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CLUES AND CONSEQUENCES OF DNA BENDING IN TRANSCRIPTION

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ABSTRACT

This review attempts to substantiate the notion that nonlinear DNA structures allow prokaryotic cells to evolve complex signal integration devices that, to some extent, parallel the transduction cascades employed by higher organisms to control cell growth and differentiation. Regulatory cascades allow the possibility of inserting additional checks, either positive or negative, in every step of the process. In this context, the major consequence of DNA bending in transcription is that promoter geometry becomes a key regulatory element. By using DNA bending, bacteria afford multiple metabolic control levels simply through alteration of promoter architecture, so that positive signals favor an optimal constellation of protein-protein and protein-DNA contacts required for activation. Additional effects of regulated DNA bending in prokaryotic promoters include the amplification and translation of small physiological signals into major transcriptional responses and the control of promoter specificity for cognate regulators.

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INTRODUCTION

The nucleotide sequence of DNA is the ultimate physical support of the information for the buildup of proteins and higher structures. In spite of a growing number of exceptions, the unidirectional flow DNA → RNA → proteins remains one of the basic pillars of modern biology (176). But DNA not only encodes proteins; regulatory sequences without any structural information play a pivotal role in many biological processes as well. Furthermore, beginning in the early 1980s, observations showing an entirely new aspect of DNA started to emerge: the role of nonlinear DNA structures in replication, recombination, and transcription was not directly linked to the proteins encoded or bound by the nucleotide sequence. In this respect, the DNA molecule is unique: it is able to encode in its sequence at least two independent levels of functional information. The first one (what might be called digital information) is for encoding proteins, whether structural or regulatory, and sequence targets for DNA-binding factors. A second level of information is contained in the physical and structural properties of the DNA molecule itself, which—as is reviewed in detail in this article—plays a central role in the coregulation of essential cellular processes. Although such properties are ultimately determined in each case by the nucleotide sequence, they are exploited by the cells in a fashion in which the sequence itself plays no role other than to support or facilitate a certain spatial structure. Along with RNA, this double feature of DNA has no parallel in other biological macromolecules.

DNA bending has now been identified in an entire collection of molecular phenomena, but this review focuses on only a particular and paradigmatic aspect of the subject, namely, its role in transcriptional coregulation of prokaryotic promoters, with some extensions into eukaryotic systems. In this context, we do not address the direct role of DNA curvature and bending in transcription, a controversial issue that has been examined in many different systems (48, 86, 109, 110, 123, 147a, 150, 174, 178, 183, 204) and has been recently reviewed (19, 39, 66, 140). Instead, we concentrate on nonlinear DNA structures as auxiliary regulatory elements that allow prokaryotic cells to process and integrate complex environmental signals. To this end, bendability and intrinsic or protein-induced curvatures become regulatory assets as building blocks for a productive promoter geometry. In this way, bacteria (*a*) afford multiple

metabolic control levels by altering promoter architecture, (*b*) amplify and translate minor physiological signals into major transcriptional responses, and (*c*) control promoter specificity.

THE NATURE OF DNA BENDING

Certain nucleotide sequences spontaneously impart a preferred direction of curvature on a DNA molecule, i.e. originate an intrinsic bend (72, 106, 190). Both for natural and artificially designed sequences, any short homopolymeric run of A or T nucleotides longer than 3 bp, when repeated in phase with the helical screw, can introduce a detectable degree of curvature in an isolated DNA molecule. Contiguous AA dinucleotides making minor wedge angles may sum up within the DNA helix and generate a curved structure (72, 190). In addition to AA pairs, certain dinucleotides such as AG, CG, GA, or GC can induce or contribute significantly to DNA curvature (16). Although A or T tracts are believed to be major determinants of DNA curvature, the sequence context also plays a critical role (33). In addition, polyA/polyT tracts are particularly rigid and bending may occur preferentially at the intervening sequences or at the A/T boundaries (180a, 182a). Alternatively (or at the same time), axial deflections may also arise from structural discontinuities at the boundaries between the A tracts assembled in B-DNA and the rest of the nucleotide sequence (23, 73, 90).

Bendability: Consequences for DNA-Protein Interactions

Apart from intrinsic DNA bends, a critical aspect of the structural diversity of DNA is its bendability. DNA does not behave as an isotropic rod: Depending on the sequence, it might bend more easily in one plane than another, indicating it possesses a degree of anisotropic flexibility. In any bending event, both the major and the minor groove of the helix surface inside the curve must narrow to some extent, because of the compression associated with bending, while those on the outside of the curve become correspondingly wider. It is the ability of specific short sequences to assume these conformations that enables the DNA structure to accommodate the deformation associated with bending and, thus, determines the bendability of DNA (34, 49, 87, 188). One important difference between curved DNA and bendable DNA arises from different physical dynamism. Statically curved DNA is deformed even in the absence of external forces, thereby resulting in a very rigid structure. On the other hand, bendable DNA allows a mixture of many different conformational states, the equilibrium of which can be displaced toward one specific form by external forces such as proteins interacting with them (49, 188). This is critical, since interactions between static structures are insufficient to explain DNA-protein recognition events (74). In fact, the specificity of DNA-protein contacts is the result not only

of direct recognition of certain bases within the DNA helix by particular amino acids, but also of an array of interactions between protein and DNA surfaces not necessarily linked to a particular nucleotide sequence. This type of interaction has been termed indirect readout (124) or analog recognition (35, 36) as opposed to the direct recognition of individual base pairs. Indirect readout involves protein contacts (e.g. amino acids and their side-groups) with the sugar-phosphate backbone of DNA through ionic bridges, hydrogen bonds, and hydrophobic interactions. Direct recognition requires a mutual DNA-protein reading to form bonds between the amino acid chains and the hydrogen atoms presented by the bases on the major or minor grooves of the double helix. Both types of interaction contribute to the specificity and stability of the complex and require the protein and the DNA to accommodate each other in order to set up adequate contacts. In other words, both the protein and DNA partners seek regions of the other that maximize their interaction, but conformational distortions may also be required in both partners to achieve this optimum fit (181, 182).

Regardless of the type of DNA-binding motif, the number of proteins known to bend their DNA target increases every year. But how is bending actually effected? In some cases it can be explained by the ability of proteins with typical major-groove recognition motifs (for instance, helix-turn-helix structures) to neutralize charges in the DNA backbone (179) or to set up extended DNA-protein contacts (181, 182). Interestingly, some regulators, such as the Cro protein of phage λ, seem to bend DNA efficiently without specific sequence recognition (42, 160). For proteins that interact with the minor groove, a model of the bending mechanism has been proposed that involves the intercalation of a protein side chain in the minor groove that acts like a wedge and eventually causes a deformation of the DNA helix (199). In the case of the integration host factor (IHF) protein, this effect is heavily dependent on indirect readout via the phosphodiester backbone (152a).

The extent to which sequence-dependent bendability of DNA plays a key role in DNA-protein interactions is clearly reflected in the affinity of a number of regulatory proteins for precurved or bending-prone sequences. The pioneering work with the catabolite gene activator protein (CAP) or cyclic AMP receptor protein (CRP) 10 years ago showed the correlation between the bendability of a CAP site within a DNA sequence and the affinity of CRP for the target site (53, 101). Similarly, the *Drosophila* protein Su (HW) requires an intrinsically bent DNA sequence in its target site (173). Also in the prokaryotic world, the bacterial σ^{70}-RNA polymerase induces a strong bend in the promoter sequence upon binding (92, 139), and the correlation between promoter strength and the presence of upstream curved DNA has been extensively shown (see 140 for review). Along the same line, the effects of a DNA bend on the binding of eukaryotic TBP or holo-TFIID to the TATA box were examined (175) with

the conclusion that DNA bending is an important component of the eukaryotic promoter.

CLUES OF DNA BENDING

DNA Bending as an Inducer or Inhibitor of Protein-DNA Interactions

Given the importance of DNA conformation for its recognition by some proteins, it is easy to anticipate that additional intrinsic or protein-induced bends may synergize or antagonize the interactions with proteins required to bend their targets for optimal binding. This effect, called structural synergy (70), was first shown in experiments in which an intrinsic bend was placed in different helical phases relative to a CAP-binding site (85). CAP binding to its target sequence increased by two orders of magnitude when the precurved DNA was in the same orientation as the CAP-induced bend. On the other hand, CAP binding was impaired when the bends were located in opposite directions. Moreover, a separate study pointed out that such an effect could even occur at considerable distances (98). A remarkable example of structural synergy involving different regulators is that of the interplay between the *Escherichia coli* repressor PutA and the integration host factor (IHF, see below), which binds to and bends two sequences adjacent to the PutA sites in the intergenic region between the *putA* and the *putP* promoters (Figure 1), thereby facilitating the binding of the repressor to the promoters targets (118). IHF is one major architectural element for nucleoprotein complexes in bacteria (50, 66), and its co-crystal with DNA has recently been resolved (152a). Because IHF participates in a variety of otherwise very different molecular events, its roles and properties are discussed in separate sections of this article.

The effect of DNA bending on the interaction of proteins and DNA has been addressed also in a number of eukaryotic promoters. Insertion of an intrinsically curved DNA upstream of the TATA box was shown to increase the DNA-binding affinity of the TBP protein in the context of a minicircle by a factor of 100 relative to linear DNA (128). Interestingly, this increase was strictly dependent on the specific phase of the curved DNA relative to the TATA box. A similar instance has been described for the eukaryotic histone-like DNA-bending protein HMG1, which facilitates the binding of the human progesterone receptor to its cognate promoter by inducing a structural change in the target DNA (120).

The notion of structural synergy has shed new light on the intimate mechanism of traditional regulatory devices such as down-regulation of prokaryotic promoters by repressors that bind DNA in the proximity of the RNA polymerase. For instance, the CopG protein (formerly named RepA) of the streptococcal

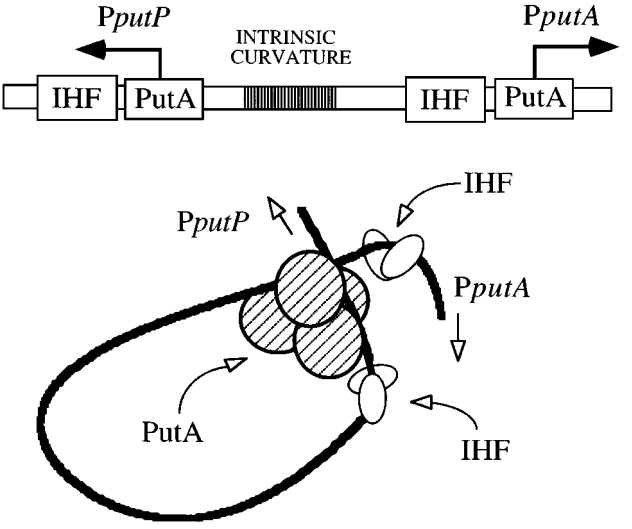

Figure 1 Organization of the PutA/integration host factor (IHF)-mediated repression complex of proline-uptake promoters of *E. coli*. The *PputA/PputP* divergent promoter region includes not only binding sites for the endogenous repressor of the system (PutA) but also an array of intrinsic DNA curvatures and IHF sites. In the absence of proline, a strong repression complex is formed, as sketched in the figure, which involves interactions between PutA molecules bound to distant sites, which are favored and sustained by both intrinsic and IHF-induced DNA bends within the region.

plasmid pLS1 is a small transcriptional repressor that bends its target DNA sharply at the plasmid replication origin (29, 136). A RepA-operator sequence placed artificially approximately 90 bp upstream of a standard *E. coli* promoter enhanced or decreased transcription in a side-of-the-helix–dependent fashion relative to the RNAP-binding site both in vitro and in vitro (137). This suggested that repression follows trapping the DNA region in a conformation not amenable to RNA polymerase binding instead of being the result of a competition between RepA and the enzyme for the same target sites. The notion that factors that bend promoter sequences in a direction unfavorable for RNAP binding can effect transcription repression (93, 205) has been substantiated in other cases. These include the repression exerted by the p4 protein from the *Bacillus subtilis* phage Φ29 on PA2b, a major promoter of early viral genes (156, 157), and the down-regulation caused by IHF on the *ilvPG1* promoter of *E. coli* (125, 196). In these and other (138) cases, there seems to be a transcriptional switch between two divergent or tandem overlapping promoters, an issue that has been reviewed separately (140).

DNA Bending as a Catalyst of Protein-Protein Interplay

Readout of the digital information encoded in DNA is preceded by the assembly of higher-order structures comprising multiple proteins, an event that is particularly complex in eukaryotic promoters (40, 70). Transcriptional factors frequently bind to distinct, sometimes distant, sites in such a way that protein-protein interactions require the distortion of the DNA structure in order to shorten the distance between the factors already bound to DNA. Recruitment of proteins to their specific targets face a dilemma. If the binding sites are too far away from each other, then the probability of contacts between two or more proteins decreases in parallel to the distance. This effect has a thermodynamic basis: For interactions to occur, the free energy resulting from the loss of entropy of the DNA when the sites are brought together through protein-protein contacts should be balanced by the free energy of association between the proteins themselves. However, "the closer the better" is not true, because below a specific DNA distance (called persistence length) the DNA stiffness makes interprotein contacts involving DNA distortions energetically unfavorable (195). Despite these >considerations, the fact is that interactions do occur between proteins bound to DNA sites ranging from few nucleotides to many kilobases. How can this occur?

In prokaryotic promoters, interactions between nonadjacent DNA-bound proteins require systematically the formation of a loop, which is energetically disfavored if the binding sites are separated by 30–140 nucleotides. DNA fragments of that size range display a limited flexibility, and the stiffness of the intervening DNA sequence may prevent the DNA from acquiring the curvature needed to juxtapose nonadjacent binding sites (170, 195). In these cases, one solution consists of altering the conformation of the DNA. The sequence between both protein-binding sites may be already curved in the appropriate direction or may show an increased and directed flexibility (i.e. bendability). Although both curved DNA and bendable DNA may show the same final effect (increasing interactions between proteins bound at both ends of the region), the statically bent DNA originates a very stable conformation, while the bendable DNA is anisotropically flexible and tolerates a more dynamic structure (see above). This concept has been directly tested in an artificial system based on the p4 protein of phage Φ29, a regulator that activates transcription at a certain distance from its cognate promoter (155). In a series of elegant experiments, Serrano et al (167) showed that activation occurred only when the intervening sequence between the binding sites for the RNA polymerase and the p4 protein was engineered to contain either a static DNA bend or a sequence with high bendability, thus facilitating productive protein-protein contacts.

A second solution to the problem of bringing about interactions between proteins bound to distant sites is the participation of extra factors that either bend DNA or increase its flexibility. These proteins may recognize specific sequences in the DNA, as it occurs with the bacterial proteins CAP or IHF or the eukaryotic factor LEF-1, some properties of which are discussed below. The binding of these proteins to DNA provokes a conformational change that has a precise directionality and is thought to line up nonadjacent binding sites, thereby enabling otherwise unlikely protein-protein interactions. A role for the DNA bending induced by these proteins is inferred from various bend swapping experiments, in which the binding site of the DNA-bending protein is exchanged either by the recognition site of unrelated DNA-bending proteins (55, 63, 64, 153) or by curved DNA (63, 112, 141). Other factors that facilitate interactions between proteins bound to distant sites may lack sequence specificity, as is the case for the bacterial histone-like proteins HU or, in eukaryotes, the high-mobility group of histone-like proteins such as HMG1 (see below).

The existence of auxiliary proteins capable of bending the DNA appears to be particularly useful in eukaryotic genomes, where distances between promoters and regulatory sites are frequently measured in kilobases. This is probably one of the many roles of the nucleosomes, the organization of which brings into physical proximity sequences that otherwise would be far apart. This concept has growing experimental support. For example, activation of the promoter of the *hsp26* gene of *Drosophila* requires that two binding sites for the heat-shock transcription factor are brought together by an intervening nucleosome (41, 186). Similarly, addition of reconstituted nucleosomes to a transcription system for the *Xenopus* vitellogenin gene results in a five- to ten-fold stimulation of its activity. This is due to the positioning of the nucleosomes between a distant recognition element for the estrogen receptor and a proximal promoter element that results in the enhancement of interactions between proteins bound to faraway sites (161; see below).

DNA Chaperones: DNA-Bending Proteins Working as Enzymes

One effect of DNA-bending proteins is to stabilize an otherwise loose structure in a particular conformation that is able to sustain the assembly of additional proteins into a higher-order complex. In these cases, the role of DNA-bending proteins resembles the function of certain chaperones, which stabilize polypeptides in a folding step that is critical for subsequent tridimensional assembly of the entire protein (189). Similarly, DNA-bending proteins play an early role in the assembly of nucleoprotein complexes by organizing a longer or shorter DNA segment into a curved configuration, where other proteins may later become docked. At that point, DNA-bending proteins may be displaced from the DNA by those that form the final assembly. The net effect of protein-induced bending is, therefore, that of facilitating formation of the nucleoprotein complex. For

this reason, DNA-bending proteins behave operatively in this context as virtual enzymes. To this end, the DNA chaperones should (*a*) bind the nucleotide sequences with little or no specificity, (*b*) induce bending, and finally (*c*) form complexes with the DNA unstable enough to allow their displacement by the components of the final complex (116, 189).

Proteins with DNA chaperone properties exist in both prokaryotic and eukaryotic cells. The archetype of the bacterial proteins of this class is HU, which binds to the minor groove of the DNA without apparent specificity and is able to induce an array of conformational changes in the nucleotide sequence (37, 80). In addition, HU binds to unusual DNA structures, such as cruciforms (18, 147). Although the best-known case of HU protein is that from *E. coli* (37, 114), HU equivalents have been found in virtually every bacterium in which it has been searched for (28, 76), and it has been particularly well studied in the case of *Bacillus stearothermophilus* (184) and *Bacillus subtilis* (6, 7). The eukaryotic counterpart of HU (14) appears to be the chromatin-associated proteins that belong to the high mobility group, namely HMG1 and HMG2 (11). Similarly to HU, these proteins bind and bend DNA with limited (if any) sequence selectivity (144). Both HU and HMG1/HMG2 play crucial roles not only in transcription but also in an entire collection of cellular processes, including DNA replication transposition and recombination, which is consistent with their primary role as DNA chaperones (22, 71).

Despite the functional relationships between HU proteins and HMG1/HMG2 proteins, they are very different structurally. The most salient structural motif of HU proteins is a β-sheet protruding from the rest of the protein in an arm-like configuration (114, 184), whereas the DNA-binding domain of HMG1 consists of three L-shaped α-helices (151, 198). These structural differences hint at a case of divergent evolution for the same function as DNA chaperones. Interestingly, the presence of HMG-type DNA binding domains has been reported in the transcriptional regulator CarD of *Myxococcus xantus* (117), thus suggesting that there is not a barrier for these structural motifs in the eukaryotic and prokaryotic worlds. Along the same line, the yeast mitochondrial histone HM has been suggested as the missing link between bacterial HU and the nuclear HMG1 proteins (108).

According to the view that DNA chaperones act as enzyme-like elements, transient complexes between these proteins and DNA would be finally displaced by the components of the final higher-order structure. This notion has been directly tested in the assembly of the nucleoprotein complex involved in the Hin invertasome, which mediates site-specific gene inversion in *Salmonella* (129), and in which the structural role of the IHF protein could be substituted by either HU or HMG1 proteins. However, while it was possible to detect the presence of IHF in the final complex, DNase I footprinting failed to reveal HU bound to DNA. In fact, only the use of very sensitive agents for detection of

short-lived complexes could indicate interactions of HU and the target DNA (99). These results suggest that in contrast to IHF, which acts as an authentic structural element to sustain the complex, the role of DNA chaperones is mostly transient.

The cellular functions of HU/HMG1-like chaperones are not restricted to being catalysts of protein-protein interactions; they seem also to stimulate DNA-protein interactions, at least in some cases. For instance, binding of the CAP protein and the LacI repressor to their target sites can be enhanced 10- to 20-fold by the HU protein in vitro, although HU does not appear to be present in the final DNA-protein complex (49). Similarly, HU assists the binding of IHF to the *E. coli* origin of chromosomal replication, *oriC* (17), in a remarkable case where the effect of HU is not to replace IHF (see next section) but to increase its binding to a target DNA.

Functional Substitutions Between DNA-Bending Proteins

An important property of HU- and HMG1-type DNA chaperones is their ability to replace, at least in part, specific DNA-bending proteins at their target sites. Such an ability was first tested in the assembly of the protein complex termed intasome, which mediates site-specific integration of the λ phage to the bacterial chromosome (64), in which IHF could be replaced by HU. More recently, functional substitutions of IHF by HU have been extended to promoters in which IHF acts as either a negative or positive coregulator by virtue of its structural effect on binding and bending DNA. In one case, both HU and HMG1 could substitute IHF in vitro during the formation of the repression complex of the Mu phage (13). A more intriguing case is the substitution of IHF by HU in the coactivation of σ^{54}-dependent promoters (20, 133, 135). This is one of the few cases in the prokaryotic world of control at a distance, since the activators bind to upstream activating sequences (UAS) placed at considerable distances ($>$100 bp) in respect to the RNA polymerase binding site (see below). In a class of σ^{54}-dependent promoters, contacts between the enzyme and the activator bound to the UAS is facilitated by the binding and bending of IHF to a site present within the intervening region (see below). At least in two cases (20, 135), HU and HMG1 could mimic the stimulatory effect of IHF protein during transcription of these promoters in vitro. Interestingly, the substitution of IHF by HU occurs in vitro also in the σ^{54}-dependent promoter *Pu* of *Pseudomonas putida* (1, 26, 141, 131), although the replacement is not fully efficient. Furthermore, in other σ^{54}-dependent promoters lacking an IHF site, HU appears to be the authentic coregulator of the system (20, 133). The corollary to these observations is that the functional substitution of IHF by HU in vivo and in vitro for coactivation of this class of promoters (and perhaps in other systems as well) may not be simply a side effect of their intrinsic properties as DNA

chaperones. It could reflect also a functional redundancy of their activities that ensures the basal functioning of the many systems that require the concourse of static or protein-induced bending. In addition, this effect raises interesting possibilities for understanding the evolution of transcriptional control systems (27; see below).

An important issue on the redundancy between specific DNA-bending proteins and DNA chaperones is how nonspecific proteins are able to replace the bends at distinct positions. Studies on the integration of phage λ (166) suggest that although DNA chaperones (such as HU) randomly associate to DNA, only the small fraction of complexes in which the binding sites involved in the final complex are properly aligned will result in a productive nucleoprotein formation. This effect, named structural trapping, is anticipated to be more significant if the DNA site involved is already bent or prone to bend in a particular orientation; the physical properties of the sequence act then as a guiding element for curving the DNA in a certain direction. Along the same line, the mechanism of IHF-mediated stabilization of the nucleoprotein complexes that repress phage Mu (13) reveals the requirement of a such a guiding element, i.e. intrinsically bent DNA, for the preferential action of heterologous DNA chaperones (HU or HMG1) on specific DNA segments.

CONSEQUENCES OF DNA BENDING

Channeling Signals Through Promoter Architecture

An important regulatory consequence of the requirement for proteins acting as architectural elements is that they become regulatory assets through their ability to sustain or inhibit an active promoter configuration. In this way, regulation assisted by protein-induced DNA bending allows the superimposition of different regulatory levels for the control of a single promoter. This is particularly useful for individual prokaryotic promoters, where their relatively simple organization and the few protein targets available (as compared to their eukaryotic counterparts) would not allow responses to simultaneous signals unless coregulation elements such as DNA bending are present in the system.

CAP AS AN ARCHITECTURAL ELEMENT An archetypical case of the proteins that channel environmental signals into promoters by altering their geometry is that of CAP (cAMP receptor protein or CRP) of *E. coli*, some aspects of which have been discussed above. Along with IHF, CAP is the best-studied example of the proteins that introduce a bend at the site of interaction with DNA (39, 89). This protein has been the subject of intensive studies, because of its involvement in the phenomenon called catabolite repression, which consists of the lack of expression (i.e. lack of activation) of a number of operons when

cells face glucose in the medium. In the absence of this sugar, cAMP levels increase and dimers of the CAP-cAMP complex bind to specific sequences at target promoters, bringing about a sharp bend in the bound DNA (201). In most cases, CAP activates promoters through direct protein-protein contacts with the RNA polymerase (19) and some contribution of the upstream DNA bend caused by the binding of the regulator (see 89, 140 for reviews). However, there are promoters in which catabolite repression is just one of the various signals the system has to integrate and in which altering DNA architecture is the only possibility of entering an additional regulatory level. The diverse ways by which the DNA-bending ability of CAP enters a level of catabolite control in promoters regulated primarily by other signals is truly remarkable. Some of these systems are discussed below.

The activity of the divergent *malEp* and *malKp* promoters of the maltose operon of *E. coli* depends both on MalT, the cognate regulator of the system, and CAP (165). The intervening region between the two promoters (217 bp long) is a nearly continuous stretch of operators for both proteins, in which two series of MalT sites appear separated by various CAP sites (148). Analysis of the mechanism whereby CAP and MalT coactivate both promoters has revealed that CAP induces the repositioning of MalT within the proximal region of *malEp* and *malKp* from nonproductive but high-affinity sites to productive but low-affinity sites, which are displaced by 3 bp from the former (154). In this context, CAP promotes the assembly of the active complex by merely bending the DNA in the intervening region. This facilitates the cooperative binding of MalT and the shifting of the equilibrium toward occupation of the productive MalT sites (Figure 2). This notion has been confirmed by the successful replacement of the CAP binding sites by either intrinsically curved DNA or by IHF-binding sites (153).

Another interesting example of CAP as a coregulator is its role in the repression complex that controls expression the divergent *nagE-nagBACD* operons of *E. coli*, which are involved in the uptake and metabolism of *N*-acetylglucosamine (145). In this system (Figure 3), *nag* genes are induced by growth on *N*-acetylglucosamine or glucosamine but are also subject to catabolite control. In the absence of inducers, the repressor NagC binds to cognate sites overlapping the *nagE* and *nagB* promoters, which are themselves separated by 130 bp, thus resulting in the looping-out of the intervening sequence. A strong CAP-binding site appears positioned between the two NagC operators in such a way that CAP binding and subsequent DNA bending stabilize the loop formed between the two NagC binding sites, thus improving the repression of *nag* genes and placing the system under the control of CRP-cAMP (146).

The repressor called CytR regulates transcription initiation from a number of promoters involved in the metabolism of nucleotides in *E. coli*. Various of these

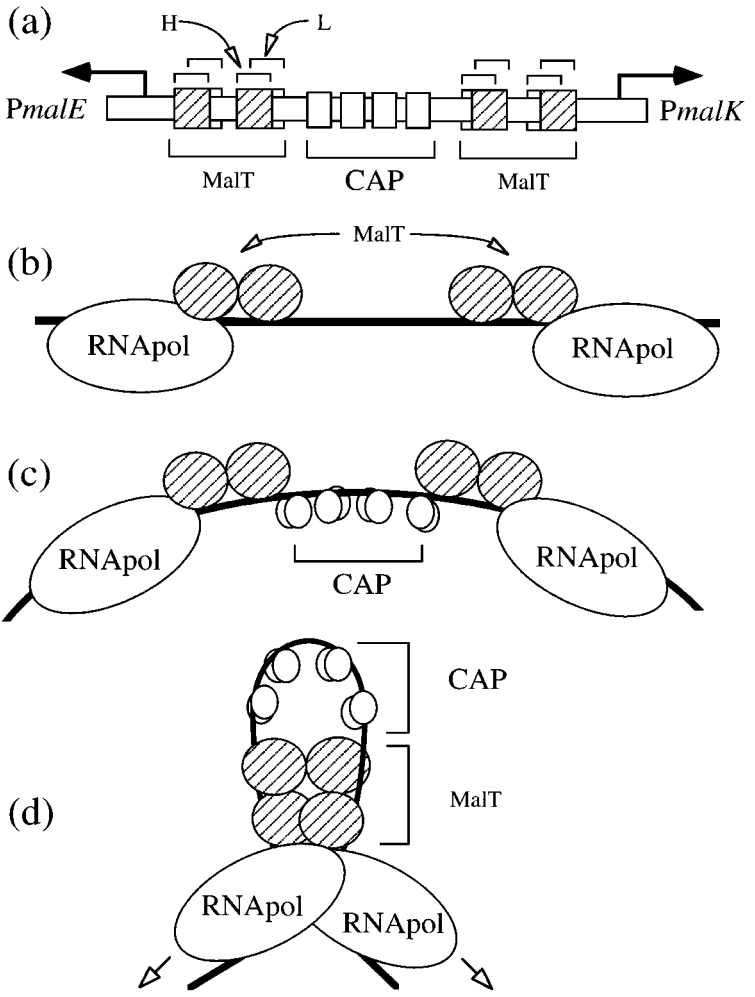

Figure 2 Activation of divergent promoters *malEp* and *malKp* of *E. coli* by MalT and CAP. (*a*) The linear arrangement of all control elements. These include catabolite gene activator protein (CAP) and MalT sites, the later bearing high-affinity (*H*, diagonal lines) or low-affinity (*L*, open) sequences for the regulator. *H* and *L* sites are separated by three nucleotides, so they overlap to an extent. All four schemes summarize the sequence of events leading to the activation of the divergent promoters in the presence of maltose and absence of glucose. To this end, both MalT and CAP must bind the region. The effect of CAP is that of bending the DNA so that MalT becomes repositioned at the productive sites (*L*) that guide its interactions with the RNA polymerase (RNApol).

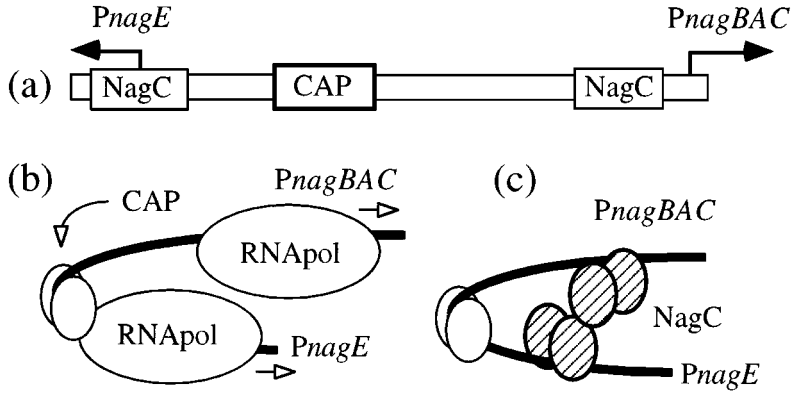

Figure 3 Assembly of a repression/activation complex at the *nag* promoters of *E. coli*. (*a*) The divergent promoter region *PnagE/PnagBAC* includes two sites for the NagC repressor, along with a catabolite gene activator protein (CAP) site more proximal to *PnagE*. (*b*) The CAP protein bound to its site activates *PnagE* by contacting the RNA polymerase (RNApol), whereas it has no effect on *PnagBAC*, which is expressed constitutively. (*c*) The DNA bend caused by CAP binding assists the formation of a repression complex that involves NagC molecules bound to distant sites and prevents the entry of the polymerase.

promoters also bear CAP sites, so the combination of CytR sites with CAP sites originates an entire repertoire of regulatory possibilities (54, 191). One case is that of the *cytR* gene promoter itself. P*cytR* is activated by CAP, the effect of which is counteracted by CytR (130). To this end, CAP binds to a region located around position −64, whereas CytR binds to sequences immediately downstream. Both proteins can bind simultaneously and cooperatively the P*cytR* promoter, a process that implies direct interactions between them. Interestingly, both proteins bend DNA at their target sites, but in different orientations, in such a way that the repression efficiency of CytR lies, at least in part, in its ability to counteract the CAP-induced bend (130). In a further level of complexity, CRP seems also to change the sequence-specificity of CytR for its target site, an effect that could be induced by protein-protein interactions between the two regulators (130a).

The control of the P*araBAD* promoter of the arabinose system of *E. coli* is one of the best-known paradigms of prokaryotic gene expression (162). 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* (Figure 4). Alternatively, AraC binds in the presence of arabinose to a complete site (*araI1/araI2*) next to −35 box of P*araBAD* (77). The repression loop

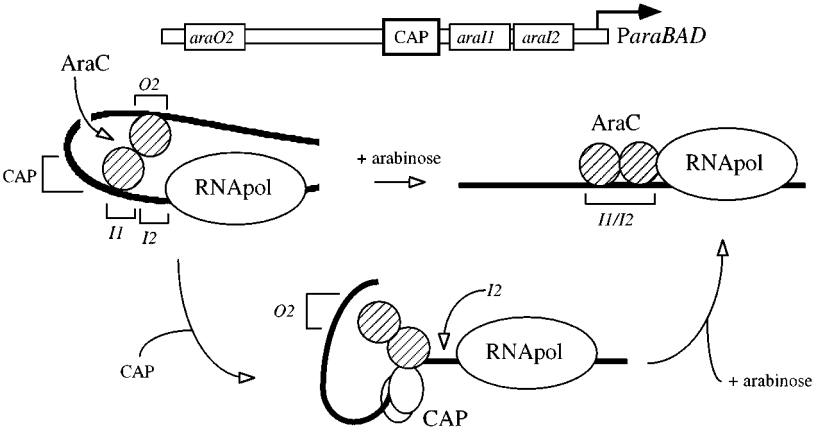

Figure 4 Antirepression of the arabinose promoter of *E. coli* by a catabolite gene activator protein (CAP). The P*araBAD* promoter region contains proximal and distal binding sites for AraC, as well as a CAP-sequence adjacent to the AraC proximal site. In the absence of arabinose, a repression loop is formed between AraC molecules bound to distant half-sites, which prevents occupation of the AraC half-site more proximal to RNA polymerase (RNApol). Arabinose destroys such a loop and allows occupation of the *I2* half-site and the ensuing AraC-RNApol contacts required for transcriptional activation. The absence of glucose further contributes to this effect, since CAP binding bends and misorients the upstream region involved in the repression loop, thereby indirectly favoring occupation of *I2*.

plays an anti-induction role, thus preventing AraC from binding to the activator site *araI1/araI2* (102). Besides the AraC-binding sites, the regulatory region includes a CAP site just upstream from the *araI1* half-operator involved in the loop. The CAP site is centered along the face of the DNA helix opposite *araI1*. It is believed that the role of the bend induced by CAP is not that of helping formation of an activating nucleoprotein complex. On the contrary, CAP inhibits the repression loop formed by AraC by misorienting the intervening sequence, thus stimulating P*araBAD* activity in conditions where glucose is not present (103).

COREGULATION OF σ^{54} PROMOTERS THROUGH DNA BENDING The positive and negative control of promoter architecture by DNA-bending proteins may become quite sophisticated in some σ^{54}-dependent promoters. These promoters are generally involved in expression of functions for adaptation to harsh metabolic and environmental situations (95), and they are unique in that the sequence recognized by the σ^{54}-RNA polymerase holoenzyme includes GG and GC doublets at positions -24 and -12, respectively, instead of the typical -10 and -35 hexamers of the σ^{70}-promoters. As mentioned above, σ^{54}-promoters

are activated at a distance (100–200 bp) by specific regulators bound to upstream enhancer-like sequences that must loop out for productive contact with the polymerase (see 94 for a review). This arrangement of elements makes the intervening DNA sequence between the binding sites for the activator (UAS) and the σ^{54}-RNA polymerase an ideal target for factors that promote or inhibit the correct promoter geometry required for activation.

In most σ^{54}-promoters examined, an IHF site is found at such intervening regions. Since the absence of IHF results in a drastic drop in promoter activity, the role of this protein in these systems appears to be to assist in the formation of a DNA loop between the UAS-bound regulators and the polymerase (1, 21, 26, 60, 62, 81). This notion is supported by the possibility of effecting functional substitutions of the IHF sites by intrinsically curved DNA in the σ^{54}-dependent promoter *Pu* of *Pseudomonas putida* (141) and the *PnifH* promoter of *Klebsiella pneumoniae* (112).

More recently, the intervening region between the UAS and $-12/-24$ of σ^{54}-dependent promoters has been shown to be the target of negative coregulation. One example of this is the down-regulation that the regulator Nac of *Klebsiella aerogenes* exerts on its own transcription. Nac is the activator of a set of genes involved in the metabolism of several amino acids, but it is also the repressor of other genes required for nitrogen assimilation in the absence of amino acids (12). Expectedly, the *nac* gene is transcribed from a σ^{54}-promoter activated by the prokaryotic enhancer-binding protein NtrC, which responds to nitrogen limitation (44). For autoregulation, Nac happens to bind to a specific site located exactly between those for σ^{54}-RNA polymerase and NtrC. Binding of the Nac protein to its target at the *nac* promoter results in the bending of the DNA, but it has no effect on the binding of either σ^{54}-RNA polymerase or NtrC to the region. These results indicate that Nac down-regulates the *nac* promoter through an antiactivation mechanism that prevents the interactions between the activator and the polymerase by misorienting the DNA in a nonproductive geometry (45).

A second example of negative coregulation of σ^{54}-systems through DNA bending is provided by the mechanism at work in the catabolite repression of some *Bacillus subtilis* promoters, which differs substantially from its counterparts in *E. coli*. For instance, expression of the levanase operon of *B. subtilis* is driven by a σ^{54}-promoter that requires the activation of the regulator LevR bound at a distant site (25, 180). In addition, transcription of the levanase genes needs the inactivation of the CcpA repressor, which mediates the catabolite control of the system (107). Since the CcpA protein binds to the intervening region between the LevR binding sites and the $-12/-24$ sequences, it has been suggested that its repressive effect lies in its ability to counteract the activator loop required for transcription of the σ^{54}-dependent promoter (107).

EUKARYOTIC COUNTERPARTS? As opposed to prokaryotic systems, the complexity of the transcriptional machinery of eukaryotes makes available a large number of potential targets for regulation. However, in these cases, the DNA is employed also as a regulatory asset to channel signals into a particular system. Protein LEF-1, a lymphoid-specific member of the family of factors containing HMG-type DNA-binding domains, binds one specific site in the center of the human TCRa enhancer (55, 57). LEF-1 cannot stimulate transcription by itself but collaborates with other enhancer-binding factors (56). On the basis of the observation that LEF-1 induces a sharp bend in the DNA helix, it has been proposed that, similarly to the prokaryotic counterparts, this protein acts as an architectural element that facilitates the interaction between the proteins bound at the sequences flanking the LEF-1 site on either site (70).

The c-*fos* promoter provides another paradigm for the role of protein-induced DNA bends in eukaryotic transcriptional regulation. One binding site for the Zn-finger protein YY1 is located in this promoter between the TATA box and the upstream site for the CREB protein, a factor that is partly responsible for promoter activation (115). In this system, YY1 bends the DNA and represses promoter function, presumably by preventing the interactions between the activator CREB protein and the components of the basal transcription machinery, in a fashion similar to that described above for the repressors in σ^{54}-dependent promoters (115).

Nucleosomes broaden the repertoire of regulatory devices available to the eukaryotic cells by employing the DNA-bending ability of the histone octamer. As mentioned above, the *Xenopus* vitellogenin B1 promoter (161) contains a nucleosome positioned between an estrogen-responsive enhancer and the binding sites for two transcription factors: the hepatocyte nuclear factor 3 (HNF3) and the nuclear factor 1 (NF1). This nucleosome creates a static loop that brings together the enhancer with the promoter proximal elements, stimulating transcription as a result. An intriguing aspect of this process is the nature of the signal(s) that guide the histones to associate with a particularly strategic position. A recent study (4) has shown that the transcription factor NF1 interacts specifically with the histone H3, thereby directing the positioning of the entire nucleosome in a particular location. These results open the possibility that proteins unable to bend DNA guide the effect of other DNA-bending proteins in order to stimulate transcription as part of an extended regulatory nucleoprotein complex.

DNA Bending and Transcriptional Promiscuity

Typically, enhancers (whether eukaryotic or prokaryotic) control transcription at a distance, in different orientations, and even located downstream from the expressed gene (75, 94). As a consequence, it can be anticipated that the same

enhancer sequence may act on various promoters. This is not necessarily a disadvantage, since a degree of nonspecificity is an evolutionary asset for adaptation to novel environments (27). At other times, however, this may become a problem, since nonspecific activation of many promoters would be deleterious (113). Therefore, it seems necessary for cells to suppress, or at least control, what has been termed transcriptional noise (27).

A number of examples can be found in the literature where transcriptional cross-regulation is restrained through structural DNA elements and DNA-bending proteins. The bacterial σ^{54}-dependent promoters provide again an example of the role of protein-induced bending in the suppression of cross-activation in vitro. As mentioned above, it is currently believed that the role of IHF in σ^{54}-promoters is to facilitate contact between the activator protein attached to the UAS and the σ^{54}-polymerase prebound to the promoter. The same loop sustained by IHF that brings the two proteins in close proximity may, at the same time, restrict the flexibility of the region. 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 (21). Santero et al (159) suggested that IHF-induced bends in σ^{54}-promoters ensure high fidelity and high efficiency of activation by the cognate regulator while disfavoring the action of heterologous activators from solution. This hypothesis has been tested in the σ^{54}-promoter *Pu* from *P. putida*. The *Pu* promoter is activated at a distance by the XylR protein (132, 134, 149). Similarly to other σ^{54}-systems (see above), the intervening DNA segment between the UAS and those for RNA polymerase binding contains an IHF site that is required for full transcriptional activity (1, 26). When placed in *E. coli* cells lacking IHF, the *Pu* promoter not only drops its activity, it can also be cross-activated by other members of the family of regulatory proteins that act in concert with σ^{54} (46, 131). Such illegitimate activation does not require the binding of the heterologous regulators to DNA and can be suppressed by bent DNA structures, either static or protein-induced, between the UAS and RNAP recognition sequence (131). The term "restrictor" has been proposed to describe this specificity-enhancing function assigned to protein-induced or static bends in σ^{54}-promoters (27, 46, 131), although the concept may have a more general value.

Indications of the restrictor effect of IHF on other σ^{54} promoters can be traced in the literature, even if the phenomenon was not identified as such at that time. For instance, Claverie-Martín & Magasanik (20a) reported that deletion of the UAS of the IHF-dependent promoter *glnHp2* of *E. coli* resulted in a promoter variant that could be activated from solution in vitro by NR_I (NtrC), but only in the absence of IHF.

Similarly, IHF inhibited transcriptional activation of *PnifH* by *Azotobacter*'s NifA on a supercoiled DNA plasmid lacking the UAS but still containing an

IHF binding site (8a). Along the same line, Kustu and coworkers detected that activation of the *PnifH* promoter of *Klebsiella* by the central domain of NifA (i.e. an active form of the protein unable to bind DNA) was inhibited by IHF (12a). On the contrary, IHF stimulated activation of the same promoter by full-size NifA (12a).

We think DNA bending has evolved to control the degree of promiscuity of certain promoters, in particular those of the σ^{54} type. This is based on the observation that some of these promoters have optimal IHF sites (i.e. have a restrictor element), that others have suboptimal binding sequences, and that others do not have IHF sites at all. This suggests that protein-induced bending provides these promoters with an additional level of control for fine-tuning their physiological activity (Figure 5). This concept is consistent with the observations made on the regulation of two σ^{54}-promoters of the TOL plasmid of *P. putida* (149). Both *Pu* and *Ps* are positively regulated by XylR, but *Pu* contains an IHF site (see above) whereas *Ps* does not. Interestingly, the requirement

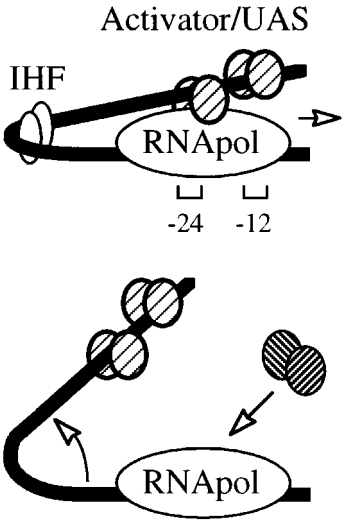

Figure 5 Restrictor effect of integration host factor (IHF) on σ^{54}-dependent promoters. Most (but not all) promoters of this class contain an IHF site placed between the binding sequences for the σ^{54}-containing RNA polymerase (bound to $-12/-24$) and the upstream activating sequences (UAS) for the cognate activator. Besides assisting the assembly of an optimal promoter geometry, IHF seems to sustain a restricted conformation of the entire promoter region that prevents an illegitimate activation of the RNApol by other regulators of the NtrC family. In the absence of IHF, some of these noncognate regulators may gain access to the promoter from solution and cause a degree of basal transcription.

for the products they transcribe are different: In the absence of *m*-xylene, the restrictor-containing *Pu* promoter remains totally silent, while the restrictor-less *Ps* promoter still has a considerable level of basal transcription, owing in part to cross-activation by other regulators of the NtrC family (46, 133). This allows growth on *m*-xylene through transcription of the upper and the *meta* operons due to the XylR-dependent activity of *Pu* and *Ps*. But also, cells can grow on *m*-toluate alone through the activity of the *meta* pathway caused by the XylR-independent transcription of XylS from *Ps*, while keeping the upper pathway totally silent. This effect is due also to the presence of a low-constitutive σ^{70} promoter in front of the *xylS* gene (149). In any case, at least in the TOL system, the presence or absence of the DNA bend caused by IHF seems to displace the responsiveness of each promoter, *Pu* and *Ps*, either toward specificity or toward promiscuity for the sake of a proper physiological balance.

One potential case of restrictor elements in eukaryotic systems seems to be present in the assembly of the three-dimensional complex of the virus-inducible enhancer of the human β-interferon gene. This enhancer is bound by the transcription factors IRF (interferon regulatory factor), by NF-κB, by ATF-2/c-Jun, and by the DNA-bending protein HMG1(Y). Within this complex, HMG-I(Y) recognizes sequences present in the center of the NF-κB site and flanking the nonadjacent ATF-2/c-Jun binding site through contacts with the minor groove of the DNA (185). Unlike IHF in σ^{54}-systems, HMG1(Y) contacts directly with the other proteins, i.e. NF-κB and ATF2, and appears to induce in them conformational changes that increase their interaction with the DNA and with each other (38). In this case, HMG1(Y) acts as an architectural protein that contributes to formation of a higher-order structure by optimizing DNA geometry and protein conformation. Interestingly, synthetic enhancers lacking HMG1(Y) binding sites were still able to activate transcription but they displayed unusually high levels of basal promoter activity and became less responsive to viral infection than the enhancer with HMG1(Y) sites. Furthermore, the synthetic enhancers were activated also by other non-viral inducers (185), thus suggesting a restrictor effect of HMG1(Y) in the system. It should be noted, however, that in this case such an effect has not been correlated with the DNA-bending ability of HMG1(Y).

DNA Bending in Response-Amplification Mechanisms

A final regulatory aspect of DNA bending is its role in the amplification of minor environmental signals into major transcriptional responses. An interesting example of this type 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 (65). *Pe* is an early promoter that drives expression of genes required for lytic growth, whereas the *Pc*

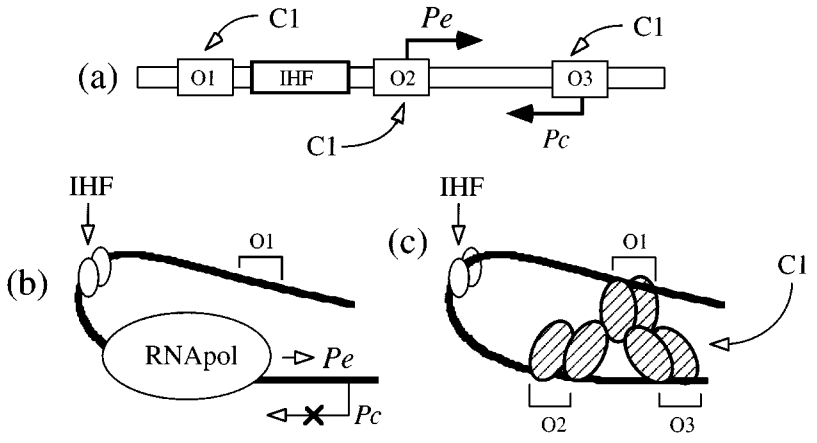

Figure 6 Stimulation of lysis/lysogeny of phage Mu by DNA bending. (*a*) The DNA segment spanning the promoter region *Pe*/*Pc* contains sites for RNA polymerase (RNApol) binding within the divergent promoters, which overlap two sites (O2 and O3) for the Mu repressor (the C1 protein). The region also contains an IHF site as well as an additional sequence for C1 binding (O1). When C1 concentration is low (*b*), the DNA bending caused by IHF stimulates transcription of lysis genes from the *Pe* promoter, while inhibiting transcription from *Pc*. IHF also assists in relieving the structural repression caused by H-NS on the *Pe* promoter. On the other hand, an increase in C1 levels leads to the formation of a repression complex (*c*) that includes C1 molecules bound to distant sites. This complex is assembled with the structural assistance of the same DNA bend produced by IHF.

promoter transcribes the gene *c1* for the Mu repressor (Figure 6). Normally, this repressor prevents transcription from both *Pe* and *Pc* by binding to cognate sites that overlap in each case the respective promoter sequences, thereby fixing the phage in a lysogenic state. Although the two promoters (along with their cognate operator sequences for the repressor, O1 and O2) are separated within the Mu genome, the repressor must bind both of them simultaneously to shut down transcription from *Pe*. Binding of the repressor to O1 and O2 sites is very cooperative and seems to occur simultaneously rather than sequentially. As is seen in many other cases, bringing two distant elements into close proximity must involve 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 the binding of IHF to a specific site also present in the sequence. In turn, IHF binding results in a tighter binding of the repressor proteins to O1 and O2 and, therefore, in a decrease of promoter activity. This effect is due to the stabilization by IHF of a loop that engages the occupied O1 and O2 sites, allowing protein-protein interactions between repressor molecules (3, 13, 52).

However, in the absence of Mu repressor, IHF stimulates transcription from *Pe* and inhibits transcription from *Pc*, thus favoring lytic growth (91). Therefore, depending on the status of the repressor, IHF-induced bends may stimulate either lysis by activating *Pe* or lysogeny through stabilization of repressor complexes. 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, mainly those affecting repressor concentration. Recent observations (193) indicate that under conditions of lytic growth, IHF assists also to relieve the repression of the *Pe* promoter caused by the histone-like protein H-NS. Since H-NS is involved in the maintenance of chromosomal superhelicity (see below), the antagonistic effect of IHF could be one way to bring the system under one more coregulation check.

CHROMOSOME ORGANIZATION AND DNA STRUCTURES

The chromosome of *E. coli* is a single, supercoiled circular molecule of about 4.7 Mb (i.e. about 1 mm long) that is condensed into a nucleoid structure. It has been generally believed (143, 200) that such a nucleoid was organized in large superhelical independent domains, the structure of which is influenced by the action of topoisomerases and the binding of histone-like proteins. There is increasing evidence, however, that the building of a higher-order chromosomal structure is basically a stochastic process (79). Various attempts have been made (111, 129a, 172) to identify in vivo regions of differential supercoiling in the chromosome of *E. coli* by using genetic probes (transposons or minitransposons) bearing *lacZ* fusions to supercoiling-responsive promoters such as those for the genes of the DNA gyrase subunits (*gyrA* and *gyrB*). Every study has consistently reported that the variable expression of promoters placed at different positions in the chromosome is due exclusively to their proximity to the replication origin and not to the effect of differential supercoiling. Although barriers to supercoil diffusion exist (200), the DNA segments engaged in formation of superhelical domains vary from cell to cell or within one cell over time (79). Therefore, a sequence-specific overall architecture of the bacterial chromosome does not seem to exist as such (at least in *E. coli* and *Salmonella*).

DNA Supercoiling and Coregulation of Gene Expression

At a more local level, however, the chromosomal context and the physical structure of the DNA region can influence the architecture of transcription complexes. Supercoiled DNA appears in vitro mostly as a rod of two interwound duplex chains (2, 15). This configuration predicts the appearance of curved apices in which the DNA folds back upon itself. Obviously, such apices are favored to

become coincident with intrinsically or protein-induced bent DNA sequences (97). The tendency of bent DNA to nucleate DNA folds in supercoiled DNA suggests an interesting link between DNA bending, supercoiling, and the assembly of transcription complexes, because the assembly of productive geometries may be enhanced or inhibited by supercoiling (152). Since chromosomal superhelicity varies during growth depending on the ATP/ADP ratio (194), the resulting changes in local DNA geometry might be a mechanism of sensing physiological conditions and channeling such general signals into specific promoters. In this respect, it is interesting to note that the movement of interwound supercoils might be affected by sequence-specific and sequence-nonspecific, nucleoid-associated DNA-binding/bending proteins such as HU, IHF, FIS (factor for inversion stimulation), and H-NS (79; see below). Furthermore, there is a change in the abundance of these proteins along the growth curve (9, 10, 30, 32), suggesting that the interplay between supercoiling and DNA-bending proteins provides a major level of gene expression control in connection with the physiological status of the cells. Another level of general metabolic control of promoter activity could be related to the overall effect that internal K^+ glutamate pools could have on the affinity of DNA-binding proteins to chromosomal DNA. It has been reported that the external levels of ammonium determine not only the regulation of nitrogen-related genes in *Salmonella*, they also alter drastically the internal pool of potassium glutamate (201a). It is tempting to speculate that, in turn, this may influence the binding of an entire collection of regulatory DNA-bending and nonbending proteins to their respective targets. In this way, such a major metabolic condition (nitrogen limitation) could end up having an overall effect on virtually every promoter.

Nucleoid-Associated Proteins and DNA Bending

It is remarkable that most bacterial proteins known to bend the DNA are generally associated with the nucleoid, thus suggesting the existence of nucleosome-like structures in the prokaryotic chromosome. The best-studied proteins of this type in *E. coli* (that many authors design generically as histone-like proteins) include not only the above-mentioned HU and IHF factors, but also H-NS and an entire collection of additional small proteins able to bind and/or bend DNA with a variable degree of sequence specificity. These include FIS (47), LRP (leucin-responsive protein; 24), and Dps (DNA-binding protein for starved cells; 5) and their functional equivalents in other genera (76, 168). In this section, we deal exclusively with aspects of some histone-like proteins that are relevant to this article.

One major class of nucleoid-associated proteins includes HU and IHF of *E. coli* (and their functional equivalents in many other genera), as well as the TF1 protein of *Bacillus* phage SP01. The structure and properties of HU and

IHF have been reviewed (50, 114), and some of their coregulation roles are addressed above. The HU protein (158) is a heterodimer of two very similar, but nonidentical, subunits of 9.5 kDa in *E. coli* and *Salmonella typhimurium* but a homodimer of similar size in many other prokaryotic species (28, 163). Interestingly, IHF homodimers of *E. coli* are not synthesized in an active form, whereas HU homodimers are perfectly active, suggesting that bacteria of this genus can tolerate the loss of one of the two HU genes (called *hupA* and *hupB*). In addition, HU displays a significant structural homology with IHF, which makes possible the construction of chimeric active HU-IHF hybrids (61, 67). However, as mentioned above, HU binds DNA, apparently without much sequence specificity, increasing the flexibility of the bound DNA and thus facilitating formation of bent structures (80, 105, 169). It is tempting to speculate on the evolutionary meaning of such similarities in light of the various observations (see above) on the functional substitutions of IHF by HU in vitro and in vivo. It looks as if bacterial cells had evolved a hierarchy of nucleoid-associated factors that cover all needs of DNA bending, with each spanning a different range of sequence specificity, so that in case of need the less specific ones act as functional backups of the more specific ones. An extreme case of specificity would be IHF, which requires the assembly of its two subunits for activity in vitro. In the absence of IHF, HU takes over the basic cellular needs for DNA bending, albeit less efficiently. But even if one of the two HU subunits fails, the other can still work by forming homodimers. The pleiotropism of HU mutations (126) and the presence of HU-like proteins in many bacterial genera (76) pinpoints this type of protein as one key element of overall chromosome functioning. The properties of TF1 are similar to those of IHF and HU, but this factor binds preferentially to SP01 virus DNA, which has 5-hydroxymethyl-uracil instead of thymine (83), thereby showing a strong binding preference for sites at the SPO1 genome (69). TF1 bends sharply its target sequence (164), thus resulting in the preferential packaging of the viral genome into phage heads, although a role in the transcription of SP01 promoters has not been proved.

Recent work made with IHF has revealed two novel aspects of the protein that are important for its role as a global coregulator of transcriptional activity and that could be extended to other nucleoid proteins. One of them is its responsiveness to temperature. Giladi et al (58) have shown that IHF binds to its target sequence at the λP_L promoter more avidly at low temperatures, thus resulting in an increase in transcriptional activity in that particular promoter, where IHF acts directly on the RNA polymerase. If, in general, IHF binding to DNA is dependent on temperature (as with many other proteins), it is likely that such environmental signals can be channeled into every nucleoprotein complex where IHF acts as an auxiliary factor (see above), thus imposing a level of physiological control on the activity of individual promoters. Another interesting

finding is the ability of IHF to activate at a distance (−80/−100 nucleotides) the *ilvPG* promoter of *E. coli* in the absence of any protein-protein interaction, even when the relative orientation of the IHF-caused bend and the RNA polymerase is reversed (127). That the effect is not observed in a linear template suggests that the combination of negative supercoiling and a severe distant bend causes a conformational change at the downstream promoter site that facilitates transition from a closed to an open complex. IHF can be replaced in the same system by the eukaryotic protein LEF-1 (see above) along with a cognate site, which indicates that besides architectural effects, DNA-bending proteins may coregulate transcription of certain promoters through a structural transmission event separate from their action on DNA geometry.

Another major nucleoid-associated protein is H-NS, an abundant neutral histone-like polypeptide of 15.5 kDa (37, 82, 142, 163). H-NS is not a strong DNA bender, but it does bind and stabilize curved structures without much sequence specificity. H-NS participates actively in the control of chromosomal supercoiling (78, 82) and seems to perform as a general transcriptional silencer (8, 43, 84, 119, 122, 171, 187), acting sometimes over long DNA segments. Since changes in growth conditions (temperature, osmolarity, nutrients) determine DNA topology (30, 82), H-NS protein may bind to local DNA structures characteristic of a certain physiological status, thus activating subsets of genes and silencing others by virtue of its effect on promoter geometry.

FIS is another small (98 amino acids) nucleoid-associated, basic and dimeric protein, which is involved in a whole set of recombination, replication, and transcription reactions. Although it is structurally unrelated to IHF or HU, it shares some of their properties, in particular the ability to bend sharply target DNA, albeit with a relatively low sequence specificity. The role of FIS in transcription is best known for its direct stimulation of ribosomal promoters (51, 68, 100, 151, 203). However, its activity on events unrelated to transcription, such as the stimulation of recombination by DNA invertases (47, 88), indicated also a role as an architectural element in the assembly of nucleoprotein complexes. Similarly to IHF, FIS levels in *E. coli* vary dramatically in response to nutritional conditions, but in an opposite direction: While IHF increases its intracellular concentration at the onset of the stationary phase (9, 32), FIS levels become undetectable during slow growth but quickly reach $>30{,}000$ dimers per cell when bacteria are shifted to rapid growth in rich medium (10, 76).

Another ubiquitous DNA-bending factor in *E. coli* is the leucine-responsive regulatory protein (Lrp), which is increasingly recognized as a general regulator of gene expression (24). Similarly to the other nucleoid-associated proteins, Lrp activates the expression of some operons and represses the activity of others (96, 192), and it seems to bind and bend target sequences located at various sites within the corresponding promoters with a loose specificity (197). Lrp

is a homodimer (19 kDa each subunit), thus resembling various histone-like proteins. Similarly to IHF, Lrp can regulate transcription through the specific activation of a large collection of individual promoters and by affecting the maintenance of chromosome structure (24, 122).

REP and RIP Sequences

REP sequences, also termed PU (palindromic units), are conserved repetitive extragenic palindromic sequences that account for as much as 1% of the *E. coli* chromosome (104). A REP sequence consists of 30–40 bp arranged as an imperfect inverted repeat separated by a loop of up to 4 bp. They appear in clusters, and over 100 of them have been located on the *E. coli* chromosome (31). Their function is not very clear. They stabilize certain transcripts (177), but they can also play a role in chromosome folding (59). Interestingly, HU stimulates the binding of DNA gyrase to REP sequences (202). Successive REPs within a REP cluster always alternate with respect to their orientation. REP clusters contain additional conserved DNA motifs and were labeled BIMEs (bacterial interspersed mosaic elements). The motifs of the REP clusters allow BIMEs to be divided into classes. One particular motif (called L) is particularly well conserved. Oppenheim et al showed (121) that such an L motif is, in fact, an IHF-binding site. There is, therefore, a subclass of REP clusters (designed RIP sequences) in which two REP elements are separated by a single IHF recognition site. There seem to be about 70 of these RIP elements in the *E. coli* chromosome (121). Although they have not been found in other microorganisms, it is possible that the IHF-binding site can be replaced by another DNA-bending protein or by intrinsic DNA bends. Interestingly, all RIP sequences occur in transcribed but not translated regions of the chromosome (i.e. at the untranslated 3′ ends of mRNAs), suggesting a role in influencing transcript stability. It is possible also that the RIP elements are DNA gyrase-binding sites that help to maintain negative superhelicity in these regions. This would add one more function to the many roles of a simple protein such as IHF, whose only special characteristic is that of bending sharply DNA at distinct sites.

EPILOGUE

A consistent body of evidence supports the concept that static and protein-induced DNA bending has allowed prokaryotic cells to evolve complex signal integration devices for environmental inputs. One raison d'être of regulatory cascades is the possibility of insertion of additional checks, either positive or negative, at every stage of the signal transduction pathway. Since, unlike eukaryotes, the number of channels to enter physiological signals in prokaryotic promoters is limited, bacteria seem to have thoroughly exploited the physical

properties of DNA to accomplish additional levels of regulation through changes in promoter architecture. The major consequence of DNA bending in transcriptional regulation is, therefore, that promoter geometry becomes an authentic regulatory asset. In this way, bacteria afford multiple metabolic control levels by just altering promoter architecture, so that positive signals favor an optimal assembly of protein-protein and protein-DNA contacts required for activation, while negative inputs prevent such a productive geometry. Additional effects of regulated DNA bending in prokaryotic promoters include the possibility of amplifying physiological signals and also of increasing or decreasing promoter specificity or promiscuity for cognate regulators. Both aspects appear to play an important role in evolution and adaptation to novel environments.

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