could still be affected by some contribution from the machine background. The kaersutites are most susceptible because they contain the least intrinsic hydrogen. It is difficult to correct quantitatively for this background contribution other than to apply a constant correction, an unquestionably inaccurate approach. Because adsorbed water is characterized by a low D/H (δD ≈ −200) value, any background correction will increase the reported δD values. To take a conservative approach, no background correction was made. If a correction were made, however, we estimate it would raise the δD values of the Chassigny kaersutites by no more than ~500 per mil, taking an average background contribution of ~1/5 the total H^+ count rate. In addition, the small size of the kaersutites can also contribute to lower H^+ count rates as a result of overlap of the primary beam onto neighboring anhydrous phases. This problem was observed (by microscopic examination of the samples after measurement) to be most pronounced in the shergottite kaersutites [they are the smallest measured (~10 μm)]. Thus, the shergottite kaersutites had lower H^+ count rates than the Chassigny samples and could need a maximum correction of ~+1500 per mil, taking an average background contribution of ~2/5 of the total H^+ count rate. Again we emphasize that these corrections represent the maximum that could be needed, and the similarity in measured D/H of coexisting biotite and kaersutites described in the text suggests that the actual effects are much smaller. Moreover, any correction will not eliminate the variable nature of the δD values observed in the kaersutite and would not significantly change the interpretations presented.

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Identification of the Target of a Transcription Activator Protein by Protein-Protein Photocrosslinking

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Here it is shown, with the use of protein-protein photocrosslinking, that the carboxyl-terminal region of the α subunit of RNA polymerase (RNAP) is in direct physical proximity to the activating region of the catabolite gene activator protein (CAP) in the ternary complex of the *lac* promoter, RNAP, and CAP. These results strongly support the proposal that transcription activation by CAP involves protein-protein contact between the carboxyl-terminal region of the α subunit and the activating region of CAP.

Escherichia coli CAP is a structurally characterized transcription activator protein (*1*, *2*). Genetic studies have indicated that amino acids 156 to 164 of CAP constitute an "activating region" that is essential for transcription activation at the *lac* promoter but not essential for DNA binding by CAP or DNA bending by CAP (*3*). Further genetic studies have indicated that the 100 COOH-terminal amino acids of the α subunit of RNAP are essential for transcription activation by CAP at the *lac* promoter but are not essential for CAP-independent transcription (*4*). The simplest interpretation of the genetic results with CAP and RNAP is that transcription activation at the *lac* promoter involves protein-protein contact between the activating region of CAP and the COOH-terminal region of the α subunit of RNAP.

In this study, we used protein-protein photocrosslinking to determine directly—without preconceptions—the site on RNAP that is in proximity to the activating region of CAP in the ternary complex of the *lac* promoter, RNAP, and CAP. Our approach had five steps: (i) We covalently attached a photoactivatible crosslinking agent at a single, defined amino acid within the activating region of CAP. (ii) We verified that the resulting CAP derivative retained the ability to activate transcription. (iii) We formed the ternary complex. (iv) We irradiated the ternary complex with ultraviolet (UV) light. (v) We determined the site at which crosslinking occurred (Fig. 1). To facilitate identification of the site at which crosslinking occurred, we used a photoactivatible crosslinking agent that contained a radiolabel and that was attached to CAP through a disulfide linkage (Fig. 1A). This permitted, after UV irradiation, cleavage of the crosslink and transfer of the radiolabel to the site at which crosslinking occurred (Fig. 1, B and C).

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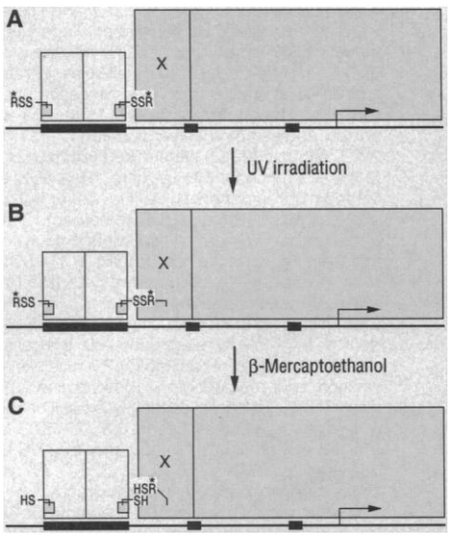
We constructed and analyzed a CAP derivative with a photoactivatible crosslinking agent incorporated at amino acid 161: [^{125}I]-{[*S*-[*N*-(3-iodo-4-azidosalicyl)cysteaminyl]-Cys^{161}; Ser^{178}]CAP} (IAC^{161}CAP) (Fig. 2). Amino acid 161 is located within the activating region of CAP (*3*) but is not essential for transcription activation (*5*, *6*). Saturation-mutagenesis studies have indicated that position 161 tolerates a wide range of amino acid substitution without loss of ability to activate transcription (*6*). We reasoned that position 161 might likewise tolerate incorporation of a photoactivatible crosslinking agent without loss of ability to activate transcription.

To construct IAC^{161}CAP, we used a two-step procedure consisting of (i) introduction of a unique surface cysteine residue at position 161 of CAP and (ii) cysteine-specific chemical modification. In step (i), we used site-directed mutagenesis to replace the preexisting surface cysteine residue at position 178 with serine and to replace the aspartic acid residue at position 161 with cysteine (*7*). In step (ii), we reacted the resulting CAP derivative with [^{125}I]-{*S*-[*N*-(3-iodo-4-azidosalicyl)cysteaminyl]-2-thiopyridine} (*10*) (Fig. 3, A and B) under conditions that resulted in highly efficient, highly selective, derivatization of surface cysteine (*12*, *13*) (Fig. 3C).

To assess the ability of IAC^{161}CAP to activate transcription, we did abortive initiation in vitro transcription experiments with a derivative of the *lac* promoter having a consensus DNA site for CAP: *lacP*(*ICAP*) (*14*). The results establish that IAC^{161}CAP retains nearly full ability to activate transcription (≈70% of the level of transcription activation shown by



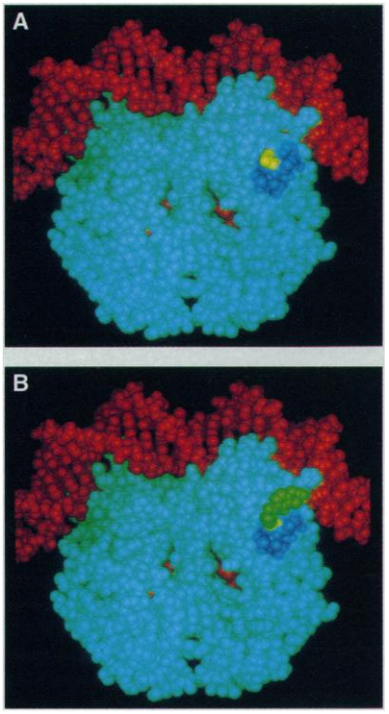


Fig. 1 (left). Identification by photocrosslinking and radiolabel transfer of the site on RNAP that is closest to the activating region of CAP in the promoter-RNAP-CAP ternary complex. (**A**) Formation of a ternary complex with a CAP derivative having a photoactivatible crosslinking agent incorporated at a single, defined amino acid within the activating region. The photoactivatible crosslinking agent contains a radiolabel (asterisk) and is attached to CAP through a disulfide linker (SS). (**B**) Photocrosslinking. (**C**) Cleavage and radiolabel transfer. **Fig. 2 (right).** (**A**) Crystallographic structure of the CAP-DNA complex (*1*) [coordinates obtained from Brookhaven Protein Data Bank (accession code 3GAP)]. CAP is blue; DNA and CAP-bound cAMP are red. The activating region of CAP is dark blue (*3*). Amino acid 161 is yellow. (**B**) Model for the structure of the IAC^{161}CAP-DNA complex. The [^{125}I]-[*N*-(3-iodo-4-azidosalicyl)cysteaminyl] moiety is green.

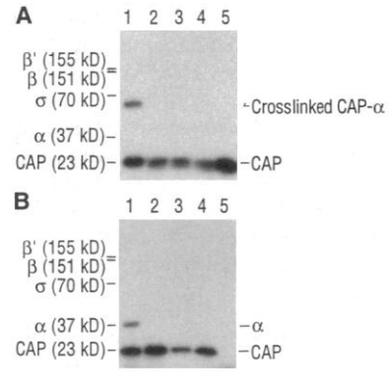


Fig. 4. Photocrosslinking experiments (*17*). (**A**) Photocrosslinking. (**B**) Photocrosslinking followed by cleavage and radiolabel transfer. Lane 1, photocrosslinking reaction; lane 2, control reaction omitting promoter DNA; lane 3, control reaction omitting RNAP; lane 4, control reaction omitting cAMP; lane 5, control reaction omitting UV irradiation. Radiolabeled CAP in (B) is the product of intramolecular, intrasubunit self-photocrosslinking; radiolabeled α in (B) is the product of intermolecular, CAP → α photocrosslinking (Fig. 1).

underivatized CAP) (*16*). We infer that incorporation of the photoactivatible crosslinking agent at position 161 does not prevent formation of the promoter-RNAP-CAP ternary complex and does not grossly alter the conformation of the promoter-RNAP-CAP ternary complex.

To assess photocrosslinking with IAC^{161}CAP, we formed the ternary complex using the same reaction conditions as were used in the in vitro transcription experiments, and we irradiated the ternary complex with UV (*17*). We then analyzed the products, either directly (Fig. 4A), or after cleavage and radiolabel transfer (Fig. 4B). The results establish that CAP → RNAP crosslinking occurs efficiently (efficiency ≈20%). The results further establish that crosslinking occurs exclusively within the α subunit of RNAP; no detectable crosslinking occurs within the β, β′, or σ subunits of RNAP. Control experiments established that crosslinking requires UV irradiation, promoter DNA, RNAP, and adenosine 3′,5′-monophosphate (cAMP) (the allosteric effector required for specific DNA binding by CAP) (Fig. 4). Additional control experiments established that crosslinking requires that the promoter DNA fragment contain a properly positioned DNA site for CAP.

To define the site within the α subunit at which crosslinking occurs, we did proteolytic mapping with hydroxylamine, which cleaves α into fragments consisting of amino acids 1 to 208 and amino acids 209 to 329 (*18–20*) (Fig. 5). The results establish that crosslinking occurs exclusively within amino acids 209 to 329.

We conclude that the COOH-terminal region of the α subunit of RNAP is in direct physical proximity to the activating region of CAP in the promoter-RNAP-CAP ternary complex (≤16 Å from the α carbon of amino acid 161 of CAP). The combination of (i) genetic results indicating that the COOH-terminal region of the α subunit is essential for transcription activation at the *lac* promoter (*4*), and (ii) the present biochemical results indicating that the COOH-terminal region of the α subunit is in direct physical proximity to the activating region of CAP at the *lac* promoter, constitutes strong evidence that transcription activation at the *lac* promoter involves protein-protein contact between the COOH-terminal region of the α subunit and the activating region of CAP.

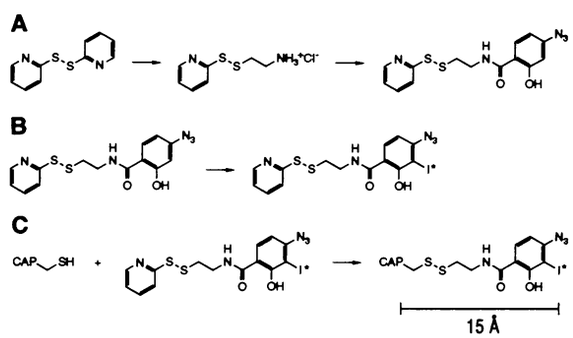


Fig. 3. Construction of IAC^{161}-CAP. (**A**) Synthesis of *S*-[*N*-(4-azidosalicyl)cysteaminyl]-2-thiopyridyl. (**B**) Radioiodination of *S*-[*N*-(4-azidosalicyl)cysteaminyl]-2-thiopyridyl (*10*). (**C**) Reaction of [Cys^{161};Ser^{178}]CAP with [^{125}I]-{*S*-[*N*-(3-iodo-4-azidosalicyl)-cysteaminyl]-2-thiopyridyl} (*12, 13*). The resulting linker arm between the α carbon of position 161 and the photoreactive atom is 15 Å. The linker arm is suitable for analysis of interactions occurring at or just beyond side chain contact distance.

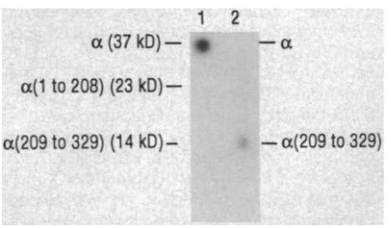


Fig. 5. Proteolytic mapping (*18*). Lane 1, radiolabeled α; lane 2, hydroxylamine digest of radiolabeled α.

Protein-protein contact between the COOH-terminal region of the α subunit and activator proteins may be a widely used mechanism for transcription activation in prokaryotes (*21*). Protein-protein contact between the σ subunit and activator proteins appears to be a second widely used mechanism (*22*). The approach used here should be generalizable to analysis of other prokaryotic and eukaryotic transcription complexes.

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7. Plasmids pXZCRP (*5*), pXZCRP178S (*8*), and pXZCRP161C;178S encode, respectively, CAP, [Ser^{178}]CAP, and [Cys^{161};Ser^{178}]CAP. Plasmid pXZCRP161C;178S was constructed from plasmid pXZCRP178S by site-directed mutagenesis (*9*). CAP, [Ser^{178}]CAP, and [Cys^{161};Ser^{178}]CAP were prepared as described in (*8*) and were purified from trace contaminating RNAP by centrifugal ultrafiltration at 2000*g* for 8 min at 4°C with the use of 100,000 NMWL polysulfone Ultrafree-MC filter units (Millipore).
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10. Reaction mixtures (120 μl) contained 125 μM *S*-[*N*-(4-azidosalicyl)cysteaminyl]-2-thiopyridine (Fig. 3A), 124 μM ^{125}I-KI [12.4 becquerels (Bq) per femtomole], 90 mM sodium borate (pH 8.4), and 0.4% dimethyl sulfoxide. Reactions were initiated by transfer to a vial containing 120 nmol 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (*11*) [IODO-GEN (Pierce); plated onto the vial walls by evaporation of a 1.2 mM solution in chloroform]. Reactions were terminated after 30 s at 22°C by transfer to a vial containing 15 μl of 8 mM methionine and 1 mM tyrosine. All operations in this and subsequent steps were performed in darkness or under safe-lamp illumination.
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12. Reaction mixtures (100 μl) contained 4 μM [Cys^{161};Ser^{178}]CAP (*7*), 70 μM [^{125}I]-{*S*[*N*-(3-iodo-4-azidosalicyl)cysteaminyl]-2-thiopyridine} (radioiodinated immediately before use) (Fig. 3, A and B), 50 mM sodium borate (pH 8.3), 8 mM tris-HCl (pH 8.3), 80 mM KCl, 0.4 mM EDTA, 5 mM methionine, 0.6 mM tyrosine, 2% glycerol, and 0.2% dimethyl sulfoxide. Reactions were carried out for 20 min at 22°C. IAC^{161}CAP was purified by chromatography on Bio-Gel P6DG (Bio-Rad) and was stored at −70°C in 40 mM tris-HCl (pH 8.0), 100 mM KCl, 10 mM $MgCl_2$, and 5% glycerol.
13. Quantitation of ^{125}I indicated that the reaction was 100% complete: 1.0 mol of [^{125}I]-[*N*-(3-iodo-4-azidosalicyl)cysteaminyl] was incorporated per mole of [Cys^{161};Ser^{178}]CAP subunit. Control experiments with [Ser^{178}]CAP (*7, 8*) indicated that the reaction was 100% site-specific: <0.01 mol of [^{125}I]-[*N*-(3-iodo-4-azidosalicyl)cysteaminyl] was incorporated per mole of [Ser^{178}]CAP.
14. Abortive initiation in vitro transcription experiments were done as described in (*5*). Reaction mixtures (20 μl) contained 5 nM *lacP(ICAP)* DNA fragment [base pair −121 to +102; prepared by polymerase chain reaction amplification of M13mp2-ICAP replicative-form DNA (*5*)], 40 nM RNAP [prepared as in (*15*); desalted into assay buffer (by Bio-Gel P6DG chromatography) immediately before use], 0 to 40 nM IAC^{161}CAP or CAP, 0.2 mM cAMP, 15 μM [α-^{32}P]UTP (0.3 Bq/fmol), 0.6 μM ApA, 40 mM tris-HCl (pH 8.0), 100 mM KCl, 10 mM $MgCl_2$, and 5% glycerol.
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16. IAC^{161}CAP and CAP showed identical concentration dependences for stimulation of open-complex formation (half-maximal stimulation at 5 nM; saturation at ≈10 nM). At saturating concentrations, IAC^{161}CAP and CAP stimulated open-complex formation 7-fold and 10-fold, respectively.
17. Reaction mixtures (800 μl) contained 5 nM *lacP-(ICAP)* DNA fragment (*14*), 40 nM RNAP (*14*), 10 nM IAC^{161}CAP, 0.2 mM cAMP, 40 mM tris-HCl (pH 8.0), 100 mM KCl, 10 mM $MgCl_2$, and 5% glycerol. Reaction vessels were polystyrene microcentrifuge tubes held inside borosilicate glass culture tubes (13 mm by 100 mm); these reaction vessels exclude wavelengths <290 nm. Reaction mixtures were incubated for 15 min at 37°C. Reaction mixtures then were UV-irradiated for 20 s at 37°C (350 nm; 1×10^5 erg mm^{-2} s^{-2}) in a Rayonet RPR100 photochemical reactor (Southern New England Ultraviolet). After UV irradiation, 6 mg of solid iodoacetamide was added, samples were incubated for 15 min at 22°C, and samples were concentrated to 20 μl by centrifugal ultrafiltration at 2000*g* for 7 min at 22°C with the use of 30,000 NMWL regenerated-cellulose Ultrafree-MC filter units (Millipore). After concentration, 20 μl of 80 mM iodoacetamide and 15 M urea were added, and samples were incubated for 15 min at 22°C. Aliquots (20 μl) were mixed with 5 μl of 0.5 M tris-HCl (pH 6.8), 50% glycerol, 10% SDS, and 0.1 mg/ml bromophenol blue (Fig. 4A) or with 5 μl of the same solution plus 10% β-mercaptoethanol (Fig. 4B) and were analyzed by SDS–polyacrylamide gel electrophoresis (PAGE) [0.1% SDS, 5 to 15% polyacrylamide gradient gels (Bio-Rad)] followed by autoradiography.
18. Crosslinking and nonreducing SDS-PAGE were done as in (*17*). After electrophoresis, the gel slice corresponding to crosslinked CAP-α was excised, washed, and reacted with hydroxylamine as described (*20*). The gel slice then was equilibrated in 500 μl of 100 mM tris-HCl (pH 6.8), 2% SDS, and 2% β-mercaptoethanol for 30 min at 37°C and analyzed by SDS-PAGE (0.1% SDS, 14% polyacrylamide) followed by autoradiography.
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Mediation of Epstein-Barr Virus EBNA2 Transactivation by Recombination Signal–Binding Protein $J_κ$

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The Epstein-Barr virus (EBV) transactivator protein, termed Epstein-Barr virus nuclear antigen 2 (EBNA2), plays a critical role in the regulation of latent viral transcription and in the immortalization of EBV-infected B cells. Unlike most transcription factors, EBNA2 does not bind directly to its cis-responsive DNA element but requires a cellular factor, termed C-promoter binding factor 1 (CBF1). Here, CBF1 was purified and was found to directly interact with EBNA2. CBF1 is identical to a protein thought to be involved in immunoglobulin gene rearrangement, RBPJ$_κ$. Contrary to previous reports, CBF1-RBPJ$_κ$ did not bind to the recombination signal sequences but instead bound to sites in the EBV C-promoter and in the CD23 promoter.

Epstein-Barr virus infects human B lymphocytes and epithelial cells and immortalizes B cells. In these cells, the EBV genome establishes latency and is stably maintained in the nucleus as an episome in which only a subset of the viral genome (about 10 genes) is expressed (*1, 2*). A virally encoded transcription factor, EBNA2, is required for both B cell immortalization and the establishment of latency (*3, 4*). EBNA2 transactivates latent viral genes and certain cellular genes that have been implicated in B cell activation (*5–10*). EBNA2 activates gene expression through a common cis-regulatory element found in both viral and cellular promoters (*11*), yet EBNA2 is unable to bind directly to these regulatory elements. In order to associate with target genes, EBNA2 requires a DNA binding protein encoded by the host cell. This

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