C . Crystals were hexagonal and had space-group symmetry $P6_22$, $a = b = 176$ Å, $c = 158$ Å, $\alpha = \beta = 90^{\circ}$, $\gamma = 120^{\circ}$.
30. Data were collected at the X25 beamline [National Synchrotron Light Source (NSLS), Brookhaven National Laboratory] and processed with DENZO and SCALEPACK (51). Initial phasing was derived by molecular replacement with AMORE (52) using the structure of the CAP-DNA complex at a resolution of 2.2 Å [Protein Data Bank (PDB) accession number 2CGP; residues 9 to 205 of the CAP protomer and nucleotide pairs 13 to 25 of DNA; initial $R = 47.1\%$]. Additional DNA was modeled into electron density in $F_{\text{obs}} - F_{\text{calc}}$ maps, and analysis of difference-Fourier

electron density from diffraction data collected on a 5-bromouracil derivative (5-bromouracil at positions 11, 23, and 32') was used to confirm the placement of DNA. Helical segments of the solution structure of α CTD (PDB accession number 1COO) were modeled into electron density in $F_{\text{obs}} - F_{\text{calc}}$ maps, additional residues of CAP and α CTD were modeled into electron density in successive cycles of Fourier refinement, and the structure was refined by using CNS (53) with anisotropic initial B -factor refinement and bulk solvent correction, incorporating water molecules conservatively, following strict criteria as described (5). The completeness of the highest resolution shell (3.2 to 3.1 Å) was 0.4; reflections from the highest resolution shell were included in the refinement. Omit maps, combined with simulated annealing, were used to confirm the placement and conformation of each residue in the structure. The asymmetric unit contains one CAP protomer and two α CTD protomers (2688 nonhydrogen atoms), one DNA half-site (895 nonhydrogen atoms), and 32 water molecules (Fig. 1, B and C). Atomic coordinates have been deposited in the PDB (accession number 1LB2).

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32. DNA helical parameters were analyzed with 3DNA (54).
33. The DNA bend between CAP and α CTD^{CAP,DNA} (15° roll, 13 bp from the center of the DNA site for CAP in each half-complex) is not in phase with the DNA bend induced by CAP (44° roll, 5 bp from the center of the DNA site for CAP, and -9° roll, 10 bp from the center of the DNA site for CAP, in each half-complex). Therefore, the overall DNA bend angle in the present complex is similar to that in the CAP-DNA complex (45° versus 46° in each half-complex for PDB accession number 1RUN) (6), but the out-of-plane component of DNA bending in the present complex is greater (-39° versus -21° in each half-complex for PDB accession number 1RUN) (6).
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38. The presence of the experimental water molecule (cyan in Fig. 2, D and E) is unequivocal (largest peak in $F_{\text{obs}} - F_{\text{calc}}$ difference map at 4 σ ; clear in a simulated annealing omit map at 4 σ and clear in 2 $F_{\text{obs}} - F_{\text{calc}}$ map at 1 σ ; B factor = 36.5).
39. Expected positions of first-shell minor-groove-spine water molecules were calculated as described (55). The position of the experimental water molecule (cyan in Fig. 2, D and E) corresponds to the expected position of a first-shell minor-groove-spine water molecule. Expected positions of second-shell minor-groove-spine water molecules were calculated analogously (56). The position of the Arg²⁶⁵ NH1 atom corresponds to the expected position of a second-shell minor-groove-spine water molecule.
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Figs. S1 and S2

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Identification of a Potential Ejaculation Generator in the Spinal Cord

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We tested the significance of a population of lumbar spinothalamic cells for male sexual behavior in rats. These cells are positioned to relay ejaculation-related signals from reproductive organs to the brain, and they express neurokinin-1 receptors. Ablation of these neurons by the selective toxin SSP-saporin resulted in a complete disruption of ejaculatory behavior. In contrast, other components of sexual behavior remained intact. These results suggest that this population of spinothalamic cells plays a pivotal role in generation of ejaculatory behavior and may be part of a spinal ejaculation generator.

Male sexual behavior is a complex behavior dependent on intrinsic and extrinsic factors, including olfactory, somatosensory, and visceral cues (1). The pathways that relay somatosensory and visceral sensory information from the reproductive organs to the brain are not well understood. Recent studies have used expression of the protein product Fos of the immediate early gene *c-fos* to map neural activation in the brain related to the expression of ejaculation in male rodents (2). Ejaculation-related Fos induction is restricted to a few brain regions, including areas within the medial amygdala, the bed nucleus of the stria terminalis, and a medial portion of the parvocellular subparafascicular nucleus (SPFp) within the posterior thalamus (3). The thalamus receives direct sensory inputs from the spinal cord and may thus be an important relay for genital sensory inputs to other areas of the brain important for sexual behavior. Indeed, the SPFp receives unique inputs from a population of spinothalamic neurons located in laminae VII and X in lumbar segments 3 and 4 (L3 and L4) containing galanin, cholecystokinin (4-6), and enkephalin (7). Here we refer to this population of lumbar SPFp-projecting neurons as LSt (lumbar spinothalamic) cells. These cells are specifically activated with ejaculation but not with other components of male sexual behavior (5, 6). Thus we hypothesized that LSt neurons are

involved in the relay of ejaculation-specific information, although the behavioral significance of these neurons is unknown.

To test the behavioral significance of LSt neurons, effects of lesions of the LSt population on sexual behavior were investigated. LSt neurons are sparsely distributed lateral to the central canal in lamina X and in the medial portion of lamina VII of L3 and L4 and are difficult to lesion by traditional methods. We thus identified a membrane target located on the LSt neurons. It was demonstrated that $93.0 \pm 1.7\%$ of LSt neurons express neurokinin-1 receptor (NK-1R) and conversely $84.7 \pm 2.47\%$ of NK-1R-containing cells in the area surrounding the central canal at L3 and L4 express galanin (Fig. 1). We therefore used the targeted toxin SSP-saporin (SAP), which consists of the toxin SAP conjugated to SSP, a substance P analog with high affinity for NK-1R (8). SSP-SAP was infused into the L3 and L4 spinal cord at the location of the LSt cells in sexually experienced or sexually naïve male rats (9). Control animals were injected with unconjugated equimolar concentrations of SAP. The doses used selectively ablate NK-1R-containing cells in vivo without producing nonselective lesions (8). Sexual behavior was first tested 10 days after lesion surgery, and during five subsequent twice-weekly tests.

After the final behavior test, animals were perfused, and spinal cord tissue was immunoprocessed for galanin, NK-1R, or neuronal marker N (NeuN) (9). Labeled cells were counted in a standard area surrounding the central canal of L3 and L4 sections representative of the location of LSt cells (Fig. 2A, area 1).

Of the 19 rats included in the behavioral

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