Bending of DNA by Transcription Factors

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Summary

An increasing number of transcription factors both from prokaryotic and eukaryotic sources are found to bend the DNA upon binding to their recognition site. Bending can easily be detected by the anomalous electrophoretic behaviour of the DNA-protein complex or by increased cyclization of DNA fragments containing the protein-induced bend. Induction of DNA bending by transcription factors could regulate transcription in various ways. Bending may bring distantly bound transcription factors closer together by facilitating DNA-looping or it could mediate the interaction between transcription factors and the general transcription machinery by formation of large nucleoprotein structures in which the DNA is wrapped around the protein complex. Alternatively, the energy stored in a protein-induced bend could be used to favour formation of an open transcription complex or to dissociate the RNA polymerase in the transition from initiation to elongation. Modification of the bend angles and bending centers, caused by homodimerization or heterodimerization of transcription factors, may well turn out to be an important way to enlarge the range of interactions required for regulation of gene expression.

Introduction

Initiation of transcription requires the ordered assembly of large multiprotein-DNA complexes. The proteins in these complexes contact DNA at several different positions which requires flexibility of the DNA and leads to distortions of the regular B-DNA helical structure[1]. One example of distorted B conformation which may be instrumental in the ordered formation of these large nucleoprotein structures is DNA bending*.

In *E. coli* extensive DNA bending has been observed for Fis and IHF that act in recombination. Bending by these proteins was shown to be functional in the biological process[2]. Also, the general bacterial DNA binding protein HU, involved in DNA replication, recombination and transcription, causes DNA to bend. Due to space limitations, this review will be restricted to sequence-specific DNA-binding proteins involved in transcriptional control. Examples include the activators CAP, Cro, ϕ29 p4 protein and several repressors from prokaryotic sources as well as eukaryotic transcription factors such as TFIID, TFIIIA, TFIIIB and TFIIIC, HSTF, SRF, NF-kB, Fos, Jun and the POU-protein family (see Table 1). Other reviews including the role of IHF can be found elsewhere[52-56].

*DNA bending is defined as a deformation of DNA to a smooth curvilinear shape, caused by an external force such as a protein bound to the DNA. This should be distinguished from curved DNA, i.e. DNA that has a curvilinear shape independent of any external force[1].

How to Detect Protein-Induced DNA Bending?

Three different methods are currently being employed. These are:

1. Circular permutation analysis
2. Phasing analysis
3. Cyclization analysis

Other, less frequently used methods include electron microscopy[3] and measurement of rotational relaxation times of protein-DNA complexes[4].

1. Circular permutation analysis

As shown by Wu and Crothers[5], bent DNA can be detected by its anomalous migration in polyacrylamide gels. The position of the protein-induced bend determines the electrophoretic mobility of the protein-DNA complex, since the mobility of a DNA fragment is dependent on the mean square end-to-end distance. Reduction of migration is maximal when the bend is located at the centre of a DNA fragment and minimal when it is located near the end. Several vectors have been designed in which a unique restriction site is located between direct repeats of a range of other restriction sites. An example is pBend2[6,7]. Cloning of the octamer recognition sequence into the unique XbaI site followed by digestion of the resulting plasmid with several restriction endonucleases generates fragments of identical size and base composition but with variant positions of the protein recognition sequence (Fig 1A). Oct-2A-DNA complexes display electrophoretic migration dependent on the position of the binding site which indicates that Oct-2A induces DNA bending (Fig 1 B,C). However, this interpretation is subject to a number of assumptions. The mobility can be strongly influenced by temperature, fragment length and gel composition. Moreover, the assay is sensitive to changes in DNA flexibility caused by binding of protein and thus may not only detect bending of the DNA. Even more importantly, anomalous migration of the complex may also be caused by an aberrant shape of the protein itself, as shown for the yeast transcriptional activator GCN4 which does not bend DNA but nevertheless exhibits anomalous electrophoretic mobility in the permutation assay[8]. Therefore an assay has been developed that circumvents these problems.

2. Phasing analysis

Using isomers with varied helical phasing between the two bends, Zinkel and Crothers[9] introduced an elegant method to determine the relative bend direction. This method is inde-

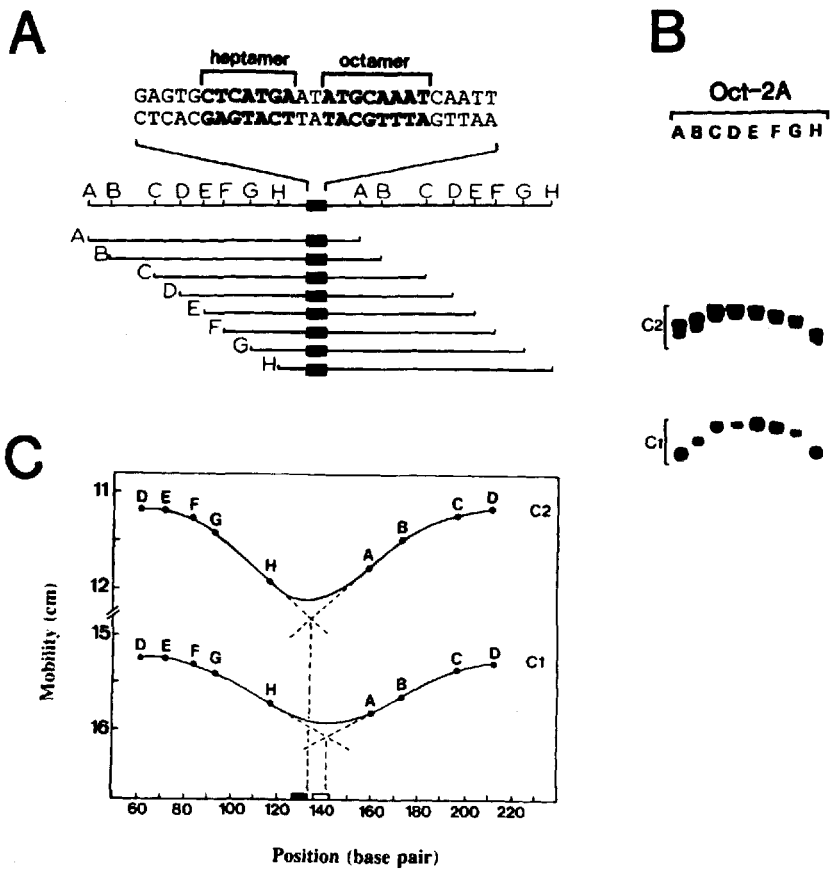


Fig 1. Circular permutation assay, A. A plasmid containing a protein binding site flanked by permutated restriction sites (A-H) is used. Restriction enzyme digestion generates a family of fragments of identical length and base composition in which the position of the binding site is variable. Electrophoresis of DNA-protein complexes will lead to mobility differences depending on the position of the bound protein, with the lowest mobility present when the protein is bound to the center of the fragment. The binding site shown is the heptamer-octamer site in the IgH promoter. B. An example is presented for binding of Oct-2A which leads to 2 complexes (C1 and C2) binding to the octamer (C1) and both octamer and heptamer (C2)[11]. C. Plotting of the relative mobility as a function of the position of the binding site shows two minima indicative for the centers of bending. Here is shown that these bending centers differ for the two complexes.

pendent of the shape of the protein and can discriminate between directed bends and other distortions of the B-DNA structure. DNA fragments containing an intrinsic, adenine-tract directed DNA curvature as well as a protein binding site are employed, separated by a set of linkers varying in length between 10 and 20 basepairs (Fig 2). If proteins induce bending by binding to their recognition site the mobility of the complexes will depend on the linker length since this determines the relative orientation of the curvature and the protein-induced bend. The mobility of the complex should be minimal for the cis-isomer where two in-phase bends cooperate to increase the overall bend angle. Out-of-phase bends counteract each other thereby reducing the overall bend angle which leads to a maximal mobility. Fig 2 shows an example for the Oct-1 protein using the SB 10-20 DNA series containing a small (38°) intrinsic curvature consisting of two GCA_6CG tracts[10]. When the relative mobilities of the protein-DNA complex are divided by the relative mobilities of the free DNA and plotted against the linker length, a clear variation with a 10 bp periodicity is observed, indicating bending. Calculation of the number of helical turns between the intrinsic curvature and the protein-induced bend suggests that in this particular case bending is caused by compression of the major groove[11]. Phasing analysis may be particularly useful for larger complexes, for example combinations of transcription factors and RNA-polymerase binding to the same fragment[12].

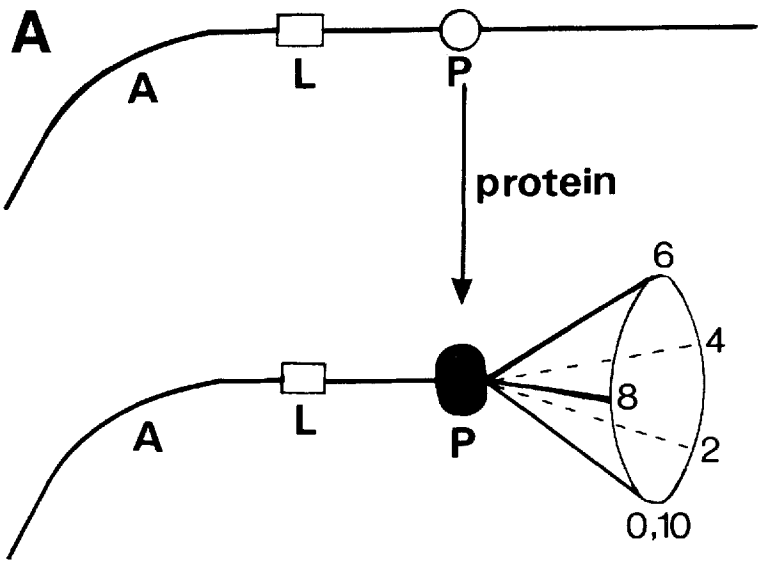
3. Cyclization analysis

An independent way to examine bending is to measure the rate of circularization of DNA fragments by DNA ligase as a function of protein binding[13]. A protein-induced bend will decrease the distance in space between the ends of a DNA fragment and hence increase the possibility of ring closure. This effect will depend on the position of the bend. Furthermore, the rate of cyclization depends strongly on the relative orientation of the 5′-phosphate and 3′-OH groups of the opposing ends to be ligated. Therefore, a protein-induced change in helical twist will also influence ring closure. It is possible to distinguish between twisting and bending by employing several fragments differing in fractional twist due to the insertion of a limited number of basepairs.

Although this method is relatively simple, reliable results are usually only obtained within a narrow range of protein-DNA ratios. Below saturation of the binding site the method becomes less sensitive whereas excess protein may interfere with ring closure, presumably by non-specific binding to the ends[11,14].

Bend Angles

The bend angle α is defined as the angle by which a segment of the DNA departs from linearity. Although a clear theory for calculating protein-induced bend angles is lacking, α can be estimated from gel mobilities determined during circular



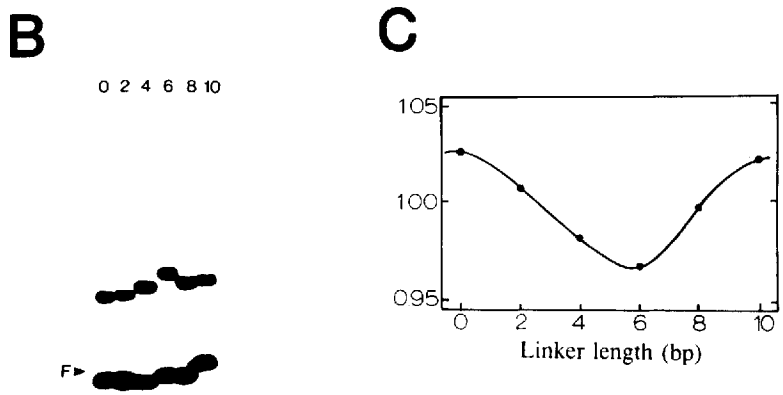


Fig 2. Phasing analysis. A. Schematic representation of the assay. Different fragments containing an A-tract directed curvature (A) and a protein binding site (P) are connected with a linker (L) of variable length spanning one helical turn (2-10 bp). Upon binding of a protein that bends DNA, fragments of different geometry are obtained that can be separated electrophoretically. The in-phase cis-isomers (here designated as 0,10) will have a reduced mobility compared to the out-of-phase trans-isomer (6). B. An example is presented for the Oct-1 POU domain[11]. Phasing of mobility is observed as a function of the linker length when the data are plotted (C).

permutation analysis. An experimental equation $\mu_M/\mu_E = \cos(\alpha/2)$ has been formulated[15] in which μ_M is the mobility of the complex with the protein bound in the middle of the fragment and μ_E is the mobility with the protein bound at the end. This formula has been adapted recently to correct for differences in temperature, gel composition, fragment length and electrical field strength[16]. It is important to note, however, that measurements of bend angles along these lines are obscured by the effects on migration of other DNA structural distortions such as changes in DNA flexibility. For these reasons, Kerppola and Curran[16] have proposed to designate this empirical bend angle the flexure angle, which includes both protein-induced bends and local DNA distortions. The values of flexure angles are generally higher than those of bend angles obtained from phasing analysis. For the CAP-DNA complex which contains a 90° bend in the crystal structure[19], a value of 140° was obtained by permutation analysis[15]. Phasing analysis appeared more in agreement with the crystal structure value since α was estimated at 100° by this method[20]. Nevertheless, the bend angle of the λ cro-operator complex observed in the crystal structure (40°)[17] is in reasonable agreement with values obtained by permutation analysis (30°)[6] or estimated from cyclization assays (45°)[18]. One should keep in mind, however, that the path of DNA in the crystal may be dependent on crystal packing forces.

As shown in Table 1, a wide range of bend angles is observed varying between 15° and 150°. Bend angles may be important parameters in regulation of gene expression, but the rules governing the relation between α and transcription rates are far from clear. In several cases, bend angles can be influenced by binding of other proteins. For CAP, the combination with RNA polymerase bends the DNA further (up to about 180°) and the bending center shifts towards the position of the RNA polymerase (20, see Fig 3B). For NF-κB, the complex of the 50kDa and 65kDa subunits has a higher bend-

Table 1. *Transcription factors that bend DNA*

	Protein	source	Methods used to study bending*	Bend angle† α	Reference
A. Prokaryotes	CAP	E.Coli	A,B,C,D,E	90°[D]	19
	Cro	λ, 434	A,C,D	40°[D]	17
	MerR	E.Coli	A	25°[A]	39
	RepA	pLS1	A,C	nd	44
	p4	φ29	A	85°[A]	32
	repressor	λ,434	A,B,C	25°-46°[A][D]	6,45
	gal repressor	E.Coli	A	105°[A]	7
B. Eukaryotes	HSTF	Drosophila	A	nd	46
	fos-jun	human	A,B	23°[1][B]	16
	jun-jun	human	A,B	27°[B]	16
	POU proteins	human	A,B,C	37°[A]	11
	NF-κB	human	A	75°-110°[2][A]	21
	TUF/GRF1	yeast	A	nd	31
	TFIIIA	X.laevis	A,C,E	30°[A]	14
	TFIIIB	yeast	A,B	95°-115°[A]	47
	TFIIIC	yeast	A,B	90°-100°[A]	47
	TFIID	human, yeast	A	nd	36
	SRF	human	A	nd	48
	E2	BPV,HPV	A	nd	49
	VETF	vaccinia	A	nd	50
	LEF-1	human	A	130°	51
	mSRY	human	A	85°	51

*A=Circular permutation, B=Phasing analysis, C=Circularization analysis, D=Crystal structure of DNA-protein complex, E=Electron microscopy.
†Average values are given. The method used to obtain α are given in brackets.
nd=not determined
Note that for values obtained by the circular permutation assay the effects of protein-shape and DNA flexibility are included and that this flexure angle does not need to correspond to the real bend angle.
1. fos-jun and jun-jun bend DNA in opposite directions. Flexure angles obtained by permutation analysis are 94° for fos-jun and 79° for jun-jun[16].
2. Dependent on the subunit composition (ref. 21 and S. Nedospasov, personal communication).

ing angle (21, S. Nedospasov, personal communication). A most dramatic effect is observed for fos and jun. Dependent on the subunit composition not only the magnitude but also the orientation of the bend is changed. Binding of jun-jun homodimers bends DNA towards the minor groove while Fos-jun heterodimers, binding to the same site, bend DNA towards the major groove[22]. These changes in magnitude, orientation and position of the bends may well represent an additional level of regulation of transcription.

Bending Relies on Bendability of the DNA

Many of the proteins listed in Table 1 employ a helix-turn-helix motif for DNA recognition and in particular the prokaryotic transcription factors bind as dimers. Despite the similar mode of binding the induced bend angles vary considerably. Eukaryotic helix-turn-helix proteins like the homeodomain proteins that bind as monomers do not bend DNA at all[11,23,24,25], which indicates that it is not the helix-turn-helix motif by itself that is responsible for the induction of DNA bending. So why these differences? From studies of the crystal structures of the helix-turn-helix protein CAP in complex with DNA, it appears that the 90° bend results almost entirely from two 40° kinks that occur between two TG basepairs that are unstacked. The energy required for bending, estimated at 17-19 kcal/mole[19] is largely supplied by hydrogen bonds and electrostatic interactions with the DNA backbone of two regions flanking the central 10-bp segment. The ability of certain DNA sequences to adopt a particular structure required for protein-binding can reduce the free energy cost of the interaction and thus increase specificity. So, differences in bend angles induced by different proteins could be explained by differences in DNA bendability, i.e. the ability to yield to an external force. In the crystal structures of 434 Cro and repressor the DNA is overwound in the center between the two major groove contacts. This requires a narrowed minor groove in the center caused by changes in propeller twist that optimize stacking interactions in bent DNA. These changes, including minor groove compression, can only be readily adopted by basepairs with two hydrogen bonds (A-T and I-C). Therefore the base pairs connecting the two sites which are in direct contact with the protein can determine the ease with which the operator can be overtwisted. Extensive mutagenesis of binding sites in the 434-repressor[26] and CAP[27] support the view that sequence-dependent differences in DNA bendability and torsional rigidity are important factors in determining the binding affinity of the protein-DNA complexes.

Protein Domains Required for Bending

Many eukaryotic transcription factors have a modular structure in which a DNA-binding domain and an activation domain can be distinguished that can function independently (for review, see ref. 28). DNA binding domains are not always highly ordered but can undergo disorder to order transitions upon recognition of their DNA sites as exemplified by the bZip proteins GCN4 and AP-1 (for review, see ref. 29).

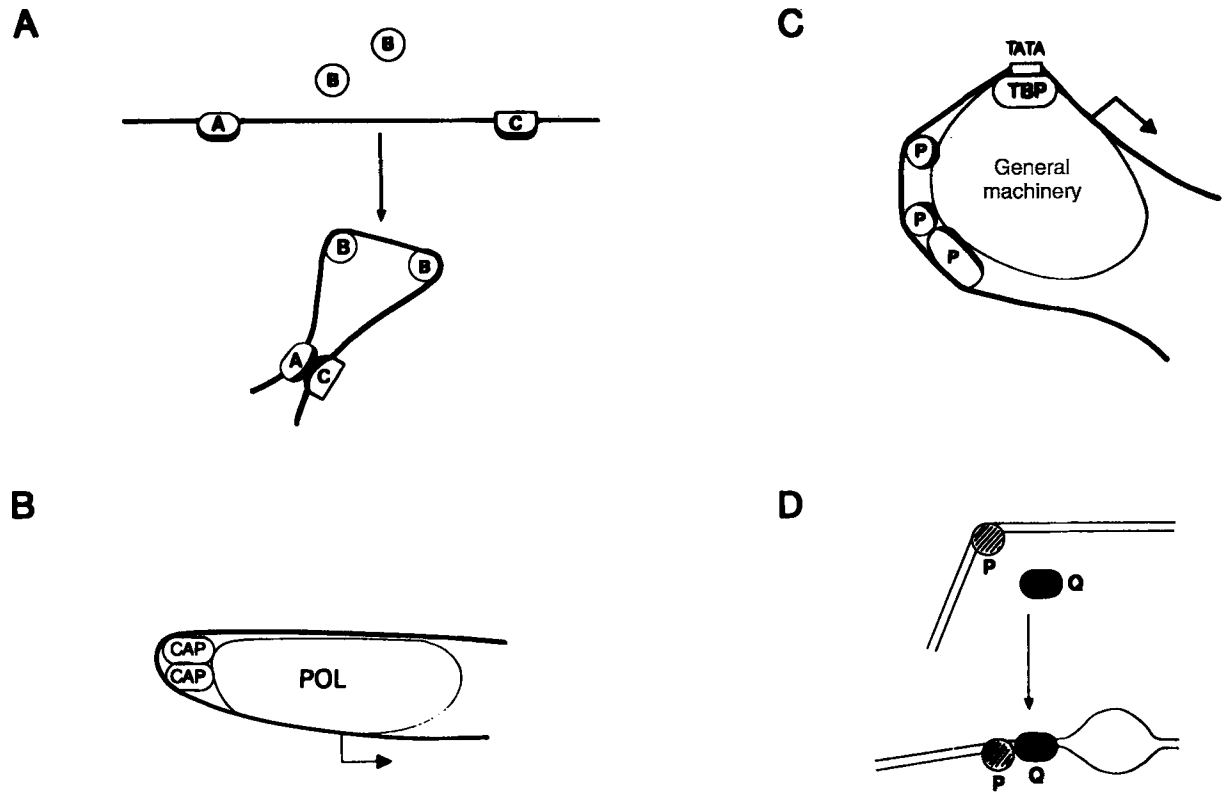


Fig 3. Models to explain the role of bending in transcription activation. A. *Looping*. Bending of the DNA by proteins B facilitates the interaction of distantly bound proteins (A and C). B. *Wrapping*. DNA bending facilitates binding of a large nucleoprotein complex including RNA polymerase (pol) in which the DNA is wrapped around the surface of the complex. As an example the CAP-RNA-polymerase-DNA complex is shown[9]. The arrow indicates the transcription start site. C. *Short-range contacts*. When properly spaced, proteins that bend DNA could facilitate interactions between transacting factors and the TATA binding protein (TBP) or other components of the general transcription machinery, including TBP-associated factors (TAFs). It should be stressed that this is just an example of how TBP bending could act. It is equally feasible that TBP assists in unwinding of the DNA. D. *Distortion of DNA structure*. Protein-DNA binding free energy is used for local DNA distortions, here drawn tentatively as unwinding. A protein (P) that bends DNA will, upon interaction with another protein (Q), release energy leading to unwinding and possibly resolution of the bend. Local unwinding may facilitate or stabilize binding of other transcription proteins or assist in open complex formation. Bending free energy might also be used for enhanced protein dissociation (not shown, see text).

Simultaneously the DNA structure becomes modified, e.g. bent to obtain the energetically most favourable conformation of the DNA-protein complex. In general, the protein domains involved in bending are buried within the DNA binding domain but in some cases separate domains have been distinguished. In the bipartite DNA binding domain of POU-proteins, the highly conserved POU-specific domain, which contributes additional contacts[30], is required for bending whereas the POU-homeodomain does not bend DNA but contributes most to the binding energy[11,30]. For fos and jun, peptides containing only the DNA-binding domain bend DNA to smaller angles than the intact protein[16] and for TUF/GRF, proteolysis leads to loss of bending without affecting the binding affinity suggesting additional domains required for bending[31]. A clearly separated domain is present in the bacteriophage Φ29 p4 protein in which a stretch of 12 C-terminal amino acids enhances the existing curvature without significantly affecting the binding constant[32]. Since this C-terminal domain has a net positive charge, a model is proposed in which this basic domain makes non-specific electrostatic interactions with the DNA, thereby inducing a bend. In conclusion, bending can be modulated in several instances by domains outside the core DNA binding domain.

How Does Bending Stimulate Transcription?

Apart from being the energetically most favourable conformational state of the DNA-protein complex, bent DNA could function in several ways to enhance transcription. Bending might mediate the interaction of proteins bound to dispersed sites. Bent DNA close to the transcription-initiation site could enhance the interaction between transcription factors and the general transcription machinery during formation of complex nucleoprotein structures. In both cases, bending facilitates protein-protein interactions. Alternatively, energy stored in protein-DNA bends could cause local structural changes in the promoter that in turn enhance or stabilize

binding of other proteins or facilitate opening of the DNA helix. Finally, this stored energy could also be used after initiation to promote the transition from initiation to elongation by increasing the dissociation of RNA polymerase from an initiation complex. These possibilities, which are not mutually exclusive, will be discussed below (see also Fig 3).

Bending facilitates protein-protein interactions

Long-distance interactions between proteins via looping (Fig 3A) could be enhanced by bending of the DNA. Examples are the RepA protein for which two sites are required for transactivation that are brought together by IHF[33], the Lymphoid Enhancer Factor LEF-1[51] and the NtrC protein that contacts RNA polymerase leading to a stable initiation complex[34]. Interestingly, the HMG domain of LEF-1 which bends DNA sharply, can functionally substitute for bacterial IHF that also bends DNA extensively[51] indicating a structural role for these proteins.

Since promoter and enhancer regions generally contain multiple factor binding sites, the orientation- and magnitude of the bend angle could provide a means to select among different protein-protein interactions. This could explain opposite effects of transcription factors, for instance in the interaction of the glucocorticoid receptor with fos-jun heterodimers (negative effect) or jun-jun homodimers (positive effect) (for review, see ref. 35).

Bending might also influence short-range interactions between proteins without DNA looping. At short distance from the transcription initiation site, DNA bending could help to position the activator properly for direct interaction with the RNA polymerase or other components of the general transcription machinery like TFIID of TFIIB (Fig 3C). In this respect it is interesting that also TFIID bends DNA[36]. Evidence for such a mechanism is obtained so far only in prokaryotic systems. ϕ29 p4 protein interacts better with RNA polymerase if the C-terminal sequences required for bending are present[37]. Also, as suggested for CAP (Fig 3B), wrapping the DNA around the protein complex could provide additional contacts between RNA polymerase and DNA sequences upstream of the CAP binding site.

Local Structural Changes in DNA Induced by Bending

The free energy of protein-induced DNA bending might be used for local deformation of the DNA structure such as underwinding without strand separation, or unwinding (Fig 3D). This induced torsional strain could then be used to activate transcription by increasing the binding or stability of the general transcription-machinery or to promote formation of an open transcription complex. Energetic coupling between DNA bending and basepair opening is theoretically possible[38] and has been detected recently for the mercury-inducible repressor MerR[39]. Upon activation of this allosteric effector with Hg(II), the bend angle does not change but local underwinding of the spacer region of the promoter occurs. The positive free energy change occurring during this underwinding may come from a decrease in protein-DNA binding free energy.

DNA distortions might also be sensed by proteins sliding along the DNA and thereby lead to pausing, which could assist in formation of multi-protein structures ('traffic-jam effect'). Alternatively, distorted DNA may prevent nucleosome formation or change the nucleosome position slightly to influence transcription. However, convincing evidence for these mechanisms has not yet been obtained.

DNA bending could facilitate dissociation of nucleoprotein complexes

Energy stored in DNA-protein complexes could not only be used for enhanced association but also for dissociation of interacting proteins. I-κB increases the dissociation of NF-κB DNA complexes which might be facilitated by bend energy[21]. The same could be true for the interaction of I-POU with the POU-domain protein Cf1A[40]. Evidence that bend energy can be used to help counteract protein-DNA or protein-protein interactions comes from recent results by Zinkel and Crothers[12]. They noticed that during *lac*-promoter directed transcription *in vitro* the CAP-induced bend is maintained and even increased upon formation of closed and open initiation-complexes. This excludes the possibility that the bend energy is used for unwinding and they propose that it could be used to assist in escape of RNA polymerase from the promoter after initiation. DNA unwinding could then be mediated by the formation of a writhing structure in which the DNA is wrapped around the CAP-RNA polymerase complex.

Is Bending Sufficient for Transcription Activation?

If protein-induced bending just serves to bring dispersed binding sites closer together one could envisage that intrinsically curved DNA could fulfill the same function. Indeed, synthetically curved DNA can act as a transcriptional activator replacing the CAP-activated part of the gal promoter[41] or the *lac* promoter[42] both *in vivo* and *in vitro*. The proper position of the center of curvature is important suggesting that contacts between upstream activators and RNA polymerase are phase-dependent, in agreement with functional spacing in natural promoters. DNA curvature can also substitute for Φ29 p4 induced repression[43]. Conversely, substitution of curved DNA upstream of a promoter by a straight DNA fragment containing the binding site for a repressor (rep A) that bends DNA results in dependence of transcription on this protein suggesting that bending in the natural promoter is important[44]. These examples indicate that under certain conditions intrinsic bends can substitute for protein-induced bends in prokaryotes, presumably by facilitating protein-protein interactions. For eukaryotic transcription factors this has not yet been reported. The presence of activation domains that are supposed to contact the general transcription machinery by direct protein-protein interactions may prevent functional substitution by curved DNA. Moreover, in those situations in which stored bend energy is used for unwinding, it is difficult to envisage how unstressed,

permanently curved DNA lacking expendable free energy could substitute for protein-induced bending.

Conclusions and Perspectives

The development of several non-crystallographic techniques to reveal DNA bending has expanded our understanding of protein-DNA interactions. It now appears that bending is a common, although not universal, property of transcription factors. The occurrence of protein-induced bending, in addition to existing DNA curvature in promoters and at a considerable energetic cost, indicates that a compact shape of the DNA is of particular advantage to the cell to control gene expression. Since even basal transcription in eukaryotic cells requires the interaction of at least 17 different polypeptides, DNA bending may be only a relatively cheap investment to stabilize these large multi-protein-DNA structures. Similar complex structures may exist during DNA replication and considering this, it does not come as a surprise that several transcription factors are also functional in DNA replication[11]. The various intermediate steps in the process of assembly, function and disassembly of initiation complexes will certainly be accompanied by structural changes in these complexes, both in proteins and in DNA. In particular, phasing analysis, which is least dependent on protein shape, may turn out to be a relatively accessible tool to study changes in DNA geometry occurring during transcription initiation.

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