Action at a Distance Along a DNA

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A number of ways are known by which an event at one location on a DNA molecule can affect an event at a distant location on the same molecule. Three classes of mechanisms are described for such distal actions: tracking or translocation of a protein along a DNA, the association of two proteins bound at separate sites to form a DNA loop in between, and distal interactions that are affected by the topology of the DNA. The basic characteristics of each type of mechanism are discussed in terms of the known physicochemical properties of DNA. The various modes of action at a distance are often interrelated. Examples include the formation of positively and negatively supercoiled DNA loops by tracking and the strong effects of DNA topology on looping.

A RECURRING THEME IN MOLECULAR BIOLOGY AND DNA enzymology is that of "action at a distance" along a DNA: an event at a site (sequence) a on a DNA molecule is able to affect an event at a distant site b on the same molecule. Examples of such cis-acting distal effects are the transcriptional enhancers in eukaryotes and the phenomenon of transcriptional activation of replication origins in prokaryotes. How does an enhancer sequence affect the transcription of a gene thousands of base pairs away? How does transcription of one region of a DNA affect the initiation of replication at a separate site connected to this region?

In this article, we discuss three classes of examples of action at a distance: tracking or movement of a protein along the DNA, looping or the formation of a DNA loop by bringing two sites together, and distal actions through influences on the topology of a DNA ring or loop. By focusing on selected examples, we illustrate the diversity of the modes of distal actions and show that they are often interrelated; some of the characteristics of each class are also discussed.

Tracking

By tracking it is meant that a protein or an assembly of proteins A binds to a site a and translocates to a site b along the DNA to affect an event at b. It is not obligatory that A leave a to reach b. There can be two sites on A, one remains bound to a and the other affects translocation; in such a case, tracking is accompanied by the gradual enlargement of a DNA loop between a and the mobile site interacting with the translocation site on A. We describe below three different examples of action at a distance by tracking: the helicases, the polymerases, and the type I restriction enzymes. In each case, translocation of the protein along the DNA is an active process tightly coupled to the hydrolysis of an energy cofactor. Passive diffusion or "sliding" of a protein along a DNA will not be discussed in this article.

The helicases. The RecBCD helicase/nuclease (exonuclease V) is one of the best documented cases of tracking along the DNA (*1, 2*). The enzyme requires a DNA free end for its entry and can move along the DNA using its adenosine triphosphate (ATP)–dependent DNA helicase activity. Its action at sites distal to the entry site has been demonstrated in vitro: the enzyme preferentially cleaves at asymmetric sequences termed Chi sites, when approaching from one side of such sequences (*2*). In vivo, Chi sites in phage λ mutants have been shown to be hot spots in the RecA-RecBCD recombination pathway (*3*).

In replication, the importance of helicase actions has recently been demonstrated at the molecular level. In the initiation of *Escherichia coli* and phage λ DNA replication, an ordered assembly of several proteins at a unique DNA sequence precedes the action of the helicase, which unwinds the duplex DNA (*4, 5*). In the case of λ DNA replication in vitro (*5*), for example, several molecules of λ O protein bind first their recognition sequences at the origin of replication for λ (*ori*λ). The λ P protein and *E. coli* DnaB protein then bind to this nucleoprotein core complex. When the *E. coli* proteins DnaK, DnaJ, and Ssb are added to the O-P-DnaB/*ori*λ complex, an ATP-dependent unidirectional unwinding of the DNA occurs. The detailed roles of the individual proteins in this unwinding reaction are not clear. DnaB is presumably the helicase, and DnaK and DnaJ are thought to activate DnaB by unlocking it from protein P; Ssb presumably prevents the reversal of the unwinding reaction by its specific binding to single-stranded DNA. The unwinding reaction requires that the *ori*λ-containing DNA is in a negatively supercoiled conformation; thousands of base pairs of DNA can be unwound if DNA gyrase and excess Ssb are present.

In contrast to the complexity of protein assembly that precedes the helicase action in the initiation of *E. coli* and phage λ replication, in the replication of the animal virus SV40, origin recognition and unwinding are carried out by a single protein. The virus-encoded large tumor or T antigen can bind specifically to duplex DNA at the origin of replication and unwind the DNA (*6–8*). Both reactions can occur with either linear or negatively supercoiled *ori*SV40-containing DNA, and the unwinding reaction proceeds bidirectionally (*8*).

The polymerases. The polymerases are so well known for their tracking along the DNA that they are rarely considered in the context of action at a distance. An insightful example of their roles in distal actions is the initiation of replication of the plasmid ColE1 by an initiator RNA (*9*). The initiator RNA starts at a promoter 550 bp upstream of *ori*. As the RNA chain extends to *ori*, there is a certain probability that the RNA will form an RNA-DNA hybrid with the template. Once such a hybrid is formed, the sequential actions of

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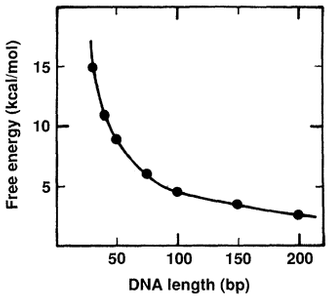

Fig. 1. The dependence of the free energy of bending a DNA segment smoothly into a semicircle on its length. Values are calculated from Eq. 1. The persistence length is taken as 500 Å, and the contour length of the DNA is taken as 3.4 Å times the number of base pairs.

ribonuclease (RNase) H and DNA polymerase I lead to the initiation of replication. Thus an event at one point (initiation of transcription) is affecting another event (initiation of replication) at a distal point in cis. An important message from the series of elegant experiments by Tomizawa and colleagues (*9*) on this system is that the detailed structure of the RNA itself is critical. Mutations between the RNA start site and *ori* can prevent the initiation of replication by changing the secondary or tertiary structure of the spanning RNA, or both.

Type I restriction enzymes. These enzymes bind to their unique recognition sequences and cleave the DNA at sites that can be thousands of base pairs away (*10*). It appears that the type I restriction enzyme remains bound to its recognition sequence and then uses a second DNA binding site in the protein to translocate along the DNA; a distant site is reached by the enlargement of the DNA loop between the stationary recognition sequence and the mobile second site (*10, 11*).

Entry site. In this and the next several paragraphs, we discuss some of the characteristics of the tracking mechanism. The supercoiling of DNA accompanying a tracking process will be discussed in the section on DNA topology. In all of the examples cited earlier, there are unique sites of initiation or entry. The RecBCD entry site is specified by a structure (the ends of a duplex DNA), and the entry sites in the other examples are specified by sequences.

Directionality of movement. Because of the dyad symmetry of the DNA double helix, the directionality of tracking is usually determined by the asymmetry of the protein, although the binding of a protein with a dyad symmetry to an asymmetric DNA sequence might bias the direction of tracking. For RNA polymerase, the movement is clearly unidirectional; the direction is fixed by the binding of the asymmetric protein to the asymmetric promoter sequence. In the case of DNA unpairing at the replication origins, bidirectional separation of the strands is observed in vitro for *E. coli oriC* (*4*) and *ori*SV40 (*8*), whereas unidirectionality is observed in vitro for *ori*λ (*5*). It is plausible that the protein-DNA assembly for phage λ replication has a twofold symmetry, and the unidirectionality is due to the difference in the (A + T) contents of the regions next to the assembly (*5*). For the type I restriction enzymes, the Eco K enzyme appears to act bidirectionally in vitro, whereas the analogous Eco B enzyme appears to act unidirectionally (*10*). This difference is probably due to a difference in the subunit makeup of the purified enzyme preparations.

The continuity of DNA between the sites. Distal effects involving active tracking are presumably dependent on the continuity of the DNA connecting the sites. In special cases such as that of RecBCD, severing the DNA between the sites may generate new entry sites. When a and b are present on a circular plasmid, cutting at one position may not prevent bidirectional movement as it is conceivable that the protein could reverse its direction when reaching an end.

Elements along the DNA between the sites. When a protein actively tracks along a DNA, its motion is expected to be affected by many elements along the path. Some of the natural and artificial roadblocks include particular sequences or structures, bound proteins, DNA strand cross-links, and chemical modifications.

In the case of initiation of ColE1 DNA replication by transcription, the structure of the transcript is instrumental in primer formation at *ori*. Thus many sequence changes along the path can affect the initiation of replication. More often, interaction between the tracking protein and the sequences along its path affects the process. The movement of RNA polymerase along the DNA, for example, is known to be hindered by pause sites (*12*). In a particular instance, an alternating CG sequence has been shown to block the passage of a transcribing polymerase when the sequence is in the left-handed Z-helical structure, but not when it is in the right-handed B-helical structure (*13*).

Bound proteins may also hinder tracking. In vitro, the advancement of a replication fork is stopped by a single bound RNA polymerase (*14*). Interestingly, the helicase dda has been shown to effectively derail bound polymerases and probably other proteins as well (*14*). The effects of nucleosome core particles in slowing down transcribing polymerases have also been reported (*15*).

If the tracking of a particular assembly along the DNA is accompanied by DNA strand separation, as in the cases of the helicases and polymerases, cross-linking of the DNA strands may severely interfere with translocation. Psoralen cross-links, for example, are believed to effectively block the movement of RecBCD along the DNA (*16*).

Looping and Supranucleoprotein Structure

Here we consider first the simplest case: proteins A and B, respectively, bound at sites a and b of a linear DNA, come together to form a complex A·B, closing a DNA loop in between (A and B could be identical or the two domains of a single protein).

The DNA loop held together by the proteins forms a topological domain. If the protein link is fairly rigid, the DNA loop is subject to the same topological constraints as those in a covalently closed DNA ring. Recent work on the in vitro binding of phage λ repressor to neighboring operator sites convincingly demonstrates that looping of the DNA between the sites can occur (*17*). Cooperative binding of repressors to a pair of operator sites is observed if the centers of the sites are separated by 5 or 6 helical turns, but not if they are separated by 4.6, 5.5, or 6.4 turns. These results are consistent with the model of two repressors bound on the same side of the DNA double helix coming together and forming a smooth bend in between. If the sites are separated by nonintegral turns, twisting or writhing of the DNA between the sites is necessary in order to bring together the bound repressors, which is thermodynamically less favorable than a smooth bend in a plane (discussed in a later section). Looping has also been implicated in the regulation of the *gal* and L-arabinose operons in *E. coli* (*18*) and in the regulation and expression of the early promoter of SV40 (*19*).

When sites a and b are located on a circular plasmid or in a chromosomal loop, bringing sites a and b together divides the DNA ring or loop into two topological domains. Many examples of site-specific recombination fall into this category. Depending on the detailed path of the DNA within the A·B/a·b complex, the topology of this situation may be very different from the case when a and b are on a linear DNA.

Many proteins may assemble along a long stretch of DNA to form a functional complex. In the case of protein assembly at the phage λ attachment site, about a dozen protein molecules cover a DNA segment 230 bp in length (*20*). Although an even larger number of proteins may span a longer DNA region to form a supranucleoprotein structure, in most of these cases long-range effects occur

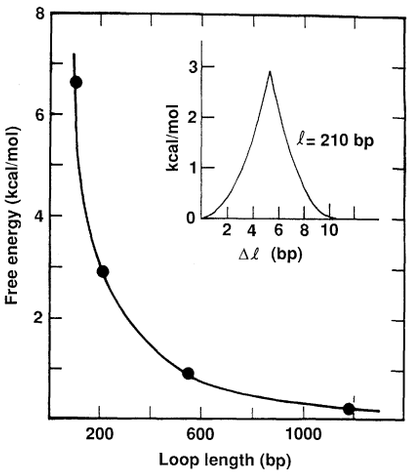

Fig. 2. The extra free energy of bringing two sites on a DNA together to form a loop if the two sites are angularly misaligned by 180°. Values as a function of length are calculated from the data in Horowitz and Wang (*29*). In the inset, the quadratic rise and fall of the free energy is shown when the length of a DNA loop, approximately 200 bp long, is increased or decreased to cause angular misalignment.

through multiple neighboring actions and therefore do not fall in the category of "action at a distance."

An example of the supranucleoprotein structure model in action at a distance is the one proposed for gene repression by a higher order structure of nucleosomes (*21*). A local change may affect the organization and structure of a large chromatin domain and thus exert distal effects within this domain. The binding of a few control proteins to a folded chromatin loop, for example, might unfold the entire loop (*22*). The participation of chromatin structure in the regulation of gene expression has been suggested in a number of cases (*21–23*).

The energetics of bending. For a long isotropic rod of length ℓ, bending it into a loop with an average radius of curvature r requires a free energy

$$\Delta G_b = RT(p\ell/2r^2) \quad (1)$$

where p is a parameter termed the persistence length, which describes the stiffness of the rod (*24*), R is the gas constant, and T is the absolute temperature. For DNA in solution, various physicochemical measurements yield values of p around 500 Å (*25*). We plot in Fig. 1 the calculated value of ΔG_b required to bend an originally straight DNA of length ℓ into a semicircle as a function of ℓ.

The values plotted in Fig. 1 provide a qualitative indication of the difficulty of bending a short DNA segment. For a number of reasons, however, calculations based on Eq. 1 are probably unreliable in estimating the energetics of DNA looping by protein binding. First, if a protein is bending the DNA, the DNA is also bending the protein; we often view the protein as a rigid body only because of our ignorance of its moduli of deformation. The assumption of a rigid protein may thus overestimate the extent of DNA deformation. Second, Eq. 1 was derived for a weakly bent isotropic rod; more experimental data are needed to test its validity as a model for DNA, which is not isotropic, especially for bends of large curvature. Third, for bends of large curvature, there is often insufficient information on the sequence dependence of the bending free energy. Some DNA sequences are naturally curved rather than straight (*26*). Fourth, Eq. 1 also gives the false impression that little free energy is required to bring two widely separated sites together. When a and b are far apart, their coming together greatly reduces the number of configurations the DNA chain can assume. The configurational entropy loss associated with this reduction is not accounted for in Eq. 1.

The configurational entropy of bringing together two widely separated sites. When the two sites a and b are widely separated, the unfavorable free energy corresponding to the configurational entropy loss of the DNA when the sites are brought together by the association of proteins bound to them may diminish or override the favorable free energy of association between the DNA bound proteins. An indicator of the difficulty of bringing two sites on the same DNA molecule together is the parameter j, which is the probability density or the concentration of site a in the neighborhood of site b on the same DNA. The smaller the magnitude of j, the more difficult it is to bring a and b together. In solution, j has been measured experimentally for DNAs ranging from 250 to 40,000 bp (*27*). For a and b 1 kb apart, j is about $6 \times 10^{-8} M$; if they are separated by 10 kb, j drops to about $3 \times 10^{-9} M$. The decrease in j as the distance between the sites increases reflects the larger configurational entropy loss in bringing more distant sites together; when the sites are widely separated, it can be shown that j is inversely proportional to the 3/2 power of the distance in between (*27*). Although the measured values of j are for linear DNA molecules, it can be shown that j for bringing two particular sites separated by ℓ base pairs along a circular DNA of sizes L is $j_\ell j_{L-\ell}/j_L$ where j_ℓ, $j_{L-\ell}$ and j_L are the j factors for linear DNAs of length ℓ, $L - \ell$, and L, respectively. When $l << L$, $j_{L-\ell}$ and j_L are about equal and j for loop formation is about the same whether the two points are on a circular or linear DNA.

The magnitude of j is useful in judging whether DNA looping by the association of DNA bound proteins is energetically favorable. If j is lower than the concentration of DNA molecules, an A protein bound to one DNA is more likely to encounter a B protein bound to a different DNA rather than to the same DNA; intermolecular association would therefore be more favorable than intramolecular DNA loop formation. If K_d is the dissociation constant of the complex A·B, with A and B bound to different DNA molecules, the concentration of DNA molecules must be higher than K_d in order to form a stable intermolecular complex. The magnitude of j must be larger than the concentration of DNA molecules and therefore larger than K_d if looping is to occur.

From the discussions above, association between proteins bound to sites far apart on the same DNA is unfavorable in vitro because of the small magnitude of j. In vivo, however, the probability of two sites coming together is influenced strongly by the binding of proteins or other molecules to the DNA spanning the sites. In such a situation, it is difficult to distinguish the simple looping model from the supranucleoprotein model.

The energetics of twisting and writhing in a loop. For a number of cases in which looping has been implicated, a striking characteristic is that the interaction between A and B appears to be an oscillatory function of the distance between sites a and b (*17–19*). The periodicity of this function is close to the 10.5-bp periodicity of the DNA double helix in solution (*28*).

If a DNA segment of length ℓ corresponds to an energy minimum for loop formation, then changing ℓ by a few base pairs puts the sites out of angular alignment, and twisting or writhing of the DNA loop is necessary to bring the sites together. The additional energy required can be calculated from the free energy of supercoiling, ΔG_τ, which has been measured as a function of DNA length (*29*):

$$\Delta G_\tau = K(\alpha - \alpha^\circ)^2 \quad (2)$$

where K is a length-dependent constant, and $(\alpha - \alpha^\circ)$ is the linking difference (*30*). The smallest DNA for which K has been measured is about 200 bp. Theoretical considerations for twisting and bending of a DNA, however, indicate that for smaller DNAs the product of K and the length of the DNA ℓ should approach a limiting value corresponding to twisting as the only mode of deformation. This limiting value is about 10% greater than the product $K\ell$ when ℓ is 200 bp (*31*). In Fig. 2, we plot ΔG_τ for an equivalent angular distortion of half a turn (*32*), as a function of the loop size. Below a length of 100 bp, the cost of angular misalignment by 180° is high:

Fig. 3. Supercoiling of a DNA segment by a protein tracking along the double helix. The ends of the DNA segment are shown as attached to X and Y, and the tracking protein is depicted as a bar (P) moving in the direction of the arrow. See the text for conditions that lead to the positive supercoiling (+ signs) of the DNA ahead of the translocating protein and the negative supercoiling (− signs) of the DNA behind it. Taken from (*39*).

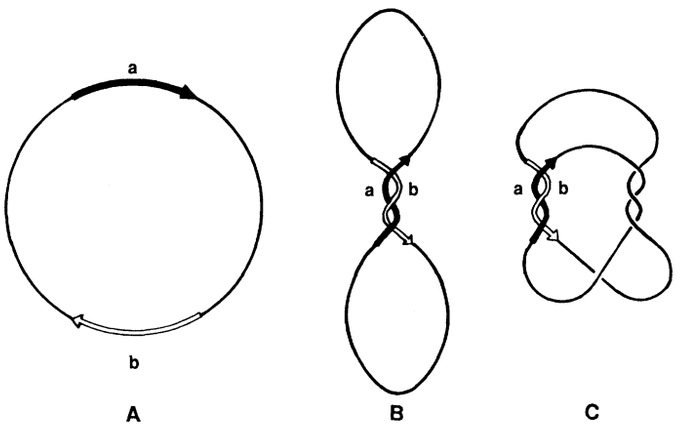

Fig. 4. Two asymmetric sequences a and b, representing by a solid and a hollow arrow, are tandemly oriented in a relaxed double-standard circular DNA (**A**). Interwrapping of a and b (**B**) due to the formation of a complex with a protein, for example, does not involve the entanglement of the two DNA loops outside the complex. In (**C**), the two sites are oppositely oriented along an initially relaxed DNA ring; formation of the same interwrapped structure shown in (B) leads to entanglement of the loops. Redrawn from figure 5 in Gellert and Nash (*46*).

about 7 kcal at a length ℓ of 100 bp and 13 kcal at a length ℓ of 50 bp. In the inset in Fig. 2, the quadratic rise and fall in ΔG_τ is shown when the length of a loop approximately 200 bp long is changed by a few base pairs to cause misalignment.

The free energy values calculated above are likely to be upper bounds; it might be less costly to deform the protein holding a short DNA loop. There is also some indication that different sequences may show different torsional as well as bending stiffness (*33*). Structural transitions for particular sequences may also occur when ΔG_τ is sufficiently high.

DNA Topology

DNA supercoiling is the best known and most extensively studied aspect of DNA topology (*30, 34*). Supercoiling affects the entire DNA ring or loop; both structural transitions and interactions between DNA and other molecules can be strongly influenced by supercoiling. In bacteria, the global effects of supercoiling of intracellular DNA are responsible for some of the pleiotropic phenotypes of DNA topoisomerase mutants (*35*). In the context of action at a distance along the DNA, several mechanisms are plausible in a supercoiled DNA. Severing or nicking the DNA at any point, or the binding of a topoisomerase to a particular site, or a combination of the two, would affect the entire topological domain. Similarly, a structural change at one site may modulate the degree of supercoiling of the DNA, thus affecting events elsewhere in the same topological domain.

A protein tracking along a DNA may also generate supercoiled loops. The basic idea is illustrated in Fig. 3. The vertical bar P represents a protein moving along a DNA in the direction of the arrow, and the ends of the DNA are depicted as being attached to two supports X and Y. P could be moving along one strand or one groove of the DNA, or it could be unwinding the DNA strands in front of it and rewinding the strands behind it. In either case, the helical geometry of DNA requires a rotation of P relative to the DNA, at a rate of about one turn per 10 bp. Several situations can lead to the positive supercoiling of the DNA ahead of P and the negative supercoiling of the DNA behind it: (i) when P, X, and Y are all anchored on a large cellular structure (*36*); (ii) when X and Y are integral parts of P or are proteins interacting with P (*11, 36–38*); and (iii) when the frictional force against the rotation of P relative to X and Y is large (*39*). The generation of positively and negatively supercoiled DNA loops by tracking was initially postulated for DNA gyrase (*37*). Although gyrase acts by a different mechanism (*35*), the tracking-supercoiling model is likely to be relevant in other cases. Electron microscopic examination of the intermediates in the cutting of DNA by the type I restriction enzyme Eco R·K suggests that DNA supercoiling accompanies translocation of the enzyme (*11*); tracking of a protein along a DNA was also postulated to be a way of supercoiling a DNA locally (*38*). A case of particular interest is the possibility of DNA supercoiling by transcription (*36, 39*). Recent analysis suggests that in prokaryotes at least, transcription is an important determinant of the degrees of supercoiling of intracellular DNAs (*39*).

Supercoiling is one important aspect of the topology of a DNA ring. There are, however, topological aspects other than supercoiling. To emphasize this point we return to the topological problem of bringing two sites together in a ring. In Fig. 4, two sites represented by arrows are shown to wrap around each other in the protein-DNA complex [the arrows indicate the asymmetry inherent in the sequences, and the arrows are assumed to be antiparallel in the complex (Fig. 4A)]. In Fig. 4B, the arrows are in tandem when traced along the DNA, and the loops outside the complex are not intertwined. In Fig. 4C, the arrows are opposing each other when traced along the DNA, and the formation of the same complex in this case leads to entanglement of the loops, which would in turn introduce an unfavorable free energy term. Evidence has been obtained recently in a number of studies on site-specific recombination that the detailed topology of the enzyme-DNA complexes has a strong influence on the reactions (*40, 41*). The topological requirement explains why in some cases site-specific recombination requires tandemly oriented sites, whereas others require opposing sites. Base pairing between two single-stranded gaps of complementary sequences on a DNA ring could also cause a similar entanglement of the two loops separated by the synaptic region.

Circular versus linear DNA. The supercoiling of a closed circular DNA (or a loop) is the best understood example that sets circular DNAs apart from their linear counterparts. There are additional features unique to closed loops. As described above, in certain cases of site-specific recombination the entanglement of loops accompanying the synapsis of sites a and b may prevent the occurrence of recombination. It is particularly significant that Boocock *et al.* (*41*) showed that under conditions where a linear DNA containing two opposing *res* sites can undergo Tn3 resolvase–catalyzed site-specific recombination between the sites, the same DNA in the nicked circular form cannot. This shows that topological features other than DNA supercoiling are important here in distinguishing circular from linear molecules.

Differential actions of the topoisomerases. Although in a number of processes the type I and type II topoisomerases can substitute for each other (*35, 42*), in other processes they may not be interchangeable. In eubacteria, it is well established that DNA topoisomerase I relaxes supercoiled DNA only negatively (*43*), unless there are single-stranded regions in the DNA (*44*). If a tracking process is

generating a positively supercoiled loop and a negatively supercoiled loop at the same time, DNA topoisomerase I and DNA gyrase may act differentially in the loops (*39*).

Another important difference between the type I and type II topoisomerases is that only the type II enzyme can pass two double-stranded segments without nicks or gaps through each other. The requirement of the type II enzyme during mitosis is believed to reflect the ability of the type II, but not the type I, enzyme to segregate intertwined duplex DNA after replication (*45*). This difference in the two types of topoisomerases may be manifested in other processes as well. When entanglement of DNA loops occurs as a result of synapsis, whether mediated through protein-protein interactions or through base pairing between complementary sequences, DNA topoisomerase II may be the only activity capable of relieving the unfavorable free energy barrier.

Concluding Remarks

We have summarized a number of examples in which an event at one site can affect an event at a distant site on the same DNA. Action at a distance can occur in a number of ways, and different mechanisms of distal actions are often interrelated. DNA looping, for example, requires a consideration of the topology of the loop or loops. Tracking and supercoiling are also closely related. A perspective view of the various possibilities, as well as knowledge of the characteristics of various modes of action, should therefore be helpful in understanding the mechanisms of many well-known but little understood phenomena in molecular biology.

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