MICROBIOLOGICAL REVIEWS, June 1994, p. 268–290
0146-0749/94/$04.00+0

Vol. 58, No. 2

Promoters Responsive to DNA Bending: a Common Theme in Prokaryotic Gene Expression

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INTRODUCTION

One of the most remarkable outcomes of the last decade of research on gene expression is the realization that DNA not only contains information for structural proteins and sequences for binding cognate regulators but also has intrinsic structural properties which play an active role in many cell functions. Intrinsically bent or curved DNA molecules most frequently appear when recurrent short sequences including several A residues occur in phase with the B-DNA helical repeat, 10.5 bp per turn. A tracts are believed to be the major determinants of DNA curvature, yet the ultimate explanation for such an effect is still the subject of some controversy. Axial deflections of contiguous AA dinucleotides may sum up within the DNA helix, making minor NN wedge angles (N is any nucleotide) located at the same helix side generate a planar curvature (86, 244). Axial deflections may also arise from structural discontinuities at the boundaries between the A tracts (which seem to adopt an unusual structure named B′-DNA) and the rest of the B-DNA sequence (42, 123). In any case, phasing of A tracts results in the formation of an intrinsically bent DNA molecule. In addition to AA pairs, certain dinucleotides such as AG, CG, GA, or GC can induce or contribute significantly to DNA curvature (20).

Early evidence of the existence of DNA bending was obtained from experiments carried out during the study of a minicircle DNA in the kinetoplast body of *Leishmania tarentolae* (149). Catabolite activator protein (CAP) was the first prokaryotic protein shown to direct a sharp bending of its target DNA sequences (265), but it was not until 1984 that the

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presence of an upstream static bend could be associated with the activity of a bacterial promoter (23). Following the widespread utilization of the circular permutation assay to identify curved DNA sequences (265), the participation of curved DNA sequences at nearly every stage of the transcription process has been found to be the rule rather than the exception. This article reviews current data on the role of DNA curvature in the regulation of transcription in a number of prototypical bacterial promoters.

DNA BENDS WITHIN BACTERIAL PROMOTERS

We start our survey of the role of DNA curvatures in promoter activity by examining the structural features of promoters (121) and how these features are ultimately recognized by bacterial RNA polymerase (RNAP). As discussed below, both intrinsic and RNAP-induced DNA bends play a determinant role at every stage of the process leading to the formation of a transcriptionally competent complex. It is worth mentioning at this point that in the absence of detailed RNAP and RNAP-promoter cocrystal structural data, our understanding of how the enzyme interacts with cognate DNA sequences is based mainly on indirect evidence.

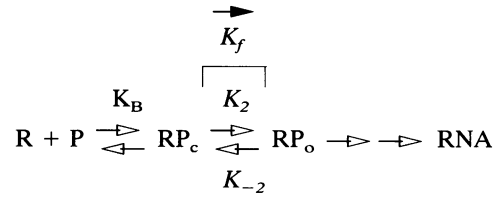
Bacterial RNA Polymerase Bends DNA

The first observations suggesting that *Escherichia coli* RNAP could induce the bending of its target DNA sequences was made during the course of in vitro studies on the promoter of the *gal* operon (128). Applying the circular permutation assays, Kuhnke et al. (129) showed that σ^{70}-RNAP bound to the promoter bends DNA. Furthermore, at least in two more cases (92, 187), binding of σ^{70}-RNAP to target DNA sequences has been shown to result in a significant protein-induced bending in vitro. Although such a change in DNA has been examined only with purified σ^{70}-RNAP of *E. coli*, it probably has widespread value and could be intimately associated with the transcription mechanism in a wide range of systems, including mitochondrial promoters of yeasts (222).

The way σ^{70}-RNAP bends DNA appears to be quite distinct, since the nucleotides seem to wrap around part of the protein (27, 187). DNase I footprints with purified holoenzyme show protections up to −50 bp upstream of the transcription initiation site (29, 243). Cross-linking of σ^{70}-RNAP with the *lacUV5* promoter has shown that there are multiple contacts of the DNA with the β, β′, and σ subunits (36), which can be envisioned as RNAP being a globular protein with DNA wrapped around it. An additional clue to the way σ^{70}-RNAP interacts with DNA is given by data on its three-dimensional structure at 27-Å (2.7-nm) resolution (46). The most prominent feature is the presence of a protruding arm surrounding a sort of channel of 25 Å (2.5 nm) in diameter, connected with a groove also 25 Å wide. This channel and groove have the optimal dimensions for binding double-helical B-DNA, and therefore it is believed that the DNA helix binds and proceeds through this structure. Since the channel-to-groove angle is about 60°, the DNA would have to bend that much to interact productively with the RNAP, thus accounting in part for the observations made by circular permutation assays and imaging by scanning force microscopy of σ^{70}-RNAP–DNA complexes (200).

Transcription Initiation Engages Several Changes in DNA Structure

To understand the role of DNA bending in promoter activity, it is worth remembering that transcription initiation is a multistep process which results in the melting of a short DNA region encompassing the site of initiation, concluding with the synthesis of an oligonucleotide and the escape from an oligonucleotide cycling state. The existing kinetic view of the process is summarized by a simple scheme (32, 153):



The process involves an initial binding of RNAP (R) and the promoter sequence (P), with a binding constant K_B, to form an inactive intermediate closed complex (RP_c). This complex then isomerizes with a rate constant K_f to form a transcriptionally active open complex (RP_o), in which the two strands are melted through a ca. 10-bp region around the transcription start site (230). The sequence of events seems, however, to be more complex. Data obtained with the σ^{70}-dependent *lacUV5* (126) and T7A1 (221) promoters, as well as with the σ^{32}-dependent *groE* promoter (154), suggest a mechanism for RNAP binding to the promoter and subsequent transcription initiation which deserves some comments relevant to DNA bends. In this model (Fig. 1), RNAP initially anchors to the −35 region of the promoter to form the first closed complex, RP_{c1}, and remains attached to that sequence through the whole process of isomerization and open-complex formation. The RP_{c1} complex produces a distinct pattern of protection from hydroxyl radical (OH*) and DNase I nicking, which spans positions −60 to −1 relative to the transcription start point. Subsequent contacts result in a second closed complex, RP_{c2} (intermediate complex), in which the enzyme interacts with the −10 region. RP_{c2} displays a characteristic pattern of protection from nicking by OH* and DNase I spanning from positions −60 to +20. Upstream contacts produced in RP_{c1} are therefore extended downstream in RP_{c2}. Significant changes in footprints can be detected in the downstream region during transition from RP_{c1} to RP_{c2}. These changes reflect modifications in the DNA-RNAP interface and are particularly noticeable at the −35/−10 spacer region, where several bands hypersensitive to DNase I appear. This is interpreted as the result of a bend imposed by the simultaneous interaction of RNAP with the two −35 and −10 sequences, which would require a certain distortion of the DNA (154). DNase I-hypersensitive bands are placed at the opposite DNA side to that bound by RNAP, suggesting that promoter recognition is concomitant with wrapping of the DNA around the holoenzyme. Simultaneously, RNAP realigns the −35 and −10 hexamers, thus stressing the spacer sequence; this effect is thought to play an essential role in driving formation of the open complex (11). Some transcriptional regulators such as MerR, which activates transcription of an operon for resistance to mercuric salts when cells are exposed to Hg^{2+}, underwins the promoter sequence at the MerR operator to realign the −35/−10 hexamers into a conformation which can contact cognate surfaces on the RNAP (10).

As illustrated in Fig. 1, isomerization from RP_{c2} to the open complex RP_o engages exclusively the so-called melting domain of the promoter (158). In this step, a region of 11 to 17 bp is melted, resulting in the exposure of a short stretch of single-stranded DNA (66, 230, 254). How is the melting actually effected? DNase I footprints of RP complexes at different

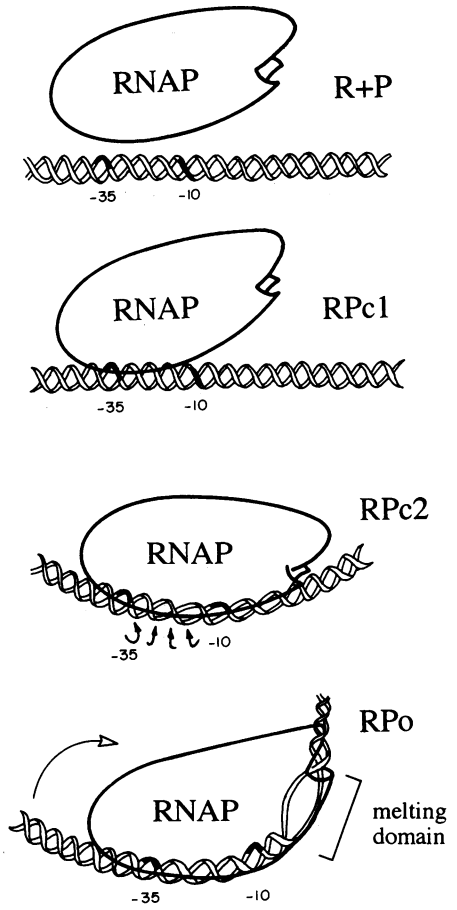


FIG. 1. Events leading to transcription initiation in promoters with canonical −35 and −10 hexamers. The subsequent steps which determine formation of a transcriptionally competent open complex (RP_o) are summarized in the figure. Recognition of promoter sequences and early binding of the RNAP seem to start through the interaction of a channel-shaped surface of RNAP with the −35 hexamer to form a first closed complex (RP_{c1}). This complex then proceeds into an intermediate complex, RP_{c2}, which engages DNA sequences further downstream (down to +20) and provokes the realignment of the −10 box with respect to the −35 hexamer. Such torsion helps drive the complex into an open form (RP_o) concomitantly with a significant increase in DNA bending of the region, which may also engage sequences farther upstream (see text for explanation). The figure was inspired by reference 221.

temperatures (221) suggest that contacts of the enzyme with the −10 hexamer and the bases downstream spanning the melting domain may result from embracing of the DNA helix by two protruding protein domains (46, 221). Furthermore, these data also suggest that a protein channel around the DNA interacts loosely in RP_{c1} and more tightly in RP_{c2}. If this channel suggested by footprinting data corresponded to the protruding protein arm detected by electron microscopy, DNA following the path from the channel to the groove would have to bend 60° (see above). Strand separation may then result from the release of the torsional stress accumulated at the spacer region between −35 and −10 by the combination of the extensive bending and realignment caused by initial binding of RNAP with the somewhat sharp bend produced by the channeling of the DNA through specific domains of RNAP surface. This notion is supported by theoretical predictions and experimental evidence on the coupling between DNA bending and A · T base pair opening (33, 198).

Promoters Include Intrinsic DNA Curvatures

An important prediction of the model for transcription initiation mentioned above is that promoter strength is closely related to the quality of the DNA-RNAP interactions. Given the major changes experienced by the DNA during the process, such interactions are probably determined not only by the nucleotides directly involved in the contacts but also by the three-dimensional structure of the whole region. Regardless of how close the −35 and −10 regions are to the consensus promoter sequence, the intrinsic spatial distribution of the promoter DNA may predetermine the ease with which the region adapts to the structural transitions discussed above. Structural readiness of the promoter will certainly influence initiation kinetics, a bottleneck particularly critical for genes which have to be expressed at very high levels (51).

If DNA must wrap the RNAP for transcription initiation, promoter activity may then be limited by the orientation of intrinsic curvatures present in the sequence. This general notion is substantiated by abundant data. For instance, the presence of extra T residues just upstream of the −35 hexamer results in a major decrease of promoter activity both in ribosomal promoters (62) and in artificial constructions (145). This decrease is thought to occur because the two T residues included in the consensus hexamer (TTGACA) are misoriented as a result of the narrowing of the minor groove caused by the whole T tract. Similarly, the structure of the so-called spacer region located between the −35 and −10 sequences also plays a determinant role in promoter strength. The interaction between RNAP and the spacer is largely nonspecific, but whether the sequence is intrinsically bent in the proper orientation (or at least has the flexibility required to bend adequately) may determine the overall activation energy of promoter activation (41, 145). A GC→TA transversion at position −19 in the *galP1* promoter which creates a run of six thymines on the same strand (one of the combinations that allow DNA bending) increases the transcription rate from this promoter and also enhances contacts with the RNAP at around −50 (29).

In some cases (41), divergence from the consensus −35 and −10 hexamers can be compensated with a spacer sequence predicted to have a properly oriented static bend or increased flexibility in the appropriate direction. By the same token, bends that are present in spacer regions and that misplace protein-DNA interfaces do decrease promoter activity (41, 145). Finally, the minor groove at the center of the −10 hexamer must be placed on the inside of a curvature for efficient recognition by RNAP (41, 53, 241). Structural requirements for promoter activity do not cease at the −10 region but seem to span the melting domain (nucleotides −7 to +2) also. In the A1 promoter of T7 phage, introduction of single nucleotide gaps at positions between −8 and +2 overcomes the temperature requirement for open-complex formation (260). Since the gap is expected to result in a more flexible local structure, such a result may mean that native DNA bends strategically distributed throughout the promoter region not only energetically favor the wrapping of DNA around the RNAP but also may lower the energy required for unwinding the −10 region.

INTRINSIC CURVATURES UPSTREAM OF THE −35 HEXAMER

As discussed above, the major determinants of promoter strength are the −10 and −35 hexamers and the conformation of their spacer sequence. However, sequences outside this region but in *cis* to it can strongly influence its activity (51, 120, 135). A survey of nucleotide sequences of 43 *E. coli* promoters (191) indicates a clear relationship between promoter strength and the presence of upstream regions of curved DNA. A significant percentage of *E. coli* promoters have a poly(A) sequence predicted to confer an intrinsic bend centered around −44 bp (64). Similarly, a survey of nonlinear structures in the data bases of DNA of gram-positive and gram-negative bacteria showed that about one-half of the sharpest bends were located near promoter sequences (250), the centers of bending being at positions around −50. Most randomly cloned curved DNAs from *E. coli* are located in regions immediately upstream of coding sequences, and, as expected, these curved DNA fragments contain promoters. In most cases, the bends were located upstream of the −35 region (237). Furthermore, some types of promoters, such as those dependent on the σ^s factor (a σ^{70}-like factor required for late expression of some genes in *E. coli*) seem to be particularly prone to being located in regions containing static curvatures (57).

Curved DNA and Transcriptional Activity

Besides indications from the surveys discussed above, the relationship between intrinsic DNA curvature and transcriptional activity in vivo has been suggested in a number of cases, including certain *E. coli* ribosomal and tRNA promoters (14, 81, 101, 216, 270), the *bla* promoter from pUC19 (173), the *Alu156* promoter from *B. subtilis* phage SP82 (151), and the streptococcal plasmid promoter PII (187). This correlation is also observed in vitro with RNAP alone, thus indicating that upstream curved DNA may by itself have the ability to enhance transcriptional activity (17, 101, 139, 151, 187). Furthermore, heterologous sequences directing intrinsic bends are sometimes exchangeable without loss of transcriptional activity (160, 165).

The location of the intrinsically bent DNA upstream of the RNAP-binding site does vary, ranging from −235 in the distal activating region of streptococcal promoter PII (recently renamed PctII) (187) to −40 in the early promoters of T5 and T7 phages (28). In most cases known, only one curved upstream region is present, but it is not infrequent to find examples in which two regions placed in phase contribute to promoter activation (14, 101, 152, 187). In these cases, the proximal curvature is located between −38 and −70 and the distal curvature is at different positions upstream from −60. In the few cases studied with two upstream elements, each of them stimulates transcription by a different mechanism. In the *argT* promoter of *E. coli* (proximal element between −60 and −38 [101]) and in the streptococcal promoter PII (center of proximal element around −55 [187]), the proximal curved DNA favours RNAP binding, i.e., increase of K_B. In contrast, in the same *argT* promoter, the distal element (−130 to −60 [101]) increases K_2 by facilitating isomerization to the open complex, whereas the distal element of the PII promoter (centered at −235 [187]) seems to increase later steps of the initiation process (Table 1). In this last case, however, binding of additional host-encoded proteins cannot be dismissed.

TABLE 1. Prokaryotic promoters containing intrinsic DNA bends[a]

Origin	Promoter	Intrinsic curvature[b]	Effect[c]	Reference(s)
E. coli	*argT*	−38 to −130	25-fold (total)	101
		−38 to −60	K_B	
		−60 to −130	K_f	
E. coli	*gal*p1	−60 to −90	K_f	138
E. coli	*rrnB* P1	−40 to −60[d]	K_B, K_f	139, 199
E. coli	*ada*p	−50[e]	K_B*	17
pLS1[f]	PII	−50 region	K_B	187
		−230 region	K_f	
SP82 phage	p*Alu156*	−40 to −70	K_B	151
E. coli	*lac*pI (synthetic)	−50 to −80	K_f*	68

[a] Compilation of some prokaryotic promoters containing intrinsic DNA curvatures for which the step of the transcription initiation process which is affected by DNA bending has been determined.

[b] Position of the curved DNA sequences within the promoter region relative to the transcription initiation start site.

[c] Kinetic parameters affected by the curved DNA within the promoter indicated in each case: K_B means an enhancement of the binding of RNAP to cognate DNA sequences (closed complex), while K_f indicates a stimulation of the open complex, i.e., DNA melting and subsequent transcription initiation. *, effect on supercoiled templates.

[d] The −40 to −60 (UP) element, thought to overlap with a intrinsically curved DNA structure (81), may actually be independent of the major statically bent DNA sequence of the region at about −100 (63).

[e] Obtained through mutation.

[f] Promiscuous plasmid of streptococcal origin.

Orientation of DNA Curvature and Transcription Activation

A remarkable feature of activation mediated by intrinsically curved sequences is the dependency on its orientation relative to the RNAP-binding site. Phasing experiments in which the distance between the curved region and the promoter is changed by insertions of integral and nonintegral helical turns show that insertions of 11 or 21 bp produce mutant promoters which maintain most of the activity of the wild-type promoter, whereas insertions of 5 or 15 bp do not (152, 162, 187). Furthermore, the gross geometry of the curvature influences the degree of transcription stimulation (173, 187). In summary, when two or more curved regions are present upstream of the same promoter, the relative orientation between them and with respect to the promoter determines the final outcome of promoter activity.

Mechanism of Transcription Stimulation by Curved DNA

How can an upstream static bend influence promoter activity? An early model suggested that curved sequences could act as docking regions for RNAP, so that the local concentration of the enzyme in the proximity of the promoter is increased (191, 242). This notion, however, did not explain a number of experimental results mentioned above, such as the effect of curved DNA on steps following closed-complex formation and data on phasing. Actually, many results suggest a far more complex scenario, in which DNA curvatures play an active role in the formation of a transcriptionally competent complex (Fig. 2). Table 1 summarizes some data on the kinetic step(s) affected during transcription initiation by the presence of curved DNA within the upstream promoter region in a number of well-characterized cases.

As mentioned above, promoter-proximal intrinsic bends could facilitate the initial binding of RNAP. A+T-rich sequences found at nearby positions upstream of many promoters have been found to stimulate their activity (12, 28, 52, 83). There is extensive evidence that RNAP contacts include

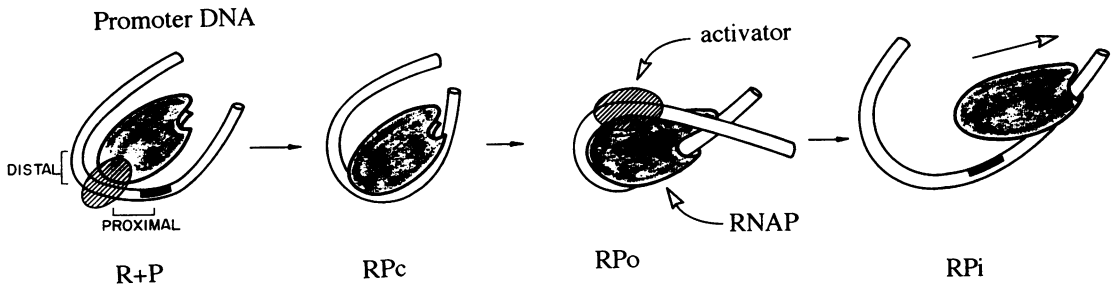


FIG. 2. General model for bacterial promoters responsive to DNA bending. Analysis of a variety of cases reveals a general trend which is summarized in this scheme. Statically bent DNA or protein-induced bends within promoter regions are frequently located either relatively close to the RNAP-binding sequence (proximal sites, from −40 to −80) or further upstream (−80 to −240). In most cases, proximal bends help the formation of closed complex (RP_c), whereas more distal curvatures are frequently also involved with the isomerization into an open complex (RP_o). A simple explanation of this could be the existence of several RNAP surfaces which interact actively with DNA through sequences significantly upstream of the promoter core. Proximal sites may help the initial docking of the polymerase into the promoter by facilitating the initial wrapping of the DNA around the back of the enzyme (Fig. 1). DNA sequences farther upstream may also interact with a different protein surface aided by intervening static or protein-induced DNA bends, resulting in a stressed overall conformation which is eventually relieved by driving the complex into an open form and subsequent transcription initiation (RP_i). Potential distribution of static and/or activator-induced bends throughout the promoter region is symbolically indicated. The figure was inspired by reference 272.

regions upstream of −35 and −10. Footprinting data obtained from ribosomal promoters (163) indicate that RNAP interacts with the DNA up to around position −50, distinctly upstream of the −35 hexamer. Phasing experiments with the proximal region of the *rrnB* P1 promoter of *E. coli* (162) suggested a relationship between factor-independent activation of the promoter and the interactions of the RNAP with the −53 region. Furthermore, recent results have shown that in *rrnB* P1, direct contacts of the A+T-rich region at −40 to −60 (the UP element [199]) with the α subunit of the RNAP (210) account for the stimulation of transcription by 30-fold in vivo and in vitro with respect to promoter activity devoid of the UP element. This A+T-rich sequence has the same effect when placed upstream of a heterologous promoter such as P*lacUV5* (210). The presence of a third promoter recognition element interacting with the RNAP besides the −10 and −35 hexamers may explain the presence of A+T-rich DNA upstream of many prokaryotic promoters (210). The extended promoter sequences interacting with RNAP are not exclusive of ribosomal promoters. In the *tyrT* promoter, the region footprinted by the RNAP extends up to nucleotides −61 to −63, somewhat further upstream of the average position of intrinsic bends (243).

Although there may not be a general, clear-cut boundary between proximal and distal positions within the promoter region (depending on the specific case, they may even have some overlap), the most frequent trend is that the more distal the DNA bends are, the more they are involved in later stages of the transcription activation process, namely in the formation of the open complex. Such distant curvatures probably allow the DNA to contact the back side of RNAP during the transition from closed to open complex. After RNAP becomes bound to −35 and −10 hexamers and to the promoter-proximal sequences, the distal bend might facilitate further wrapping of the DNA helix around the enzyme. Studies on the *lacUV5* promoter (8, 27) suggested that distal bends could enhance wrapping of DNA around RNAP, which contributes to the formation of single-stranded regions at the isomerization step. How could this happen? As mentioned above, DNA unwinding at the melting domain seems to be the final result of releasing the torsional strain of the intermediate complex. Such a metastable intermediate is formed because the −10 and −35 sequences are not optimally oriented relative to the surface of the RNAP. The enzyme must therefore distort the DNA to contact both regions (21). Within this scheme, it is plausible that distal DNA bends bring additional contact sites to the vicinity of the RNAP, thus driving the enzyme on the promoter into a more stressed intermediate and energetically favoring open-complex formation. Such distribution of interactions may facilitate the unwinding process (i.e., increase K_2) and/or fix the RNAP in the conformation of the open complex disfavouring the reverse reaction (i.e., decrease K_{-2}).

In spite of the results discussed above, the presence of curved sequences upstream of promoters should not be granted automatically as regulatory elements per se, independent of protein-protein or protein-DNA contacts. A very significant static curvature found about −100 of the ribosomal *rrnB* P1 promoter of *E. coli* does not correlate with the sequences responsible for stimulating transcription (63). In other cases, it is still possible that upstream curved sequences, even those placed quite upstream from the RNAP-binding site, simply act as docking sites for unidentified proteins interacting with the enzyme for transcription stimulation.

Promoter Clearance

Increasing the stability of the RNAP-DNA complexes may also hinder promoter clearance, i.e., the escape of RNAP from the promoter after initiation of the transcript and subsequent synthesis of the RNA chain (82). In these cases, increased stability is predicted to result in a net loss of promoter efficiency. For instance, the activity of the *Bal129* promoter of *B. subtilis* phage SP82, which has a sharp proximal intrinsic bend, is limited at the step of promoter clearance and not at the level of open-complex formation (151). The effect of distal curvatures may, however, be very different. Further upstream contacts might actually help clearance by springing the RNAP off the promoter after formation of the open complex. Some results support this notion: Bujard et al. (28) noticed that the strength of some hybrid phage-*E. coli* promoters depends on the presence at their distal upstream region of A tracts, which turned out to favor the escape of RNAP from the promoter. In fact, the effect of upstream bends on promoter clearance was first envisioned for CAP in the *lac* promoter, although in this case there is no evidence that such effect may occur in the absence of protein-protein contacts (272; also see below).

In Vitro versus In Vivo Effects

In a limited yet significant number of cases, the effect of intrinsic DNA bends upstream of the −35 hexamer has been examined in parallel in vitro versus in vivo with quite consistent results (23, 101, 151, 152, 187). However, there are also a number of reports involving exclusively in vivo experiments in which the effects of mutations and deletions at the upstream region on the expression of a reporter gene has been studied in detail (25, 135, 173). In a remarkable report, Bracco et al. (25) showed that an intrinsic bend upstream of the *gal* promoter could mimic in vivo the activation effect of CAP. These data suggested that a properly oriented curved DNA may by itself have the ability to activate a downstream promoter even in the absence of protein-protein contacts between the RNAP and its cognate activator. The results of Bracco et al. (25), however, should not be overinterpreted. Not all curved sequences tested had an activating effect, and exchanges which were active in vivo had no activity in vitro; it is likely that the precise geometry adopted by the DNA curvature is critical for the activation effect. Whether functional substitutions of this type have a general meaning remains an open question. Auxiliary factors and/or a particular degree of superhelicity of the DNA templates in vivo may play a major role in promoter activity in these cases. The H-NS histone-like protein (see below), which binds to intrinsically curved DNA sequences (266), affects in vivo and in vitro the expression of synthetic promoters carrying upstream curved DNA sequences (274).

Physiological Role of Upstream Statically Bent DNA

The effect of intrinsic curvatures located upstream of the −35 sequence seems to be somewhat nonspecific in the sense that activation may occur once a certain spatial distribution of nonlinear DNA structures has been properly assembled within the promoter region. It is even possible, yet not proven in all cases, that such activation takes place regardless of the origin of the nucleotide sequence providing the structure (25, 68, 160, 165). There are a number of situations in which such permissiveness might be a useful evolutionary device for proper regulation of transcription, in particular when the promoter cannot accept changes in its nucleotide sequence, i.e., promoters included within structural genes. In these cases, degeneration of the genetic code could allow formation of curved DNA structures to control the strength of downstream promoter sequences without affecting the primary structure of the protein encoded by the cognate mRNA. This could be particularly true for cases in which short-lived regulatory antisense RNAs are required in large amounts, typically in control of plasmid replication (167). This notion is actually supported by data on the structure of the DNA regions controlling expression of the antisense RNA, which regulate the copy number of streptococcal plasmid pLS1 (187) and enterobacterial plasmid R1 (18). Besides these few examples, the role of intrinsic curvatures frequently found upstream of prokaryotic promoters (101, 151, 173) is not well understood. It is possible that these nonlinear structures play a role in cases when maintenance of the sequence of the core element (−35/−10 region) is essential for optimal promoter activity (31, 213).

DNA BENDS INDUCED BY ACTIVATORS OF TRANSCRIPTION

The first report of the DNA-bending capacity of the CAP (265) provided a hint at understanding not only the mechanism of action of activators binding promoter-proximal sequences but also effects at long distance exerted by proteins on regions far from their contact sites. Besides CAP, a number of transcriptional regulators have been reported to direct bending of the DNA helix at the contacted sequences. However, curvature and transcriptional regulation have been investigated in only a few cases. A central question, which remains largely unanswered, is whether protein-induced bending plays an active role in transcription initiation or is just a consequence of protein binding. Indeed, various results suggest that, at least in some cases, DNA bending may suffice to activate transcription from a downstream promoter (see above). Protein-induced bending may act in transcriptional activation independently of (but in addition to) protein-protein communication between the activator and the RNAP. Even in the cases studied in which protein-protein contacts appear to be the critical step for activation, activator-induced DNA bending resulting from the binding to cognate sites is likely to facilitate activator-polymerase contacts (67, 227, 257; for reviews, see references 4 and 87).

Catabolite Activator Protein

The cyclic AMP (cAMP) receptor protein of *E. coli* (CAP or CRP) is the best-studied example of a protein introducing a bend at the site of interaction with DNA. The physiological role of cAMP in bacteria has been recently reviewed (24, 122). When intracellular cAMP levels raise, dimers of the CRP-cAMP complex bind to specific sequences at target promoters (24, 47), bringing about a sharp bend in the bound DNA (265). X-ray crystallography of CRP-cAMP-DNA complexes (227) indicate that bending is the result of two discontinuous kinks of about 45° at the central TpG sites of each of the TGTGA sequences, which form the inverted repeat recognized by the protein. Such an arrangement leads to a total bending angle within the region of at least 90°. CAP binding to DNA can be further tightened 200-fold if the CAP site is within an in-phase intrinsically curved stretch of DNA (116). This suggests that the energy required to maintain the CAP-induced DNA bend arises not only from specific interactions (67, 227) but also from accommodation of the nucleotides surrounding the core target DNA sequence to the surface of the CAP protein (227, 257).

CAP activates RNAP at several promoters through protein-protein interactions (15, 56, 93, 271) and concomitant conformational changes of DNA at the promoter region (27). The specific contribution of each of these two effects to transcription initiation seems, however, to vary in different CAP-dependent promoters, where the activator may bind to proximal or distant sites, thus affecting distinct kinetic bottlenecks (69, 90, 147, 235).

Does the CAP-directed curvature explain by itself transcription activation at cognate promoters? CAP-binding sites may be functionally replaced by statically curved DNA in vivo (25) and in vitro (68). It is also possible to substitute functional CAP sites by DNA sequences which are targeted by heterologous DNA-bending proteins (186). These data do not, however, give us the whole picture. Single-site CAP mutants which are as perfectly able to bind and to bend DNA as the wild-type protein but are unable to activate transcription exist (15, 56, 271), thus indicating a requirement for protein-protein contacts. A significant synergy between CAP and RNAP in the formation of the open complex in the *lac* promoter has also been reported, suggesting the existence of close RNAP-CAP interactions (202, 235). Furthermore, cAMP-CAP and σ^{70}-RNAP holoenzyme interact in solution (93, 190), even in the absence of promoter DNA (93). Mutational analysis of CAP has permitted identification of a patch on the surface of the

protein which is distant from the DNA-binding domain but available for contacts with the RNAP (271). Also, mutations in the α subunit of RNAP make the enzyme insensitive to activation by CAP at the *lac* promoter in vitro (108, 111, 273).

The corollary of these data is that activation by CAP can be envisioned as the result of the simultaneous effects of protein-induced bending and protein-protein interactions. Bending can facilitate and stabilize CAP-RNAP contacts to form a productive nucleoprotein transcription initiation complex. The degree of DNA bending caused by the combination of CAP and RNAP in vitro is far greater than that caused by CAP alone and is further exacerbated during formation of the open complex (272). The role of the CAP-binding DNA sequence is not just to increase the local concentration of the activator. Unlike other regulatory proteins (91, 258), cAMP-CAP does not stimulate transcription in a "catenane" assay in which RNAP and CAP are placed in linked circular DNAs so that they are in close proximity but still in *trans* (5). Besides protein-protein interactions directed by induced DNA curvature, CAP-induced bending might stimulate contacts of upstream DNA sequences with the back of the RNAP during the transition from a closed to an open complex, thus enhancing the unwinding process in the fashion which has been discussed above for static bends.

As mentioned above, CAP sites do appear at different distances upstream from cognate promoters, although some restrictions in their phasing and distance from the −35/−10 region seem to apply. This issue has been examined in detail in vitro with hybrid promoters containing CAP-binding sites at different distances (69, 247). These experiments showed that maximal activation was obtained when CAP was located at position −61.5 or −41.5 bp upstream of the transcription start site. A plausible explanation for this is that CAP dimers have two activating domains (one per subunit) and that therefore, depending on the distance, either one or the other domain productively contacts the RNAP bound to the promoter DNA. In addition, CAP interacts with a different region of the RNAP when located at −41.5 or at −61.5 (108, 110). This is fully consistent with the observation that −41.5 and −61.5 are the actual distances in natural CAP-dependent promoters and that the stages of the transcription initiation process affected by the activator are the same as well (122).

Two good examples of CAP sites placed at different distances from the transcription initiation point are the *gal* promoter (CAP site at −41.5) and the *lac* promoter (CAP site at −61.5). Although the CAP subunit actually making the contact with the RNAP would be different in each case, in both instances the activating domains of the regulator could be oriented similarly with respect to the enzyme anchored to the DNA. However, the location of CAP sites seems to make a difference in the respective activation mechanism. These are more noticeable when linear DNA templates are used. In the case of the *lac* promoter, CAP favors formation of the closed complex, i.e., RNAP binding (147), whereas in the *gal* promoter, both binding and isomerization to the open complex are stimulated (90). How does one explain this difference? The activator bound to CAP sites like that of *lac* promoter may just enhance the binding of RNAP to the promoter, mostly through protein-protein interactions. In the *gal* promoter, in addition to this effect, the DNA bending caused by CAP bound to the site at −41.5 also favors the contact of upstream DNA sequences with the back of the enzyme, leading to open-complex formation in the fashion discussed above for intrinsic bends. Such stimulation by CAP of RP_o in the *gal* promoter depends on the presence of a short poly(A) curved sequence just upstream of the activator-binding site (138) which is absent (or irrelevant) in the *lac* promoter. If this A tract is deleted, CAP activates the *gal* promoter at a different formal step of the initiation process, namely by just increasing the affinity of the RNAP for the promoter (138). Furthermore, CAP can stimulate isomerization to the open complex in the *lac* promoter if the template DNA is sufficiently supercoiled (156, 235). This suggests that when the CAP site is located at position −61.5, linear templates lack a particular topology required for stimulation of upstream DNA-RNAP contacts which would favor open-complex formation. In summary, the presence of a DNA bend upstream of the promoter core seems to determine the way CAP works; i.e., the presence of upstream curved DNA facilitates the transition from closed to open complexes in linear templates.

Protein p4 of Phage Φ29

The p4 protein of *B. subtilis* phage Φ29 is another well-characterized example of a prokaryotic transcriptional activator which bends DNA. Transcription in phage Φ29 takes place in two stages, early and late. Early genes are transcribed from a number of promoters, the best characterized being *PA2b*. The viral protein p4, which is produced at early stages of infection, is responsible for the switch from early to late transcription. Late genes are transcribed from a single promoter named *PA3*, which is inactive in the absence of protein p4. *PA2b* and *PA3* are located close to each other but in opposite orientations. Protein p4 is a small (12.5-kDa) dimeric activator which binds to a region of *PA3* spanning positions −58 to −104 relative to the transcription start site, producing a sharp bend of 80 to 85° (13, 157, 207, 209). The protein p4-binding site at the *PA3* promoter partially overlaps with the early *PA2b* promoter, and, as a consequence, activation of *PA3* by protein p4 is concomitant with the repression of the *PA2b* promoter (208). p4 activates transcription from *PA3* by stabilizing the binding of *B. subtilis* RNAP to the promoter as a closed complex (169). In this process, both p4-induced DNA bending and protein-protein contacts between RNAP and p4 are believed to be essential for full promoter activation. Truncated protein p4 derivatives that can bind to DNA but have a reduced DNA-bending ability are unable to activate transcription from *PA3* and do not stabilize the binding of RNAP to the promoter (209). The parallel between the stability of the DNA bends induced by different p4 mutants and their ability to activate transcription from *PA3* led to the proposal that the p4-induced bend plays a determinant role in the activation process. Nevertheless, the scenario seems to be more complex: mutations that reduced DNA bending were shown to affect a p4 domain that probably interacts with RNAP to activate transcription (157). Indeed, several lines of evidence indicate that protein p4 interacts directly with RNAP at the *PA3* promoter (157, 168, 169, 229). These protein-protein contacts seem to be essential for promoter activation since p4 mutants which induce normal DNA bending but are unable to either activate transcription or contact the RNAP are available (157). Productive p4-RNAP contact requires an adequate orientation of the p4-activating domain with respect to the RNAP, which should be brought about by the p4-induced bend. Therefore, the p4-induced bend is necessary but not sufficient for activation. It is likely that, similarly to CAP, p4-induced DNA bending in *PA3* may have two somewhat independent but concomitant roles: to stimulate activator-RNAP contacts and to induce a DNA conformation that favors the transcription initiation process (see above).

FIS Protein

FIS (factor for inversion stimulation) is a small (11.2-kDa) protein of *E. coli* involved in processes such as stimulation of recombination by DNA invertases (113, 115), DNA replication (74), and transcription of rRNA and tRNA operons (164, 212, 269). The FIS protein occurs as an homodimer, and its amino acid sequence includes a typical helix-turn-helix motif for binding DNA. FIS-binding sites are quite diverse, although some consensus sequences have been proposed (58, 105, 251). Biochemical data (238), three-dimensional computer simulation, and X-ray crystallography (124, 268) clearly indicate that, upon binding, FIS bends DNA by 90° at cognate target sites. This notion has also been substantiated by cryoelectron-microscopic studies (125).

Because of its DNA-bending properties, binding of FIS to ribosomal promoters is predicted to bring about a particular conformation at the promoter region which may enhance RNAP binding and further promoter activation (252). More recent data (80) indicate, however, that FIS-induced DNA bends on *rrnB* P1 may actually be just one component of a more complex scenario. Similarly to CAP and p4, FIS mutants which bind and bend their cognate sites at the *rrnB* promoter but cannot activate transcription of the *rrnB* P1 promoter have been found (80, 174), indicating that direct FIS-RNAP contacts are most essential for activation. Mutations in the α subunit of RNAP which make the enzyme insensitive to other regulators which bend DNA, such as CAP (on the *lac* promoter [see above]) or OmpR (110), have no effect on FIS-mediated activation (211). This suggests that FIS-RNAP contacts occur at a different RNAP surface from that proposed to mediate the activating effect of other regulatory proteins.

FIS-dependent promoters seem to have similar structures (251). All of them share a FIS-binding site centered at ca. −70, and some of them (typically the *rrnB* P1 promoter [80, 212]) have additional FIS sites placed in phase at −102 and −143. The intervening region between the FIS site(s) and the −10 and −35 hexamers includes an A+T-rich sequence (the UP element at −40 to −60) that itself possesses activating capacity (199). Although earlier work (81) associated the presence of such an activating element at −40 to −60 with a static bend in the vicinity of *rrnB* P1, more recent results (63) indicate that the actual major static bend is at about −100, i.e., well upstream of the elements which account for almost all effects of the upstream activation region, namely the sequence from −40 to −60 and FIS-binding site I (Fig. 3).

The FIS sites and the element from −40 to −60 seem to have properties which affect promoter activity independently (162). The intervening sequence (the UP element) has been shown to interact directly with the α subunit of the RNAP regardless of the presence of upstream FIS sites (210), and, similarly, a functional UP element is not absolutely essential for stimulation of *rrnB* P1 by FIS (199). This suggests that there is little connection between FIS-dependent and UP element-dependent activation of the promoter and that protein-protein interactions between FIS and RNAP may be the critical event for FIS-dependent activation of *rrnB* P1, rather than affecting promoter activity directly through DNA bending or indirectly by locking interactions of the RNAP with the intervening UP sequence.

Integration Host Factor

Integration host factor (IHF) of *E. coli* is an archetype of proteins whose major function seems to be generating sharp bends at distinct positions of DNA sequences, regardless of their final physiological effect (161). IHF is a heterodimer of

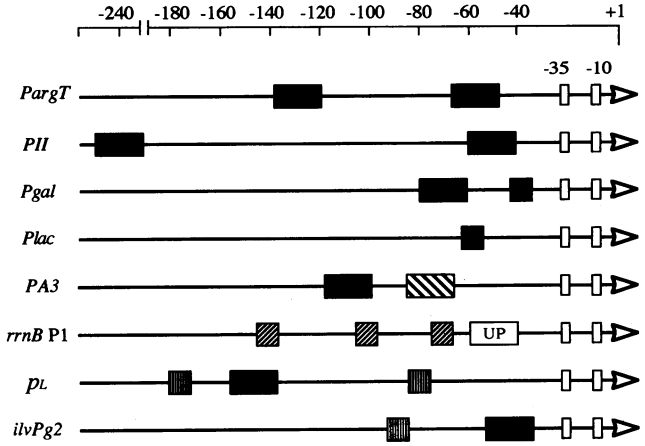


FIG. 3. Positioning of statically curved DNA and/or protein-induced bends in various prokaryotic promoters. The drawing summarizes several prototypical cases of promoters responsive to DNA bending. In most examples, promoters possess one proximal element and one to three distal elements, all represented in relation to the transcription initiation site (+1). The functions of the A+T-rich UP element within the *rrnB* P1 promoter are discussed in the text. The contribution to promoter activity of each segment of DNA and/or its cognate binding protein is also examined in the text. Symbols: ■, curved DNA; ▨, P4; ▨, FIS; ▥, IHF; ■, CAP.

two basic peptides encoded by the *himA* and *himD* (called also *hip*) genes; it is involved in a variety of processes including transcription of certain promoters (see reference 61 for a review). IHF is one of the few examples known of regulatory proteins binding the minor groove of the target DNA sequences, the result being a sharp bend (>140°) at the cognate site (238). IHF may repress or stimulate transcription directly (71, 73, 85, 104, 127, 130, 131, 249) or in concert with other regulatory proteins. The mostly structural role of IHF is indicated by the fact that its function can be mimicked in some cases (76, 188) by substituting its binding site by a DNA sequence endowing an approximately equivalent static bend. In this section we discuss exclusively the activation mediated by IHF alone. The cases in which IHF acts as a coregulator will be examined later in this review.

Perhaps the best-studied instance of direct involvement of IHF in transcription activation is its effect on the p_L promoter of phage λ (71, 73). IHF binds strongly to two sites upstream of p_L, one proximal at ca. position −80 and one distal at around position −180. As is the case with other activators (see above), IHF facilitates the formation of a closed complex on linear templates (71), whereas in covalently closed circular DNA templates, both supercoiling and IHF synergistically stimulate the isomerization of p_L into the open complex (73). Promoter activity in vivo is increased fourfold in IHF-plus strains compared with that in IHF-minus counterparts, the positive effect being face-of-the-helix dependent (73). As is the case with other activators that induce bends in DNA, IHF could stimulate p_L by bringing farther-upstream sequences into contact with the RNAP and/or by undergoing direct interactions with the enzyme that might stimulate formation of the closed complex. Activation of p_L by IHF requires an intact α subunit of RNAP (72), suggesting a requirement of additional protein-protein or protein-DNA interactions. Interestingly, there is no evidence of IHF-mediated contacts of upstream DNA with the back side of the RNAP (72, 73). Although the final picture is still unclear, stimulation of p_L by IHF may

involve formation of a distorted DNA loop in the region between the sites bound by IHF and RNAP.

The *ilvPg2* promoter of *E. coli* is also regulated by IHF. Two upstream regions independently enhance *ilvPg2* transcription (179), the more proximal (at ca. −50) spanning an intrinsic DNA bend and the more distal (at −90) containing an IHF site (179). The proximal activating sequence stimulates promoter activity 7-fold, whereas the distal sequence stimulates activity ca. 4-fold, the two of them together causing an increase of about 28-fold in promoter activity. The mechanisms proposed to explain these observations (179) are not very different from those discussed previously: IHF and the intrinsic bend may cooperate to bring further upstream DNA into contact with the RNAP; alternatively, the intrinsic bend may facilitate essential protein-protein contacts of IHF with the enzyme. As with the p_L promoter, this question deserves further clarification.

A third example of direct transcriptional regulation by IHF is the activation of the *Pe* promoter of phage Mu, which determines the entry of the phage into the lytic cycle (249). *Pe* activity requires both IHF and a certain degree of supercoiling. Mu can lysogenize but not lyse IHF mutants or *gyrB* mutants at normal frequencies (227, 267). It utilizes IHF to control transcription of the genes which trigger lysis or lysogeny. The mechanism by which IHF regulates *Pe* is not completely known. The promoter contains an IHF-binding site located around −80. Higgins et al. (97) have suggested that DNA sequences bent by IHF may form superloops under negative superhelical strain. If that is the case, *Pe* would be located between the end of the superloop and the first supercoiled node, thus providing a good spatial arrangement for binding of the RNAP and further wrapping of DNA around the enzyme (97).

Do All Transcriptional Activators Bend DNA?

Although not every regulatory protein in bacteria has been examined with respect to its DNA-bending capacity, it seems to be the rule (yet with remarkable exceptions [see below]) that transcriptional activators bend the DNA of their target sequences to different extents, frequently exacerbating an intrinsic curvature already present within or near the binding site (141, 215). However, the sign of the effect caused by DNA bending is different depending on the type of regulator. For example, regulators belonging to the LysR family control expression of cognate promoters in a fashion likely to also involve DNA bending (220). In most cases, regulators of the LysR family respond to the presence of specific effectors in the medium, but such effectors do not significantly affect the affinity of the regulators for their target DNA sequences. Footprints available for OxyR (233), TrpI (34), OccR (255), CysB (100), and NahR (103) indicate that prebound DNA undergoes a significant conformational change in the presence of the effector of the regulatory protein. The nature of such change is difficult to interpret. There are at least two examples of proteins of this family (CysB and OccR) in which addition of the inducer to the system results in an apparent decrease (rather than increase) of the DNA bending caused by prebound activator (100, 103, 255) as examined by circular-permutation assays. The intimate mechanism of transcriptional activation by this type of regulator remains unknown.

Another intriguing example of activation associated with DNA bending is that of the leucine-responsive regulatory protein (Lrp), which is increasingly recognized as a major, general regulator in *E. coli* (45). Similarly to IHF, Lrp activates the expression of some operons, represses the activity of others, and seems to bind and bend target sequences located at various sites within the corresponding promoters (256). Unlike IHF, however, such sites do not necessarily share a consensus sequence, but they may share some structural features. Lrp is a dimer containing two identical subunits of ca. 19 kDa, thus resembling various histone-like proteins. Whether the role of Lrp is to act mostly as the common activator of a large collection of individual promoters involved in global responses or, rather, to exert its effect through the maintenance of chromosome structure and organization (or both) still remains an open question (45).

Other types of transcriptional regulators have also been shown to bend DNA, but there are cases in which significant bending does not occur (119). At least for this type of regulator, such as *c*I of phage λ, proximal DNA bending does not seem to greatly affect the promoter activity; i.e., activation is triggered primarily through protein-protein contacts with the RNAP. The DNA-bending activity of other types of activators (for example, the MalT and AraC families of proteins) is uncertain and deserves further study.

DNA Bending and Protein-Protein Contacts Are Required for Promoter Activation

The data discussed above, which include mostly results on σ^{70}-RNAP promoters, indicate that (i) upstream curved DNA has by itself the capacity to activate certain promoters even in the absence of any other known regulator and that (ii) activator-dependent initiation of transcription frequently involves formation of protein-directed upstream DNA bends and/or protein-protein contacts. Regarding the specific stage at which either intrinsic or protein-induced DNA bending and activator-RNAP contacts affect the initiation process, examples of virtually all possible combinations are known, making it difficult to produce a general model to account for all observations made in the different systems. Some definite trends can, however, be drawn.

In most promoters, activation elements are located at one of two positions relative to the start of transcription (Fig. 3). The proximal site (−40 to −80) is typically the target for activator proteins which contact RNAP directly, although in some cases curved DNA also appears. Distal sites might be located within the next 200 bp upstream, and they frequently include intrinsically curved DNA or target sites for proteins which bend DNA (typically, IHF or FIS). In these distal sites, curved DNA or protein-induced DNA bends could create an appropriate conformation, helping the surrounding DNA (or other proteins bound to different sites) to contact RNAP. The way distal sites control promoter activity is not completely understood, but the current view of the transcription initiation machinery as a large nucleoprotein complex involves far-upstream DNA contacts eventually brought into proximity with the RNAP back surface to favor open-complex formation (Fig. 2). At the isomerization step, the intermediate complex would be a metastable structure. Perhaps upstream contacts (either protein or DNA mediated) provide a third contact site to stabilize RNAP on the promoter. This could enhance the ability of RNAP to distort the DNA prior to open-complex formation. Such upstream contacts might be stimulated by the changes in local DNA structure brought about by distal DNA bends or by the interaction of RNAP with proteins (such as FIS or IHF) bound to farther-upstream sites. Interestingly, a synthetic *lac* promoter bearing a second CAP site quite far upstream of the natural binding site (i.e., centered around −93.5) was strongly induced by the activator in a fashion which did not depend on cooperative binding of CAP to the two sites but, rather, on the

simultaneous contact of the protein with two independent activation surfaces of the RNAP (114).

The effect of the proximal site seems to be more complex. Activators which bend DNA near RNAP-binding sites always favor formation of the closed complex and, in some but not all cases, formation of open complex as well. Within proximal sites, the independent contributions to transcription initiation of DNA curvature alone and of protein-protein contacts alone are difficult to separate. As mentioned above, in the case of CAP (in vivo and in vitro), intrinsic curvatures may substitute to some extent for the effect of the corresponding activators, suggesting that induced bending participates directly in promoter activation. Nevertheless, the bulk of the activating effect is lost when the amino acid residues thought to contact RNAP are mutated. At the same time, an excess of activator cannot meet the need for a cognate curved DNA to effect productive RNAP-regulator contacts. The corollary of this rule is that both DNA curvature and protein-protein contacts are required for full activation of the promoter by proximal sites. Although the role of the proximal bending is mostly to generate an efficient spatial distribution of protein and DNA surfaces within the promoter region for an efficient docking of RNAP, a direct effect of the curvature itself may also help the early stages of transcription initiation. Since RNAP-activator contacts are extremely weak in the absence of DNA, activator binding and concomitant DNA bending could be a prerequisite for closed-complex formation (i.e., binding of the RNAP to the promoter sequence) but bending alone would not be sufficient for full promoter activity, which seems to be determined instead by protein-protein contacts. The combination of DNA bending for modulation of promoter activity, along with sharp on-off switches effected by RNAP-activator contacts, seems to be at the basis of the fine tuning of promoter activity throughout a wide range of physiological conditions and environmental changes.

REPRESSION AND DNA BENDING

As discussed above, structural variability of DNA (ultimately dependent on its nucleotide sequence) permits the generation of local stretches with intrinsic curvatures or an increased flexibility in a distinct orientation (102). This allows the DNA to play a dynamic role in the process of gene regulation, facilitating or even determining the binding of regulatory proteins to target sequences (87, 242). Since binding sites for most transcriptional repressors overlap RNAP-binding sites within promoters, it is generally believed that negative regulators inhibit transcription by steric hindrance, i.e., by preventing the access of RNAP to promoter sequences. Recent findings, however, challenge this somewhat trivial concept and suggest that DNA could play a more dynamic role, leading to an increase in repression efficiency. First, RNAP binding to promoter sequences is not mutually exclusive with simultaneous binding of certain repressors, such as LacI (234) or KorB (262), even though the corresponding target sequences do overlap. Furthermore, some "classical" repressors such as LacI, GalR, DeoR, and NagC turn out to have multiple binding sites located at a certain distance from the RNAP-binding site (9, 37, 109, 129, 171, 192). In these cases, protein-protein interactions between repressor molecules bound at the different operators generate higher-order structures which prevent productive access of RNAP to promoter sequences. Alternatively, multiple repressor binding may cause a kind of DNA looping that maintains promoter DNA in a conformation unsuitable for transcription initiation (37, 109, 275). Repression mediated by DNA loops has been reviewed recently (2, 150, 224) and will not be covered here. Another finding suggesting that the DNA shape has an active role in repression is the fact that many repressors bend the DNA at their target sites (119). This bending could contribute to repression efficiency in one or more of mechanisms discussed below.

Assembly of Complex Repression Structures

Bending may just arise as a consequence of, or a prerequisite for, protein-DNA interactions. The specificity of DNA-protein contacts is the result not only of direct recognition of certain bases within the DNA helix by particular amino acid residues but also of a constellation of interactions between protein and DNA surfaces not necessarily linked to a particular nucleotide sequence (indirect recognition). Both effects contribute to the specificity and stability of the complex and require the protein and DNA to accommodate each other for setting up adequate interactions. This is well documented for activators such as CAP, for which interactions of this kind are thought to help to maintain the protein-induced DNA bend (see above). There are also some examples involving repressors, among which those of LexA and ArgR are particularly interesting. LexA binds to several *E. coli* promoters, acting as the repressor of the SOS regulon. Binding to its operator at the *caa* promoter of *E. coli* produces a sharp DNA bend, whereas its interaction with the *recA* promoter does not induce a significant curvature (141). This difference is probably the result of the presence of T tracts flanking the LexA operator at *Pcaa*, which are absent in the *PrecA* counterpart and which, for *Pcaa*, would facilitate wrapping of the DNA around the repressor, thus stabilizing the interaction. Since, unlike *PrecA*, *Pcaa* contains a LexA operator which deviates significantly from the consensus LexA boxes, it is possible that extra protein-DNA contacts facilitated by the repressor-induced DNA bending at *Pcaa* compensate for a poor protein-DNA recognition (142).

The case of ArgR is somewhat different. The arginine regulon contains nine transcriptional units, all of which contain two imperfect target sites for ArgR, a hexameric protein (43, 140). In the presence of L-arginine, a single hexameric repressor molecule binds to the two adjacent "Arg boxes," inducing a bend of about 70° and covering a region of about four helical turns through only one side of the DNA helix (240). The repressor can also bind to a single box but with 100-fold-lower affinity. In the presence of all Arg boxes, a nucleoprotein complex in which the DNA seems to wrap around the protein hexamer, masking the promoter core and hindering access of RNAP, is formed (35). Within such a steric-hindrance model, formation of this complex would require a considerable distortion of the DNA, which is expected to be compensated by a wide array of interactions between the repressor and the DNA. Since RNAP and ArgR must compete for their overlapping recognition sites, the stability of the repressor-DNA complex is critical for repression efficiency.

In certain cases, cooperative binding of repressor proteins to different sites within a promoter is a frequent means of increasing repression efficiency when the affinity of the proteins for their target sequences is low. Under these conditions, DNA bending or DNA bendability (i.e., ease of bending) can facilitate DNA-protein and protein-protein interactions between the components of the repression complex. The RepA repressor (recently renamed CopG) is an example in which DNA bending favors protein-DNA contacts within a complex to enhance repression efficiency. RepA (CopG) is a small protein (5.1 kDa) which is involved in the control of the copy number of the streptococcal plasmid pLS1 (50, 134) by repressing transcription of its own gene and that of RepB, whose

product is the cognate replication initiation protein of the plasmid (185). RepA recognizes a sequence which includes a 13-bp palindrome overlapping the −35 box of its own promoter (50), but the whole of the RepA-DNA contacts engage a segment of over 40 bp (50, 181). This region has an intrinsic curvature (182), which is increased upon RepA binding to its target sequence (183). Gel retardation assays indicate that several complexes are formed upon addition of increasing amounts of RepA, suggesting that binding of one RepA molecule to its target (and the resulting bending of the DNA sequence) may facilitate subsequent attachment of additional molecules of the repressor to flanking sequences. In this context, RepA-induced bending would facilitate adequate and otherwise unlikely interactions that stabilize the multiprotein complex (181). A RepA derivative deleted of part of its carboxyl end, which binds DNA but induces less bending than the wild-type protein (perhaps because of the lack of cooperative binding), did not form this apparent multiprotein complex in vitro and showed a reduced repression efficiency (185). The nucleoprotein complex formed by RepA seems to make promoter sequences unavailable for recognition and binding by the RNAP.

Inhibition of RNAP-Promoter Contacts by Misoriented DNA Bending

The notion that RNAP bends promoter DNA to form a transcriptionally active complex (see above) predicts that any factor which may bend the same target sequence in an opposite direction should act as an efficient repressor (275). This underlies the basis of an additional mechanism of repression which has been substantiated in a number of cases. As described above, p4 protein from *B. subtilis* phage Φ29 simultaneously activates the late *PA3* promoter and represses the divergent *PA2b*, the major promoter of early genes (208). Both effects are the result of p4 binding to a single site centered at −84 bp relative to *PA3* and overlapping the −35 hexamer of the *PA2b* promoter, which is oriented in a direction opposite to that of *PA3*. Protein p4 prevents binding of RNAP to *PA2b* while simultaneously favoring binding of the enzyme to the nearby *PA3* promoter (208). Since the protein p4-binding site overlaps the −35 box of *PA2b*, the resulting repression could be simply attributed to steric hindrance. A closer look at the system, however, reveals a more complex situation. First, the binding sites for protein p4 and RNAP at *PA2b*, although partially overlapping, are on different sides of the DNA helix. Therefore, given the small size of p4, mutual exclusion is not automatically ensured. Furthermore, the p4 operator includes a sequence-dependent curvature of about 45°, which increases to 80 to 85° in the presence of the protein (13, 209). This immediately suggests that, although steric hindrance is likely to be involved in *PA2b* repression, the p4-induced bend could help repression efficiency, since the direction of the p4-induced bending would be opposite to the bend associated to RNAP binding. This possibility was analyzed by the introduction of four point mutations in the protein p4-binding site designed to increase the intrinsic curvature in the same direction as protein p4 does, thus simulating the p4-induced bend. The mutations were combined to avoid interfering with the −35 box of *PA2b*. The resulting curvature was shown to be able to inhibit transcription from *PA2b*, impairing both promoter recognition by RNAP and transition from closed to open complexes (208). These results indicate that any event which bends promoter sequences in a direction unfavorable for RNAP binding can effect transcription repression. Given that competition between a repressor and RNAP for their respective overlapping binding sites ultimately determines repression efficiency (136), DNA bending adds (at least when the repressor and RNAP bind to opposite DNA sides) an additional dimension to such competition. If the repressor-induced curvature modifies the structure of the promoter, the ability of RNAP to compete and displace the repressor from its binding site can be efficiently reduced.

Another remarkable example of repression by spatial misorientation of promoter sequences is that of the *ilvGMEDA* operon of *E. coli*, which is transcribed from two tandem promoters, namely *ilvPG1* and *ilvPG2*. The IHF protein represses *ilvPG1* while simultaneously activating *ilvPG2*. The IHF-binding site at *ilvPG1* overlaps that of RNAP, but the two proteins bind opposite sides of the DNA helix and do not share any contact on the DNA backbone (263). Nevertheless, binding of IHF to *ilvPG1* prevents productive interactions of RNAP with promoter sequences (179). Since IHF induces substantial DNA bending at the target sequences, it is believed that IHF repression at *ilvPG1* is due to the change of DNA geometry at the promoter and not to the physical occlusion of RNAP by prebound IHF (179). Whether the same is true in other cases of repression by IHF sites overlapping promoter sequences (85, 245) deserves further examination.

To complete the range of possibilities, there are cases in which RNAP and repressors bind simultaneously to target sequences. In such instances, repression may actually occur by a "freezing" of the RNAP at the promoter site in an inactive complex. Surprising as it may seem, Thompson and Mosig (239) have suggested that IHF represses the activity of the chloroplast *Pa* promoter by binding to a site overlapping promoter sequences, leading to inhibition of open- rather than closed-complex formation. It can actually be speculated that maintaining RNAP bound to the promoter yet in an inactive state can be advantageous in situations which require a quick response to a particular stimulus. In these cases a mechanism of repression which permits closed-complex but not open-complex formation might be desirable. Bending-mediated repression would meet this need since it may permit the arrest of open-complex formation without inhibiting binding of the RNAP to target sequences. This notion is supported by the results with three repressors: LacI, GalR, and TetR, which bend DNA and regulate operons required to respond quickly to the presence of nutrients or antibiotics. These repressors form ternary RNAP-repressor-DNA complexes (129, 155, 234). In the three cases, the operator for the repressor is placed opposite the promoter core on the DNA helix.

Repression by Distant DNA Curvatures

Interestingly, the negative effect of protein-induced bends on promoter activity may occur also when the binding site is distant from the core promoter sequence. A RepA repressor operator placed artificially around −90 upstream of a standard promoter enhanced or decreased transcription in a side-of-the-helix-dependent fashion relative to the RNAP-binding site both in vitro and in vivo (184). Since at such distances direct RepA-RNAP contacts are unlikely, the effect can be attributed to RepA-caused bending, which, depending on the phase, can either favor or inhibit upstream DNA-RNAP contacts that facilitate transcription initiation (see above). Similarly, the activating effect of IHF on the p_L promoter of phage λ may be converted into a repression effect when the IHF-binding site, natively placed at −86, is artificially moved to the DNA side opposite that of RNAP binding (73). Although these are artificial situations, there are some cases in which a repressor protein naturally binds far upstream of a core promoter

sequence. A similar instance is the negative effect of IHF on expression of *ompC* of *E. coli*, in which the IHF-binding site appears quite far upstream (ca. −180 bp) of the promoter (104). Additional examples of repression at distance caused by proteins which bend DNA have been reported, including repression by RNAP itself (55).

Repression-Activation Switches

The concept of a transcriptional switch applies to situations in which two or more promoters share a common regulatory region in such a way that expression of one of them represses the activity of the other and vice versa. Switches may include sharp on-off changes (typically involved in developmental programs of phages) but also gradual transitions of transcriptional activity from one promoter to the other. This last situation is frequently observed in the regulation of metabolic operons, in which changes occur in response to availability of nutrients in the medium. The promoters involved in either case might be arranged divergently or in tandem, but in all cases they are placed at different sides of the DNA helix and have a certain degree of overlap (203). Typically, also, one of the two promoters is subjected to positive regulation by the very same factor which represses the activity of the second (and otherwise constitutive) promoter. In previous sections, we have already examined some systems which fall within the category of transcriptional switches, namely the tandem *ilvPG1* and *ilvPG2* promoters of the *ilvGMEDA* operon of *E. coli* and the divergent *PA3* and *PA2b* promoters of phage Φ29. In both cases, the activity of each promoter involved is determined by a regulatory protein (IHF and p4, respectively) which causes the target DNA to bend in an orientation such that the resulting curved DNA represses one of the promoters (or enhances the repression effect of the regulator-mediated steric hindrance on RNAP binding) while activating the other, positively controlled promoter. Similar arrangements are found at promoters which control lysis-lysogeny switches of phages λ and Mu, in which IHF and supercoiling play a determinant role in developmental decisions (97). Some examples of transcriptional switches involve the CAP protein. The tandem *gal* promoters are subjected to a mode of control by CAP in which binding of the regulator to target sequences at the cognate catabolite-responsive promoter prevents binding of the RNAP to the overlapping constitutive promoter (231). An interesting case of a CAP-driven transcriptional switch is that of the *fur* promoter of *E. coli* (48, 186). The *fur* promoter region includes two tandem overlapping σ^{70} promoters, placed at opposite sides of the DNA helix, and a distant upstream CAP site at −70 (48). When the CAP site is not occupied (i.e., under catabolite repression conditions), the promoter placed on the same side of the DNA helix as the CAP-binding sequence remains virtually silent. However, there is considerable transcription from the promoter placed on the side opposite to the CAP-binding sequence. Growth under conditions of catabolite activation (i.e., binding of cAMP-CAP to target sequences) changes this situation by triggering the activity of the promoter placed on the same side as the CAP-binding sequence and repressing transcription from the opposite promoter (48). This very same behavior is maintained when the CAP site is precisely replaced by that for the heterologous RepA protein, a repressor which strongly binds and bends DNA at target sequences (see above). Actually, it is possible to activate alternately each of the overlapping *fur* promoters by placing the RepA-binding site in phase with either of them, suggesting that the switch of activity from one promoter to the other is due exclusively to the orientation of the DNA curvature caused by RepA (or CAP in the natural case) binding (186). For tandemly located natural promoters, such as those present on the *fur* and *gal* operons, RNAP binding to the constitutive basal promoter may contribute to repression of transcription from the activator-induced promoter not only through steric hindrance but also because binding of the RNAP to the basal promoter generates a bend at the opposite side of the DNA helix. When the activator protein binds to its upstream site, the activator-induced bend counteracts the RNAP-induced bend at the constitutive promoter and may drive the RNAP into the activator-responsive promoter. The activator-induced bend, reinforced with the RNAP-induced bend at the activator-responsive promoter, could help to inhibit the binding of RNAP to the basal promoter, now located at the "wrong" face of the bend.

OVERALL DNA STRUCTURAL CHANGES AND PROMOTER ACTIVITY

The trivial visualization of promoters as isolated, short DNA stretches has been vigorously challenged over the last few years by an increasing number of reports which point out the influence of surrounding DNA structure on transcriptional activity. Whether within the chromosome or at plasmids and phages, overall DNA structure (in particular, the degree of superhelicity) modulates and sometimes determines promoter strength. The effects of proteins involved in maintenance of DNA structure (histone-like proteins) and DNA conformation itself are sometimes difficult to distinguish.

Histone-Like Proteins

Prokaryotic histone-like proteins are a miscellaneous group of polypeptides which bind DNA in a quasi-nonspecific fashion and provide the physical support for nucleoprotein structures similar to eukaryotic nucleosomes (see reference 54 for a review). Histone-like proteins include the HU protein found in several microorganisms (214, 236), the H-NS (also called H1) protein of *E. coli* (94, 96), and the transcription factor TF1 of *B. subtilis* phage SPO1 (219). IHF is also considered a histone-like protein, but it has been treated separately in this review (see above).

The HU protein (214) is a heterodimer of two similar but nonidentical subunits of 9.5 kDa in *E. coli* and *Salmonella typhimurium* but is a homodimer of similar size in many other prokaryotic species (225). HU of *E. coli* displays a significant structural homology with IHF, which makes possible, for instance, the construction of chimeric active HU-IHF hybrids (79). Unlike IHF, however, HU binds DNA, apparently without much sequence specificity, increasing the flexibility of the bound DNA and thus facilitating the formation of bent structures (98). Seemingly because of this DNA-bending and/or DNA flexibility-enhancing effect, it is possible to functionally replace IHF by HU during the process of site-specific recombination of phage λ (77). The activity of a number of promoters in vitro is greatly influenced by addition of HU, but the sign of the effect varies in each case. For instance, HU stimulates the binding of *lac* repressor and CAP to their cognate sites at the *lac* promoter, but it inhibits binding of TrpR repressor to its operator (59). In each case, the effect seems to be the result of an HU-mediated increase in DNA flexibility, which facilitates the binding of DNA-bending regulators such as LacI and CAP to target sequences while impairing the binding of other regulatory proteins such as TrpR.

H-NS (or H1) is another histone-like polypeptide (15.5 kDa), which was initially described as a neutral protein in-

volved in the packing of the *E. coli* chromosomal DNA to form nucleoid structures (54, 189, 225, 232). H-NS plays a determinant role in a number of essential cellular functions (16, 266), and therefore H-NS mutants tend to be very pleiotropic and quite unstable. H-NS binds DNA with a quite loose sequence specificity but with a preference for curved structures (266). The effect of H-NS on transcription can be direct (i.e., acting as a transcriptional repressor) or indirect (through the effect of H-NS on the control of overall chromosomal supercoiling) (95, 107). In most instances examined, H-NS behaves as a general silencer of transcription, even rendering long segments of DNA virtually devoid of any gene expression activity (78). In some cases, however, the effect of H-NS seems to be more specific. H-NS selectively represses transcription initiation from the osmoregulated *proV* promoter without affecting a closely linked *Ptac* promoter artificially introduced in the test construction, yet these results should be used with caution because of the multicopy nature of the assay system (246). A curved DNA sequence placed downstream of the *proV* promoter is required for normal regulation of *proV* expression in vivo (178), suggesting that H-NS may repress this promoter through formation of a nucleoprotein complex which occludes RNAP access to target sequences (246). These complexes are expected to be rather dynamic given the poor specificity of the protein; the extent and stability of an H-NS complex in a particular DNA region would determine its repression efficiency (274). In some cases, promoters can be activated by relieving the inhibitory effect caused by H-NS. For instance, the divergent *pap* promoters of the pilus adhesin system of uropathogenic *E. coli* become activated by cAMP-CAP from a site located at ca. 100 and 200 bp away from the promoters of *papI* and *papB* genes, respectively. Forsman et al. (60) showed that the effect of CAP is to relieve directly the repression exerted by H-NS on both promoters.

Besides having an effect on expression of specific genes, H-NS seems to be an ubiquitous and pleiotropic regulatory protein which may act either positively or negatively on totally unrelated promoters owing to its property (as mentioned above) to bind preferentially curved DNA sequences (25, 266), such as those frequently found in regions upstream of *E. coli* promoters (237). Since changes in growth conditions (temperature, osmolarity, nutrients) determine DNA topology (107), H-NS protein may bind to specific conformations characteristic of a certain physiological status, thus activating subsets of promoters and silencing other subsets.

An interesting protein displaying homology to HU and IHF is transcription factor 1 (TF1) from *B. subtilis* phage SPO1. The properties of TF1 are very similar to those of IHF, but it binds preferentially to viral DNA, which has 5-hydroxymethyluracil instead of thymine (112), showing a strong binding preference for certain sites at the SPO1 genome (84). TF1 is essential for SPO1 development, and its binding preferences are thought to determine its ability to selectively inhibit transcription of SPO1 DNA by bacterial RNA polymerases, at least in vitro. TF1 bends DNA sharply. It is thought that multiple protein-DNA contacts allow DNA to wrap around a TF1 dimer, considerably deforming the DNA helix in the process (226).

DNA Supercoiling and Bend-Mediated Activation

In agreement with the important role played by DNA topology in transcription initiation (see above), DNA supercoiling greatly influences the expression of many bacterial genes, enhancing or repressing the activity of some of them while having no apparent influence on many others (195). DNA supercoiling can play a role at every step of the transcription initiation process, i.e., binding of the RNAP to promoter sequences, binding of activator proteins to DNA, and/or enhancing activation efficiency at later steps of the process. For instance, DNA supercoiling seems to facilitate transcription initiation at the *lac* promoter of *E. coli* in a fashion independent of CAP (156). In the *malEp* and *malKp* promoters of *E. coli*, whose activation requires the formation of a complex nucleoprotein structure involving CAP and MalT activators, DNA supercoiling is required to form the activating complex, most probably to facilitate adequate interactions between the different proteins involved (204). Not surprisingly, the supercoiling effect is maximal at superhelical densities similar to those thought to exist in vivo, decreasing at higher or lower superhelical densities (22, 261). Mechanistically, DNA supercoiling could affect promoter efficiency in at least two ways: (i) modifying the structure of the promoter to a configuration that can be optimally recognized by RNAP; or (ii) reducing the energy needed to melt the DNA at the transcription initiation region, thus favoring the transition from closed to open complexes (22, 26, 139, 172, 207). Concomitantly, the topology of supercoiled DNA can modulate the influence of DNA bending on promoter activity.

The structure of supercoiled DNA can be visualized either as a toroidal ring or as a rod of two interwound duplex chains, the interwound form being the more likely form both in vivo and in vitro (6, 19). This configuration predicts the appearance of curved apices in which the DNA folds back upon itself. The energy required to form and maintain the supercoiled structure is decreased when such apices become coincident with intrinsically or protein-induced bent DNA sequences (137). Therefore, it is believed that bent DNA spontaneously tends to be localized in the apices. Consistent with the ability of the RNAP to bend or increase the flexibility of the DNA at promoters (92, 129), direct visualization of *E. coli* RNAP-promoter interactions on a supercoiled plasmid (88) confirmed that the complexes had a marked preference to be located at the extremes of the superhelical loops. Furthermore, the apical localization of a transcribing RNAP on supercoiled DNA would avoid its rotation around the template, thus preventing the transcript from becoming entangled with the template DNA (88).

The tendency of bent DNA to nucleate the formation of apical loops suggests an interesting correlation among DNA bending, supercoiling, and promoter efficiency, because the effect on transcription initiation of an intrinsic or protein-induced DNA curvature may be enhanced if it becomes stabilized as a consequence of supercoiling. There are several examples supporting this notion. For example, a point mutation in the *ada* promoter of *E. coli* increasing bending (or flexibility) of the sequence at a position around −60 had a significant stimulatory effect on the binding of RNAP to supercoiled DNA but not on further steps of the initiation process (17). Since the stimulatory effect required DNA supercoiling, it was proposed that DNA bending in conjunction with supercoiling facilitated the wrapping of DNA around RNAP, thus stabilizing its binding to the promoter. Along the same lines, Zinkel and Crothers (272) suggested that the CAP-induced DNA bend at the *lac* promoter tends to become localized at an apical loops of supercoiled DNA, thus helping the DNA to wrap around the promoter-bound RNAP. Such an arrangement could direct the energy available from DNA supercoiling into the process of DNA unwinding. Zinkel and Crothers (272) also suggested that the energy associated with the bend is instrumental in springing RNAP out of the

promoter (promoter clearance) by pulling both DNA and CAP away from RNAP.

The importance of DNA supercoiling on bend-mediated activation is further stressed by the fact that some intrinsically curved sequences placed upstream from a promoter can enhance transcription on supercoiled but not linear DNAs (272). The two effects (DNA curvature and supercoiling) may not necessarily go together since, as discussed above, DNA bending alone can sometimes modulate promoter activity on linear templates as well (138, 152). In these cases, the effect of DNA bending was also attributed to an extended wrapping of the DNA around RNAP, thus facilitating the initiation process. It is likely that this effect would be stronger in supercoiled templates, although the exact orientation of the DNA bend is probably critical for activation, and DNA supercoiling could affect this orientation.

GENE REGULATION AIDED BY BENT DNA

In previous sections we have examined the direct participation of DNA curvatures, whether intrinsic or caused by bound proteins, in transcription regulation. There are, however, many cases in which DNA structure plays an indirect yet essential role in the process, typically by bringing about protein-protein interactions between regulatory proteins and/or between these and the transcription machinery. Although looping of distant protein-binding DNA sequences can be included within this notion (2, 150, 224), the most significant role of DNA bends in coregulation occurs when DNA curvature facilitates contacts between two or more proteins bound to nonadjacent but relatively proximal sites which would be otherwise energetically impeded by the rigidity of the intervening DNA sequence (253). In these cases, intrinsic or protein-induced DNA bends act as architectural elements in the assembly of transcriptionally active complexes, and the corresponding sites of curvature themselves frequently become targets for additional levels of regulation.

DNA Structures as Corepressors

There are a few examples where the role of bending as corepressor has been examined in detail. One is the regulation of the *put* operon of *S. typhimurium*. Utilization of proline as the sole carbon and nitrogen source requires the expression of the two genes of the *put* operon, which are transcribed from a common regulatory region; these genes are *putA*, which encodes a repressor protein with an additional dehydrogenase activity which converts proline to glutamate, and *putP*, which encodes proline permease (148). The complex regulatory region contains the divergent *putA* and *putP* promoters, operator sites for PutA, IHF-binding sites, and a region of intrinsically curved DNA (176). PutA protein represses transcription of the *put* genes at low intracellular proline concentrations by binding to two sites within the regulatory region which are separated by an intervening DNA region containing a statically bent DNA sequence (177). Mutant promoters deleted in the tract of curved DNA are unable to fully repress expression of *put* genes. Furthermore, when both PutA-binding sites are present on the same DNA fragment, PutA protein binds DNA with higher affinity (175). This suggests that repression of the *put* genes may occur by DNA looping between two operator sites, facilitated by the curved DNA located between them. In addition, although IHF does not have a repression effect by itself, it facilitates PutA-mediated repression by binding two cognate sequences adjacent to the PutA sites (170). There are therefore two elements of corepression mediated by DNA bending in the *put* system, one resulting from a static curvature between the two PutA operators and the other resulting from the IHF-induced bends on flanking sites. The combination of various proteins and DNA structures within the *put* regulatory region is likely to result in the formation of a higher-order repression complex. At first sight, it may appear surprising that a simple requirement for repression requires such a complex molecular setup. Perhaps the explanation lies in the bifunctionality of PutA, which may act as a DNA-binding repressor or as a membrane-bound dehydrogenase (175). It is possible that the two activities conflict, since in the presence of proline, the PutA protein is targeted to the inner membrane to interact with components of the electron transport chain, whereas in the absence of proline, the DNA-binding activity can be stabilized within a nucleoprotein complex. The apparent competition between the two potential physical targets of PutA (i.e., DNA or membrane) is pointed out by experiments in which overproduction of some flavin adenine dinucleotide dehydrogenases which compete with PutA for the same sites in the membrane causes a reduction in *put* operon expression (148). This may not be the whole story, however. Since DNA can be anchored to the inner membrane through cotranscriptional synthesis of membrane proteins (146), the ability of PutA to simultaneously bind the membrane and the DNA cannot be ruled out.

An interesting example of CAP as a coregulator participating in a repression complex appears in the regulation of the divergent *nagE-nagBACD* operons of *E. coli*, involved in the uptake and metabolism of *N*-acetylglucosamine. Expression of *nag* genes is induced by growth on *N*-acetylglucosamine or glucosamine. In the absence of inducers, the repressor NagC binds to cognate sites overlapping the *nagE* and *nagB* promoter sequences, which are themselves separated by 130 bp (193). Since the intervening DNA sequence is intrinsically curved, it is believed that NagC binds cooperatively to the two sites, enhancing the static curvature (192) and looping out the intergenic sequence between the divergent promoters (192). In addition, a strong CAP-binding site is placed asymmetrically between the two NagC operators. When CAP and NagC bind simultaneously to the respective target sites, a CAP-NagC-DNA complex, which is far more stable than the equivalent binary NagC-DNA complex, is formed. Comparison of the cylindrical projections of the CAP and NagC sites on the surface of a DNA helix with DNase I nicking patterns of the ternary complexes indicates that DNA actually wraps around a NagC-CAP protein core (192). Although it is difficult in this case to separate the different contributions of each element to the formation of the complex, it is believed that CAP binding and subsequent DNA bending stabilize the loop formed between the two NagC-binding sites, thus improving the repression of the *nag* genes.

Nucleoprotein repression complexes which include positive regulators in their molecular architecture are not at all infrequent in bacterial promoters. CAP and LacI seem to bind cooperatively to the *lac* promoter (106), and GalR proteins bound to distant operators within the *gal* promoter may be brought together by formation of a loop directed by CAP and RNAP binding to the intervening sequence (2, 44). The simultaneous and cooperative binding of repressor and activator proteins to neighboring and even overlapping control regions may actually facilitate rapid and tight responses of certain promoters to specific stimuli once the repression complex is weakened by inducer addition or changes in the physiological status of the cells.

A similar but more complex case is that of the *E. coli* CytR repressor, which regulates transcription initiation from several

promoters, including its own promoter, *cytRp*. This promoter is activated by CAP, whose effect is counteracted by CytR (70). CAP binds to a region located around position −64, whereas CytR binds immediately downstream. Both proteins can bind simultaneously and cooperatively, a process that implies direct interactions between the two proteins (180). Interestingly, both proteins bend DNA at their target sites but probably in different directions: the overall bend in the combined complex deviates from that induced by either protein alone. It is likely that the repression efficiency of CytR lies, at least in part, in its ability to counteract the CAP-induced bend.

An example of nucleoprotein repression complexes evolved to trigger sharp transcriptional responses is found in the regulation of the *Pe* and *Pc* promoters of phage Mu, the balance between their activities controlling lysis-lysogeny decisions during the developmental cycle of the phage. *Pe* is an early promoter which drives expression of genes required for lytic growth, whereas the *Pc* promoter transcribes the gene *c1* for the Mu repressor. Normally, this repressor prevents transcription from both *Pe* and *Pc* by binding to cognate sites which overlap, in each case, the respective promoter sequences, thus fixing the phage in a lysogenic state. Although the two promoters (along with their cognate operator sequences for the repressor, O_1 and O_2) are separated within the Mu genome, the repressor must bind both of them simultaneously to shut down transcription from *Pe* (127). Indeed, binding of the repressor to O_1 and O_2 sites is very cooperative and seems to occur simultaneously rather than sequentially. Both in vivo and in vitro data indicate that the repressor has a specific protein domain essential for the physical approach between the two promoters and the subsequent shutdown of *Pe* activity (65). Logically, bringing two distant elements into close proximity must be associated with changes in the conformation of the intervening DNA sequence. The region between *Pe* and *Pc* has a minor intrinsic bend, which is further exacerbated upon binding of IHF to a cognate binding site also present in the sequence. In turn, IHF binding results in a tighter binding of the repressor protein to O_1 and O_2 and therefore in a further decrease of promoter activity (7, 65). This effect is probably due to the stabilization by IHF of a loop structure which engages the occupied O_1 and O_2 sites, allowing protein-protein interaction between repressor molecules. Not surprisingly, in the absence of Mu repressor, IHF stimulates transcription from *Pe* and inhibits transcription from *Pc* (97, 248, 249), thus favoring lytic growth. Therefore, depending on the status of the repressor, IHF-borne bends may stimulate either lysis by activating *Pe* or lysogeny through stabilization of repressor-operator complexes fixed within a nucleoprotein structure (7). At least for Mu, an IHF-induced bend seems to behave as an amplifier of developmental decisions, the ultimate sign of which is determined by other factors.

DNA Looping as a Coactivation Mechanism

Similarly to corepression, curved DNA may play an indirect role in transcriptional activation when it helps to bring about contacts between proteins bound to otherwise distant sites. Not infrequently, activators bind to regions relatively distant from those bound by RNAP, thus requiring DNA to loop out to make productive contacts with the enzyme. Activators such as CAP or OmpR are expected to produce small loops when placed at certain DNA distances from the RNAP-binding site (40). These loops formed by short DNA segments may actually coregulate the system depending on whether the sequence is bent or prone to bend in the appropriate direction. This concept has been tested in an artificial system based on the p4 protein of phage Φ29 (see above), in which this activator could, at a certain distance, stimulate transcription from its cognate promoter only when the intervening sequence was engineered to contain a static DNA bend (229).

σ^{54}-Dependent Promoters

Promoters depending on the alternative σ^{54} factor are generally involved in expression of functions for adaptation to harsh metabolic and environmental situations (133). They are unique in that the sequence recognized by the RNAP-σ^{54} holoenzyme includes GG and GC doublets at positions −24 and −12, respectively (instead of the typical −35 and −10 sequences of the σ^{70} promoters) and that they are activated at a distance (up to 2 kb [201]) by specific regulators (166) bound to upstream enhancer-like sequences. These unusual properties (reviewed in references 40 and 132) are explained by the eukaryotic-like structure of the σ^{54} factor itself (218, 264) and that of the cognate regulatory proteins (132, 166, 259). In most (but not in all) cases, RNAP-σ^{54} holoenzyme spontaneously forms a closed complex with its target sequences, but the bound enzyme cannot isomerize into an open complex in the absence of protein-protein contacts between the RNAP and the corresponding activator (194).

In a subset of σ^{54}-dependent promoters, an IHF site is found between the binding sites of the RNAP-σ^{54} holoenzyme and the upstream enhancer-like sequences (1, 30, 38, 49, 75, 99). This IHF site is normally required for activity in vivo (49, 75) and in vitro on linear but not supercoiled templates (39). The major (but perhaps not the sole) role of IHF as coactivator in the σ^{54} promoters is believed to be that of assisting the formation of a DNA loop or even a nucleoprotein complex to stabilize contacts between the RNAP and the activator protein bound to the upstream enhancer-like sequences (49). The role of the IHF-induced bend as coactivator is pointed out by the functional substitution of curved DNA sequences for the IHF sites within the σ^{54}-dependent *Pu* promoter of *Pseudomonas putida* (188) and the *PnifH* promoter of *Klebsiella pneumoniae* (159). The structural effect of IHF (or an equivalent statically curved DNA sequence) is more dramatic in situations in which the closed complex is short lived; i.e., when the affinity of the RNAP for the promoter is not very strong, so that chances for RNAP-activator contacts are increased. In fact, promoter mutants able to make closed complex with RNAP are IHF independent (39, 99). IHF-induced bends in combination with promoters which make weak closed complexes with RNAP ensure high fidelity and high efficiency of activation by the physiologically appropriate positive regulator, i.e., the one with a binding site properly positioned with respect to the bend (217). In fact, an IHF-induced bend can inhibit the function of an activator bound to a site that is not correctly placed with respect to the bend (39).

The presence or absence of IHF sites in σ^{54}-dependent promoters, sometimes responsive to the same cognate regulator, is still quite intriguing and may play a different role from providing an structural aid for promoter architecture. We have observed, for instance, that in the absence of IHF, the *Pu* promoter of *P. putida* is more susceptible to cross-activation in vivo by heterologous regulators than in the presence of the histone-like protein. Furthermore, such relaxation of specificity is totally suppressed when the IHF-binding site present in the promoter is substituted by a DNA sequence endowing an equivalent static bend (188). The curved DNA element seems therefore to restrict the induction range of the promoter in vivo to respond exclusively to its cognate regulator and avoid activation by other signals.

E. coli Maltose Operon

The molecular architecture of nucleoprotein complexes for transcription initiation has one of its most complex instances in the regulation of the divergent *malEp* and *malKp* promoters of the maltose operon of *E. coli*. The activity of both promoters depends on MalT, the indigenous regulator of the system, and on CAP (228); the intergenic region between the two promoters (217 bp long) is a nearly continuous stretch of operators for both proteins (197) in which two series of MalT sites appear separated by various CAP sites. An early model to explain the combined activity of MalT and CAP proposed that the role of CAP was to provide an adequate degree of bending of the intervening DNA sequences around MalT boxes to assist MalT monomers bound to cognate sites to form a multimer around which the intervening sequence is wrapped in a nucleosome-like fashion (197). The current view is, however, far more complicated. Richet et al. (206) have shown that the promoter-proximal region of *malKp* contains two overlapping sets of three MalT-binding sites, each separated by 3 bp from the other, i.e., placed at different sides of the DNA helix. Occupation of the distant, higher-affinity sites by MalT (which occurs in the absence of CAP) is insufficient to activate *malKp*. Binding of MalT to the lower-affinity sites, proximal to the −35 box of *malKp*, occurs only in the presence of CAP and is essential for transcription activation. The effect of CAP is believed to position the two sets of MalT proteins bound to distal and proximal sites into an arrangement conducive to transcription initiation through generation of a nucleoprotein complex which engages the whole regulatory region. The role of CAP in this case might be similar to that suggested for IHF in σ^{54}-dependent promoters; i.e., CAP-borne DNA bends stabilize interactions of MalT with the lower-affinity sites by bringing about protein-protein contacts which help to fix MalT to otherwise unoccupied sites (206). Alternatively (or simultaneously), CAP binding may alter the conformation of the neighboring DNA sequences of the low-affinity MalT sites into a higher-affinity form, perhaps with a concomitant decrease of MalT binding to the distant sites. In any case, the role of the CAP sites as mostly structural elements is further substantiated by the possibility of exchanging CAP sites for equivalent IHF sites without loss of *malKp* activity (205).

Activation of the P_{araBAD} Promoter by CAP/AraC

The regulation of the P_{araBAD} promoter of the arabinose system of *E. coli* is one of the best-known paradigms of prokaryotic gene expression (223). P_{araBAD} is both positively and negatively regulated by the same protein, AraC, which, in the absence of arabinose, mediates the formation of a repressor loop by binding two half-operator sites separated by 210 bp, namely *araO2* and *araI1*. Alternatively, in the presence of arabinose, AraC binds to a complete site (*araI1*/*araI2*) next to the −35 box of P_{araBAD} (89). The repression loop plays an anti-induction role, thus preventing AraC protein from binding to the activator site *araI1*/*araI2*. The role of DNA looping in transcription of P_{araBAD} and in other systems has been reviewed elsewhere (224) and will not be addressed here. Besides the AraC-binding sites, the regulatory region includes a CAP site just upstream of the *araI1* half-operator involved in the loop. The CAP site is centered along the face of the DNA helix opposite that of *araI1*, suggesting that CAP prevents formation of the repression loop by misorienting the intervening sequence (143, 144) or by distorting the neighboring *araI1*/*araI2* sites into a conformation with higher affinity for an AraC dimer. In any case, the effect of CAP seems to discourage formation of the repression loop, thus stimulating P_{araBAD} activity. This possibility (DNA bending which hampers DNA looping) might be a sophisticated and unexpected device for gene regulation.

CONCLUSIONS

Curved DNA is an integral element of promoter architecture, and DNA bending appears to be a major component of the control of bacterial gene expression. The examples discussed in this article illustrate two strategies where the DNA geometry has evolved for fine-tuning transcriptional responses to different physiological situations. In one case, DNA bending may change (or even determine) the affinity of a regulatory protein (whether activator or repressor) to cognate sites in the neighborhood of the RNAP site. In these cases, changes in DNA structure caused by or associated with the bend (DNA allostery [3]) allow a bound regulatory protein(s) to transmit and amplify its signaling into the RNAP. The second regulatory feature that DNA bending affords is the superimposition of different regulatory circuits in the activation of a single promoter. The relatively simple organization of prokaryotic promoters would not permit a single promoter to respond to various signals unless a coregulation element such as DNA bending is present in the system. As shown extensively with CAP, IHF, FIS, and H-NS, the sites eligible for DNA bending frequently become targets for additional regulatory devices. The picture which emerges from all the data is that nonlinear DNA structures not only provide physical support for the transcription machinery but also afford additional regulation levels which connect the activity of individual promoters to the overall physiological status of the cells (196). This notion applies not only to the prokaryotic world but also to eukaryotic systems, in which DNA bending induced by transcription factors such as c-*jun* or c-*fos*, is likely to play an essential role in the assembly of the transcriptional apparatus (117, 118). One aspect of DNA structure which has not been separately addressed in this article is that of DNA bendability as something different from static or protein-induced DNA bending. Although this concept may be complex to define in physical terms, is it believed to play a major role in all cell functions which rely on DNA structure. The role of histone-like proteins which may modify the general flexibility or the proneness of a certain DNA sequence to bend or not in a particular direction remains mostly undefined and will probably become a major area of research in the future.

ACKNOWLEDGMENTS

We are indebted to A. Oppenheim, O. Raibaud, E. Santero, H. Buc, S. Adhya, R. Gourse, and P. Otrovsky de Spicer for comments and for sharing unpublished results, as well as to D. Crothers and M. Espinosa for their useful criticism. The comments and the help of J. Sokatch with the English style are also gratefully acknowledged.

The work at the Laboratory of V. de L. at the Centro de Investigaciones Biológicas in Madrid was funded by CICYT grant BIO92-1018-CO201. The work of J.P.M. was supported by EC BRIDGE contract BIOT-CT91-0293. F.R. thanks the Fundación Ramón Areces for its Institutional Grant to the Centro de Biología Molecular as well as M. Salas for her continuous support.

REFERENCES

1. **Abril, M. A., M. Buck, and J. L. Ramos.** 1991. Activation of the *Pseudomonas* TOL plasmid upper pathway operon. Identification of binding sites for the positive regulator XylR and for integration host factor protein. J. Biol. Chem. **266**:15832–15838.
2. **Adhya, S.** 1989. Multipartite genetic control elements: communication by DNA loop. Annu. Rev. Genet. **23**:227–250.

3. **Adhya, S., and S. Garges.** 1990. Positive control. J. Biol. Chem. **265:**10797–10800.
4. **Adhya, S., M. Gottesman, S. Garges, and A. Oppenheim.** 1993. Promoter resurrection by activators—a minireview. Gene **132:** 1–6.
5. **Adhya, S., S. Ryu, and S. Garges.** 1992. Positive control of transcription initiation by cAMP-CRP in *lac* promoter of *E. coli*, p. 48. *In* M. Salas and L. Rothman-Denes (ed.), Workshop on transcription initiation in prokaryotes. Instituto Juan March, Madrid.
6. **Adrian, M., B. ten Heggeler-Bordier, W. Wahli, A. Z. Stasiak, A. Stasiak, and J. Dubochet.** 1990. Direct visualization of supercoiled DNA molecules in solution. EMBO J. **9:**4551–4554.
7. **Alazard, R., M. Bétermier, and M. Chandler.** 1992. *Escherichia coli* integration host factor stabilizes bacteriophage Mu repressor interactions with operator DNA *in vitro*. Mol. Microbiol. **6:**1707–1714.
8. **Amouyal, M., and H. Buc.** 1987. Topological unwinding of strong and weak promoters by RNA polymerase. A comparison between the *lac* wild-type and the UV5 sites of *Escherichia coli*. J. Mol. Biol. **195:**795–808.
9. **Amouyal, M., L. Mortensen, H. Buc, and K. Hammer.** 1989. Single and double loop formation when *deoR* repressor binds to its natural operator sites. Cell **58:**545–551.
10. **Ansari, A. Z., M. L. Chael, and T. V. O'Halloran.** 1992. Allosteric underwinding of DNA is a critical step in positive control of transcription by Hg-MerR. Nature (London) **355:**87–89.
11. **Ayers, D. G., D. T. Auble, and P. L. deHaseth.** 1989. Promoter recognition by *Escherichia coli* RNA polymerase. Role of the spacer DNA in functional complex formation. J. Mol. Biol. **207:**749–756.
12. **Banner, C. D. B., C. P. Moran, Jr., and R. Losick.** 1983. Deletion analysis of a complex promoter for a developmentally regulated gene from *Bacillus subtilis*. J. Mol. Biol. **168:**351–365.
13. **Barthelemy, I., and M. Salas.** 1989. Characterization of a new prokaryotic transcriptional activator and its DNA recognition site. J. Mol. Biol. **208:**225–232.
14. **Bauer, F. B., E. G. Kar, R. M. Elford, and W. M. Holmes.** 1988. Sequence determinants for promoter strength in the *leuV* operon of *Escherichia coli*. Gene **63:**123–134.
15. **Bell, A., K. Gaston, R. Williams, C. Chapman, A. Kolb, H. Buc, S. Minchin, J. Williams, and S. Busby.** 1990. Mutations that alter the ability of the *Escherichia coli* cyclic AMP receptor protein to activate transcription. Nucleic Acids Res. **18:**7243–7250.
16. **Bertin, P., P. Lejeune, C. Laurent-Winter, and A. Danchin.** 1990. Mutations in *bglY*, the structural gene for the DNA-binding protein H1, affect expression of several *E. coli* genes. Biochimie **72:**889–891.
17. **Bertrand-Burggraf, E., J. Dunand, R. P. P. Fuchs, and J. F. Lefèvre.** 1990. Kinetic studies of the modulation of *ada* promoter activity by upstream elements. EMBO J. **9:**2265–2271.
18. **Berzal-Herranz, A., E. G. H. Wagner, and R. Diaz-Orejas.** 1991. Control of replication of plasmid R1: the intergenic region between *copA* and *repA* modulates the level of expression of *repA*. Mol. Microbiol. **5:**97–108.
19. **Bliska, J. B., and N. R. Cozzarelli.** 1987. Use of site specific recombination as a probe of DNA structure and metabolism *in vivo*. J. Mol. Biol. **194:**205–218.
20. **Bolshoy, A., P. McNamara, R. E. Harrington, and E. N. Trifonov.** 1991. Curved DNA without A-A: experimental estimation of all 16 DNA wedge angles. Proc. Natl. Acad. Sci. USA **88:**2312–2316.
21. **Borowiec, J. A., and J. D. Gralla.** 1986. High-resolution analysis of Lac transcription complexes inside cells. Biochemistry **25:** 5051–5057.
22. **Borowiec, J. A., and J. D. Gralla.** 1987. All three elements of the *lac* p^s promoter mediate its transcriptional response to DNA supercoiling. J. Mol. Biol. **195:**89–97.
23. **Bossi, L., and D. M. Smith.** 1984. Conformational change in the DNA associated with an unusual promoter mutation in a tRNA operon of *Salmonella*. Cell **39:**643–652.
24. **Botsford, J. L., and J. G. Harman.** 1992. Cyclic AMP in prokaryotes. Microbiol. Rev. **56:**100–122.
25. **Bracco, L., D. Kotlarz, A. Kolb, S. Diekmann, and H. Buc.** 1989. Synthetic curved DNA sequences can act as transcriptional activators in *Escherichia coli*. EMBO J. **8:**4289–4296.
26. **Brahms, J. G., O. Dargouge, S. Brahms, Y. Ohara, and V. Vagner.** 1985. Activation and inhibition of transcription by supercoiling. J. Mol. Biol. **181:**455–465.
27. **Buc, H.** 1986. Mechanism of activation of transcription by the complex formed between cyclic AMP and its receptor in *Escherichia coli*. Biochem. Soc. Trans. **14:**196–199.
28. **Bujard, J., M. Brunner, U. Deuschle, W. Kammerer, and R. Knaus.** 1987. Structure-function relationship of *Escherichia coli* promoters, p. 95–104. *In* W. S. Reznikoff, et al. (ed.), RNA polymerase and the regulation of transcription. Elsevier Science Publishing, Inc., New York.
29. **Busby, S., A. Spassky, and B. Chan.** 1987. RNA polymerase makes important contacts upstream from base pair −49 at the *Escherichia coli* galactose operon P1 promoter. Gene **53:**145–152.
30. **Cannon, W., W. Charlton, and M. Buck.** 1991. Organization and function of binding sites for the transcriptional activator NifA in the *Klebsiella pneumoniae nifE* and *nifU* promoters. J. Mol. Biol. **220:**915–931.
31. **Carpousis, A., J. E. Stefano, and J. D. Gralla.** 1982. 5′ nucleotide heterogeneity and altered initiation of transcription at mutant *lac* promoters. J. Mol. Biol. **157:**619–633.
32. **Chamberlin, M. E.** 1974. The selectivity of transcription. Annu. Rev. Biochem. **43:**721–775.
33. **Chan, S. S., K. J. Breslauer, M. E. Hogan, D. J. Kessler, R. H. Austin, J. Ojemann, J. M. Passner, and N. C. Wiles.** 1990. Physical studies of DNA premelting equilibria in duplexes with and without homo dA · dT tracts: correlations with bending. Biochemistry **29:**6161–6171.
34. **Chang, M., and I. P. Crawford.** 1991. In vitro determination of the effect of indoleglycerol phosphate on the interaction of purified TrpI protein with its DNA-binding site. J. Bacteriol. **173:**1590–1597.
35. **Charlier, D., M. Roovers, F. Van Vliet, A. Boyen, R. Cunin, Y. Nakamura, N. Glansdorff, and A. Piérard.** 1992. Arginine regulon of *Escherichia coli* K12. A study of repressor-operator interactions and of *in vitro* binding affinities versus *in vivo* repression. J. Mol. Biol. **226:**367–386.
36. **Chenchik, A., R. Beabealashvili, and A. Mirzabekov.** 1981. Topography of interaction of *E. coli* RNAP subunits with *lacUV5* promoter. FEBS Lett. **128:**46–50.
37. **Choy, H. E., and S. Adhya.** 1992. Control of *gal* transcription through DNA looping: inhibition of the initial transcribing complex. Proc. Natl. Acad. Sci. USA **89:**11264–11268.
38. **Claverie-Martin, F., and B. Magasanik.** 1991. Role of integration host factor in the regulation of the *glnHp2* promoter of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **88:**1631–1635.
39. **Claverie-Martin, F., and B. Magasanik.** 1992. Positive and negative effects of DNA bending on activation of transcription from a distant site. J. Mol. Biol. **227:**996–1008.
40. **Collado-Vides, J., B. Magasanik, and J. D. Gralla.** 1991. Control site location and transcriptional regulation in *Escherichia coli*. Microbiol. Rev. **55:**371–394.
41. **Collis, C. M., P. L. Molloy, G. W. Both, and H. Drew.** 1989. Influence of the sequence dependent flexure of DNA on transcription in *E. coli*. Nucleic Acids Res. **17:**9447–9468.
42. **Crothers, D. M., T. E. Haran, and J. G. Nadeau.** 1990. Intrinsically bent DNA. J. Biol. Chem. **265:**7093–7096.
43. **Cunin, R., N. Glansdorff, A. Pierard, and V. Stalon.** 1986. Biosynthesis and metabolism of arginine in bacteria. Microbiol. Rev. **50:**314–352.
44. **Dalma-Weiszhausz, D. D., and M. Brenowitz.** 1992. Interactions between DNA-bound transcriptional regulators of the *Escherichia coli gal* operon. Biochemistry **31:**6980–6989.
45. **D'Ari, R., R. T. Lin, and E. B. Newman.** 1993. The leucine responsive regulatory protein: more than a regulator? Trends Biochem. Sci. **18:**260–263.
46. **Darst, S. A., E. W. Kubalek, and R. D. Kornberg.** 1989. Three-dimensional structure of *Escherichia coli* RNA polymerase holoenzyme determined by electron crystallography. Nature (London) **340:**730–732.
47. **de Crombrugghe, B., S. Busby, and H. Buc.** 1984. Cyclic AMP

receptor protein: role in transcription activation. Science **224:** 831–838.
48. **de Lorenzo, V., M. Herrero, F. Giovannini, and J. B. Neilands.** 1988. Fur (ferric uptake regulation) protein and CAP (catabolite activator protein) modulate transcription of *fur* gene in *Escherichia coli*. Eur. J. Biochem. **173:**537–546.
49. **de Lorenzo, V., M. Herrero, M. Metzke, and K. N. Timmis.** 1991. An upstream XylR- and IHF-induced nucleoprotein complex regulates the σ^{54}-dependent Pu promoter of TOL plasmid. EMBO J. **10:**1159–1167.
50. **del Solar, G., J. Pérez-Martín, and M. Espinosa.** 1990. Plasmid pLS1-encoded RepA protein regulates transcription from *repAB* promoter by binding to a DNA sequence containing a 13-base pair symmetric element. J. Biol. Chem. **265:**12569–12575.
51. **Deuschle, U., W. Kammerer, R. Gentz, and H. Bujard.** 1986. Promoters of *Escherichia coli*: a hierarchy of *in vivo* strength indicates alternate structures. EMBO J. **5:**2987–2994.
52. **Diekmann, S.** 1987. Temperature and salt dependence of the gel migration anomaly of curved DNA fragments. Nucleic Acids Res. **15:**247–265.
53. **Drew, H., and A. A. Travers.** 1985. DNA bending and its relation with nucleosome positioning. J. Mol. Biol. **186:**773–790.
54. **Drlica, K., and J. Rouviere-Yaniv.** 1987. Histone-like proteins of bacteria. Microbiol. Rev. **51:**301–319.
55. **Duval-Valentin, G., and C. Reiss.** 1990. How *Escherichia coli* RNA polymerase can negatively regulate transcription from a constitutive promoter. Mol. Microbiol. **4:**1465–1475.
56. **Eschenlauer, A. C., and W. S. Reznikoff.** 1991. *Escherichia coli* catabolite gene activator protein mutants defective in positive control of the *lac* operon transcription. J. Bacteriol. **173:**5024–5029.
57. **Espinosa-Urgel, M., and A. Tormo.** 1993. σ^{s}-dependent promoters in *Escherichia coli* are located in DNA regions with intrinsic curvature. Nucleic Acids Res. **16:**3667–3670.
58. **Finkel, S. E., and R. C. Johnson.** 1992. The Fis protein: it's not just for DNA inversion anymore. Mol. Microbiol. **6:**3257–3265.
59. **Flashner, Y., and J. D. Gralla.** 1988. DNA dynamic flexibility and protein recognition: differential stimulation by bacterial histone-like HU. Cell **54:**713–721.
60. **Forsman, K., B. Sonden, M. Goransson, and B. E. Uhlin.** 1992. Antirepression function in *Escherichia coli* for the cAMP-cAMP receptor protein transcriptional activator. Proc. Natl. Acad. Sci. USA **89:**9880–9884.
61. **Friedman, D. I.** 1988. Integration host factor: a protein for all reasons. Cell **55:**545–554.
62. **Gaal, T., J. Berkel, R. R. Dickson, H. A. deBoer, P. L. deHaseth, H. Alavi, and R. L. Gourse.** 1989. Saturation mutagenesis of an *E. coli* rRNA promoter and initial characterization of promoter variants. J. Bacteriol. **171:**4852–4861.
63. **Gaal, T., and R. L. Gourse.** Unpublished data.
64. **Galas, D. J., M. Eggert, and M. S. Waterman.** 1985. Rigorous pattern-recognition methods for DNA sequences. J. Mol. Biol. **186:**117–128.
65. **Gama, M. J., A. Toussaint, and N. P. Higgins.** 1992. Stabilization of bacteriophage Mu repressor-operator complexes by the *Escherichia coli* integration host factor protein. Mol. Microbiol. **6:**1715–1722.
66. **Gamper, H. B., and J. E. Hearst.** 1982. A topological model for transcription based on unwinding angle analysis of *E. coli* RNA polymerase binary, initiation and ternary complexes. Cell **29:**81–90.
67. **Gartenberg, M. R., and D. M. Crothers.** 1988. DNA sequence determinants of CAP induced bending and protein binding affinity. Nature (London) **333:**824–829.
68. **Gartenberg, M. R., and D. M. Crothers.** 1991. Synthetic DNA bending sequences increase the rate of *in vitro* transcription initiation at the *Escherichia coli lac* promoter. J. Mol. Biol. **219:**217–230.
69. **Gaston, K., A. Bell, A. Kolb, H. Buc, and S. Busby.** 1990. Stringent spacing requirements for transcription activation by CRP. Cell **62:**733–743.
70. **Gerlach, P., P. Valentin-Hansen, and E. Bremer.** 1990. Transcriptional regulation of the *cytR* repressor gene of *Escherichia coli*: autoregulation and positive control by the cAMP/CAP complex. Mol. Microbiol. **4:**479–488.
71. **Giladi, H., M. Gottesman, and A. B. Oppenheim.** 1990. Integration host factor stimulates the phage lambda *pL* promoter. J. Mol. Biol. **213:**109–121.
72. **Giladi, H., K. Igarashi, A. Ishihama, and A. B. Oppenheim.** 1992. Stimulation of the phage λ pL promoter by integration host factor requires the carboxy terminus of the α-subunit of RNA polymerase. J. Mol. Biol. **227:**985–990.
73. **Giladi, H., S. Koby, M. E. Gotesman, and A. Oppenheim.** 1992. Supercoiling, integration host factor, and a dual promoter system, participate in the control of bacteriophage lambda *pL* promoter. J. Mol. Biol. **224:**937–948.
74. **Gille, H., J. B. Egan, A. Roth, and W. Messer.** 1991. The FIS protein binds and bends the origin of chromosomal DNA replication, *oriC*, of *Escherichia coli*. Nucleic Acids Res. **19:**4167–4172.
75. **Gober, J. W., and L. Shapiro.** 1990. Integration host factor is required for the activation of developmentally regulated genes in *Caulobacter*. Genes Dev. **4:**1494–1504.
76. **Goodman, S., and H. A. Nash.** 1989. Functional replacement of protein-induced bend in a DNA recombination site. Nature (London) **341:**251–254.
77. **Goodman, S. D., S. C. Nicholson, and H. A. Nash.** 1992. Deformation of DNA during site specific recombination of bacteriophage lambda: replacement of IHF by HU protein or sequence directed bends. Proc. Natl. Acad. Sci. USA **89:**11910–11914.
78. **Goransson, M., B. Sonden, P. Nilson, B. Dagberg, K. Forsman, K. Emanuelsson, and B. E. Uhlin.** 1990. Transcriptional silencing and thermoregulation of gene expression in *Escherichia coli*. Nature (London) **344:**682–685.
79. **Goshima, N., Y. Inagaki, H. Otaki, H. Tanaka, N. Hayashi, F. Imamoto, and Y. Kano.** 1992. Chimeric HU-IHF proteins that alter DNA binding ability. Gene **118:**97–102.
80. **Gosink, K. K., W. Ross, S. Leirmo, R. Osuna, S. E. Finkel, R. C. Johnson, and R. Gourse.** 1993. DNA binding and bending are necessary but not sufficient for Fis-dependent activation of *rrnB P1*. J. Bacteriol. **175:**1580–1589.
81. **Gourse, R., H. A. de Boer, and M. Nomura.** 1986. DNA determinants of rRNA synthesis in *E. coli*: growth rate dependent regulation, feedback inhibition, upstream activation, antitermination. Cell **44:**197–205.
82. **Gralla, J. D.** 1990. Promoter recognition and mRNA initiation by *Escherichia coli* $E\sigma^{70}$. Methods Enzymol. **185:**37–54.
83. **Graves, M. C., and J. C. Rabinowitz.** 1986. In vivo and in vitro transcription of the *Clostridium pasteurianum* ferredoxine gene. J. Biol. Chem. **261:**11409–11415.
84. **Greene, J. R., and E. P. Geiduscheck.** 1985. Site-specific binding by a bacteriophage SPO1-encoded type II DNA-binding protein. EMBO J. **4:**1345–1349.
85. **Griffo, G., A. B. Oppenheim, and M. Gottesman.** 1989. Repression of the lambda *pcin* promoter by integrative host factor. J. Mol. Biol. **209:**55–64.
86. **Hagerman, P. J.** 1990. Sequence-directed curvature of DNA. Annu. Rev. Biochem. **59:**755–781.
87. **Harrington, R. E.** 1992. DNA curving and bending in protein-DNA recognition. Mol. Microbiol. **6:**2549–2555.
88. **Heggeler-Bordelier, B., W. Wahli, M. Adrian, A. Stasiak, and J. Dubochet.** 1992. The apical localization of transcribing RNA polymerases on supercoiled DNA prevents its rotation around the template. EMBO J. **11:**667–672.
89. **Hendrickson, W., and R. B. Schleif.** 1984. Regulation of the *Escherichia coli* L-arabinose operon studied by gel electrophoresis DNA binding assay. J. Mol. Biol. **174:**611–628.
90. **Herbert, M., A. Kolb, and H. Buc.** 1986. Overlapping promoters and their control in *Escherichia coli*: the *gal* case. Proc. Natl. Acad. Sci. USA **83:**2807–2811.
91. **Herendeen, D. R., G. A. Kassavetis, and E. P. Geiduschek.** 1992. A transcriptional enhancer whose function imposes a requirement that proteins track along DNA. Science **256:**1298–1303.
92. **Heumann, H., M. Ricchetti, and W. Werel.** 1988. DNA-dependent RNA polymerase of *Escherichia coli* induces bending or an increased flexibility of DNA by specific complex formation. EMBO J. **7:**4379–4381.

93. **Heyduk, T., J. Lee, Y. Ebright, E. Blatter, Y. Zhou, and R. Ebright.** 1993. CAP and RNA polymerase interact in solution in the absence of promoter DNA. Nature (London) **364**:548–549.
94. **Higgins, C. F.** 1992. Protein H-NS (H1a), chromatin structure and gene expression. Nucleic Acids Mol. Biol. **6**:67–81.
95. **Higgins, C. F., C. J. Dorman, D. A. Stirling, L. Waddell, I. R. Booth, G. May, and E. Bremer.** 1988. A physiological role for DNA supercoiling in the osmotic regulation of gene expression in *S. typhimurium* and *E. coli*. Cell **52**:569–584.
96. **Higgins, C. F., J. C. D. Hinton, C. S. J. Hulton, T. Owen-Hughes, G. D. Pavitt, and A. Seirafi.** 1990. Protein H1: a role for chromatin structure in the regulation of bacterial gene expression and virulence? Mol. Microbiol. **4**:2007–2012.
97. **Higgins, N. P., D. A. Collier, M. W. Kilpatrick, and H. M. Krause.** 1989. Supercoiling and integration host factor change the DNA conformation and alter the flow of convergent transcription in phage Mu. J. Biol. Chem. **264**:3035–3042.
98. **Hodges-Garcia, Y., P. J. Hagerman, and D. E. Pettijohn.** 1989. DNA ring closure mediated by protein HU. J. Biol. Chem. **264**:14621–14623.
99. **Hoover, T. R., E. Santero, S. Porter, and S. Kustu.** 1990. Integration host factor stimulates interaction of RNA polymerase with NifA, the transcriptional activator for nitrogen fixation operons. Cell **63**:11–22.
100. **Hryniewicz, M. M., and N. M. Kedrich.** 1991. The *cysP* promoter of *Salmonella typhimurium*: characterization of two binding sites for CysB protein, studies of in vivo transcription initiation, and demonstration of the anti-inducer effects of thiosulfate. J. Bacteriol. **173**:5876–5886.
101. **Hsu, L. M., J. K. Giannini, T. W. C. Leung, and J. C. Crosthwaite.** 1991. Upstream sequence activation of *Escherichia coli argT* promoter *in vivo* and *in vitro*. Biochemistry **30**:813–822.
102. **Htun, H., and J. E. Dahlberg.** 1988. Single strands, triple strands and kinks in H-DNA. Science **241**:1791–1796.
103. **Huang, J., and M. A. Schell.** 1991. *In vivo* interactions of the NahR transcriptional activator with its target sequences. J. Biol. Chem. **266**:10830–10838.
104. **Huang, L., P. Tsui, and M. Freundlich.** 1990. Integration host factor is a negative effector of in vivo and in vitro expression of *ompC* in *Escherichia coli*. J. Bacteriol. **172**:5293–5298.
105. **Hübner, P., and W. Arber.** 1989. Mutational analysis of a prokaryotic recombinational enhancer element with two functions. EMBO J. **8**:577–585.
106. **Hudson, J. M., and M. G. Fried.** 1990. Co-operative interactions between the catabolite gene activator protein and the lac repressor at the lactose promoter. J. Mol. Biol. **214**:381–396.
107. **Hulton, C. S. J., A. Seirafi, J. C. D. Hinton, J. N. Sidebotham, L. Waddel, G. Pavitt, T. Owen-Hughes, A. Spassky, H. Buc, and C. F. Higgins.** 1990. Histone-like protein H1 (H-NS), DNA supercoiling and gene expression in bacteria. Cell **63**:631–642.
108. **Igarashi, K., and A. Ishihama.** 1991. Bipartite functional map of the *E. coli* RNA polymerase α subunit: involvement of the C-terminal region in transcription activation by cAMP-CRP. Cell **65**:1015–1022.
109. **Irani, M. H., L. Orosz, and S. Adhya.** 1983. A control element within a structural gene: the *gal* operon of *Escherichia coli*. Cell **32**:783–788.
110. **Ishihama, A.** 1992. Role of the RNA polymerase α subunit in transcription activation. Mol. Microbiol. **6**:3283–3288.
111. **Ishihama, A.** 1993. Protein-protein communication within the transcription apparatus. J. Bacteriol. **175**:2483–2489.
112. **Johnson, G. G., and E. P. Geiduschek.** 1977. Specificity of the weak binding between the phage SPO1 transcription-inhibitory protein, TF1 and SPO1 DNA. Biochemistry **16**:1473–1485.
113. **Johnson, R. C., M. F. Bruist, and M. I. Simon.** 1986. Host protein requirements for in vitro site-specific DNA inversion. Cell **46**: 531–539.
114. **Joung, K., L. U. Le, and A. Hochschild.** 1993. Synergistic activation of transcription by *Escherichia coli* cAMP receptor protein. Proc. Natl. Acad. Sci. USA **90**:3083–3087.
115. **Kahmann, R., F. Rudt, C. Koch, and G. Mertens.** 1985. G inversion in bacteriophage Mu DNA is stimulated by a site within the invertase gene and a host factor. Cell **41**:771–780.
116. **Kahn, J. D., and D. M. Crothers.** 1992. Protein induced bending and DNA cyclization. Proc. Natl. Acad. Sci. USA **89**:6343–6347.
117. **Kerppola, T. K., and T. Curran.** 1991. Fos-Jun heterodimers and Jun homodimers bends DNA in opposite orientations: implications for transcription factor cooperativity. Cell **66**:317–326.
118. **Kerppola, T. K., and T. Curran.** 1991. DNA bending by Fos and Jun: the flexible hinge model. Science **254**:1210–1214.
119. **Kim, J., C. Zwieb, C. Wu, and S. Adhya.** 1989. Bending of DNA by gene-regulatory proteins: construction and use of a DNA bending vector. Gene **85**:15–23.
120. **Knaus, R., and H. Bujard.** 1988. P_L of coliphage lambda: an alternative solution for an efficient promoter. EMBO J. **7**:2919–2923.
121. **Knaus, R., and H. Bujard.** 1990. Principles governing the activity of *E. coli* promoters. Nucleic Acids Mol. Biol. **4**:110–122.
122. **Kolb, A., S. Busby, H. Buc, S. Garges, and S. Adhya.** 1993. Transcriptional regulation by cAMP and its receptor protein. Annu. Rev. Biochem. **62**:749–795.
123. **Koo, H.-S., H. M. Wu, and D. M. Crothers.** 1986. DNA bending at adenine/thymine tracts. Nature (London) **320**:501–506.
124. **Kostrewa, D., J. Granzin, C. Koch, H.-W. Choe, S. Raghunathan, W. Wolf, J. Labhan, R. Kahmann, and W. Saenger.** 1991. Three-dimensional structure of the *E. coli* DNA-binding protein FIS. Nature (London) **349**:178–180.
125. **Kostrewa, D., J. Granzin, C. Sandmann, U. Lücken, D. Stock, and W. Saenger.** 1992. Three-dimensional structure of the *E. coli* DNA-binding protein FIS, p. 58–61. *In* A. Klug and J. Subirana (ed.), Workshop on DNA structure and protein recognition. Instituto Juan March, Madrid.
126. **Kovacic, R. T.** 1987. The 0° C closed complexes between *Escherichia coli* RNA polymerase and two promoters, T7-A3 and *lac*UV5. J. Biol. Chem. **262**:13654–13661.
127. **Krause, H. M., and N. P. Higgins.** 1986. Positive and negative regulation of a Mu operator by Mu repressor and *E. coli* integration host factor. J. Biol. Chem. **261**:3744–3752.
128. **Kuhnke, G., H.-J. Fritz, and R. Ehring.** 1987. Unusual properties of promoter up mutations in the *E. coli* galactose operon and evidence suggesting RNA polymerase-induced DNA bending. EMBO J. **6**:507–513.
129. **Kuhnke, G., C. Theres, K.-J. Fritz, and R. Ehring.** 1989. RNA polymerase and *gal* repressor bind simultaneously and with DNA bending to the control region of the *Escherichia coli* galactose operon. EMBO J. **8**:1247–1255.
130. **Kur, J., N. Hasan, and W. Szybalski.** 1989. Physical and biological consequences of interactions between integration host factor (IHF) and coliphage lambda late P'_R promoter and its mutants. Gene **81**:1–15.
131. **Kur, J., N. Hasan, and W. Szybalski.** 1989. Repression of transcription from the b2-att region of the coliphage lambda by integration host factor. Virology **168**:236–244.
132. **Kustu, S., A. K. North, and D. S. Weiss.** 1991. Prokaryotic transcriptional enhancers and enhancer-binding proteins. Trends Biochem. Sci. **16**:397–402.
133. **Kustu, S., E. Santero, J. Keener, D. Popham, and D. Weiss.** 1989. Expression of σ^{54} (*ntrA*)-dependent genes is probably united by a common mechanism. Microbiol. Rev. **53**:367–376.
134. **Lacks, S., P. Lopez, B. Greenberg, and M. Espinosa.** 1986. Identification and analysis of genes for tetracycline resistance and replication functions in the broad-host-range plasmid pLS1. J. Mol. Biol. **192**:753–765.
135. **Lamond, A. I., and A. A. Travers.** 1983. Requirement for an upstream element for optimal transcription of a bacterial tRNA gene. Nature (London) **305**:248–250.
136. **Lanzer, M., and H. Bujard.** 1988. Promoters largely determine the efficiency of repression action. Proc. Natl. Acad. Sci. USA **85**:8973–8977.
137. **Laundon, C. H., and J. D. Griffith.** 1988. Curved helix segments can uniquely orient the topology of supertwisted DNA. Cell **52**:545–549.
138. **Lavigne, M., M. Herbert, A. Kolb, and H. Buc.** 1992. Upstream curved sequences influence the initiation of transcription at the *Escherichia coli* galactose operon. J. Mol. Biol. **224**:293–306.
139. **Leirmo, S., and R. L. Gourse.** 1991. Factor independent activa-

tion of *Escherichia coli* rRNA transcription. I. Kinetic analysis of the roles of upstream activator region and supercoiling on transcription of the *rrnBP1* promoter *in vitro*. J. Mol. Biol. **220**:555–568.

140. **Lim, D., J. D. Oppenheim, T. Eckhardt, and W. K. Maas.** 1987. Nucleotide sequence of the *argR* gene of *Escherichia coli* K12 and isolation of its product, the arginine repressor. Proc. Natl. Acad. Sci. USA **84**:6697–6701.
141. **Lloubes, R., M. Granger-Schnarr, C. Lazdunski, and M. Schnarr.** 1988. LexA repressor induces operator dependent DNA bending. J. Mol. Biol. **204**:1049–1054.
142. **Lloubes, R., C. Lazdunski, M. Granger-Schnarr, and M. Schnarr.** 1993. DNA sequence determinants of LexA-induced DNA bending. Nucleic Acids Res. **21**:2363–2367.
143. **Lobell, R. B., and R. F. Schleiff.** 1990. DNA looping and unlooping by AraC protein. Science **250**:528–532.
144. **Lobell, R. B., and R. F. Schleiff.** 1991. AraC-DNA looping: orientation and distance dependent loop breaking by cyclic AMP receptor protein. J. Mol. Biol. **218**:45–54.
145. **Lozinsky, T., K. Adrych-Rozek, W. T. Markiewicz, and K. L. Wierzchowski.** 1991. Effect of DNA bending in various regions of a consensus-like *Escherichia coli* promoter on its strength *in vivo* and structure of the open complex *in vitro*. Nucleic Acids Res. **19**:2947–2953.
146. **Lynch, A., and J. Wang.** 1993. Anchoring of DNA to the bacterial cytoplasmic membrane through cotranscriptional synthesis of polypeptides encoding membrane proteins or proteins for export: a mechanism of plasmid hypernegative supercoiling in mutants deficient in DNA topoisomerase I. J. Bacteriol. **175**:1645–1655.
147. **Malan, T. P., A. Kolb, H. Buc, and W. R. McClure.** 1984. Mechanism of CRP-cAMP activation of lac operon transcription initiation activation of the P1 promoter. J. Mol. Biol. **180**:881–909.
148. **Maloy, S.** 1987. The proline utilization operon, p. 1513–1519. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
149. **Marini, J. C., S. D. Levene, D. M. Crothers, and P. T. Englund.** 1982. Bent helical structure in kinetoplast DNA. Proc. Natl. Acad. Sci. USA **79**:7664–7668.
150. **Matthews, K. S.** 1992. DNA looping. Microbiol. Rev. **56**:123–136.
151. **McAllister, C. F., and E. C. Achberger.** 1988. Effect of polyadenine-containing curved DNA on promoter utilization in *Bacillus subtilis*. J. Biol. Chem. **263**:11743–11749.
152. **McAllister, C. F., and E. C. Achberger.** 1989. Rotational orientation of upstream curved DNA affects promoter function in *Bacillus subtilis*. J. Biol. Chem. **264**:10451–10456.
153. **McClure, W.** 1980. Rate limiting steps in RNA chain initiation. Proc. Natl. Acad. Sci. USA **77**:5634–5638.
154. **Mecsas, J., D. W. Cowing, and C. A. Gross.** 1991. Development of RNA polymerase-promoter contacts during open complex formation. J. Mol. Biol. **220**:585–597.
155. **Meier, I., L. V. Wray, and W. Hillen.** 1988. Differential regulation of the Tn*10*-encoded tetracycline resistance genes *tetA* and *tetR* by the tandem *tet* operators O1 and O2. EMBO J. **7**:567–572.
156. **Meiklejohn, A. L., and J. D. Gralla.** 1989. Activation at the *lac* promoter and its variants. Synergistic effects of catabolite activator protein and supercoiling *in vitro*. J. Mol. Biol. **207**:661–673.
157. **Mencía, M., M. Salas, and F. Rojo.** 1993. Residues of the *Bacillus subtilis* phage Φ29 transcriptional activator required both to interact with RNA polymerase and to activate transcription. J. Mol. Biol. **233**:695–704.
158. **Metzger, W., P. Schickor, and H. Heumann.** 1989. A cinematographic view of *Escherichia coli* RNA polymerase translocation. EMBO J. **8**:2745–2754.
159. **Molina-López, J. A., F. Govantes, and E. Santero.** Geometry of the process of transcription activation at the σ^{54}-dependent *nifH* promoter of *Klebsiella pneumoniae*. Submitted for publication.
160. **Nachaliel, N., J. Melnick, R. Gafny, and G. Glaser.** 1989. Ribosome associated protein(s) specifically bind(s) to the upstream activator sequence of the *E. coli rrnA* P1 promoter. Nucleic Acids Res. **17**:9811–9822.
161. **Nash, H. A.** 1990. Bending and supercoiling of DNA at the attachment site of bacteriophage λ. Trends Biochem. Sci. **15**:222–227.
162. **Newlands, J. T., C. A. Josaitis, W. Ross, and R. Gourse.** 1992. Both Fis-dependent and factor-independent upstream activation of the *rrnB P1* promoter are face of the helix dependent. Nucleic Acids Res. **20**:719–726.
163. **Newlands, J. T., W. Ross, K. K. Gosink, and R. L. Gourse.** 1991. Factor-independent activation of *Escherichia coli* rRNA transcription. II. Characterization of complexes of *rrnBP1* promoters containing or lacking the upstream activator region with *Escherichia coli* RNA polymerase. J. Mol. Biol. **220**:569–583.
164. **Nilsson, L., A. Vanet, E. Vijgenboom, and L. Bosch.** 1990. The role of FIS in *trans* activation of stable RNA operons of *E. coli*. EMBO J. **9**:727–734.
165. **Nishi, T., and S. Itoh.** 1986. Enhancement of transcriptional activity of the *Escherichia coli trp* promoter by upstream A+T rich regions. Gene **44**:29–36.
166. **North, A., K. E. Klose, K. Stedman, and S. Kustu.** 1993. Prokaryotic enhancer-binding proteins reflect eukaryote-like modularity: the puzzle of nitrogen regulatory protein C. J. Bacteriol. **175**:4267–4273.
167. **Novick, R. P.** 1987. Plasmid incompatibility. Microbiol. Rev. **51**:381–395.
168. **Nuez, B., F. Rojo, I. Barthelemy, and M. Salas.** 1991. Identification of the sequences recognized by phage Φ29 transcriptional activator: possible interaction between the activator and the RNA polymerase. Nucleic Acids Res. **19**:2337–2342.
169. **Nuez, B., F. Rojo, and M. Salas.** 1992. Phage Φ29 regulatory protein p4 stabilizes the binding of the RNA polymerase to the late promoter in a process involving direct protein-protein contacts. Proc. Natl. Acad. Sci. USA **89**:11401–11405.
170. **O'Brien, K., G. Deno, P. Otrovsky de Spicer, J. F. Gardner, and S. R. Maloy.** 1992. Integration host factor facilitates repression of the *put* operon in *Salmonella typhimurium*. Gene **118**:13–19.
171. **Oehler, S., E. R. Eismann, H. Krämer, and B. Müller-Hill.** 1990. The three operators of the *lac* operon cooperate in repression. EMBO J. **9**:973–979.
172. **Ohlson, K. L., and J. D. Gralla.** 1992. DNA melting within stable closed complexes at the *Escherichia coli rrnBP1* promoter. J. Biol. Chem. **267**:19813–19818.
173. **Ohyama, T., M. Nagumo, Y. Hirota, and S. Sakuma.** 1992. Alteration of the curved helical structure located in the upstream region of the β-lactamase promoter of plasmid pUC19 and its effect on transcription. Nucleic Acids Res. **20**:1617–1622.
174. **Osuna, R., S. E. Finkel, and R. C. Johnson.** 1991. Identification of two functional regions in FIS: the N-terminus is required to promote Hin-mediated DNA inversion but not lambda excision. EMBO J. **10**:1593–1603.
175. **Otrovsky de Spicer, P., and S. R. Maloy.** 1993. Regulation of proline utilization in *Salmonella typhimurium*: autogenous regulation by a membrane-associated dehydrogenase. Submitted for publication.
176. **Otrovsky de Spicer, P., and S. R. Maloy.** 1993. PutA protein, a membrane-associated flavin dehydrogenase, acts as a redox-dependent transcriptional regulator. Proc. Natl. Acad. Sci. USA **90**:4295–4298.
177. **Otrovsky de Spicer, P., K. O'Brien, and S. R. Maloy.** 1991. Regulation of proline utilization in *Salmonella typhimurium*: a membrane-associated dehydrogenase binds DNA in vitro. J. Bacteriol. **173**:211–219.
178. **Owen-Hughes, T. A., G. D. Pavitt, D. S. Santos, J. M. Sidebotham, C. S. J. Hulton, J. C. D. Hinton, and C. F. Higgins.** 1992. The chromatin associated protein H-NS interacts with curved DNA to influence DNA topology and gene expression. Cell **71**:255–265.
179. **Pagel, J. M., J. W. Winkelman, C. W. Adams, and G. W. Hatfield.** 1992. DNA topology-mediated regulation of transcription initiation from tandem promoters of the *ilvGMEDA* operon of *Escherichia coli*. J. Mol. Biol. **224**:919–935.
180. **Pedersen, H., L. Soogard-Andersen, B. Holst, P. Gerlach, E. Bremer, and P. Valentin-Hansen.** 1992. cAMP-CRP activator complex and the CytR repressor protein bind co-operatively to

the *cytRP* promoter in *Escherichia coli* and CytR antagonizes the cAMP-CRP-induced DNA bend. J. Mol. Biol. **227**:396–406.
181. **Pérez-Martín, J.** 1992. Ph.D. thesis. Universidad Autónoma de Madrid, Madrid, Spain.
182. **Pérez-Martín, J., G. H. del Solar, A. G. de la Campa, and M. Espinosa.** 1988. Three regions in the DNA of plasmid pLS1 show sequence-directed bending. Nucleic Acids Res. **16**:9113–9126.
183. **Pérez-Martín, J., G. H. del Solar, R. Lurz, A. de la Campa, B. Dobrinski, and M. Espinosa.** 1989. Induced bending of plasmid pLS1 DNA by plasmid encoded protein RepA. J. Biol. Chem. **264**:21334–21339.
184. **Pérez-Martín, J., and M. Espinosa.** 1991. The RepA repressor can act as a transcriptional activator by inducing DNA bends. EMBO J. **10**:1375–1382.
185. **Pérez-Martín, J., and M. Espinosa.** 1992. A genetic system to study the *in vivo* role of transcriptional regulators in *Escherichia coli*. Gene **116**:75–80.
186. **Pérez-Martín, J., and M. Espinosa.** 1993. Protein-induced bending as a transcriptional switch. Science **260**:805–807.
187. **Pérez-Martín, J., and M. Espinosa.** Submitted for publication.
188. **Pérez-Martín, J., K. N. Timmis, and V. de Lorenzo.** Transcriptional coregulation by bent DNA: functional substitutions of the IHF site at the σ^{54}-dependent promoter *Pu* of the upper TOL operon by intrinsically curved DNA sequences. Submitted for publication.
189. **Pettijohn, D. E.** 1988. Histone-like proteins and bacterial chromosome structure. J. Biol. Chem. **263**:12793–12796.
190. **Pinkey, M., and J. G. Hoggtey.** 1988. Binding of the cyclic-AMP receptor protein of *Escherichia coli* to RNA polymerase. Biochem. J. **250**:897–902.
191. **Plaskon, R. R., and R. M. Wartel.** 1987. Sequence distributions associated with DNA curvature are found upstream of strong E. coli promoters. Nucleic Acids Res. **15**:785–797.
192. **Plumbridge, J., and A. Kolb.** 1991. CAP and Nag repressor binding to the regulatory regions of the *nagE-B* and *manX* genes of *Escherichia coli*. J. Mol. Biol. **217**:661–679.
193. **Plumbridge, J. A.** 1989. Sequence of the *nagBACD* operon in *Escherichia coli* K12 and pattern of transcription with the *nag* regulon. Mol. Microbiol. **3**:506–515.
194. **Popham, D. L., D. Szeto, J. Keener, and S. Kustu.** 1989. Function of a bacterial activator protein that binds to transcriptional enhancers. Science **243**:629–635.
195. **Pruss, G. J., and K. Drlica.** 1989. DNA supercoiling and prokariotic transcription. Cell **56**:521–523.
196. **Ptashne, M.** 1986. Gene regulation by proteins acting nearby and at a distance. Nature (London) **322**:697–701.
197. **Raibaud, O., D. Vidal-Ingigliardi, and E. Richet.** 1989. A complex nucleoprotein structure involved in activation of transcription of two divergent *Escherichia coli* promoters. J. Mol. Biol. **205**:471–485.
198. **Ramstein, J., and R. Lavery.** 1988. Energetic coupling between DNA bending and base pair opening. Proc. Natl. Acad. Sci. USA **85**:7231–7235.
199. **Rao, L., W. Ross, J. Appleman, T. Gaal, S. Leirmo, P. Schlax, T. Record, and R. L. Gourse.** 1994. Factor-independent activation of *rrnB* P1: an extended promoter with an upstream element that dramatically increases promoter strength. J. Mol. Biol. **235**:1421–1435.
200. **Rees, W. A., R. W. Keller, J. P. Vesenka, G. Yang, and C. Bustamante.** 1993. Evidence of DNA bending in transcription complexes images by scanning force microscopy. Science **260**: 1646–1649.
201. **Reitzer, L. J., and B. Magasanik.** 1986. Transcription of *glnA* in E. coli is stimulated by activator bound to sites far from the promoter. Cell **45**:785–792.
202. **Ren, Y. L., S. Garges, S. Adhya, and J. S. Krakow.** 1988. Cooperative binding of heterologous proteins: evidence for contact between the cyclic AMP receptor protein and RNA polymerase. Proc. Natl. Acad. Sci. USA **85**:4138–4142.
203. **Reznikoff, W. S., K. Bertrand, C. Donnelly, M. Krebs, L. E. Maquat, M. Peterson, L. Wray, J. Yin, and X.-M. Yu.** 1987. Complex promoters, p. 105–113. *In* W. S. Reznikoff, C. A. Gross, R. R. Burgess, M. T. Record, J. E. Dahlberg, and M. P. Wickens (ed.), RNA polymerase and the regulation of transcription. Elsevier Science Publishing, Inc., New York.
204. **Richet, E., and O. Raibaud.** 1991. Supercoiling is essential for the formation and stability of the initiation complex at the divergent *malEp* and *malKp* promoters. J. Mol. Biol. **218**:529–542.
205. **Richet, E., and L. Søgaard-Andersen.** Unpublished results.
206. **Richet, E., D. Vidal-Ingigliardi, and O. Raibaud.** 1991. A new mechanism for coactivation of transcriptional activation: repositioning of an activator triggered by the binding of a second activator. Cell **66**:1185–1195.
207. **Rojo, F., B. Nuez, M. Mencía, and M. Salas.** 1993. The main early and late promoters of *Bacillus subtilis* phage Φ29 form unstable open complexes with σ^{A}-RNA polymerase that are stabilized by DNA supercoiling. Nucleic Acids Res. **21**:935–940.
208. **Rojo, F., and M. Salas.** 1991. A DNA curvature can substitute phage Φ29 regulatory protein p4 when acting as a transcriptional repressor. EMBO J. **10**:3429–3438.
209. **Rojo, F., A. Zaballos, and M. Salas.** 1990. Bend induced by phage Φ29 transcriptional activator in the viral late promoter is required for activation. J. Mol. Biol. **211**:713–725.
210. **Ross, W., K. Gosink, J. Salomon, K. Igarashi, C. Zhou, A. Ishihama, K. Severinov, and R. L. Gourse.** 1993. A third recognition element in bacterial promoters: DNA binding by the α subunit of RNA polymerase. Science **262**:1407–1413.
211. **Ross, W., J. T. Newlands, L. Rao, K. Gosink, A. Appleman, A. J. Bokal, M. Barlet, J. Salomon, C. A. Josaitis, I. Çastano, and R. L. Gourse.** 1992. Upstream activation of ribosomal RNA transcription in *E. coli*, p. 23–25. *In* M. Salas and L. Rothman-Denes (ed.), Workshop on transcription initiation in prokaryotes. Instituto Juan March, Madrid.
212. **Ross, W., J. F. Thompson, J. T. Newlands, and R. L. Gourse.** 1990. *E. coli* FIS protein activates ribosomal RNA transcription in vitro and in vivo. EMBO J. **11**:3733–3742.
213. **Rossi, J. J., X. Soberon, Y. Marumoto, J. McMahon, and K. Itakura.** 1983. Biological expression of an *E. coli* consensus sequence promoter and some mutant derivatives. Proc. Natl. Acad. Sci. USA **80**:3203–3207.
214. **Rouiviere-Yaniv, J., and M. Yaniv.** 1979. *E. coli* DNA binding protein HU forms nucleosome-like structure with circular double-stranded DNA. Cell **17**:265–274.
215. **Ryder, K., S. Silver, A. L. De Lucia, E. Fanning, and P. Tegtmeyer.** 1986. An altered DNA conformation in origin region I is a determinant for the binding of SV40 large T antigen. Cell **44**:719–725.
216. **Sander, P., W. Langer, and K. Mueller.** 1993. Mechanisms of upstream activation of the *rrnD* promoter P1 of *Escherichia coli*. J. Biol. Chem. **268**:16907–16916.
217. **Santero, E., T. R. Hoover, A. K. North, D. K. Berger, S. C. Porter, and S. Kustu.** 1992. Role of Integration host factor in stimulating transcription from the σ^{54}-dependent *nifH* promoter. J. Mol. Biol. **227**:602–620.
218. **Sasse-Dwigth, S., and J. D. Gralla.** 1990. Role of eukaryotic type functional domains found in the prokaryotic enhancer receptor factor σ54. Cell **62**:945–954.
219. **Sayre, M. H., and E. P. Geiduschek.** 1988. TF1, the bacteriophage SPO1-encoded type II DNA-binding protein, is essential for viral multiplication. J. Virol. **62**:3455–3462.
220. **Schell, M.** 1993. Molecular biology of the LysR family of transcriptional regulators. Annu. Rev. Microbiol. **47**:597–626.
221. **Schickor, P., W. Metzger, W. Werel, H. Lederer, and H. Heumann.** 1990. Topography of intermediates in transcription initiation of *E. coli*. EMBO J. **9**:2215–2220.
222. **Schinkel, A., M. J. Groot-Koerkamp, A. Teunissen, and H. F. Tabak.** 1988. RNA polymerase induces DNA bending at yeast mitochondrial promoters. Nucleic Acids Res. **16**:9147–9163.
223. **Schleif, R.** 1987. The L-arabinose operon, p. 1473–1481. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
224. **Schleif, R. F.** 1992. DNA looping. Annu. Rev. Biochem. **61**:199–223.
225. **Schmidt, M. D.** 1990. More than just "histone-like" proteins. Cell **63**:451–453.

226. **Schneider, G. J., M. H. Sayre, and E. P. Geiduschek.** 1991. DNA-bending properties of TF1. J. Mol. Biol. **221:**777–794.
227. **Schultz, S. C., G. C. Shields, and T. A. Steitz.** 1991. Crystal structure of a CAP-DNA complex: the DNA is bent by 90°. Science **253:**1001–1007.
228. **Schwartz, M.** 1987. The maltose regulon, p. 1482–1502. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
229. **Serrano, M., I. Barthelemy, and M. Salas.** 1991. Transcription activation at a distance by phage Φ29 Protein p4. Effect of bent and non-bent intervening DNA sequences. J. Mol. Biol. **219:**403–414.
230. **Siebenlist, M.** 1979. RNA polymerase unwinds an 11-base pair segment of a phage T7 promoter. Nature (London) **279:**651–652.
231. **Spassky, A., S. Busby, and H. Buc.** 1984. On the action of the cyclic AMP-cyclic AMP receptor protein complex at the *Escherichia coli* lactose and galactose promoter regions. EMBO J. **3:**43–50.
232. **Spassky, A., S. Rimsky, H. Garreau, and H. Buc.** 1984. H1a, an *E. coli* DNA-binding protein which accumulates in stationary phase, strongly compacts DNA in vitro. Nucleic Acids Res. **12:**5321–5340.
233. **Storz, G., L. A. Tartaglia, and B. N. Ames.** 1990. Transcriptional regulator of oxidative stress-inducible genes: direct activation by oxidation. Science **248:**189–194.
234. **Straney, D. C., and D. M. Crothers.** 1987. Lac repressor is a transient gene-activating protein. Cell **51:**699–707.
235. **Straney, D. C., S. B. Straney, and D. M. Crothers.** 1989. Synergy between *Escherichia coli* CAP protein and RNA polymerase in the lac promoter open complex. J. Mol. Biol. **206:**41–57.
236. **Tanaka, I., K. Applet, J. Dijkt, S. W. White, and K. S. Wilson.** 1984. 3Å resolution structure of a protein with histone-like properties in prokaryotes. Nature (London) **310:**376–381.
237. **Tanaka, K., S. Muramatsu, H. Yamada, and T. Mizuno.** 1991. Systematic characterization of curved DNA segments randomly cloned from Escherichia coli and their functional significance. Mol. Gen. Genet. **226:**367–376.
238. **Thompson, J. F., and A. Landy.** 1988. Empirical estimation of protein-induced DNA bending angles: applications to lambda site-specific recombination complexes. Nucleic Acids Res. **16:**9687–9705.
239. **Thompson, J. F., and G. Mosig.** 1988. Integration host factor (IHF) represses a *Chlamydomonas* chloroplast promoter in *E. coli*. Nucleic Acids Res. **16:**3313–3326.
240. **Tian, G., D. Lim, J. Carey, and W. K. Maas.** 1992. Binding of the arginine repressor of *Escherichia coli* K12 to its operator sites. J. Mol. Biol. **226:**387–397.
241. **Travers, A. A.** 1987. The structure and function of *E. coli* promoter DNA. Crit. Rev. Biochem. **22:**181–219.
242. **Travers, A. A.** 1989. DNA conformation and protein binding. Annu. Rev. Biochem. **58:**427–452.
243. **Travers, A. A., A. I. Lamond, H. A. F. Mace, and M. L. Berman.** 1983. RNA polymerase interactions with the upstream region of the *E. coli* tyrT promoter. Cell **35:**265–273.
244. **Trifonov, E. N.** 1985. Curved DNA. Crit. Rev. Biochem. **19:**89–106.
245. **Tsui, P., L. Huang, and M. Freundlich.** 1991. Integration host factor binds specifically to multiple sites in the *ompB* promoter of *Escherichia coli* and inhibits transcription. J. Bacteriol. **173:**5800–5807.
246. **Ueguchi, C., and T. Mizuno.** 1993. The *Escherichia coli* nucleoid protein H-NS functions directly as a transcriptional repressor. EMBO J. **12:**1039–1046.
247. **Ushida, C., and H. Aiba.** 1990. Helical phase dependent action of CRP: effect of the distance between the CRP site and the −35 region on promoter activity. Nucleic Acids Res. **21:**6325–6330.
248. **van Rijn, P. A., N. Goosen, S. C. H. J. Turk, and P. van de Putte.** 1989. Regulation of phage Mu repressor transcription by IHF depends on the level of the early transcription. Nucleic Acids Res. **17:**10203–10212.
249. **van Rijn, P. A., N. Goosen, and P. van de Putte.** 1988. Integration host factor of *Escherichia coli* regulates early and repressor transcription of bacteriophage Mu by two different mechanisms. Nucleic Acids Res. **16:**4595–4605.
250. **Van Wye, D., E. C. Bronson, and J. N. Anderson.** 1991. Species-specific patterns of DNA bending and sequence. Nucleic Acids Res. **19:**5253–5261.
251. **Verbeek, H., L. Nilsson, G. Baliko, and L. Bosch.** 1990. Potential binding sites of the trans-activator FIS are present upstream of all rRNA operons and of many but not all tRNA operons. Biochim. Biophys. Acta **1050:**302–306.
252. **Verbeek, H., L. Nilsson, and L. Bosch.** 1992. The mechanism of trans-activation of the *Escherichia coli* operon *thrU*(*tufB*) by protein FIS. A model. Nucleic Acids Res. **20:**4077–4081.
253. **Wang, J. C., and G. N. Giaever.** 1988. Action at a distance along a DNA. Science **240:**300–304.
254. **Wang, J. C., J. H. Jacobsen, and J. M. Saucier.** 1977. Physicochemical studies on interactions between DNA and RNA polymerase. Unwinding of the DNA helix by Escherichia coli RNA polymerase. Nucleic Acids Res. **4:**1225–1241.
255. **Wang, L., J. D. Helmann, and S. C. Winans.** 1992. The A. tumefaciens transcriptional activator OccR causes a bend at a target promoter, which is partially relaxed by a plant tumor metabolite. Cell **69:**659–667.
256. **Wang, Q., and J. Calvo.** 1993. Lrp, a major regulatory protein in *Escherichia coli*, bends DNA and can organize the assembly of a higher-order nucleoprotein structure. EMBO J. **12:**2495–2501.
257. **Warwicker, J., B. P. Engelman, and T. A. Steitz.** 1987. Electrostatic calculations and model building suggest that DNA bound to CAP is sharply bent. Proteins **2:**283–289.
258. **Wedel, A., D. S. Weiss, D. Popham, P. Dröge, and S. Kustu.** 1990. A bacterial enhancer functions to tether a transcriptional activator near a promoter. Science **248:**486–490.
259. **Weiss, D. S., J. Batut, K. E. Klose, J. Keener, and S. Kustu.** 1991. The phosphorylated form of the enhancer binding protein NTRC has an ATPase activity that is essential for activation of transcription. Cell **67:**155–167.
260. **Werel, W., P. Schickor, and H. Heumann.** 1991. Flexibility of the DNA enhances promoter affinity of *Escherichia coli* RNA polymerase. EMBO J. **10:**2589–2594.
261. **Whitehall, S., S. Austin, and R. Dixon.** 1992. DNA supercoiling response of the σ^{54}-dependent *Klebsiella pneumoniae nifL* promoter *in vitro*. J. Mol. Biol. **225:**591–607.
262. **Williams, D. R., M. Motallebi-Wescharen, and C. M. Thomas.** 1993. Multifunctional repressor KorB can lock transcription by preventing isomerization of RNA polymerase-promoter complexes. Nucleic Acids Res. **21:**1141–1148.
263. **Winkelman, J. W., and G. W. Hatfield.** 1990. Characterization of the integration host factor binding site in the *ilvPG1* promoter region of the *ilvGMEDA* operon of *Escherichia coli*. J. Biol. Chem. **265:**10055–10060.
264. **Wong, C., and J. D. Gralla.** 1992. A role for the acidic trimer repeat region of transcription factor σ54 in setting the rate and temperature dependence of promoter melting *in vivo*. J. Biol. Chem. **267:**24762–24768.
265. **Wu, H. M., and D. M. Crothers.** 1984. The locus of sequence-directed and protein-induced DNA bending. Nature (London) **308:**509–513.
266. **Yamada, H., T. Yoshida, K. Tanaka, C. Sasakawa, and T. Mizuno.** 1991. Molecular analysis of the *Escherichia coli hns* gene encoding a DNA-binding protein, which preferentially recognizes curved DNA sequences. Mol. Gen. Genet. **230:**332–336.
267. **Yoshida, R. K., J. L. Miller, H. I. Miller, D. I. Friedman, and M. M. Howe.** 1982. Isolation and mapping of Mu mutants which grow in *him* mutants of *E. coli*. Virology **120:**269–272.
268. **Yuan, H., S. E. Finkel, J. Feng, M. Kaczor-Grzeskowiak, R. Johnson, and R. E. Dickerson.** 1991. The molecular structure of wild-type and a mutant Fis protein: relationship between mutational changes and recombinational enhancer function or DNA binding. Proc. Natl. Acad. Sci. USA **88:**9558–9562.
269. **Zacharias, M., H. U. Göringer, and R. Wagner.** 1992. Analysis of the Fis-dependent and Fis-independent transcription activation mechanism of the *Escherichia coli* ribosomal RNA P1 promoter. Biochemistry **31:**2621–2628.

270. **Zacharias, N., G. Theissen, C. Bradaczek, and R. Wagner.** 1991. Analysis of sequence elements important for the synthesis and control of ribosomal RNA in *E. coli*. Biochimie **73**:699–712.
271. **Zhou, Y., X. Zhang, and R. H. Ebright.** 1993. Identification of the activating region of CAP: isolation and characterization of mutants of CAP specifically defective in transcription activation. Proc. Natl. Acad. Sci. USA **90**:6081–6085.
272. **Zinkel, S. S., and D. M. Crothers.** 1991. Catabolite activator protein-induced DNA bending in transcription initiation. J. Mol. Biol. **219**:201–215.
273. **Zou, C., N. Fujita, K. Igarashi, and A. Ishihama.** 1992. Mapping the cAMP receptor protein contact site on the α-subunit of *Escherichia coli* RNA polymerase. Mol. Microbiol. **6**:2599–2605.
274. **Zuber, F., D. Kotlarz, S. Rimsky, and H. Buc.** Upstream synthetic curved DNA sequences and promoter expression. Mol. Microbiol., in press.
275. **Zwieb, C., J. Kim, and S. Adhya.** 1989. DNA bending by negative regulatory proteins: Gal and Lac repressors. Genes Dev. **3**:602–611.