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DNA Looping

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INTRODUCTION

DNA contains coding information for the entire repertoire of proteins produced in a particular organism. However, the specific constellation of proteins synthesized varies over the lifetime of an organism and with environmental circumstances; this variation is generated by a diverse set of regulatory mechanisms that govern the recombination of DNA, production and processing of mRNA, and subsequent translation of nucleotide sequence into amino acid sequence. Although the regulatory systems may appear complex, these mechanisms derive from a combination of fundamentally simple strategies. One major target of control is initiation of transcription by RNA polymerase, as regulating this step affords the greatest economy in energy expenditure by the cell. The complexity in control systems that regulate mRNA initiation is derived from two basic mechanisms: positive control, in which initiation of transcription is facilitated (28), and negative control, in which initiation is precluded (53). In both cases, the effect is mediated by the interaction of proteins specific for cognate sites on the DNA, and a complex network of interactions that ultimately determines the degree of transcription of a particular sequence in a temporal and tissue-specific manner can be generated. Modulation is introduced by effector molecules that alter DNA recognition by a specific protein; for example, either a negative regulator can be activated to bind its cognate site or binding can be disrupted by interaction with a cellular metabolite (e.g., *trp* repressor binding to tryptophan to form the complex with high affinity for its DNA site or *lac* repressor binding to allolactose to yield a complex that exhibits low specificity in DNA binding).

Interwoven with the recognition of specific DNA sites by regulatory proteins is the capacity of these proteins to form oligomer species and to interact with one another. Thus, cooperative interactions at adjacent sites can influence regulatory outcome, and interactions of subunits or different proteins bound at remote sites on the DNA can form loops of intervening DNA that affect transcriptional status. DNA looping can be intimately related to other mechanisms by which distant sites within a DNA molecule can affect one another (113). Looped structures are clearly important in the effects of enhancer sequences found in eukaryotic organisms (80), and these assemblies also have a profound influence on transcriptional regulation in prokaryotic organisms. DNA loop formation has significance for proteins that may ultimately bind at a single site, as the formation of loops may result in transfer of the protein from one segment to another and thereby facilitate the search for the target site within the DNA. Several reviews provide different perspectives on the evidence for and requirements of DNA-looping mechanisms (1, 10, 33, 86, 92, 93, 113).

Although the influence of enhancers on transcriptional initiation at promoter sites many kilobases removed in the primary sequence of the DNA was well documented (reviewed in reference 80), experimental evidence for the importance of DNA looping in transcriptional regulation came initially from studies in prokaryotic systems, as indicated in the following discussion (25, 42). More recently, evidence has accumulated that loop formation is involved not only in regulating transcription but also in other processes involving DNA and proteins. This review is not intended as an exhaustive survey of the literature, but, rather, the objective is to present an overview of the wide variety of systems and processes in which DNA looping has been demonstrated as an important regulatory feature, from the first systems that suggested that this structure was involved in transcriptional regulation to recent work that demonstrates the importance of looping in all aspects of genetic expression, replication, and recombination.

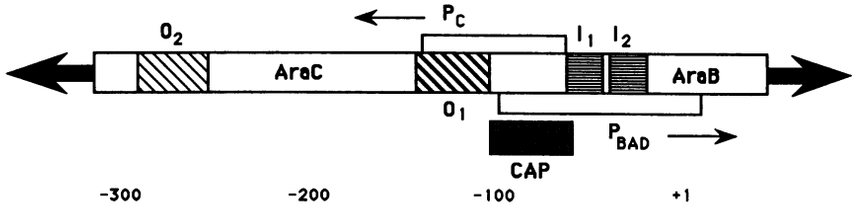

FIG. 1. Arabinose operon regulatory region. The organization of the regulatory sequences in the arabinose operon involves divergent transcription of *araC* and *araBAD* from a common control region utilizing the P_C and P_{BAD} promoters (38, 48, 63). AraC recognition sequences are indicated as O_1, O_2, I_1, and I_2. The cAMP-CAP site is also indicated. Loop formation between O_2 and I_1 negatively regulates expression from both P_{BAD} and P_C. The presence of arabinose disrupts the O_2/I_1 loop, allows loop formation between O_2 and O_1 to repress AraC mRNA synthesis, and results in occupation of the two *araI* sites to promote transcription from P_{BAD}. Numbers indicate the distance from the transcriptional start site for *araBAD* mRNA. Small arrows indicate the direction of transcription.

PROKARYOTIC SYSTEMS

ara Operon

Early evidence to suggest that looped DNA structures might be generated as a part of the regulatory mechanism for transcriptional initiation came from studies of the *ara* operon (25, 36, 37). This operon encodes proteins that are involved in the transport and metabolism of arabinose as an energy source. Regulation of mRNA production for the proteins in this operon involves DNA recognition at multiple operator sites by the AraC protein (Fig. 1), with its regulatory effect (positive or negative) depending upon the specific sites occupied, a function in turn influenced by arabinose itself (25, 36–38, 48, 63, 65, 70, 94). The *araC/araBAD* promoter region and associated structural genes form a complex transcriptional unit with an elaborate control system. Transcription of *araBAD* mRNA, encoding proteins involved in metabolism, is positively regulated by AraC more than 100-fold in the presence of arabinose, but negatively regulated by AraC in the absence of arabinose. AraC negatively autoregulates production of its own mRNA without regard to the presence of sugar. Two sites separated by ca. 210 bp (designated $araO_2$ and *araI*) are required for the negative regulatory activity of AraC and were originally detected by deletion mutation and DNase/methylation protection studies (25). These studies also demonstrated that insertions of bases corresponding to half-integral turns of the DNA impaired repression of *araBAD* expression, whereas insertions of integral turns did not affect function (25). From these results, it was evident that protein occupation of sites on the same side of the DNA, a necessary criterion for DNA looping of short segments, was important for the repressor function of AraC. Footprinting in vivo indicated occupancy of both the $araO_2$ and *araI* (later shown to be a subsite, $araI_1$) sites by AraC protein in the presence and absence of arabinose (70), corresponding to the regions known to be required for repression of *araBAD*. In addition, this regulatory loop prevents synthesis of mRNA encoding the AraC protein (autoregulation) (38). Repression of AraC in the presence of arabinose involves a loop between $araO_2$ and $araO_1$ (38, 48, 65). Supercoiling of the DNA is required for loop formation as demonstrated in vitro; linear DNA did not exhibit cooperative binding to distant sites (37, 65).

The use of alternative DNA loops and adjacent binding for regulation in the arabinose operon serves as an example of complexity generated from a fundamentally simple strategy: the binding of protein to specific sites on DNA. The "unliganded" AraC protein forms a negative repression loop between $araO_2$ and $araI_1$, inhibiting *araBAD* expression; the presence of sugar disrupts the $araO_2$-$araI_1$ loop, and arabinose-AraC complex binds to two sites within *araI*, $araI_1$ and $araI_2$, to activate transcription of *araBAD* (38, 48, 63, 65). Autoregulation of *araC* mRNA synthesis involves the $araO_2$-$araI_1$ loop in the absence of sugar and the $araO_1$-$araO_2$ loop in the presence and absence of sugar (38, 48, 63, 65). The relative occupancy of each of these sites determines the extent of mRNA synthesis from each promoter, and arabinose modulates binding to a subset of these target DNA sequences. Interaction of AraC protein at multiple sites is thus an essential feature of its regulatory activity. AraC mutants that are truncated have been found to activate transcription even in the absence of arabinose, suggesting that the conformational change elicited by arabinose exposes an otherwise shielded activation domain (72). The inability of these truncated mutant proteins to repress, despite demonstrated binding to both operator sequences $araO_2$ and $araO_1$, suggests that binding alone is not sufficient for precluding mRNA synthesis and that loop formation mediated by protein-protein interactions that involve missing regions of these mutant proteins is required for regulation (72).

The *ara* operon regulatory network is further complicated by the participation of the cyclic AMP (cAMP) receptor/activator protein (CAP) in positive regulation of the expression of *araBAD* and *araC* (66, 101). Evidence from in vitro studies indicates that the cAMP-CAP complex destabilizes the $araO_2$-$araI_1$ loop in an orientation- and distance-dependent manner (66). This disruption may result from the bending of DNA (presumably in a direction not conducive to loop formation) elicited by cAMP-CAP binding (120). cAMP-CAP has been shown to facilitate the closure of linear DNA containing its target site; this ring formation does not occur in the absence of the protein and derives from the bending induced by cAMP-CAP association rather than effects on twisting (23). Thus, cAMP-CAP binding per se does not preclude loop formation and in fact brings segments of the DNA into closer proximity. The effects of cAMP-CAP on AraC-mediated loop formation in the *ara* system suggest that the relative positions of the binding sites and the orientation of the bend with respect to these sites determines whether loop formation is favored or disrupted. This example illustrates the effect of additional DNA-binding proteins on the ability of a specific repressor or activator to form stable loops; in addition to effects on loop formation, ancillary proteins can compete for specific sites, bind to the regulator to alter its specificity or block its DNA-binding site, etc. The binding of multiple proteins to a regulatory region (either at the promoter directly or at distant sites)

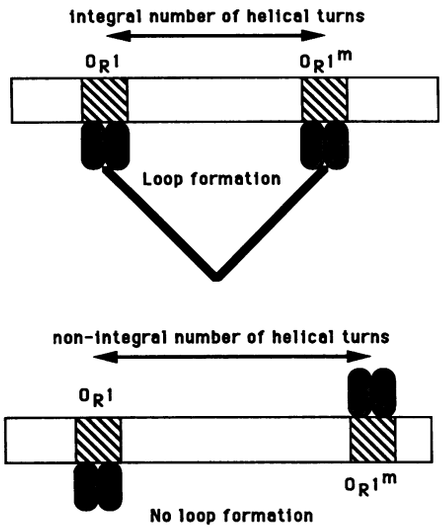

FIG. 2. Lambda repressor loop formation. Lambda repressor recognizes sites at O_R1 and O_R1^m on a single DNA and generates loops provided that the spacing between the sites is an integral number of helical turns (five to six were examined in this study [42]). Spacing that results in phasing that places the repressors on opposite sides of the helix (nonintegral turns) precludes loop formation.

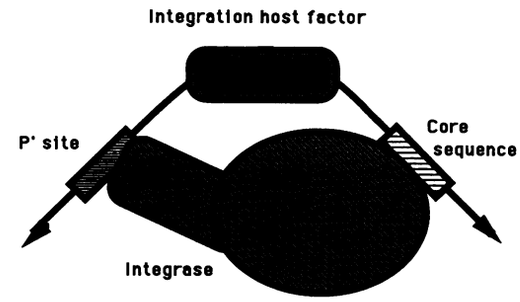

FIG. 3. Bending of DNA by integration host factor to facilitate loop formation (73). The binding of λ integration host factor results in a bent DNA; this alteration in the topology of the DNA facilitates the interaction of integrase with two sites on the DNA, the core site and the P′ site. There is no direct interaction between integration host factor and integrase in this complex. Bending of DNA can have a significant effect on the ability of proteins to form looped structures.

provides additional layers to determine the transcriptional response to environmental or organismal signals.

PHAGE REPRESSORS

Lambda repressors bind cooperatively to adjacent sites within the two operators, O_L and O_R, that control lysogenic vs lytic response (54). Hochschild and Ptashne (42) demonstrated, by using footprinting methods, that cooperative binding could also occur to two operator sites (O_R1 and O_R1^m) separated by five to six turns of the DNA helix, with loss of cooperativity when the operator centers were separated by nonintegral turns (Fig. 2). DNA loop formation was proposed to explain these results based on the dependence of cooperative binding on the presence of the protein domain that mediates subunit interactions. In addition, periodic hypersensitivity to DNase I, characteristic of backbone strain, was observed in the region between operator sites (42). Examination of these complexes by electron microscopy revealed the presence of the expected looped DNA structures, with the size of the loop corresponding to the length of the intervening sequence between operators (34). Similar loop formation has been observed with the *Salmonella* phage P22 repressor protein, with cooperative binding to sites separated by an integral number of helical turns (111). Mutant P22 repressors that exhibit defects in cooperativity for binding to both adjacent and remote sites have been isolated (111).

The ability of DNA loops to alter transcriptional activity was demonstrated by introduction of an additional operator site at six to seven helical turns away from the normal target sequence (adjacent to the promoter) at which λ repressor binding activates transcription (43). The introduction of the second operator sequence results in inhibition of repressor activation function; on the basis of both in vivo and in vitro observations, this inhibition was deduced to be associated with DNA loop formation. The demonstration that a mutant protein which bound noncooperatively to operator sites did not inhibit activation in the presence of upstream operator sequences confirmed that protein-protein interactions are crucial to DNA loop formation in the λ repressor system (43).

Another protein produced by λ phage, initiation protein O, will also form DNA loops between its recognition sites, in this case replication origin sequences, constructed to be ca. 1 kb apart (95). Looping occurs with both supercoiled and linear DNA, and a fragment of protein O is sufficient to elicit loop formation (95). It would be anticipated that torsional stress in DNA is introduced by loops; indeed, by cross-linking protein O-looped DNAs with psoralen and UV irradiation followed by denaturation, it was possible to demonstrate the increased thermostability expected for torsionally constrained sequences (95). Looping in this case is presumably related to the functional role of protein O in replication, possibly involving microloop formation at the origin. Thus, looping mechanisms not only affect transcriptional processes but also are involved more generally in DNA metabolism. Site-specific recombination in λ also involves looping of DNA by protein integrase, a monomeric protein with two independent DNA-binding domains that exhibit differing sequence specificity (73). The action of integrase is facilitated by integration host factor (Fig. 3), which bends DNA by binding at specific sites to form a complex of DNA-integrase-integration host factor. The integration host factor facilitation of integrase binding to its DNA sites (some of lower affinity) is mediated solely by alterations in the DNA structure, with no evidence for any protein-protein contact in this case (73). This example indicates that "cooperation" between proteins need not involve direct physical interaction, but the consequences of protein binding at one site can influence the interactions of a remote DNA site with a different protein. Another protein involved in site-specific recombination in λ is Xis, which is required for excisive recombination and inhibits integrative recombination (74). Xis bends DNA and forms a specific looped structure that involves both cooperative and competitive interactions at a distance between integrase, integration host factor, and Xis (74). The specific protein-DNA complex formed determines the nature of the recombination events.

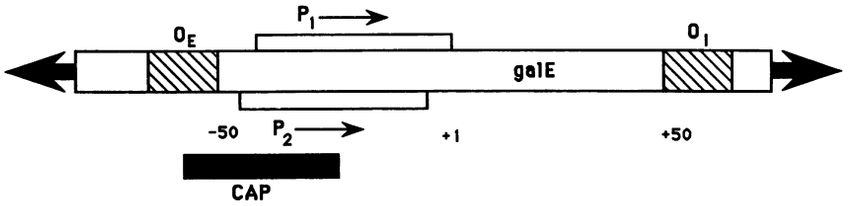

FIG. 4. Galactose operon regulatory region. Two overlapping promoters, P_1 and P_2, transcribe in the same direction. The binding site(s) for cAMP-CAP overlaps P_2 and inhibits transcription from this promoter while facilitating transcription from P_1 (1, 67). The operators are located just upstream of the promoter regions (O_E, where E stands for external) and downstream internal to the coding region for *galE* (O_I, where I stands for "internal"). The spacing between these operator sites is 114 bp. Loop formation between these sites appears to account for the observed regulatory activity, although this occurrence has not been directly demonstrated. Numbers indicate the distance from the transcriptional start site for *galETK* mRNA. Small arrows indicate the direction of transcription.

gal Operon

Two operator sequences in the *gal* operon of *Escherichia coli* were found by using a combination of genetic and sequence analysis (50, 67). One (O_E) is located immediately upstream of the two overlapping promoters in this operon, whereas the other (O_I) is found within the coding region for the first structural gene (50, 67); these sites are separated by 114 bp (Fig. 4). Binding of isolated *gal* repressor to these sequences confirmed their importance in regulation of *gal* expression, but binding data and footprinting results indicated that binding to the two sites is noncooperative (31, 68). Other studies indicated that the O_E operator alone is sufficient for negative control of the cAMP-CAP-activated promoter (P_1), whereas repression of P_2 requires both operator sequences (60). However, individual conversion of either of these two operators, O_I or O_E, to a lactose operator sequence resulted in loss of repression in vivo, despite the occupation of these sites by *gal* and *lac* repressors, respectively (35). These data suggested that both sites were required for repression and that the ability of proteins at these sites to interact, with presumed loop formation, is required for control of transcription, perhaps to deform the DNA (35). RNA polymerase and *gal* repressor can bind simultaneously to DNA, eliminating regulatory models that require competition between these proteins (61). Conversion of both *gal* operator sequences into *lac* operator sites results in repression by the tetrameric lactose repressor, whereas a dimeric mutant *lac* repressor that occupies both sites in vitro is unable to repress transcription in vivo (69), further indicating a requirement for a looped segment. Examination of DNA-protein complexes by electron microscopy confirmed the presence of DNA loops with tetrameric *lac* repressor and the absence of loops with the dimeric mutant (69). Thus, these data demonstrate directly that loop formation is requisite for repression in the *gal* operon by the *lac* repressor; these results presumably can be extrapolated to deduce that a looped DNA is responsible for repression observed with the *gal* repressor in vivo. The presence of a cAMP-CAP site in the promoter region for the *gal* operon suggests that cAMP-CAP binding and/or RNA polymerase may exert a significant influence on the ability of the *gal* repressor dimers to associate and thereby form repression loops between O_I and O_E in vivo (69). Such an effect is supported by evidence suggesting that RNA polymerase, cAMP-CAP, and *gal* repressor can each induce bending at the *gal* promoter (59, 61), although there is no direct evidence that this bending affects loop formation.

Weak cooperativity between *gal* repressor dimers in binding to two *gal* operators spaced similarly to the in vivo distance has been observed in gel retardation assays at low temperature in vitro, suggesting a weak association of dimer to tetramer (14). These studies also demonstrated that monomer-monomer interactions to form the dimeric *gal* repressor may constitute an important level of regulation, as only dimeric species can bind to DNA (14). This, specific recognition of a DNA sequence may depend not only on the thermodynamic parameters that determine its occupancy by the oligomer, but also on the relative affinity of the monomers, as binding requires the multimer. This additional level of control can be exerted by modulation of regulatory protein concentration; in cases in which mixed oligomers are found (e.g., many eukaryotic systems), relative concentrations of the species determine the sites bound and hence the regulatory outcome.

lac Operon

The lactose operon in *E. coli* was the system in which negative mechanisms of genetic regulation were first detected and examined (53). A wide range of studies of *lac* repressor binding to its target sites has provided significant insight into the mechanism of regulatory action in this system. The operon consists of the *lacI* gene, encoding the *lac* repressor protein, sites involved in transcriptional regulation of proteins involved in lactose metabolism, and the structural genes for β-galactosidase (*lacZ*), lactose permease (*lacY*), and thiogalactoside transacetylase (*lacA*). Regulation of expression of the *lac* enzymes involves interaction of repressor protein at the primary operator site (O) to prevent initiation of *lacZYA* mRNA synthesis (Fig. 5). The repressor-operator complex is destabilized by binding of specific sugar inducer molecules as a result of a conformational change that diminishes protein affinity for its DNA target site to a level comparable to nonspecific DNA sequences. The high affinity between *lac* repressor and operator suggested that this interaction alone was sufficient for the observed repression.

However, secondary operator sequences, termed pseudooperators, in the *lac* operon were identified upstream in the *lacI* gene (O_I) and downstream from the primary operator in the *lacZ* gene (O_Z) (83, 88); these sites exhibited lower affinity for the isolated repressor (119) and were presumed to be alternate binding sites. However, examination of the structure of the *lac* repressor protein had suggested the potential for two DNA-binding sites, with one potentially occupied by operator and the other by secondary operator or nonspecific sequences (55, 100). Stoichiometry measure-

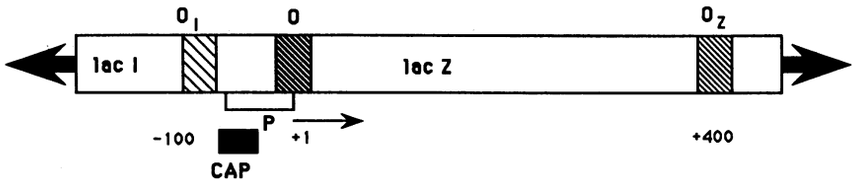

FIG. 5. Lactose operon regulatory region. The primary operator, O, is positioned over the initiation site for *lacZYA* transcription. A cAMP-CAP site is just upstream of this operator site. Two additional operator sites are found in this system, one upstream by 92 bp at the 3′ end of the *lacI* gene (O_I) and the other downstream by 401 bp within the *lacZ* gene (O_Z). Loop formation involves all three of these operator sequences in regulation. Numbers indicate the distance from the transcriptional start site for *lacZYA* mRNA. Small arrows indicate the direction of transcription.

ments of *lac* repressor with operator-containing DNAs in fact demonstrated that two operator sites on the protein could be occupied simultaneously in vitro (19, 20, 82, 117). Indeed, in vivo results demonstrated that DNAs containing pairs of remote operator sequences (either both synthetic or mutant/wild-type) result in greater transcriptional repression for the *lac* enzymes than was found for a single operator (12, 75); these results were interpreted in terms of DNA loop formation stabilizing the interaction and/or interfering with RNA polymerase initiation. The presence of additional operators in addition to a primary operator in linear DNA was found to stabilize the complex with repressor in vitro (12, 46, 58, 118); these data were also interpreted in terms of forming an intermolecular ternary complex, i.e., a looped DNA structure in which tetrameric repressor bound to both operator sequences, a deduction confirmed by electron microscopy (58). Spacing between operators in linear or relaxed DNAs significantly altered the stability of the complex with repressor in a manner consistent with the requirement for operator sites to be on the same face of the DNA (58). This periodicity in affinity of repressor for dual operator-containing DNAs also has been examined in vivo (see below) (9, 11).

Negatively supercoiling DNA containing multiple operators resulted in dramatic stabilization of in vitro repressor binding to operator, with an increase of ca. 1,000-fold in the half-life of the complex compared with the same linear DNA (13, 27, 57, 115, 116). This stabilization was found for all DNAs examined but varied with the interval between operator sites and the strength of the sites (57, 115, 116). Despite the increased stability of the repressor complex with supercoiled DNA, the presence of inducer resulted in rapid release of the operator (57, 116), consistent with rapid induction of *lacZYA* mRNA synthesis in vivo upon cellular exposure to lactose. Binding to multiple operator sequences has also been examined in vivo (13, 26, 90). The dependence on length between operator sites results in a periodic oscillation in repression (presumably directly related to repressor binding) that may be diagnostic for loop formation and has been used to deduce the helical repeat of the intervening sequence (9). Sites hypersensitive to DNA modification were noted between operator sites (O-O_I) separated by 93 bp (13), another indication of DNA deformation by loop formation. Cooperative binding involving the primary operator and secondary operators (O_I and O_Z) has been demonstrated both in vitro and in vivo (26, 27, 29, 81, 90). Repressor occupation of the primary operator (O) precludes initiation of transcription, and it has been shown that occupation of the O_Z operator sequence results in blocking of *lac* mRNA elongation (29). Thus, the O_Z operator contributes to inhibition of *lac* mRNA synthesis directly and indirectly by stabilizing repressor binding at the primary operator by forming looped DNA. The presence of a single operator results in a high level of background expression in the absence of inducer sugars, whereas the presence of at least two operators decreases this expression by 2- to 3-fold and a third operator decreases it by 50-fold to yield the characteristic expression in wild-type *E. coli* (81). Thus, the cooperative interaction of repressor with these three operators via competitive DNA loops is essential for the effective regulation observed in this system in vivo (29, 81, 90).

The importance of loop formation in the *lac* operon has been confirmed by in vivo and in vitro measurements with a dimeric mutant of the *lac* repressor (15, 81). Even with all three operator sequences intact, the mutant dimer yields repression characteristic of tetramer in the presence of a single operator site (i.e., the behavior is similar to occupation of a single site, although the secondary sites are occupied [81]); these results indicate that cooperative interaction to form tetramer is required for DNA loops, which are in turn essential to maximal repression. Comparison of tetrameric and dimeric repressor binding to operator sites in vitro by gel retardation assays indicates that dimer-tetramer association is directly responsible for loop formation via cooperative binding to multiple operator sites (15). Analysis of looped DNA complexes by using gel retardation methods has been given a theoretical treatment by Cann (16); this work facilitates the application of a convenient method to generate a detailed understanding of loop formation in a specific system.

The *lac* operon is also regulated by the cAMP-CAP complex, which binds to a site within the promoter for the *lacZYA* mRNA. Gel retardation methods have been used to determine that cAMP-CAP and repressor can bind simultaneously to the promoter-operator region and that this complex appears to involve cooperative interactions between these two regulatory proteins, one positive and one negative (47). Bending of the DNA by the cAMP-CAP complex may facilitate contact between these sites separated by only 71 bp, or the effect may be mediated by structural changes in the DNA elicited by binding either of these regulatory proteins. The *lac* repressor also binds cooperatively with RNA polymerase (102). The presence of inducer releases the inhibition to initiation, and the presence of cAMP-CAP and RNA polymerase, by virtue of cooperative interactions at the promoter, would result in rapid generation of mRNA encoding enzymes able to metabolize lactose. Competition among and cooperativity within the multiple interactions that occur at the promoter-operator region of the *lac* operon ultimately determine the transcriptional fate of the structural genes.

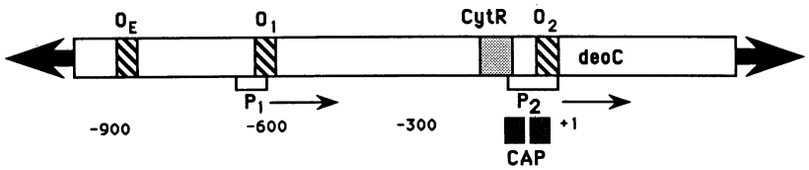

FIG. 6. *deo* operon regulatory region. Two promoters, P_1 and P_2, are used to initiate transcription of the *deoCABD* mRNA (39). The P_2 site is also regulated by cAMP-CAP binding, whereas three operator sites regulate transcriptional activity of these promoters. O_1 overlaps the P_1 promoter, and O_2 overlaps the P_2 promoter; these sites are separated by ca. 600 bp. An additional operator (O_E) is found ca. 280 bp upstream of the O_1 site. All three of these sites participate in regulation, and loop formation involving these sequences has been demonstrated (4). In addition, the CytR repressor regulates transcription from the P_2 promoter. Numbers indicate the distance from the transcriptional start site for *deoCABD* mRNA. Small arrows indicate the direction of transcription.

deo Operon

The *deo* operon encodes enzymes for nucleotide catabolism in *E. coli*, and its regulation involves negative control by DeoR repressor and CytR repressor, as well as positive control by cAMP-CAP; two promoter sites (*deo*P_1 and *deo*P_2) separated by ca. 600 bp and three DeoR repressor sites are present in this system (21, 39, 110). Two of the operator sites overlap the two identified promoter regions, while the third (O_E) is ca. 280 bp upstream of the first promoter-operator site (Fig. 6). The strongest repression in this operon derives from the DeoR repressor. Analogous to other systems examined in detail, the DeoR repressor requires multiple operator sequences for maximal repression, and regulation is diminished ca. 10-fold by deletion of O_E (110). Placement of a second operator downstream of the *deo*P_2 operator provided a slightly higher level of repression than did comparable placement of this sequence upstream (22). The degree of repression was inversely related to the distance between operators between 1200 and 4000 bp, as expected if the effect on transcription were mediated by the formation of looped DNA structures and consistent with results of DNA cyclization with segments of this size (22). It is noteworthy that these operator sites work comparably in either orientation, analogous to the effect of enhancers in eukaryotic cells.

The presence of multiple operators both increases the effective concentration of protein in the vicinity if all operators are occupied by independent protein molecules and simultaneously provides the opportunity for loop formation. The ability to compensate for operator deletion by increasing the concentration of DeoR protein in the cell (21) suggests that at least part of the effect of multiple operators is to increase the local concentration of the regulator, although loop formation has been decisively demonstrated (4). Electron-microscopic analysis has demonstrated that DeoR protein promotes single-loop formation between sets of two operators (with the size depending on the spacing), as well as double-loop formation with DNA containing three operators (4). Since the DeoR repressor is an octamer, it is theoretically possible for a single protein to bind to three operator sites to form the double loops observed by electron microscopy (4). Analogous to the additional interactions observed in the *ara*, *gal*, and *lac* operons, the CytR protein and cAMP-CAP exert their own effects on the overall expression pattern of *deoCABD* mRNA, although the details are not yet clearly defined.

Other Bacterial Systems

Nitrogen deficiency in *E. coli* results in the activation of genes whose products are involved in nitrogen metabolism from ammonia and glutamate. Expression of the *glnALG* operon is regulated at three promoters (*glnAp1*, *glnAp2*, and *glnLp*) (87). This discussion will be confined to the events which occur at *glnAp2*. Initiation at this promoter requires σ^{54} (the product of *rpoN*), NtrC (the product of *glnG*), and nitrogen deprivation (87, 103). NtrC interacts with two strong sites located 110 and 140 bp upstream from the start site of *glnAp2* promoter to increase transcription by facilitating formation of open complexes by σ^{54}-RNA polymerase (87); additionally, there are several lower-affinity NtrC sites close to the promoter region (Fig. 7). Moving the two strong sites more than 1,000 bp and changing the orientation did not alter the stimulatory action of NtrC at *glnAp2*, a characteristic resembling enhancer sequences in eukaryotic cells (87). The importance of looping in the activation function of NtrC has been indicated by the ability of this protein to activate transcription at the *glnA* promoter when its binding site is located on a separate DNA linked in a catenane structure (Fig. 8); activation does not occur when the two DNA rings are unlinked (114). In addition, electron microscopy has demonstrated the presence of looped DNA, with the length of the loop consistent with the spacing between NtrC-binding sites and the promoter (103). A similar situation exists in the xylene operon, in which the XylR protein appears to activate transcription by interacting with σ^{54}-RNA polymerase via loop formation (49).

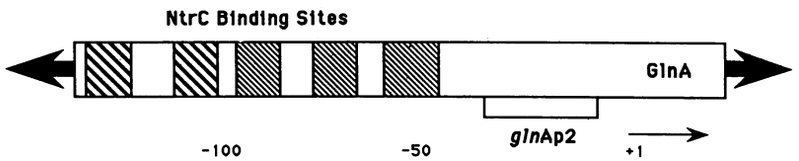

FIG. 7. NtrC regulatory region. The promoter *glnAp2* is regulated by the NtrC protein, which has five binding sites in the region ca. 150 bp upstream of the transcriptional start site (87). Two of these, indicated by dark stripes, are strong sites, whereas the three weaker sites are indicated by smaller stripes. NtrC bound at these sites interacts with the σ^{54} RNA polymerase at the *glnAp2* promoter and facilitates the initiation of transcription apparently via a looped structure.

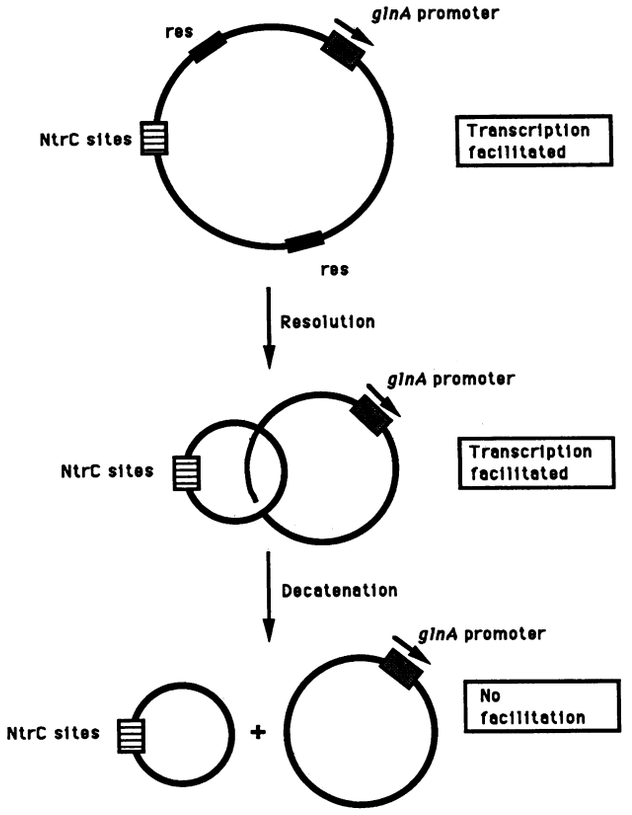

FIG. 8. Effect of catenation on NtrC regulation. A plasmid containing four NtrC sites (including the two strong sites), the *glnAp2* promoter, and resolution sites for resolvase from transposon Tn*3* was generated. Treatment of this plasmid with resolvase results in two smaller DNAs linked in a catenane. One DNA contained the NtrC sites, whereas the other included the *glnAp2* promoter. The facilitation of transcription by forming RNA polymerase open complexes was measured by using a single-cycle transcription assay. The parent plasmid containing all sites and the linked catenane DNAs exhibited NtrC-stimulated transcription, although in the latter case the sites were on separate molecules. Decatenation to produce the individual plasmid products yielded a mixture that did not facilitate transcription. Thus, the presence of the NtrC sites on a plasmid that is tethered to the DNA containing the *glnAp2* promoter is sufficient to promote transcription. These results provide strong evidence for loop formation in the action of NtrC. Adapted from reference 114 with permission (Copyright 1990, AAAS).

The *nag* operon of *E. coli* encodes genes involved in the metabolism of *N*-acetylglucosamine and has a complex regulatory region, involving two binding sites for the NagC protein with an intervening cAMP-CAP site located near the *nagE* and *nagB* divergent transcription start sites (85). The binding of NagC results in DNase I hypersensitivity between these two sites, and the cAMP-CAP–NagC ternary complex is more stable than the NagC-DNA complex alone (85). Thus, repression and activation are tightly coupled in this system, analogous in some respects to the *lac* operon. The *pap* operon, encoding proteins involved in the biogenesis of pili in *E. coli*, has a complex regulatory site with divergent transcription; three sites for the regulatory protein PapB as well as a cAMP-CAP site are present (30). This arrangement is reminiscent of the *ara* operon and suggests the involvement of multiple DNA loops in the determining gene expression in this system.

Processes involved in initiation of replication and recombination have also been shown to utilize DNA-looping mechanisms. The replication initiator protein of plasmid R6K interacts with two origin of replication sequences, either two γ sequences or an ori γ and an ori β sequence, with spacing between the γ sequences of up to 2 kb (76). In this system, techniques were developed to detect DNA looping mediated by protein molecules based on enhancement of DNA knotting and on ligase-catalyzed cyclization. The effectiveness of these methods was confirmed by electron-microscopic analysis to visualize DNA loops in the system examined (77). Physical evidence for loop formation involving the initiator protein RepA for mini-P1 plasmid replication has also been presented (18). The interaction MuA and MuB proteins of Mu phage located at different sites on the DNA has been attributed to loop formation (2).

The Hin protein in *Salmonella* spp. catalyzes the site-specific inversion of a DNA segment containing a promoter to regulate the expression of alternate flagellin genes (41) in a manner similar to bacterial phage systems, e.g., bacteriophage Mu or P1. The inversion process requires two recombination sites, *hixL* and *hixR* which are targets for Hin binding and catalysis of double-strand scission; Hin protein is covalently linked to these sites at the 5′ phosphate and is then released during religation following reorientation of the sequence. Recombination is facilitated by an enhancer sequence which contains two sites which orient two molecules of Fis protein on opposite sides of the double helix; this enhancer of recombination can be in either orientation with respect to the flagellin promoter and can facilitate recombination at greater distances from the site (up to several kilobases), although short distances (<30 bp) inhibit its activity. The structure of the catalytic complex involves proteins at four different sites and has been demonstrated by biochemical measurements and electron microscopy to have three DNA loops (41), apparently generated by Hin-Hin and Fis-Hin interactions (Fig. 9). Supercoiling of the DNA is a requirement for the inversion reaction, and relaxation of circular DNA results in a decrease in loop structures observed involving Fis-Hin, although the effects on Hin-Hin interaction were less significant (41).

The HU protein participates in a variety of DNA metabolic events, including replication, transcription, and gene regulation, and has been found to facilitate efficient recombination where the *cis*-acting sites are in close proximity. HU protein has been shown to increase the cyclization rates for short DNA fragments (44), similar to integration host factor from λ (73). This ability to stabilize increased curvature in the DNA may facilitate looping and increase interaction between remote sites that must come together for recombination.

Even endonuclease binding and cleavage can involve DNA loops; two types of target sites exist for *Nae*I endonuclease, i.e., resistant and cleavable (108). The presence of a cleavage site in a DNA, either in *cis* or in *trans*, enhances strand scission at resistant sites without affecting the apparent binding affinity for the resistant sites. Electron microscopy of DNAs containing a variable number of *Nae*I sites indicates that this enzyme can form loops only in DNAs with more than one *Nae*I site, and loop formation was reduced by competition with oligonucleotides containing the target site. The authors conclude that DNA acts as an allosteric effector for this DNA-binding protein (108). This case illustrates that

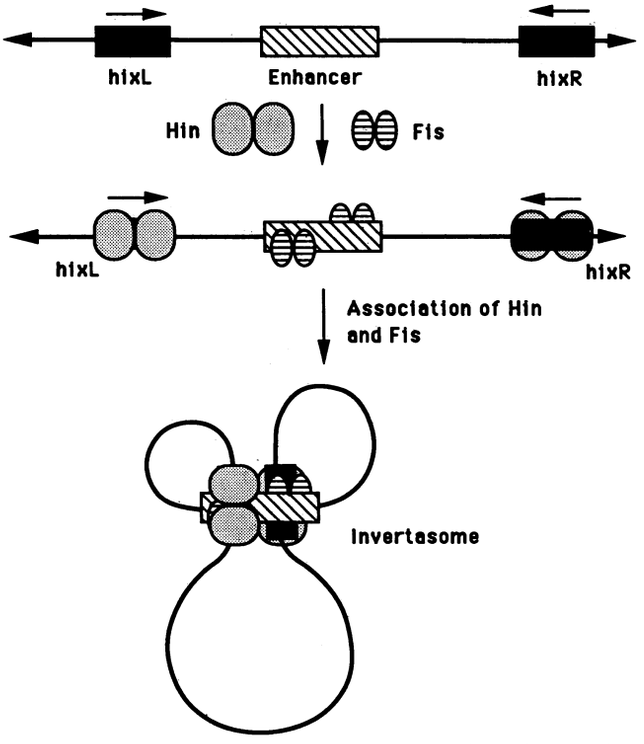

FIG. 9. Formation of the invertasome. In the inversion reaction of the flagellin genes in *Salmonella* spp., several sites are involved: two *hix* sites (*hixL* and *hixR*), which are targets for Hin protein binding, and an enhancer sequence, to which Fis protein binds. In a closed-circular DNA, the association of the Hin protein bound at different sites and the Hin protein with the Fis protein bound at the enhancer results in a trilooped DNA molecule, which has been visualized by electron microscopy and analyzed by gel electrophoresis. The structure of this complex is consistent with the data and accounts for the effects of these proteins on the inversion reaction. Adapted from reference 41 with permission (Copyright 1990, AAAS).

DNA looping is ubiquitous and can be found in any system that involves protein-DNA interaction.

Measurement of Helical Repeat in DNA

Because of the sensitivity of DNA loop formation to the distance between sites, the dependence of gene expression in vivo on the distance between regulatory sites may be used as a sensitive test of loop formation in prokaryotes (9–11). For nonintegral turns of the helix, at shorter intervals between sites, the torsional energy to place sites on the same face of the DNA diminishes (or in some cases precludes) complex formation and results in periodicity of DNA-looping behavior. The periodicity observed is related to the helical repeat in the DNA and may differ from the expected 10.4 for linear B DNA. Such variance may reflect perturbations in DNA structure elicited by protein binding, supercoiling, and sequence deformations (9, 62). The dependence of binding and hence *lac* enzyme expression in vivo on the length between *lac* operator sites has been examined in detail and exhibits the periodic behavior that may be considered diagnostic for loop formation (9). Periodicity in expression patterns has been used to deduce the helical repeat of the intervening sequence between *lac* operator sites (9) and AraC sites (62). Length dependence of *lac* repressor protein binding in vitro to dual operator-containing supercoiled DNAs of different density varied from relaxed DNA, a result interpreted as altered helical repeat in supercoiled DNA (57).

EUKARYOTIC SYSTEMS

Nucleosomes

Because DNA is found in a different physical state in eukaryotic organisms from that in prokaryotes, a brief discussion of the issues introduced by nucleosome structure and folding of DNA in eukaryotes will precede the discussion of DNA looping in these more complex systems. Nucleosomes can occur at promoter regions for specific genes and must be considered in mechanisms of activation and repression in eukaryotic systems, as transcription may require removal of these structures. Examples are the yeast *PHO5* gene (3), in which removal of four nucleosomes is required for transcription, with disassembly exposing regulatory elements for this transcription unit, and the mouse mammary tumor virus promoter response to steroid hormone receptor, in which the nucleosome structure appears to be reorganized (5, 8, 84). The packaging of DNA into nucleosomes and ultimately into chromosomes involves deformation of the backbone structure so that in nucleosomes DNA wrapping around the core histone octamer results in DNA superhelicity. The placement of nucleosomes within a particular DNA segment varies in a sequence-dependent manner, although not to the same degree as found for sequence-specific recognition by individual regulatory proteins (109). The ability to bend DNA appears to be a major determinant in positioning of nucleosomes within a sequence (109). It is interesting that sequence-dependent preferences for bending have been found to be similar between eukaryotes (e.g., nucleosome) and prokaryotes (e.g., cAMP-CAP) (109). DNA bending is requisite in looped DNAs in which significant curvature of the sugar-phosphate backbone is required; the presence of bendable versus rigid sequences in the region intervening between protein(s) bound at two sites as well as the presence of other proteins will affect the ability to form looped structures within a specific region. Therefore, proteins that alter DNA curvature (e.g., integration host factor from λ phage, histone octamer, or *E. coli* protein HU) have a significant influence on processes involving loop formation.

Transcription Factors

Transcription in eukaryotes has elements that are analogous to prokaryotic organisms, but the organization of promoter regions in general appears to be more complex, and multiple regulatory sites both in the region of the promoter and at a distance determine the transcriptional output for a particular gene. Enhancers were discovered as sequences of DNA that could significantly activate transcription from a particular promoter at a distance of >3 kb and in an orientation-independent manner (80). A wide range of enhancers have been identified, first in viruses and later as part of the eukaryotic transcriptional repertoire, and these sequences share many features with promoter sequences in eukaryotes. Both enhancer and promoter regions are composed of multiple sequence motifs that bind different transcription factors; the majority of known factors are activators, although repressors have also been identified. The potency of enhancers as activators of transcription results in

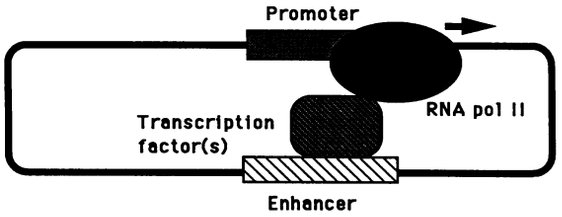

FIG. 10. Enhancer-promoter interaction via protein-mediated loop. Enhancer sequences influence initiation by RNA polymerase at promoter sequences in eukaryotic cells. These sequences function at great distances and in an orientation-independent manner. The figure is a cartoon illustrating the formation of a loop via interaction between a protein transcription factor and RNA polymerase (RNA pol II) at the promoter site. Evidence discussed in the text has been presented to suggest that this looped structure is the mechanism by which enhancers exert their transcriptional effects.

deleterious effects on an organism when these elements activate the expression of proteins normally regulated at low levels, e.g., cellular regulators that, when overproduced, results in uncontrolled cell growth (80).

Enhancers appear to act by increasing RNA polymerase binding and initiation (80), and of the several models to account for this behavior, DNA looping (Fig. 10) has found recent experimental support. Many viral systems have enhancer sequences, and these have been used to examine mechanisms of activation. One end of a linear DNA containing either the simian virus 40 or cytomegalovirus enhancer was labeled with biotin; similarly, one end of DNA containing the promoter and coding region of β-globin was labeled (79). In the absence of avidin or streptavidin, no stimulation of transcription was observed; however, in the presence of either of these proteins, which linked the two DNAs via their multivalent binding to biotin, increased transcription of β-globin mRNA was found. Examination of the DNA in the presence of streptavidin (1:1) indicated an electrophoretic size similar to that obtained with ligation of the two fragments, providing evidence that the protein linked the two fragments by binding to biotin at the end of each DNA (79). In vitro transcription of the product of 1:1 streptavidin with the two biotinylated fragments resulted in a significant increase in the amount of β-globin mRNA produced. This result indicates that the effect requires connection of DNAs, but the connection can be via a protein bridge, which would interfere with scanning mechanisms, an alternative to DNA-looping mechanisms. The most plausible interpretation of these data is that DNA looping between enhancer and promoter accounts for the effect of enhancer on β-globin transcription. A related experiment involved insertion of a complete terminator region for RNA polymerase II from the mouse β-globin^{maj} gene between the simian virus 40 enhancer and the promoter for rabbit β-globin (78). Insertion of this terminator, which would be anticipated to diminish transcription significantly in a scanning model, had no effect on enhancer activity. Again, the results support the formation of a DNA loop in transcriptional activation by enhancers. Furthermore, insertion of sequence rich in G+C content, particularly CpG and GpC dinucleotides, between enhancer and promoter resulted in diminished activation, whereas there was minimal effect on the distance between promoter and enhancer with G+C-poor sequences (up to 2.5 kb) (96). The structure of intervening DNA, i.e., its bendability, may therefore have a profound influence on the effectiveness of an enhancer sequence in increasing transcription from a particular promoter. Transcription of ribosomal genes in *Xenopus laevis* is stimulated by an enhancer located in *cis* but not in *trans*; catenation of DNAs containing promoter and enhancer on separate molecules resulted in enhanced transcription in vivo (24). This experiment is analogous to the NtrC experiment illustrated in Fig. 8 (114) and was interpreted in terms of DNA loop formation or the effect of increased local concentration of transcription factors near the promoter (24).

E2 is a transactivator from bovine papillomavirus that binds to its cognate DNA sequence as a dimer (56, 105). Maximal activation requires the presence of two E2 enhancer sites in close proximity to the promoter, while five sites are required at a distance; however, the presence of a single E2 site near the 5′ end of the initiation site increased the efficacy of E2 activation from distant sites, indicative of loop formation mediated by E2 itself (105). Indeed, E2 dimers exhibit cooperativity in binding to DNA, and stable loops are observed by electron microscopy (56). For DNAs containing three E2 sites, double-looped structures occur, and naturally occurring truncated forms of the protein that serve as repressors of transcription can bind to E2 sites in DNA but cannot form loops (56). These data strongly support the DNA-looping model for enhancer effects on transcription. Dimerization motifs have been noted in other proteins that mediate the effects of enhancer sequences on specific promoter sequences, e.g., immunoglobulin heavy chain in B cells (89), and homo- and heterooligomer formation between subunits bound to separate sites to generate intervening DNA loops provides an effective mechanism for transmitting enhancer effects. Looping mechanisms for enhancer action are also consistent with the distance dependence of action of a strongly activating GAL4 derivative which binds to its target site in a DNA and activates RNA polymerase II transcription 1.3 kb upstream and 0.32 kb downstream in nuclear extracts (17). Looping mechanisms may not universally account for enhancer activity, but the experimental evidence indicates that DNA looping is a major factor in several examples of transcriptional enhancement.

Steroid Hormone Receptors and Homeodomain Proteins

Evidence has accumulated for the importance of dimerization in the reversible *trans*-activation function of steroid hormone receptor proteins (7, 8). These protein-protein contacts can involve adjacent sequences in cooperative binding as well as connecting remote sequences to yield looped DNA structures. Protein-protein cooperativity can be homotypic or heterotypic, and interaction between receptor molecules as well as with other transcriptional factors may be required for modulating transcription from a particular promoter (7, 8). The progesterone receptor has been shown to form DNA loops via association at two different regulatory sites within a single DNA (104). Steroid hormone receptors may also have effects on chromatin structure, indicated by DNase I hypersensitivity in the vicinity of the binding site, suggestive of DNA deformation and perhaps loop formation (5, 84). A rearrangement of nucleosome structure as a consequence of receptor binding may alter the transcriptional activity of a particular site, as indicated by changes observed in yeast cells at the *PHO5* promoter (3), and expose additional sites for binding that can subsequently involve adjacent and/or remote sequences.

Homeotic genes were originally discovered as genes that specify segmental identity in the embryo during *Drosophila* development and have been found to contain a conserved

sequence, the homeobox, which encodes a protein structural motif termed the homeodomain that has been demonstrated to be involved in DNA binding and recognition (reviewed in reference 40). Evolutionary conservation of the homeobox sequence across a number of species in genes related to developmental processes indicates the importance of the homeodomain in DNA recognition by proteins involved in development (40). Interaction among homeodomain proteins has been suggested by the expression patterns of these proteins both spatially and temporally during development. Thus, combinatorial action of these proteins may be an important determinant of the effect observed on specific gene transcription and on the permanence of activation or inactivation. Synergistic action of homeodomain proteins has been observed, indicating that protein-protein interactions may contribute to the observed effects within the cell (40). The Ultrabithorax protein is a member of the family of homeotic genes, and its specificity for sites within *Drosophila* DNA has been established (6). The protein is a dimeric species, indicating that it has the potential for DNA looping, and although the stoichiometry of these complexes has not been rigorously determined, it has been reported that this protein will produce looped segments in DNA containing two sites (6). Thus, the mechanism of homeotic proteins may involve interaction of protein molecules bound at distant sites to elicit the transcriptional regulatory patterns requisite for developmental pathways.

Yeast RAP-1 Protein

RAP-1 (repressor activator) protein from yeast cells is a DNA-binding protein involved in regulation of multiple DNA-associated functions, including transcription (both activation and repression), recombination, and replication; this protein is essential, as disruption of its coding region is lethal to the cell (reviewed in reference 32). Both maintenance of mating type and the switch in mating type in yeast cells that involves DNA rearrangement require RAP-1. RAP-1-binding sites are found in the silencer (repressor) sequences in concert with other sites necessary for maintenance of transcriptional silence. Interestingly, a RAP-1 site alone serves as a transcriptional activator; thus, the context of protein binding sites in a particular locus determines the expression outcome for a particular promoter. Analysis of the structure of yeast chromosomes indicates that RAP-1 is involved in formation of the nuclear scaffold and, in particular, participates in DNA loops in the mating locus region (45). Thus, the mechanism by which RAP-1 influences transcriptional events may involve constraint of segments of DNA into looped structures. Reconstitution experiments demonstrate that RAP-1 is able to mediate loop formation in vitro in DNA fragments containing mating-type loci; the loops formed correspond to those observed in chromatin structure and coordinately regulated in vivo (45). Details of the mechanism by which this protein exerts its multiple effects have not been elucidated, but it is apparent that loop formation is integral to some aspects of its activity. These results suggest more generally that the nuclear scaffold proteins, which restrain DNA into multiple looped segments, may significantly influence processes involving DNA and are not limited to a structural role (45).

Other Eukaryotic Examples of Looping

Analysis of extrachromosomal circular DNAs in thymic cells suggested that the formation of T-cell antigen receptor involved a process of looping and excision with consequent deletion in the gene (64, 106). Similarly, in B-cell lines, class switching from immunoglobulin M to immunoglobulin G2b chains involves DNA looping as an intermediate step in generating a DNA inversion or in deletion of specific segments (51, 52, 71, 107, 112). These DNA recombinational events require binding of proteins at two separate sites on the DNA to generate a loop followed by catalytic activity to delete or rearrange the looped segment; the proteins involved in these processes have not been identified.

Simian virus 40 large tumor antigen influences a variety of cellular activities, including regulation of viral and cellular gene expression, viral DNA replication, and recombination (91). The role of T antigen in replication appears to relate to its stabilization of single-stranded DNA segments during the opening of the origin of replication and separation of strands during elongation. In addition to this activity, the isolated T antigen mediates formation of DNA loops via protein-protein interactions between antigen bound at different sites within a DNA sequence (91); this activity may be important in the recombinational processes promoted by this protein. Electron-microscopic analysis indicated that T antigen can mediate loop formation between nonspecific sites as well as specific sites and between single-stranded DNA ends and double-stranded DNA regions, an activity requisite for joining of gapped DNA molecules (91). In a different system, the selective recognition of tRNA genes in yeast cells by the transcriptional apparatus in part requires remote sequences in DNA and the complex transcriptional factor τ (97). Specific interaction of τ with target regulatory sites has demonstrated essential features of the sequences, and accommodation to a variety of intersequence distances and orientation has been noted. Examination of τ by electron microscopy has demonstrated formation of looped segments under some conditions (97). The interaction of τ with other transcription factors ultimately determines the extent of tRNA production in this system; this complexity may be anticipated for many eukaryotic systems.

These examples illustrate the diverse activities in which DNA loop formation, either transient or stable, may play a role. The ability to appose specific sequences or specific structural features of DNA may be crucial to the control of transcription at a given promoter, to initiation and possibly elongation of replication, to recombinational events, and to maintaining the packaging of DNA within a cell. The proteins which mediate looping in these varied processes will be diverse, with a wide range of ancillary activities (transcriptional activation, ligand binding, protein-protein binding specificity, catalytic activity, etc.). Achieving insight into the structure and function of these protein species and dissecting the mechanisms by which their action is exerted is an important aspect of understanding the role of DNA looping in cellular function.

DETERMINANTS OF DNA LOOPING FORMATION

The capacity to form a looped DNA structure via protein binding depends not only on the presence of specific binding sites for a given protein, but also on the distance between the sites, the orientation of the protein on the site, and the structural properties of the intervening DNA (Fig. 11). The energy gained by protein binding and interaction must exceed the energetic cost of deforming the DNA into a looped structure. This deformation may include bending of the DNA as well as twisting to bring sites into direct apposition; both are energetically costly. As the length of DNA between

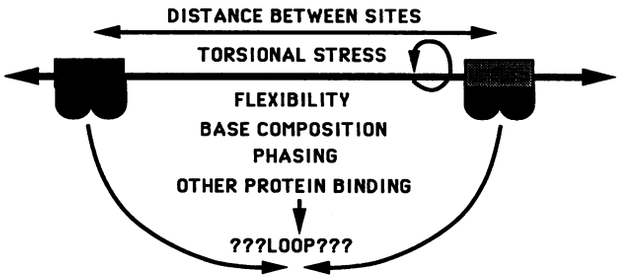

FIG. 11. Factors that affect DNA looping. A variety of factors determine whether a particular loop will form. These include the distance between the sites, the deformability of the intervening DNA (influenced by its base composition and sequence), the phasing of the two sites in terms of the face of the DNA occupied by the interacting species, and the degree to which torsional stress must be introduced to appose the protein sites. In addition, the occupation of the intervening sequence by other proteins that may also exert effects on the DNA structure has a significant influence on whether formation of a specific loop will be favored.

sites increases, the energetic requirement for both bending and twisting diminishes, but the local concentration of sites also decreases and the entropic cost increases. Initially the length dependence of the closure of linear DNAs by ligase was measured to give an estimate of the probability of two sites coming together (98, 99). An intersite distance of ca. 500 bp was optimal for cyclization, whereas at distances greater than ca. 1,000 bp between sites the enhancement of cyclization as a result of increased local concentration was diminished, presumably owing to the entropic cost of bringing these sites together (99). The influence of torsional stress (twisting potential) on the alignment of sites was also examined by closing covalent circles by using ligase (98), and, as anticipated, there is a periodicity reflecting the helical repeat, similar to observations with loop formation by protein-protein interaction. Looping can also be influenced by the binding of other proteins in the intervening sequence, as demonstrated by the effects of λ integration host factor or HU protein (see above). Such effects may be to facilitate loop formation or to prohibit it, depending on the nature and orientation of the binding interaction. The sequence of the intervening DNA segment may also influence the energy required for loop formation. The effects of sequence on nucleosome formation and the influence of intervening sequence between enhancer and promoter on the efficacy of enhancer-bound protein stimulation of transcription suggest that inherent flexibility in the DNA structure may be significantly influenced by sequence composition (see above). In addition to the effects of DNA structure on loop formation, the protein-bridging structure may introduce flexibility or rigidity. If the protein orientation with respect to the DNA site(s) must be precise and the protein structure is inflexible, the loop formed may follow a different distance dependence from that for a protein that can adopt multiple orientations or has a more pliable structure.

Loop formation appears to have multiple functions in cells, and the specific effects may derive from different mechanisms. In some cases, the increase in the effective local concentration of protein may account for the observed effects (see references 9–11, and 75 for detailed discussions), as the provision of excess protein in *trans* will yield the same physiological effect (e.g., see reference 21). However, it is apparent that the loop itself has significant physiological consequences in other cases, e.g., the *gal* repressor or RAP-1 protein in yeasts (see discussion above). Loop formation fixes the ends of a segment of DNA, isolating a specific topological domain of the sequence (e.g., see reference 57), and may thereby induce distortions in the intervening sequences, as indicated by DNase sensitivity in small loops (42).

CONCLUSION

Binding to a specific DNA target site is the basic capacity used by proteins that regulate functions involving the genetic material, including transcription, replication, and recombination. This simple, fundamental property can, however, be combined in multiple ways to generate a spectrum of outcomes depending upon other target sites in the immediate vicinity; other proteins with specificity for similar sequences or adjacent sites; the inherent capacity of a specific protein to oligomerize, to form mixed oligomers, or to interact with other proteins at adjacent or distant sites; and the structural properties of the DNA segment. Thus, cooperativity and competition between regulatory proteins yield particular patterns of expression, and interaction of proteins at sites far removed in the nucleotide sequence can exert a significant influence on the events at a particular promoter or replication origin or recombination site. The intervening sequence of DNA is also a major factor in determining the types of structure that can form. This interwoven net of protein-DNA and protein-protein interactions provides the capacity for a modulated response to a wide range of environmental and organismal signals to alter patterns of protein production, initiate replication, or influence recombination events.

DNA looping mediated by association of protein(s) bound at separate sites within a single molecule plays a significant role in transcriptional control (both activation and repression) and in other processes involved in DNA metabolism. The ubiquitous nature of this phenomenon, found from prokaryotic viral systems to immunoglobulin rearrangement in eukaryotic systems, indicates that the capacity to form DNA loops enhances the regulatory properties of proteins and expands the flexibility of systems in responding to signals that evoke cellular change. It would be anticipated that many more systems in which looping plays a role will be identified and that further studies on this phenomenon in a variety of cellular settings will decipher in greater detail the requirements for and consequences of DNA loop formation.

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