DNA Structure Changes Coupled to Protein Binding

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Protein-induced DNA distortions facilitate the interactions between proteins bound to non-adjacent sites on DNA. Correct assembly of these macromolecular complexes is necessary for DNA transcription, recombination, repair and replication.

Secondary article

Article Contents

Introduction

For historic reasons, deoxyribonucleic acid (DNA) has been primarily considered a medium for storage and transfer of genetic information. In addition to sequences encoding proteins, however, DNA contains noncoding sequences that regulate the use and propagation of that information. These regulatory processes involve proteins that ensure the correct conversion and processing of the DNA information, thus providing timely and space-integrated execution of the developmental programme. Having only four basic building blocks, DNA seems structurally monotonous and deceivingly simple compared with the structural plasticity of proteins, and was long regarded a passive partner in protein–DNA interactions. The recently solved structures of many protein–DNA complexes underscore the more active role of DNA, which is subject to various conformational changes more often than the associated proteins. It is now generally accepted that the linear sequence of nucleotides represents only one level at which the information encoded in DNA determines the outcome of developmental processes in the cell. In particular, DNA association with proteins involves not only the direct hydrogen bonding of protein side-chains with the nucleotides but also more subtle modes of indirect recognition that rely on the fine structure of the double helix and its deformability as a function of base sequence. Furthermore, DNA bending and kinking can bring into proximity proteins deployed on distant sites within a regulatory region. In this case DNA serves as a scaffold for the topological organization of multiprotein complexes in which various combinations of proteins can assemble and interact with each other. This mechanism, known as combinatorial control, provides the means for the variety of distinct functional responses using a relatively limited number of regulatory proteins.

Effects of Base Sequence on DNA Bending

DNA bending can be intrinsic or induced by some external force (Calladine and Drew, 1997). Intrinsic bending results entirely from chemical properties of base pairs and their arrangement in the DNA molecule. This type of bending should be distinguished from thermally caused DNA deformations that continually occur without preference for location or direction. As a consequence, parts of the DNA molecule in which bends arise only from thermal agitation remain straight on the average, while intrinsically bent DNA sequences produce nonlinear structures with a preferred direction of bending. Induced DNA bending is usually associated with protein binding, but can also be caused by binding of other ligands.

It has been shown using various experimental techniques that both intrinsic and protein-induced DNA bending depend on the sequence of nucleotide base pairs (Hagerman, 1990). DNA curvature was initially noted in kinetoplast DNA which contains repeated stretches of 5–6 adenines (A-tracts), because such molecules migrate more slowly on polyacrylamide gels than does DNA of the same length but composed of random sequence (see below). In parallel studies, AA/TT dinucleotides in nucleosomal DNA were shown to occur with a periodicity of about 10 base pairs. This result incited the hypothesis that AA/TT dinucleotides can produce small, local DNA bends ('wedges'), and that, because of their repetitive pattern in phase with the helical turn, they could add up and lead to macroscopic DNA curvature. AA/TT dinucleotides and A-tracts were long considered the only sequences to cause major bending, and the contributions of other DNA sequence elements were considered small and hence were ignored (Crothers *et al.*, 1992). Accordingly, the first models developed to predict the intrinsic DNA structure from its sequence accounted only for the contributions of A-tracts. More recently, however, evidence has accumulated suggesting that DNA curvature involves additional sequence elements, and more sophis-

ticated predictive models have been proposed. Nevertheless, DNA structure can be predicted from the sequence only with limited accuracy. Further development of better predictive models for DNA bending is currently restricted by the relative inability to account for differential behaviour of the same dinucleotide in various sequence contexts, which is conceptually similar to the classical problem of protein secondary structure prediction. Because of the vastly greater number of high-resolution protein structures compared with DNA sequences, the secondary structure of proteins can be predicted with roughly 75% accuracy, while such a claim cannot be made for the prediction of DNA conformation. Thus, knowledge of both intrinsic and protein-induced variations in DNA structure must rely on experimental methods.

Solution Studies of DNA Bending by Proteins

The anomalous migration of naturally curved DNA during polyacrylamide gel electrophoresis prompted the development of a method based on the gel mobility of synthetic DNA sequences ligated to form multimer repeats (Hagerman, 1990). The idea behind this approach was that local structural variations in DNA (bends), if separated by an integral number of helical turns, will retain the same global direction and their contributions will be amplified to produce a defined macroscopic shape (curve). One of the theoretical explanations for anomalous mobility of curved DNAs is that they encounter additional friction in the gel, which slows them down compared with the straight molecules of the same size. Despite the lack of a quantitative theory for anomalous gel mobility of curved DNA, gel electrophoresis has been a tremendously useful tool for identifying and characterizing both intrinsic and protein-induced DNA bends.

An important early contribution to studies of protein-induced DNA bending was the circular permutation assay. The method is based on different electrophoretic mobilities of DNA fragments of identical length and base composition, which contain the same bend either in the centre or near the ends of the molecule. Fragments with the bend located in the middle display a slower mobility on the gel, whereas fragments with the bend closer to ends of the molecule have faster mobility. If the region of curvature is small compared with the sequence length, this assay can identify the approximate centre of the bend. However, this approach has serious limitations when applied to protein-induced DNA bends. Protein binding can cause a position-dependent effect on electrophoretic mobility even in the absence of DNA bending, which limits the usefulness of this assay to studies of intrinsic DNA bends.

To overcome the limitations of circular permutation, an alternative method, designated phasing analysis, was developed. In the phasing analysis approach a reference bend of known magnitude and direction, usually consisting of several phased A-tracts, is separated from an unknown bend by spacers of different lengths spanning at least a full helical turn of DNA (Kerppola and Curran, 1991). The mobility of the protein-bound probes is determined both by the bend magnitude and its phasing with the reference bend in terms of direction. When the protein-induced and reference bends are in phase, the shape of the molecule resembles the letter U (**Figure 1c,d**), and the resulting protein–DNA complex migrates slowly in the gel. On the other hand, when two bends are out of phase, the molecules look like the letter I and move faster in the gel (**Figure 1a,f**). Since the protein is bound to the centre of each fragment, phasing analysis is not subject to the position-dependent effect of protein binding on electrophoretic mobility. Considering that both the direction and magnitude of an unknown bend can be determined by plotting the mobility anomaly of phasing probes as a function of the distance (in base pairs) between two bends,

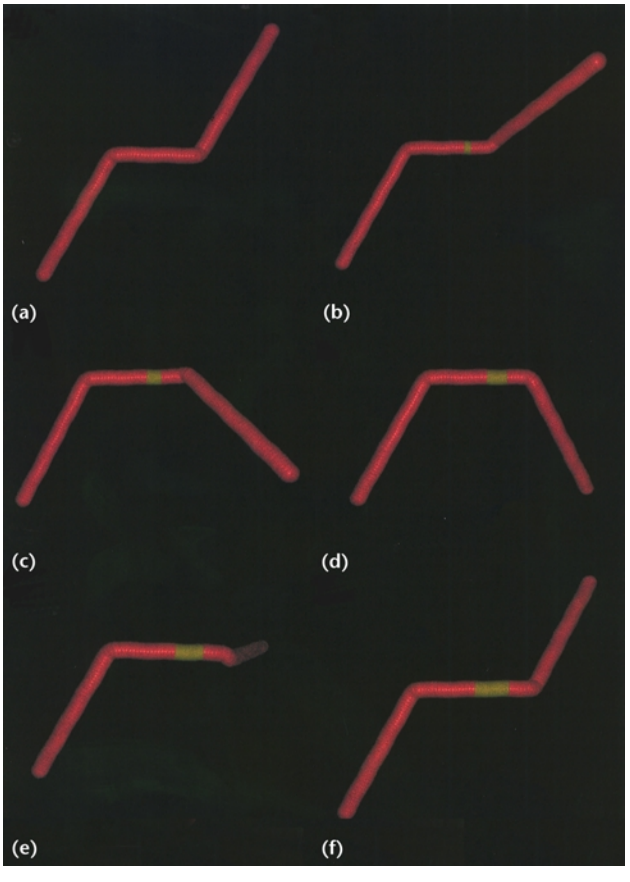

Figure 1 DNA constructs used for the phasing analysis. Intrinsic DNA bend (on the left in each part of the figure) is separated by a variable spacer (shown in yellow) from the protein-induced bend (on the right). In (a) and (f), the two bends are of opposite direction and cancel each other. Bending is additive in (c) and (d), which results in slowest gel mobility.

phasing analysis represents a method of choice for quantitative characterization of bending parameters.

Another common biochemical approach for detection of DNA bending, and arguably the one that is best understood theoretically, involves the cyclization of relatively short DNA fragments. Upon addition of DNA ligase, these fragments mostly form linear multimers and are converted to small circles inefficiently in the absence of intrinsic or protein-induced bends. When a bend is present in the sequence, however, the ends of the molecule are brought into proximity and cyclization commences proportional to the degree of bending. A quantitative measure of DNA bending that can be obtained from kinetic studies is the cyclization factor, which represents the ratio of separately obtained rate constants for cyclization and bimolecular ligation (Crothers *et al*., 1992). The mixed ligation method is a version of the cyclization approach where cyclization and bimolecular ligation data are obtained from the same reaction. This leads to considerable experimental simplifications and still produces results in good quantitative agreement with kinetic measurements. While some experimental problems, most notably the torsional misalignment of DNA ends, can hamper the interpretation of cyclization data, this method has produced results in good agreement with other experimental techniques.

Scanning probe microscopy (SPM), which includes scanning tunnel microscopy (STM) and atomic force microscopy (AFM), is a relatively new method that has gained increasing popularity in biological applications owing to its ability to image at near-atomic resolution and under physiological conditions. Both STM and AFM image surfaces by scanning over them in a raster scan pattern with a fine tip whose motion in the surface plane is precisely defined by a computer-controlled piezoelectric device. In essence, the microscope is used to measure the topography of the molecular 'terrain', meaning that molecules of interest have to be deposited on a suitable surface before imaging. The stable tethering of biological molecules to the surface and image broadening introduced by finite tip dimensions remain the most serious problems of this approach. Nevertheless, the AFM was used very successfully in many biological systems, including several where DNA bending by proteins was detected. For example, lambda Cro protein was shown to bend DNA in both specific and nonspecific complexes, while DNA bending by *Escherichia coli* RNA polymerase in open transcriptional complexes was shown to increase upon transition to elongation complexes in which RNA polymerase has synthesized a short transcript.

Nuclear magnetic resonance (NMR) spectroscopy is the most promising method for high-resolution structure determination that can be carried out in solution. Although NMR spectroscopy has been limited to the observation of relatively short-range interactions in small complexes, this method has provided very important insights into binding and DNA bending mechanisms of many proteins. In particular, the ability to study the dynamics of intermolecular interactions in solution is a unique application of this propulsive technology. Developments in the preparation of uniformly isotope-labelled DNA, multidimensional NMR resonance assignments and new refinement methods enable studies of larger nucleoprotein complexes, and hold promise for increasing the impact of NMR spectroscopy in studies of DNA structure.

Each of the methods described above has its experimental or theoretical advantages and pitfalls, and for that reason the most reliable results are obtained by the combined use of several assays. In some cases these methods might reach inconsistent conclusions, in which case it is necessary to check the validity of each result by comparing it with other independent approaches.

X-ray Crystallography Studies of DNA Bending

Recent advances in protein expression and oligonucleotide synthesis, coupled with new methods for solving the phase problem and improvements in automatic model building, have greatly reduced the time needed to solve the structure of protein–DNA complexes. As a consequence, we have been rewarded with a variety of exciting structures revealing at the atomic level the intricate network of interactions taking place in nucleoprotein complexes. In particular, these studies have revealed that protein-induced DNA bending can be achieved by several different mechanisms (Schultz *et al*., 1991; Burley and Roeder, 1996; Werner *et al*., 1996; Luger *et al*., 1997; Dickerson, 1998).

Crystallographic studies were instrumental in the evolution of our concepts regarding protein–DNA recognition. While specific protein–DNA interactions were initially thought to be mediated only by α helices recognizing the major groove of DNA, high-resolution structures of diverse protein–DNA complexes provided the evidence that the recognition can be accomplished by both α helices and β sheets, and that both minor and major grooves of DNA can be targets of sequence-specific DNA recognition (Calladine and Drew, 1997; Dickerson, 1998). Similarly, many proteins have developed the ability to bend DNA, using different structural motifs for the recognition of either groove. From a mechanistic point of view, DNA curvature can be smooth (occurring over several base pairs), or localized kinking produced by large roll angle(s) within one or two base pairs. The smooth curvature is produced by smaller positive and negative roll angles spaced half a helical turn apart, while roll angles at several consecutive base steps will result in a superhelical writhe (Dickerson, 1998). At the same time, sequence-specific DNA-binding proteins utilize two general geometric approaches to bend DNA: the protein either bends

DNA away from its surface, usually upon binding in the minor groove, or DNA bending occurs towards the protein and is generally associated with binding in the major groove. The pioneering work on catabolite activator protein (CAP) binding to DNA provided the first detailed picture of the DNA bending towards protein (Schultz *et al.*, 1991). Even when only the structure of the protein was available, it appeared that the two recognition α helices that were expected to contact the major groove were separated by less than a helical turn of DNA, therefore requiring DNA bending to allow protein recognition. The structure of the complex revealed DNA recognition in the major groove and a bend of 90° as a consequence of two major kinks at CA/TG dinucleotides separated by a helical turn (**Figure 2**). In sharp contrast, TATA box-binding protein (TBP) binds in the minor groove and bends DNA away from its surface (Burley and Roeder, 1996). The structure of TBP resembles a saddle that binds DNA on its concave side, while the upper side of the saddle is accessible for interactions with other components of the transcription complex (**Figure 3**). Examples of proteins that bend DNA are shown in Figures 2–7, and these structures reinforce the fact that in many cases the same function can be achieved by structurally divergent protein architectures.

Smooth DNA Bending, DNA Kinking by Side-chain Intercalation and Base Flipping

Smooth DNA bending in a strict sense is rarely seen because it requires very precise alternation of positive and negative roll angles between base pairs separated by half a helical turn (Dickerson, 1998). The most notable example of this type of bending can be seen in nucleosomes (**Figure 4**), where 146 base pairs are wrapped around the histone octamer in 1.65 turns, with both DNA grooves alternating between wide and narrow, approximately each 5 base pairs (Luger *et al.*, 1997).

In recent years, several structures of solved protein–DNA complexes revealed unusual DNA distortion resulting from the protein side-chain intercalation into the minor groove (Werner *et al.*, 1996). Although the intercalating side-chain is always hydrophobic, proteins that cause this type of distortion have different overall folds. Wedging the hydrophobic residue between two base pairs generates the kink, which is manifested as the partial unstacking of adjacent base pairs and local unwinding of the DNA helix. Unstacking alone would require an increase in the phosphate distances of adjacent base pairs that would cause a break in the sugar-phosphate backbone. However,

Figure 2 Homodimer of catabolite activator protein (CAP) bends DNA by 90°, mostly through two kinks separated by a helical turn. In this case, DNA kinking is achieved without side-chain intercalation. DNA trajectory is shown as a white line tracing the centres of DNA base pairs. The structure was solved in the laboratory of T. Steitz.

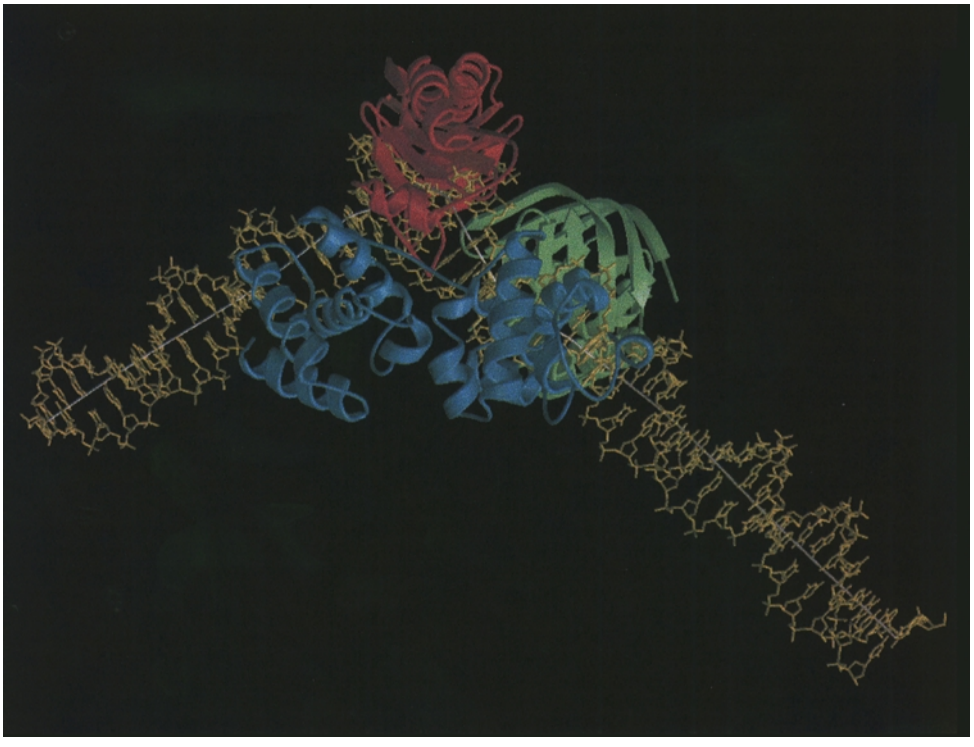

Figure 3 A complex between TATA box-binding protein (TBP) (red), transcription factor related to RNA polymerase II B (TFIIB) (blue) and transcription factor related to RNA polymerase II A (TFIIA) (green) is assembled in the initiation step of transcription. This quaternary complex has been created by combining ternary structures of TBP/TFIIB/DNA and TBP/TFIIA/DNA. A piece of ideal B-DNA is added at both sides of the original DNA structure. TBP bends DNA away from its surface by intercalating two side-chains at sites separated by 6 base pairs. The structures were solved in the laboratories of S. Burley and P. Sigler.

this structural perturbation is compensated by DNA unwinding, which relieves the backbone strain by decreasing the interphosphate distances. Overall, the net effect of the DNA kink is the local widening of the minor groove and a sharp turn in the global DNA axis. Amazingly, the DNA molecule typically assumes a normal conformation within several base pairs from the site of distortion (Burley and Roeder, 1996; Werner *et al*., 1996).

Without exception, all proteins that kink DNA by intercalation do so through the minor groove, mostly because the rolling motion between adjacent base pairs is energetically favoured in a direction that compresses the major groove (Dickerson, 1998). Given the fact that the minor groove is less suitable for specific recognition than the major groove, owing to the limited presence of potential hydrogen bond donor and acceptors, these proteins rely to a large extent on indirect modes of DNA recognition that include DNA deformability and subtle variations in minor groove width. Our understanding of the chemical principles of sequence recognition in the minor groove is still incomplete, and awaits further biochemical and structural analyses.

Base flipping is a special type of DNA structural perturbation that also involves side-chain intercalation. This extensive modification of the double helix is imposed by DNA modifying enzymes such as methyltransferases, and by DNA repair endonucleases that excise modified bases and abasic sites. Even though the reaction mechanisms of these enzymes differ, in both cases the base is completely swung out of the DNA helix and is positioned in the enzyme active site, while the amino acid side-chains intercalate at the site of modification. In this context DNA bending contributes to the correct assembly of the enzyme–DNA complex, and probably steers the flipping base into the enzyme active site.

DNA Bending by Neutralization of Phosphate Charge

On the scale of tens to hundreds of bases, unperturbed DNA behaves as a stiff chain polymer. The measure of DNA stiffness is persistence length, corresponding to the distance over which DNA tends to retain a consistent direction. Several experimental approaches estimate the persistence length to be about 150 base pairs. It should be noted that, even in such short DNA molecules, miniature helical deformations are present as a result of thermal

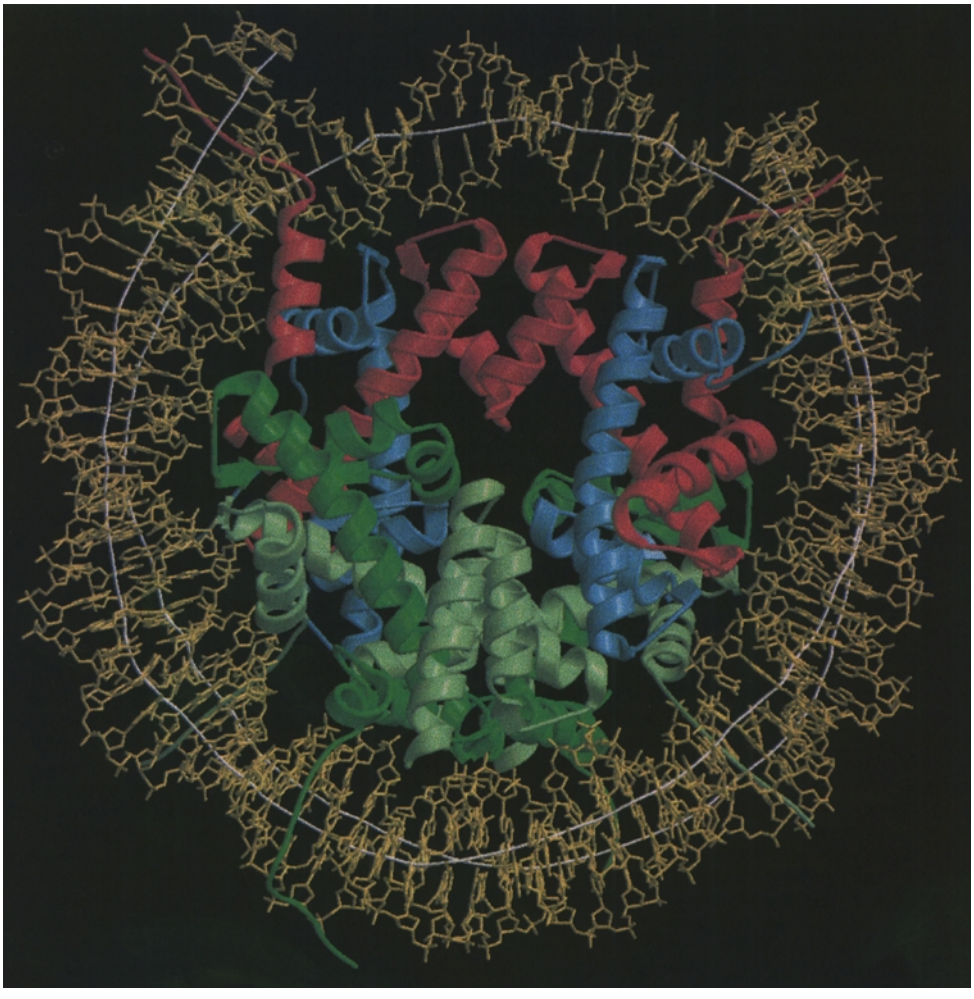

Figure 4 DNA wraps around the nucleosomal core that includes pairs of H2A, H2B, H3 and H4 histones. The structure was solved in the laboratory of T. Richmond.

fluctuations, but they do not amount to any appreciable bending owing to the lack of coherent directionality. In order to achieve the striking compaction seen in chromosomes, about 146 base pairs have to wrap almost twice around the histone octamer (Luger *et al*., 1997). It is therefore essential that the free energy released during DNA association with histones be sufficient to offset the energy expended on DNA deformation.

The specific recognition of DNA by proteins generates a network of satisfied donors and acceptors between DNA bases and protein side-chains and results in the release of free energy. This enables many specific DNA-binding proteins, especially transcription regulatory factors, to bend DNA at the expense of generated energy (Calladine and Drew, 1997). However, in a more general case of nonspecific DNA binding by histone proteins, a different strategy is employed. Early attempts to rationalize DNA wrapping around histone proteins postulated that part of the bending energy is present in the DNA molecule in the form of electrostatic repulsion between phosphate groups. Because the negative charges are evenly distributed on DNA, under normal conditions they constrain a molecule into being straight and relatively rigid. Interactions of histone proteins with DNA, in particular between cationic amino acid side-chains and the phosphate backbone, are predicted to neutralize phosphate repulsion on the side of DNA facing the proteins. As a result of the asymmetric neutralization of phosphates, DNA spontaneously bends around the histones. It should be noted that this mechanism was proposed to account only for part of the driving force for nucleosome folding; intrinsic DNA bending and bendability were thought to facilitate DNA packing in nucleosomes as well (Luger *et al*., 1997).

In principle, the contribution of phosphate neutralization to DNA bending could be tested, provided that phosphates on one side of DNA are selectively switched

off. This process was approximated experimentally by using oligonucleotides with neutral methylphosphonates in place of charged phosphate groups (Strauss and Maher, 1994). These synthetic oligomers were ligated to augment the effect of change in bending, and phased at different distances from the intrinsically curved A-tracts to determine the degree and direction of bending in a manner described above for protein-induced bends. True to theoretical predictions, the largest effect was observed when centres of neutralized DNA patches were separated from the centres of A-tracts by an integral number of helical turns, corresponding to DNA bending in the minor groove direction (Strauss and Maher, 1994).

Electrostatic interactions can also drive DNA bending by imposing directional force on the DNA helix through coulombic charge interactions. This type of bending is observed in complexes formed by Fos and Jun. The opposite directions of DNA bending by Fos and Jun are caused by their opposite charge distributions, indicating that the charge partition in DNA-binding proteins represents an important determinant of DNA bending.

Functional Role of DNA Bending

DNA bending plays an important role in chromosomal packing, transcription regulation, DNA recombination, repair and replication. In general, DNA bending can be expected to occur during macromolecular interactions that subject DNA to either directional or torsional strain. The functional role of DNA bending in transcription is likely to be similar in prokaryotes and eukaryotes, although

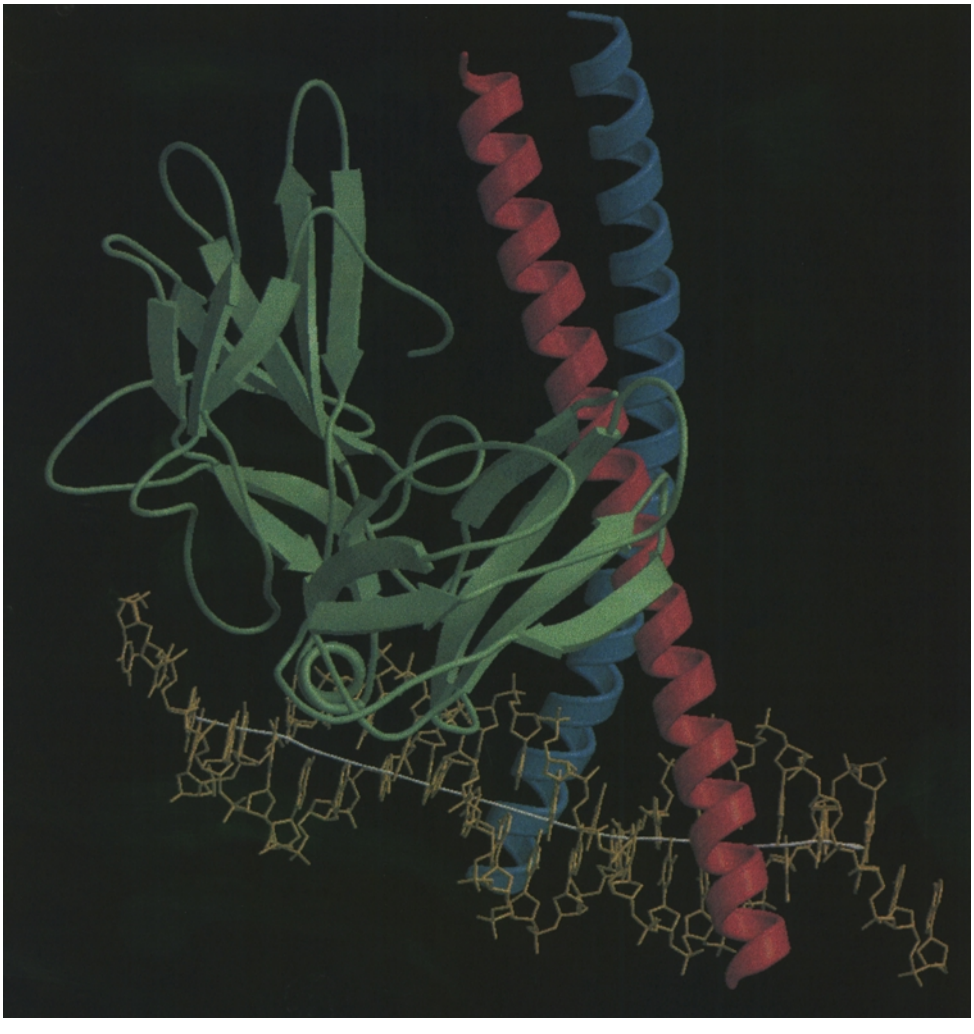

Figure 5 A quartenary complex of Fos (red), Jun (blue) and nuclear factor of activated T cells (NFAT) (green) bound to DNA from the distal antigen-receptor response element. DNA bending enables additional contacts between proteins. The structure was solved in the laboratory of S. Harrison.

differences exist primarily because of the more complex regulatory requirements in eukaryotes. The transcriptional ground state, defined as promoter activity in the absence of specific activators and repressors, is nonrestrictive in prokaryotes as ribonucleic acid (RNA) polymerase has free access to promoters, whose strength determines the transcriptional efficiency. In eukaryotes, on the other hand, promoters are inherently mostly inactive because the transcriptional ground state is maintained restrictive by the chromatin. In this context, intrinsic DNA bending in prokaryotes is a major component of the promoter geometry, and therefore a key regulatory element of transcription. This is evident in the high frequency of bent and/or bendable elements in bacterial promoters. Prokaryotic proteins that induce DNA bending are often under metabolic control. Upon activation, they trigger the cellular response by altering the trajectory of their target promoters in a way that directly determines the level of transcription. There are many cases where the introduction of a correctly phased intrinsic DNA bend can functionally replace the effects of proteins that induce DNA bending. Additionally, binding sites for one type of DNA-bending protein have been replaced by sites for other proteins that bend DNA, arguing that it is not the binding of the specific protein *per se* but rather the architectural role of DNA bending that is needed for the transcriptional regulation.

The regulation of gene expression in eukaryotes depends on a complex spatial and temporal assembly of transcriptional complexes. The precision and diversity in transcription is achieved through combinatorial control, which integrates a variety of intra- and extracellular signals by way of concerted action of activators and repressors. The stiffness of short DNA segments hinders contacts between proteins arranged on DNA in a linear fashion. DNA bending can facilitate interactions among such proteins. Eukaryotic DNA-bending proteins can be loosely classified in three categories: (1) proteins that bind DNA specifically and induce bending as an additional function; (2) 'architectural' proteins that bind DNA specifically, and whose primary function is DNA bending; and (3) nonspecific DNA-binding proteins whose only function is to produce DNA deformation.

Binding of transcriptional factors in some cases depends on their protein partners that occupy adjacent sites. DNA bending induced by one of the partners makes possible protein–protein and protein–DNA contacts that could not take place in a straight DNA fragment, and usually increases the affinity of individual proteins, both for each other and for DNA. Such DNA bending-dependent cooperation between proteins can be seen in complexes of Fos/Jun/NFAT (**Figure 5**) and MATa1/MATα2 homeodomain heterodimers (**Figure 6**). Binding sites for this group of proteins cannot be replaced either by sites for other DNA-bending proteins or intrinsically bent DNA.

Architectural proteins usually interact with the minor groove of the DNA helix and induce a large DNA bend, which serves as a nucleation site for the formation of highly structured nucleoprotein complexes. Lymphoid enhancer factor 1 (LEF-1) is an example of an architectural protein (**Figure 7**) that regulates the assembly of the T-cell receptor α enhancer. In addition to inducing a change in DNA conformation, these proteins often have additional domains that specifically interact with other proteins and recruit them into a productive transcriptional complex. Although many of these proteins are considered sequence-specific, they can often bind nonspecifically to any bent or distorted DNA. Different architectural proteins can usually be used interchangeably, provided that their binding sites are in the proper register with other parts of the enhancer. It is not clear, however, whether curved DNA can functionally replace these proteins.

The high-mobility group (HMG) domain is a DNA-binding motif that is shared between some nonhistone components of chromatin and specific regulators of

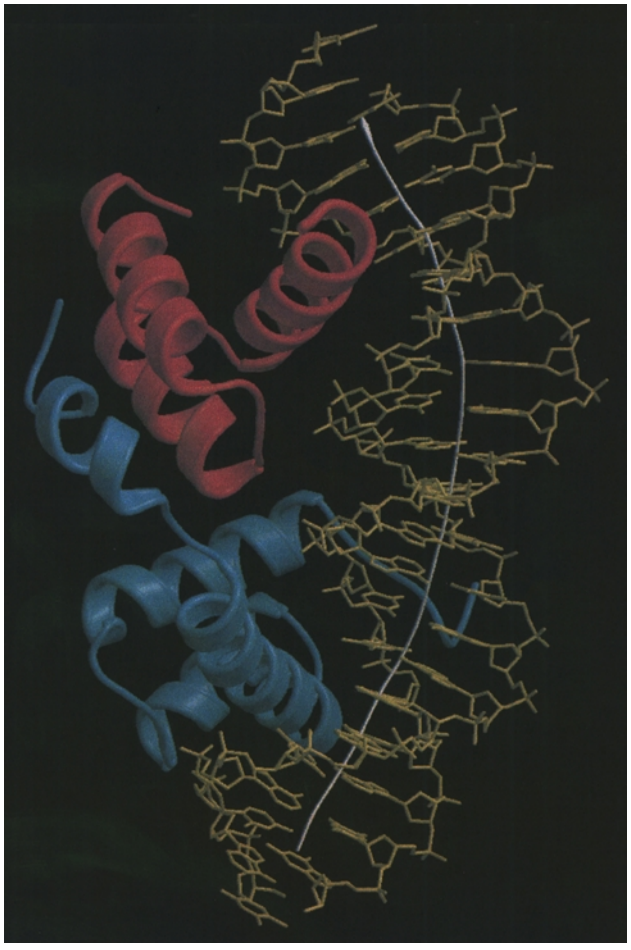

Figure 6 Ternary complex of MATa1 (red), MATα2 (blue) and DNA, which is smoothly bent towards the proteins. The structure was solved in the laboratory of C. Wolberger.

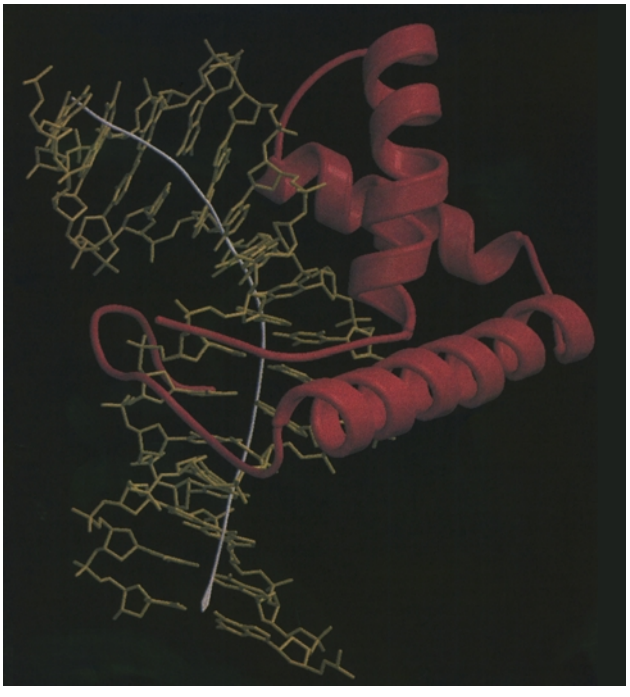

Figure 7 Lymphoid enhancer factor 1 (red) is an architectural protein that bends DNA away from its surface by side-chain intercalation. The structure was solved in the laboratories of R. Grosschedl and P. Wright.

transcription. The high mobility group proteins with multiple HMG domains bind DNA with low specificity. However, sequence-specific proteins that bind their cognate sites can trigger a subsequent binding of HMG proteins, bringing them to a defined site on DNA where they create a bend. Binding of certain nuclear receptors to hormone response elements is greatly enhanced by HMG1 and/or HMG2, even though HMG proteins do not bind these DNA sequences on their own. In a sense, these proteins provide DNA bending 'on demand', where needed.

Besides having a direct structural role in diverse cellular events, DNA bending is important for indirect recognition by DNA-binding proteins. Proteins that bend DNA have to expend part of the binding free energy on DNA deformation, and this energy requirement can be an important factor in determining their binding specificity. Intrinsic DNA bending forms 'customized' sites for protein binding, thus lowering the thermodynamic requirement for DNA deformation. In general, DNA sequences that are intrinsically bent or bendable in a way that mimics the final conformation in the complex with a protein will be recognized with higher affinity. For that reason, the knowledge of intrinsic DNA structural properties would ultimately help elucidate the code of DNA recognition by proteins (Calladine and Drew, 1997).

Although the smaller number of DNA building blocks and relatively simple secondary structure imply a more constricted range of conformations compared with proteins, subtle variations in DNA microstructure add up to produce molecules of complex shapes. It has become increasingly apparent in recent years that DNA conformational variability includes not only static sequence-directed differences in structure, but also differences in dynamics. In years to come, the challenge will be to elucidate the rules that govern DNA structure and dynamics, and to define the individual and combined roles of DNA and protein plasticity that contribute to their intricate interactions.

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