Genetics. In the article "Down syndrome phenotypes: The consequences of chromosomal imbalance" by J. R. Korenberg, X.-N. Chen, R. Schipper, Z. Sun, R. Gonsky, S. Gerwehr, N. Carpenter, C. Daumer, P. Dignan, C. Distèche, J. M. Graham Jr., L. Hudgins, B. McGillivray, K. Miyazaki, N. Ogawarama, J. P. Park, R. Pagon, S. Pueschel, G. Sack, B. Say, S. Schuffenhauer, S. Soukup, and T. Yamanaka, which appeared in number 11, May 24, 1994, of Proc. Natl. Acad. Sci. USA (91, 4997–5001), the authors request that the following correction be noted. The name of an author, L. Hudgins, was misspelled. The title and authors should read as follows:

Down syndrome phenotypes: The consequences of chromosomal imbalance


Biochemistry. In the article "An arcane role of DNA in transcription activation" by Sangreyol Ryu, Susan Garges, and Sankar Adhya, which appeared in number 18, August 30, 1994, of Proc. Natl. Acad. Sci. USA (91, 8582–8586), a printer’s error resulted in the omission of the title and edited column heads from Table 1. The correct table is shown here.

Table 1. Effect of increased distance and single-strand interruptions in the spacer on lac transcription

<table>
<thead>
<tr>
<th>Insertion interruption</th>
<th>Single-strand sequence</th>
<th>Spacer sequence</th>
<th>Cooperative RNAP binding</th>
<th>% lac transcription</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. — —</td>
<td>-38 TATTGTTAACCAGG</td>
<td>+ 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. — Nick</td>
<td>-38 TATTGTTAACCAGG</td>
<td>+ 78.5 ± 3.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. — 1-base</td>
<td>-38 CATTAGGCGCTTACAGG</td>
<td>+ 53.6 ± 8.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. — 2-base</td>
<td>-38 CATTAGGCGCTTACAGG</td>
<td>+ 17.0 ± 17.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. — 4-base</td>
<td>-38 CATTAGGCGCTTACAGG</td>
<td>+ 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. 10 bp —</td>
<td>-38 CATTAGGCGCTTACAGG</td>
<td>NT 51.0 ± 1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. 10 bp 6-base</td>
<td>-38 CATTAGGCGCTTACAGG</td>
<td>NT 0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DNA templates carried the shown variations in the spacer region. The amount of transcription of each template interrupted in the bottom strand was normalized as percentage of the transcription obtained for the corresponding isogenic intact double-stranded linear template. The last column shows the average of several experiments, including the standard deviations, obtained from independent preparations of DNA. Results shown in the line were obtained with DNA templates which were mixtures with nicks or gaps at the indicated position(s) of one or the other strand. Results were the same as shown in the last column when homogeneous single-stranded DNA circles with interruption in the bottom (as shown in column 4) or the top strand made by a bacteriophage Φ1 replication-packaging system were used as templates. +, RNAP binding to wild-type DNA templates in the presence of CRP; NT, not tested.

Biochemistry. In the article "Roles of heme iron-coordinating histidine residues of human hemopexin expressed in baculovirus-infected insect cells" by Tomoko Satoh, Hiroyuki Satoh, Shin-ichiro Iwashara, Zbynek Hrkal, David H. Peyton, and Ursula Muller-Eberhard, which appeared in number 18, August 30, 1994, of Proc. Natl. Acad. Sci. USA (91, 8423–8427), the arrow was missing from Fig. 3 due to a printer’s error. The corrected figure and legend are shown here.

![Figure 3](image_url)

Fig. 3. SDS/PAGE of recombinant wild-type Hx after each purification step. Aliquots (5 µg) were analyzed by SDS/4–20% gradient PAGE and stained with Coomassie brilliant blue R-250. Lanes: 1, molecular size markers; 2, conditioned medium 48 hr postinfection; 3, following Con A-agarose chromatography; 4, eluate from SP-Sepharose chromatography. Arrow indicates the recombinant Hx at 55 kDa.

14 21 31 45 66 97 kDa
An arcane role of DNA in transcription activation

SANGRYEOL RYU, SUSAN GARGES, AND SANKAR ADHYA*

Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Communicated by James C. Wang, April 8, 1994

ABSTRACT The mechanism by which the cAMP receptor protein (CRP) activates transcription has been investigated using the lac promotor of Escherichia coli. For transcription activation, an interaction between DNA-bound CRP and RNA polymerase is not sufficient. CRP must bind to a site in the same DNA and close to the promoter. CRP action requires an intact spacer DNA to provide a rigid support in building a CRP–RNA polymerase protein bridge or to allow a conformational change in the DNA to be transmitted to the lac promoter using the protein bridge as a structural support.

Activators are regulatory proteins that function with some promoters in response to environmental or developmental changes to overcome kinetic or energetic limitations specific to any step in transcription initiation (1). The activators act in conjunction with DNA control elements by binding with high affinity to them and converting an inadequate promoter–RNA polymerase (RNAP) combination to a more effective one. An intriguing problem for genetic chemistry is to determine the mode by which activators influence the biochemical steps. Two mechanisms for how activators function have been proposed (17–20): (a) protein–protein contact—the DNA-bound activator influences RNAP by making a direct contact with the polymerase, making the enzyme more proficient in the rate-limiting step(s)—and (b) effect through DNA—the activator binding to DNA changes the conformation of the DNA in the nearby promoter to make the promoter more optimal for RNAP action. Although there is no reason to expect that there will be a single, uniform mechanism for all activators, the following observations strongly suggest that there is a requirement for a direct contact between some of the DNA-bound activators and RNAP: (i) the binding sites of an activator and RNAP must often be separated by an intervening DNA segment with an integral number of DNA helical turns which is consistent with suitable contact between activator and RNAP while bound to the same face of DNA double helix (5–7); (ii) fluorescence spectroscopy, ultracentrifugation, and immunological studies indicate an interaction between the cAMP receptor protein (CRP) activator of Escherichia coli and RNAP (8–10); (iii) NurC activator and the cognate σ²⁴–RNAP bound at the gltA promoter in Salmonella typhimurium interact with each other by generating a loop of the intervening DNA segment (11); and (iv) mutants of CRP and bacteriophage λ cl activator protein have been described that bind normally to DNA sites but do not show cooperative binding with RNAP and do not activate transcription from the corresponding promoters (5, 12). These mutants identify likely sites of contact of these two activators with RNAP. Similarly, mutations in the α subunit of RNA polymerase which do not respond to activator have been characterized. These mutations may define the potential contact region of RNAP with some activators (13). However, such observations do not necessarily preclude that transcription activation may also involve a change in the conformation of the promoter through DNA by the binding of an activator protein to a nearby DNA site. A helix face-dependent interaction between the DNA-bound activator and RNAP creates distortion in the intervening DNA as judged by appearance of DNase-hypersensitive sites or reactivity with singlet oxygen for at least three activator–promoter combinations: integration host factor (IHF) at the phage Φ29 promoter, P4 protein at a Bacillus subtilis phage Φ29 promoter, and CRP at the lacUV5 promoter (7, 14, 15). These authors concluded that DNA distortion is the result of bending or kinking of the intervening DNA, although it was not shown if the distortion is causative or an epiphenomenon or at all related to transcription activation. Several proteins which cause initiation of DNA replication by acting at specific origin (ori) sites induce distortion in the double helix of specific DNA segments adjacent to the binding sites (16, 22).

We have used the cAMP–CRP-dependent lac promoter system to address any role of DNA structural changes in transcription activation. CRP is a dimer of identical 209-aa subunits. cAMP binding induces an allosteric change in the protein. The cAMP–CRP complex (called CRP in this article) activates transcription initiation at lac by binding to a 16-bp region of dyad symmetry located at position −61.5 relative to the transcription start site. We designed promoter constructs which would allow CRP to bind to its DNA site and to make contact with RNAP but would disrupt the spacer DNA between the DNA sites for CRP and RNAP. We show that (i) the CRP binding site must be located in cis to the lac promoter, (ii) CRP–RNAP interaction is not sufficient, and (iii) the integrity of the spacer DNA between the CRP and RNAP binding sites must be maintained for transcription activation.

MATERIALS AND METHODS

Proteins. Wild-type CRP was purified as described (17). Restriction enzymes and T4 polynucleotide ligase were purchased from New England Biolabs. Purified Int protein and IHF were gifts from Howard Nash (National Institutes of Health).

DNA. DNA catenanes were prepared by λ site-specific integrative recombination (18). Plasmid pSA450, which contains no lac sequence, was derived from pBluescript (Stratagene). pSA600 was generated by replacing the HindIII–Xba I fragment of pSA450 with that from pSA508 (19), which contains the transcription terminator from rpoC and multiple cloning sites between the Φ29 attachment site, attP, and the corresponding bacterial site, attB. pSA601 was constructed by inserting a lac promoter (−120 to +80) in pSA600 between attB and a terminator to make a 117-nt lac transcript, pSA602 by inserting a lac promoter without CRP binding site (−46 to +80) to pSA600 between attB and a terminator, and pSA603 by adding a CRP-binding site (−95 to −36) to pSA602 upstream of the attP site. Catenanes were made in vitro (20), separated by gel electrophoresis, and recovered by electro-

Abbreviations: CRP, cAMP receptor protein; IHF, integration host factor; RNAP, RNA polymerase.

*To whom reprint requests should be addressed.

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elution. The catenanes were judged pure by their migration as a single band in a gel. DNA with single-stranded nicks was prepared as follows. pSA610 was constructed by creating a Hpa I site between the CRP binding site and the promoter of pSA601 by site-directed mutagenesis. The single-strand-nicked DNA was prepared by denaturing and annealing a mixture of equal amounts of two different portions of pSA610, one cut at the Sma I site upstream of the CRP binding site and the other cut at the Hpa I site or at the HindIII site located far downstream to the lac promoter. The resulting DNA circles were mixtures containing nicks in either strand, but homogeneous nicked circles showed identical results. To make homogeneous nicked DNA circle, single-stranded DNA was made using a T1 helper phage and annealed with linearized double-stranded DNA. The nicked DNA circles were separated in a 1.2% agarose gel and electroeluted. DNA templates with single-strand gaps were made by the same principles as described for DNA with single-strand nicks described above. Base changes were made at around −45 to create a Sru I site (AGGCCCT), and the corresponding plasmids with 1, 2, and 4 extra bases in the center of Sru I site and the partner plasmids with 1, 2, and 4 extra bases were cut with Sma I. Equal amounts of these cut plasmid pairs were mixed, denatured, and renatured. The gapped DNA circles were purified from an agarose gel.

In Vitro Transcription. Transcription was performed in 50 μl containing 20 mM Tris (pH 8.0); 3 mM magnesium acetate; 200 mM potassium glutaminate; 1 mM dithiothreitol; 0.1 mM each ATP, GTP, and CTP; 0.01 mM UTP, 10 μCi of [α-32P]UTP (800 Ci/mmol; 1 Ci = 37 GBq); 2 nM DNA template; 0–40 nM CRP; 100 μM cAMP; 20 nM RNAP holoenzyme; and 5% (vol/vol) glycerol. All components except nucleotides were incubated at 37°C for 10 min. Transcription was started by addition of nucleotides and terminated after 10 min by addition of 50 μl of 80% formamide/90 mM Tris borate, pH 8.3 / 2 mM EDTA/0.05% bromophenol blue/0.05% xylene cyanol. Transcripts were quantitated with an AMBIS Systems β scanner after gel electrophoresis.

Nitrocellulose Membrane Filtration for DNA–Protein Complexes. Complexes of lac DNA with CRP and/or RNAP were assayed by filtration on nitrocellulose filters as described (21), with some modifications. Binding reactions were performed in the transcription buffer used for in vitro transcription: 50 pM labeled DNA, 120 pM RNAP, and 2 nM CRP were incubated for 15 min at 37°C, and then reaction mixtures were diluted 10-fold with transcription buffer. Samples (450 μl) were filtered at several time points through a 25-mm nitrocellulose filter (BA85; pore size, 0.45 μm; Schleicher & Schuell) pretreated with 0.4 M KOH. All experiments were repeated at least three times.

DNase I Footprinting. The experiments were performed by the method of Mukhopadhayya and Chattorraj (22) with the reactions performed in transcription buffer. The DNase I protection studies was only for the bottom strand, because the top strand carried interruptions when DNA templates with single-strand gaps were used. The primer used for primer extension hybridized to the bottom strand, just upstream of −120 of lac, within vector DNA sequences of pSA610.

RESULTS

CRP-acting CRP Binding Site. The following in vitro results show that the CRP binding site acts only in cis and close to the lac promoter; these results also confirm the in vivo observation that the site must be in an angular orientation on DNA which is facing the promoter. (i) The topological requirement for action by the CRP binding site was examined by putting the site into a separate DNA molecule—i.e., in trans to the lac promoter. The principle of the test was the same as that used to study the trans-acting role of NtrC binding site in the glnA promoter (23). The transcription mixture contained two different circular DNAs, one carrying the lac promoter and the other carrying the wild-type lac CRP binding site, as well as purified RNAP and CRP. The two circular DNAs were linked together using a catenane generated by the Φ Int protein and IHF. Such a catenated arrangement of the template DNA would fulfill any possible requirement for a DNA-induced conformational change in CRP. It also maintains the concentration of the DNA-bound CRP near the promoter higher than when the promoter and CRP binding site are in trans and unlinked. If interaction between the DNA-bound CRP and RNAP is sufficient for transcription activation, then CRP is expected to initiate lac transcription from such catenated templates, as has been shown for the NtrC-activated system (23). The amount of lac transcription in each reaction was normalized with respect to the amount of intrinsic rep RNA molecules made from the RNA I promoter of the plasmid. As a control experiment, a catenane of two DNAs, made from pSA601 in a similar fashion, was generated in which the CRP binding site was present in the same DNA circle as the lac promoter, in its natural location. In this case, the activation of transcription from the lac promoter by CRP was the same as in a larger uncatenated circle (Fig. 1A), indicating that the catenane structure does not interfere with the activation of lac transcription by CRP in our system. CRP was unable to stimulate lac transcription in trans—i.e., when the lac promoter and the CRP binding sites were present on two different DNA circles (Fig. 1B, lanes 5–8). A steric obstacle may have prevented a productive interaction between DNA-bound CRP and RNAP because of the small size (560 bp) of the lac promoter-containing DNA circle. However, CRP also failed to stimulate lac transcription when the size of the latter DNA circle was increased by 1 kb (data not shown). We note that CRP, in the trans configuration, actually inhibits the basal level of transcription that is observed in the absence of CRP when the template is supercoiled (24), suggesting that CRP may interact with RNAP in trans, albeit in a nonproductive way. Although the catenated templates used in our system theoretically may have a high degree of freedom limiting a stable CRP–RNAP contact, the results suggest that a CRP–RNAP interaction may not be sufficient for transcription activation and that a CRP binding site is actually needed in cis to the lac promoter.

(ii) We next investigated whether the CRP binding site can function when present in the same DNA molecule as the promoter if it is located much further upstream than the normal six DNA helical turns in the present system. pSA603

![Fig. 1.](image-url) (A) In vitro transcription using pSA601, containing a wild-type lac promoter (lanes 1–4), and a catenane containing a wild-type lac promoter in one circle of DNA generated from pSA601 (lanes 5–8). A pair of 107- and 108-ni rep RNA molecules from the plasmid origin of replication (the transcription of which is not dependent on CRP) provided an internal control. P. promoter. (B) In vitro transcription using pSA603, containing a lac promoter with a CRP binding site 400 bases upstream (lanes 1–4), and a catenane containing a lac promoter in one circle and a CRP binding site in the other, generated from plasmid pSA603 (lanes 5–8).
DNA, in which the wild-type CRP binding site was located 400 bp from the lac promoter, was used in the in vitro transcription assay. There was no enhancement of transcription by CRP under such conditions (Fig. 1B, lanes 1–4), demonstrating that the CRP binding site in the lac promoter, although needed in cis, cannot act from a large distance. The decrease in the ability of CRP to stimulate transcription when the distance from the promoter is increased, although the increase in distance is an integral number of DNA helical turns, has also been observed in vivo (5). Furthermore, the findings, in conjunction with the results of experiment ii also suggest that CRP’s stimulatory effect in cis is greater when the binding site is closer to the promoter. One interpretation of the observed reduction in initiation when the CRP binding site is distant is that CRP–RNAP interaction is weak and cannot withstand the increased flexibility of a longer intervening spacer DNA segment. However, the data provided below support an alternative explanation: that the bound CRP transmits to the promoter a structural change which is effective over only a short range.

**lac DNA with Interrupted Spacers.** We tested the requirement for structural integrity of the spacer DNA between the sites for CRP and RNAP by using DNA templates with specific structural aberrations. (i) DNA templates carrying a nick in one strand between positions −45 and −46 relative to the transcription start site in the lac promoter, from pSA610 and used for in vitro transcription. CRP caused stimulation of lac RNA synthesis on DNA templates carrying a nick in the region between the CRP and RNAP binding sites in either strand (Fig. 2, lanes 3 and 4). CRP-dependent stimulation of lac transcription from relaxed DNA carrying a single-strand nick elsewhere in the plasmid was like that of linear DNA, showing good activation (lanes 1 and 2). Quantitation of transcription from the lac promoter, when normalized to the amount of rep DNA, showed that CRP stimulation using templates carrying single-strand nicks at the spacer was close to 80% of that from DNA without nicks in the spacer (Table 1, lane 2). If DNA-bound CRP and RNAP contact each other, the intervening DNA segment would become an independent domain. Since nicking would relax any unstrained supercoils in this domain, we conclude that CRP action does not involve CRP-induced superhelical twists.

(ii) Using the same basic principle of construction as used to create nicked DNA, we made several DNA templates carrying 1-, 2-, or 4-base gaps at positions −46, −46 to −47, or −46 to −49, respectively, in one of the two strands of the lac promoter. Relative to stimulation of lac transcription by CRP with supercoiled DNA templates (Fig. 3A, lanes 1 and 2), CRP-mediated activation was close to 50% on DNA templates which carried a 1-base gap (lanes 3 and 4), was reduced to ~17% for a 2-base gap (lanes 5 and 6), and was undetectable for a 4-base gap (lanes 7 and 8). These results, summarized in Table 1, lanes 3–5, clearly show that CRP activation functions best with a double-stranded linkage in the spacer DNA. Control experiments performed with DNA templates with the same 2-base and 4-base single-strand gap in the lacUV5 promoter, which does not need cAMP for activation of transcription, showed that the gaps did not have a major deleterious effect on the process of lac transcription itself relative to rep.

(iii) We discussed previously that an increase of 10 bp (one DNA helical turn) in the spacer of the wild-type lac promoter permits CRP activation at a 50% level. When this template was made to contain a single-strand gap of either 6 or 10 bases within the inserted segment and used for in vitro transcription, CRP totally failed to activate transcription (Table 1, lanes 6 and 7, and data not shown). These experiments suggest that the inability of CRP to activate transcription from templates with, for example, a 4-base gap in the spacer, is not due to the destruction of a previously unknown intrinsic promoter element in the −45 region by being made single-stranded, because leaving the region intact up to −45 and placing the gap further upstream also makes CRP ineffective. In other words, our experiments clearly demonstrate the functional importance of DNA structure, not the precise sequence of the spacer DNA.

**RNAP Binding.** RNAP alone fails to bind to the wild-type lac promoter, but it binds with high affinity in the presence of CRP by cooperative action (25, 27). Cooperative binding of two protein molecules to DNA could be because of protein–protein contact (26). In principle, CRP may have failed to activate lac promoters carrying single-strand gaps because the entropy of the system was increased, thereby decreasing the chances of CRP–RNAP interaction. We studied cooperative binding of CRP and RNAP to intact and

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**Figure 2. In vitro transcription as described in the legend of Fig. 1, using 40 nM CRP with or without 100 μM cAMP. Lanes 1 and 2, transcription with DNA nicked at Smal I and HindIII sites; lanes 3 and 4, transcription with DNA nicked at Smal I and Hpa I sites.**

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**Table 1.** DNA templates carried the shown variations in the spacer region. The amount of transcription of each template interrupted in the bottom strand was normalized as percentage of the transcription obtained for the corresponding isogenic intact double-stranded linear template. The last column shows the average of several experiments, including the standard deviations, obtained from independent preparations of DNA. Results shown in each line were obtained with DNA templates which were mixtures with nicks or gaps at the indicated position(s) of one or the other strand. Results were the same as shown in the last column when homogeneous single-stranded DNA circles with interruption in the bottom (as shown in column 4) or the top strand made by a bacteriophage f1 replication–packaging system were used as templates. +, RNAP binding to wild-type DNA templates in the presence of CRP; NT, not tested.
gapped DNA templates in two ways. (i) Nitrocellulose filter binding. If two proteins bind to DNA in a cooperative fashion, there would be a net increase in total binding when both proteins are present. CRP and/or RNAP were bound to 32P-labeled intact lac DNA or DNA carrying the single-strand gaps described before, and the amount of lac DNA was determined by nitrocellulose membrane filtration (Fig. 4). RNAP alone bound poorly to either intact or single-strand-gapped lac DNA. CRP alone bound well and equally to either type of template. When both RNAP and CRP were present, the amount of protein-bound DNA was 1.4-fold greater than when CRP was the only protein present, and was equal for both intact and gapped DNA. This reflects cooperative binding of CRP and RNAP to lac DNA.

(ii) DNase I footprinting. We used a primer extension assay to measure the binding of CRP and RNAP to lac DNA template with the 4-base gap between the CRP binding site and the promoter. The CRP alone bound to both intact and 4-base-gapped DNA readily in the 6'-1.5 site (Fig. 5, lanes 2 and 3 and lanes 13 and 14). As expected, RNAP alone bound very poorly to both intact and gapped DNA (lanes 8–11 and 19–22). However, RNAP bound to the promoter in double-stranded DNA only when CRP was also present (lanes 4–7). This confirms cooperative interaction of RNAP and CRP with lac DNA (25). Similar cooperative binding was also observed in the 4-base-gapped template (lanes 15–18). In fact, on quantification, the cooperativity between CRP and RNAP seems to be somewhat enhanced for the gapped template. This last result suggests that having a single-stranded region between two protein binding sites in DNA is more conducive to CRP–RNAP interaction when the two proteins are bound to DNA. This finding is consistent with a similar finding that a 4-base single-stranded gap restores cooperative binding of Cl protein to two DNA sites which are located on two different faces of DNA (28). Both filter binding and DNase I protein assays show that having a single-strand gap in the spacer region is not a hindrance to CRP-dependent RNAP binding to lac DNA.

**DISCUSSION**

A DNA-bound activator protein can modulate transcription initiation by (i) signaling to RNAP by activator–RNAP contacts and/or (ii) signaling to the promoter by activator-mediated contortion of DNA structure. Several activators bound to DNA sites close to or far from the promoter have been shown to enhance transcription by activator–RNAP contact. In the framework of model i, the binding of an activator to a DNA site may serve (a) to increase the local concentration of the activator protein to facilitate interaction with RNA polymerase, (b) to allosterically change the activator to a conformer with potency for transcription activation, and (c) to provide a rigid platform allowing an activator-induced RNAP binding or allosteric change. In this model, DNA has a passive role. In model ii, however, DNA has a more active role and the proteins may serve here to provide a rigid platform so that DNA structure can be altered at a distance. The concept of one segment of DNA (or a ligand bound to it) influencing the dynamics or behavior of a contiguous but remote portion of the helix was first proposed by Burd et al. (3). Burd et al. (3), Dickson et al. (2), and Ivanov et al. (4) suggested that CRP may act by binding to the i-6.5 region in the lac promoter and then altering the conformation of the nearby DNA, thereby increasing the rate of open complex formation. We have shown above that the introduction of single-strand gaps in the space between the CRP and RNAP binding sites of the lac promoter prevents CRP-dependent activation of transcription. Since we have shown that there is no interference in the cooperative interaction of the two proteins on lac DNA, the failure of CRP to activate the lac promoter in the presence of a 4-base gap was not because of loss of CRP–RNAP interaction. These results

**Fig. 3.** In vitro transcription using wild-type lac (A) or lacUV5 (B) DNA templates with single-strand gaps. The size of a lac transcript was 97 bases for 1- and 2-base-gapped DNA and 94-bases for 4-base-gapped DNA. Templates homogeneous for gaps in one strand gave the same results as those homogeneous for gaps in the other strand (data not shown).

**Fig. 4.** Nitrocellulose filter assay of CRP and RNAP binding to DNA. Open bars, intact DNA; filled bars, DNA with 4-base gap. The amount of binding shown by CRP alone for intact DNA was taken as 100%.

**Fig. 5.** DNase I protection of intact (supercoiled) (Left) and 4-base-gap (relaxed) (Right) lac DNA by CRP and RNAP. Concentrations of CRP and RNAP are shown at the top. Although there is slightly more protection of the -69 band with supercoiled DNA (Left) than with the 4-base-gap DNA (Right), the DNase I protection results of intact relaxed DNA (data not shown) were identical with those of the 4-base-gap DNA (Right).
strongly support a role for the spacer DNA in CRP activation of transcription. It seems likely that both a protein–protein bridge and a bridge of intact DNA are involved in activation of CRP transcription at lac. It is possible that the spacer DNA interacts with one or both of the two interacting proteins. The C-terminal portion of the α subunit of RNAP makes contact with the DNA immediately upstream of the –35 region of rnb and lacUV5 promoters (29, 30). A reason for why CRP could not activate transcription from gapped templates could have been that the α subunit was not making the proper contacts in the gapped region. Since RNAP binding is quantitatively and qualitatively the same whether the DNA is intact or gapped, this is an unlikely explanation for the wild-type lac promoter. An alternative proposal is that CRP transmits a change in DNA conformation from where CRP is bound to the promoter and intact DNA helps this transmission. The notion of DNA allosterism is consistent with the observation that when both CRP and RNAP are bound to the lac promoter, there may be a disturbance in the DNA at position –46 of the upper strand of the spacer region, as detected by its reactivity with singlet oxygen, \( \text{O}_2^* \) (15). The effect of the proposed DNA allosterism is very likely to make a promoter better suited for transcription. The way in which this end may be achieved is discussed below.

It has also been suggested that transcription may involve an activator-induced or intrinsic bending of the DNA segment upstream of the promoter (31–33). CRP is known to induce a DNA bend (5). The degree of bending is affected by sequences as far as 16 bp from the center of symmetry of the CRP binding site (34). Although we have not measured the CRP-induced bending of the gapped DNA, we feel that lack of bending is not the reason for the inability of CRP to activate transcription. In all of the cases of gapped DNA, the DNA is double stranded at least 15 bp away from the center of symmetry (19 bp away in the case of the 10-bp insertion with 6-base gap). Furthermore, in the cases tested, CRP binding to the gapped DNA was the same as to intact DNA. Gartenberg and Crothers (34) showed that when bending was decreased, binding decreased by as much as an order of magnitude.

We speculate two possible means of CRP changing DNA conformation in nearby DNA. (a) CRP binding to its site changes the neighboring DNA from a strict B to another form which is needed for transcription initiation. CRP binding to certain specific DNA sequences causes local changes in the CD spectra of B-DNA to one that is A-DNA-like (V. Ivanov, L. Elson has also been suggested that transcription is needed to maintain the DNA conformational changes). Since transcription can induce a transition of B-DNA to an A-like form (35), it is possible that CRP can aid this transition in a cooperative fashion. (b) CRP binding might change long-range electrostatic interactions between base pairs, and the perturbation could extend from the CRP binding site to the promoter. Elson et al. (36) have shown that electrostatic interactions between base pairs extend beyond nearest-neighbor stacked bases to longer ranges. Removal of bases in one of the DNA strands very likely removes similar long-range interactions. Our observation that single-strand gaps of increasing length (1–4 bases) progressively decrease transcription activation is consistent with the idea that long-range electrostatic interactions between base pairs contribute to the proposed telecommunication. It may well be that the purpose of contact between CRP and RNAP is to anchor RNAP and corepressor so the DNA conformational change can be effected or stabilized. Such torsional stress may originate from CRP-induced DNA bending. The idea of transmitting signals between DNA segments by protein binding may be very important in the regulation of transcription of eukaryotic genes, which is generated by multiple transcrip-tion factors that act by binding to adjacent sites around the promoter.

We thank Howard Nash for valuable suggestions and gifts of purified λ Int and IHF, and Roberta Haber and Victor Zhurkin for critical reading of the manuscript.