Gene Regulation by HMGA and HMGB Chromosomal Proteins and Related Architectural DNA-Binding Proteins

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Abstract

The eukaryotic abundant high mobility group HMGA and HMGB proteins can act as architectural transcription factors by promoting the assembly of higher-order protein-DNA complexes which can either activate or repress gene expression. The structural organisation of both classes of protein is similar with either a single or repeated DNA binding domain preceding a short negatively charged C-terminal tail. In the HMGB class of proteins the HMG DNA-binding domain binds non-specifically and introduces a sharp bend into DNA whereas the AT-hook in the HMGA protein binds preferentially to A/T rich regions of DNA and stabilises a B-DNA structure. The acidic tails are hypothesised to facilitate the interaction of the proteins with nucleosomes by binding to the positively charged histone tails. Both classes of protein also interact with a large number of transcription factors that bind to specific DNA sequences.

Introduction

The eukaryotic nucleus contains three classes of abundant chromatin associated proteins - the High Mobility Group proteins of the HMGA, HMGB and HMGN classes (originally termed HMG1/Y, HMG1/2 and the HMG14/17; for recent nomenclature changes see ref. 1), so called because they were initially identified on the basis of their rapid migration through starch gels. The HMGA and HMGB classes of chromosomal proteins in general share some common characteristics, notably a conserved acidic region and the ability to interact with several different transcription factors. A major role is to organise the structure of DNA-protein complexes in the context of chromatin.

Architectural DNA Binding Proteins

In both the eukaryotic nucleus and the bacterial nucleoid the trajectory of the DNA double helix is normally tightly constrained so that not only can the DNA be compacted without entanglement but also to provide an appropriate environment for the enzymatic machinery involved in DNA transcription, replication and recombination. This organisation is normally effected by abundant DNA binding proteins, termed architectural DNA-binding proteins, that either induce DNA bending or facilitate the formation of multicomponent DNA-protein complexes. The term 'architectural' in this context implies that the protein is required for organising DNA but the proteins that fall within this definition are often otherwise functionally distinct and would include, for example, the histone octamer, abundant eukaryotic...
chromosomal proteins, such as the HMGA and HMGB proteins, abundant proteins associated with the prokaryotic nucleoid, such as FIS, H-NS, IHF and HU, as well as bona fide transcription factors exemplified by the TATA-binding protein (TBP). Some of these proteins have more than one architectural function. For example FIS can stabilise particular configurations of supercoiled DNA plasmids and also act to promote the assembly and activity of transcription, replication and recombination complexes. Many of the more generalised architectural proteins may be regarded as facilitators and are often not essential for viability while some more 'specialised' proteins, such as the histone octamer and TBP, are clearly essential.

Principles of Transcription Factor Induced Bending

The bending of DNA by transcription factors and by other protein complexes is a major component in the establishment of the overall morphology of protein-DNA complexes. This bending is usually a consequence of indirect readout, a mechanism by which the selectivity of binding is dependent not on making direct contacts between the aminoacids and bases, i.e., direct readout, but instead on the physicochemical properties of the DNA molecule itself.

Recognition of DNA by transcription factors often involves both direct and indirect readout. However, the principles of indirect readout are well illustrated by the histone octamer which, although not a transcription factor itself, completely lacks direct contacts between the aminoacid side chains and the bases of the bound DNA. The octamer binds 147 bp of DNA which are wrapped in a left-handed superhelix with a total curvature of approximate 10 radians. This curvature contrasts with the stiffness of DNA in solution where the average persistence length (P), defined as the length over which the average deflection of the polymer axis caused by thermal agitation is one radian, is 140-150 bp, i.e., the same length as that bound by the histone octamer. For DNA molecules that are not anisotropically curved the affinity of the DNA for the octamer is directly proportional to the flexibility (the inverse of the stiffness). However the dependence of the binding energy on P is some 10-fold lower than the dependence of the bending energy in solution on P. This implies that the histone octamer increases the apparent flexibility substantially to compensate for the average increase in DNA curvature on binding.

How might this change in flexibility be effected? The histone core provides a DNA binding surface in the form of a positively charged ramp. On binding to this ramp the negative charges on one side of the DNA are neutralised. This asymmetric neutralisation, which can be mimicked in free DNA, creates an imbalance in charge distribution on opposite sides of the double helix so that repulsion between the opposing sugar-phosphate backbones on the unneutralised side facilitates bending by increasing the width of the grooves. Concomitantly, the reduction in this repulsion on the inside of the bend permits greater freedom in the motions of the base-pairs, with a corresponding reduction in the width of the grooves. The greater flexibility of the motions between base-pairs is reflected in the periodic variation of twist and roll with groove width such that the ranges of values assumed for both are substantially larger than the corresponding ranges observed for DNA molecules free in solution.

The correlation between flexibility and affinity for the histone octamer only applies strictly when a DNA molecule does not possess intrinsic anisotropic curvature. When it does the affinity may be relatively higher or lower. For example, the intrinsically curved TATA DNA sequence whose curvature is compatible with the surface of the histone octamer binds with an affinity that would normally be characteristic of a substantially more flexible isotropic binding site. In this case binding is favoured by the lower entropic penalty on binding relative to an isotropically flexible molecule. However if the intrinsic bend is too great and therefore less compatible with the protein binding surface the affinity is reduced relative to an isotropically flexible molecule.

An extension of this principle of asymmetric alteration of the ionic environment of DNA is provided by the transcription factor TBP and the HMG-domain, found in HMGB proteins, a class of abundant chromosomal proteins and certain transcription factors such as SRY and LEF-1. The HMGB proteins consist essentially of a small L-shaped protein domain with a
cluster of hydrophobic residues on its inner surface and an extended unstructured basic region. When these proteins bind to DNA they produce a bend of 95-120° over about six base-pairs and decrease both the axial and torsional stiffness. On the outer surface of the bend the hydrophobic 'wedge' towards the apex of the L binds in and widens the minor groove, concomitantly untwisting the DNA. This effect is believed to be facilitated by a local reduction in the dielectric constant which increases the repulsion between opposing sugar-phosphate backbones on the approach of the protein to DNA. At the same time the basic region neutralises the phosphates bounding the major groove on the inside of the bend thus decreasing the repulsive forces and permitting the narrowing of the groove. Additionally the protein inserts, or intercalates, hydrophobic aminoacids into either a single base-step or into two base-steps that are themselves separated by a single base-step. The extent to which this intercalation increases or simply stabilises the induced bend is unclear. The bend induced by the intercalation contrasts with the smooth DNA bending induced by the histone octamer since the intercalation effectively introduces a kink in the DNA such that the stacking interactions between adjacent base-pairs are very substantially reduced.

In the TATA-binding protein this same principle of hydrophobic interactions predominates. Here two pairs of phenylalanine residues are intercalated at steps separated by 6 bases, kinking the DNA by -45° at each intercalation site. Between these pairs of phenylalanine residues a hydrophobic surface rests snugly within the minor groove. Again the minor groove is widened and untwisted. However, unlike the HMGB proteins there is no charge neutralisation on the opposing major groove face of the bent DNA and indeed the sharpness of the induced curvature is less than that for the HMGB proteins.

In other transcription factors there is substantial variation in the degree of induced bending. The Escherichia coli CAP (aka CRP) factor is a good example of mixed direct and indirect readout. This dimeric protein induces a bend of -45° per monomer. In this case the major bend occurs where the recognition helix of the helix-turn-helix motif binds in the major groove on the inside of the bend, concomitantly making direct contacts with the DNA bases and neutralising the sugar-phosphate backbone in the immediate vicinity. Flanking the central recognition palindrome is a basic ramp which binds DNA and increases the overall DNA bend by indirect readout in a manner analogous to the histone octamer.

**Biological Functions of DNA Bending**

Although one of the principal roles of DNA bending in the living cell is to maintain the compaction of DNA, it also has important functions in transcriptional control and, in particular, in the assembly of regulatory complexes. A major consequence of introducing a tight bend into DNA is to bring DNA sequences which are far apart on a linear representation of a DNA molecule into close spatial proximity. This effect, which is also characteristic of plectonemically supercoiled DNA, is mediated in chromatin by the HMG-domain transcription factors, such as TCF-1, LEF-1 and SRY. In the case of TCF-1 acting at the enhancer of the TCR promoter, the bend induced by the factor brings together a normally unstable complex of the Ets-1 and PEBP2α DNA-binding proteins and ATF/CREB activator proteins to form a stable complex. This example is probably a particular case of the more general phenomenon in which the DNA between a transcription factor and its target protein partner must be bent for protein-protein contacts to occur. The ease of bending will depend critically on the distance and the helical phase difference between the binding sites of the factor and its target. Normally unless one or both of the partner proteins are flexible contact will be facilitated when the binding sites are in helical phase, primarily because of the constraints on the torsional flexibility of DNA. However, at least in vitro, the constraints imposed by both torsional and axial rigidity can be overridden by the abundant DNA-bending proteins of the HMGB class. In the presence of these proteins a requirement for an integral number of helical turns between binding sites is no longer crucial. Furthermore the involvement of the HMGB protein in the formation of the complex need only be transient.
To what extent are variations in DNA flexibility reflected in genomic organisation? An excellent example of the dependence of biological function on DNA structure is provided by genome of the enteric bacterium *E. coli*. In this organism the strongest promoters for DNA transcription, often those directing the synthesis of rRNA and tRNA, are almost invariably associated with A/T rich, and hence flexible, DNA sequences extending upstream for 100-300 base-pairs from the transcription startpoint. The activity of many of these promoters is strongly dependent on a high negative superhelical density stored in the DNA. This would in principle favour both DNA untwisting at sequences such as TATAAT close to startpoint and also left-handed DNA wrapping around the protein complex responsible for initiating transcription. In many of these highly active promoters the DNA sequence also imparts curvature to the region, a feature that correlates both with the presence of multiple activating binding sites for the abundant DNA bending protein FIS (Factor for Inversion Stimulation). These sites are often organised in helical phase such that the binding of FIS could constrain a negative superhelical loop. Indeed in the *rrnB* P1 regulatory region the presence of a far upstream FIS site centred at position -222 from the transcription startpoint results in the constraint of an additional supercoil in the initiation complex. A primary function of this FIS-induced DNA looping is to promote the wrapping of DNA around the RNA polymerase prior to the initiation of transcription, a phenomenon that has also been proposed for the activation of the lac promoter by the CAP DNA-bending transcription factor, and consequently to facilitate the extended wrapping characteristic of the open complex. In turn the FIS-induced constraint of negative superhelicity buffers this type of promoter against changes in the unconstrained superhelical density. In other such promoters an alternative model proposes that the upstream activating sequence contains regions that are highly susceptible to DNA untwisting. For both DNA wrapping and untwisting in the upstream region both models predict that the topological unwinding is transmitted to the 'TATA' sequence and promotes its untwisting.

DNA bending may also be required for the establishment of repressive regulatory complexes. Here, a DNA loop is often formed by the binding of an oligomeric repressor to two sites that are distant from each other along the DNA sequence. This loop, which can be as tightly bent as nucleosomal DNA, prevents the binding of RNA polymerase to the regulated promoter. Examples of this mode of regulation include repression by the AraC, LacI and GalR proteins.

**HMGA Proteins**

The vertebrate HMGA proteins are small proteins of ~100-110 aminoacids and contain tandem copies, usually three, of a characteristic DNA-binding domain, the AT-hook, together with a C-terminal acidic region (Fig. 1). The AT-hook is not restricted to the HMGA proteins as such as it is also found in a related *Drosophila* chromosomal protein, D1, which contains multiple copies of the motif in the motor subunits of various ATP-dependent chromatin remodelling complexes and in certain transcription factors that also contain a primary sequence-specific DNA-binding domain where it is assumed to act as an auxiliary DNA-binding element.

The AT-hook is an unstructured short motif with the consensus sequence Arg/Lys-Pro-Arg-Gly-Arg-Pro-Arg/Lys and selectively binds in the minor groove of A/T-rich regions of DNA. The central Arg-Gly-Arg core adopts an extended conformation deep within the groove with the arginine side chains making extensive hydrophobic contacts along the base of the groove. The proline residues change the trajectory of the backbone allowing the basic residues flanking the core mediate electrostatic and hydrophobic contacts with the DNA backbone. When bound to DNA the surface of the core motif contacting the DNA is concave and resembles that of the DNA binding drug netropsin, which has a similar selectivity for A/T-rich sequences. For both netropsin and an AT-hook one consequence of DNA binding is a modest widening of the minor groove with a concomitant stabilisation of B-DNA structure. In some cases this widening results in a change in the direction of bending of DNA, particularly when that bending is dependent on a narrow minor groove width in A/T-rich sequences.
Figure 1. Comparison of the structural organisation of the HMG proteins. Regions of net negative charge outside the principal DNA binding or nucleosome binding domains are indicated in red and those of net positive charge in blue. The AT-hook DNA binding domain (AT) in HMGAl proteins and the A- and B-type HMG domains in HMGB proteins are indicated. The nucleosome binding domain (NBD) in HMGN proteins is also shown.

Thus a small intrinsic bend of -20° towards the minor groove in the IFN-β enhancer is reversed on binding HMGA1.43 This could then facilitate recognition of the opposing major groove by transcription factors binding to specific sequences. Nevertheless although the HMGA proteins induce only small changes in DNA structure they bind tightly to DNA ligands with distorted or unusual features. These include supercoiled DNA, four-way junctions and base-unpaired regions of AT-rich DNA (reviewed in ref. 34). Strikingly in vitro these proteins can also introduce supercoils into relaxed DNA, possibly by stabilising cross-overs and thereby stabilising DNA loops.44 This ability has been suggested as an explanation of the observation that in vitro HMGA1 represses the chick globin βA gene promoter in the absence of the 3' enhancer but strongly activates transcription in its presence, regardless of whether or not the substrate is free DNA or is assembled into nucleosomes.45 This stabilisation of loops could be mediated by the presence of multiple AT-hooks on each protein.

In mammals the HMGA proteins are encoded by two functional genes, HMGAl and HMGA2. Alternative splicing of the transcripts of these genes increases the variety of protein products, of which the most abundant are HMGAla (HMG-I) and HMGAlb (HMG-Y). These proteins appear to perform a variety of functions, of which the most studied are related to chromatin structure and to the facilitation or inhibition of transcription factor binding. In vitro HMGA proteins bind to nucleosomes, notably at the exit and entry points to the nucleosome core particle where they are in close proximity to histones H2A, H2B and H3.46,47 The proteins can also bind to internal sites where they can induce local changes in the rotational setting of the wrapped DNA. The binding to the nucleosomal DNA is mediated by the AT-hooks but (by analogy to the HMGB class of proteins) it is conceivable that the acidic tail may also be involved in contacting the histone octamer and could perform a similar role to that of the HMGB proteins.

In mitotic chromosomes the HMGA proteins are associated with particular bands and these proteins are localised to the base of large chromatin loops in close proximity to scaffold-attachment regions (SARs).48 As a consequence it has been suggested that the HMGA proteins are involved in the maintenance of the condensed mitotic chromosome structure in these regions.
Evidence supporting this view was adduced from the observation that synthetic 'MATH' proteins containing AT-hooks interfere with chromosome condensation during mitosis. In contrast another model suggests that co-operative binding of HMGA molecules to a looped chromatin domain in interphase nuclei will facilitate the formation of an 'open' chromatin structure that is competent for transcription by competing with histone H1. Although some experiments demonstrate that the MATH proteins can counteract the spreading of heterochromatin, as shown in particular by suppressing position-effect variegation in flies. The mechanism by which this is accomplished remains to be established. However the expression of the HMGA proteins is strongly correlated with cell growth and is characteristically high in neoplastically transformed cells.

HMGA1 has been shown to interact directly with a large variety of transcription factors including AT-1, ATF-3, NF-Y, IRF-1, SRF, NF-κB, p50, 1st-1/Oct-6 and c-Jun. In some cases the protein regulates the formation of an enhanceosome. Thus at the virus-inducible β-interferon enhancer a complex containing both HMGA proteins and transcription factors forms and then acts to recruit RNA polymerase II and its associated general transcription factors. In other cases HMGA proteins can block enhanceosome formation. This modulation of transcription factor binding may be integrated with the regulation of chromatin organisation. Thus HMGA1a enhances the binding of the ATF-3 to a site at the edge of a nucleosome positioned on the HIV-1 promoter. This combination of bound proteins can then recruit the remodelling complex hSWI/SNF. The interactions of HMGA with these factors can be modulated by covalent modifications including phosphorylation and methylation.

**HMGB Proteins**

HMGB proteins are characterised by the HMG-box, a DNA-binding domain specific to eukaryotes. A major characteristic of this domain is to introduce a sharp bend into DNA (Fig. 2). Accordingly the domain also binds preferentially to a variety of distorted DNA structures, especially those in which the distortion itself induces a bend. These include negatively supercoiled DNA, small DNA circles, cruciforms, DNA bulges and cisplatin modified DNA. The HMG-box domain is also found in several related types of protein, for example transcription factors such as SRY and LEF-1, and subunits of many chromatin remodelling complexes. All these proteins are predominantly nuclear and appear to act primarily as architectural facilitators in the manipulation of nucleoprotein complexes; for example, in the assembly of complexes involved in recombination and the initiation of transcription, as well as in the assembly and organisation of chromatin.

The archetypal HMGB proteins are highly abundant (10-20 copies per nucleosome in the mammalian nucleus) and often occur in two major forms, HMGB1 and HMGB2, originally termed HMG1 and HMG2, in vertebrates. The two distinguishing features of these highly homologous proteins are two similar, but distinct, tandem HMG-box domains (A and B), and a long acidic C-terminal 'tail', consisting of 30 (HMG1) or 20 (HMG2) acidic (aspartic and glutamic acid) residues, linked to the boxes by a short, predominantly basic linker (Fig. 1). However the most abundant HMGB-box domain proteins in Saccharomyces cerevisiae, Nhp6ap and Nhp6bp (non-histone proteins 6A and 6B respectively), contain only a single HMGB box, and lack an acidic tail. Likewise the two major HMGB-box domain proteins in Drosophila melanogaster, HMG-D and HMG-Z, have only a single HMGB box but, unlike the yeast proteins, contain a short C-terminal acidic tail in addition to a basic region (Fig. 1). These abundant proteins in yeast and Drosophila may be the general functional counterparts of HMGB1 and 2 in vertebrates.

The precise functions of the chromosomal HMGB proteins in vivo for a long time remained obscure. However there is now substantial evidence that they interact directly with both transcription factors and with the histone octamer. These interactions can affect transcription factor access to chromatin either directly or by promoting chromatin remodelling. In the latter case the proteins may facilitate repression or activation.
Figure 2. DNA bending by an HMG domain. The figure shows the DNA binding domain of the Drosophila High Mobility Group protein HMG-D binding to a short DNA fragment. The protein binds in the minor groove widening the groove and concomitantly stabilising a bend of -100° in the DNA. This is achieved by inserting the sidechains of hydrophobic aminoacid residues (space-filling representation) between adjacent base-pairs at two locations separated by one base-step. The α-helices of the protein are depicted in red and yellow. The DNA structure shown contains a 'bulge' in which two adjacent bases on one of the strands are unpaired. Reproduced with permission from ref. 88.

There are two established cases in which the assembly of nucleoprotein complexes containing sequence-specific DNA-binding proteins is promoted by the DNA-bending properties of HMGB1 and 2, i.e., the proteins have a classical architectural role. First, in V(D)J recombination the lymphocyte-specific proteins RAG1 and RAG2 (human recombination activating genes 1 and 2) appear to recruit HMGB1 and 2 to the appropriate sites in chromatin presumably by protein-protein contacts with the RAG1 homeodomain. Here they ensure the "12/23 rule". This requires that V(D)J recombination occurs only between specific recombination signal sequences (RSS). Each RSS is made up of a conserved heptamer and nonamer sequence separated by a non-conserved spacer of either 12 or 23 base pairs. HMGB1 (in concert with RAG1,2) facilitates recombination probably by bending the DNA between the two conserved sequences spaced by 23 bp and stabilising a nucleoprotein complex. The HMGB protein plays the dual role of bringing critical elements of the 23-RSS heptamer into the same phase as the 12-RSS to promote RAG binding and of assisting in the catalysis of 23-RSS cleavage. Recent footprinting experiments indicate that the HMGB1 (or HMGB2) protein is positioned 5' of the nonamer in 23-RSS complexes, interacting largely with the side of the duplex opposite the one contacting the RAG proteins. A second instance in which an abundant HMGB protein may facilitate nucleoprotein complex assembly is in the formation of an enhanceosome containing the Epstein-Barr virus replication activator protein ZEBRA and HMGB1, the two proteins bind cooperatively, HMGB1 binding to, and presumably bending, a specific DNA sequence between two ZEBRA recognition sites. Bending of DNA by HMGB1 and 2 has also been invoked to explain the essential role of these proteins in initiating DNA replication by loop formation at the MVM (minute virus of mice) parvovirus origin of replication.
In vitro HMGB proteins can enhance the binding of various transcription factors (e.g., adenovirus MLTF, Oct-1 and 2, HoxD9, p53, steroid hormone receptors, Rel proteins, p73, Dof2 and the Epstein-Barr activator Rta) to their cognate DNA binding sites (reviewed in ref. 66). Similarly rat SSRP1 has been shown to facilitate the DNA binding of serum response factor and human SSRP1 is associated with the γ isoform of p63 in vivo at the endogenous MDM2 and p21Waf1/Cip1 promoters. In most of these cases, the interaction of the HMG protein with the transcription factor has been detected in vitro and could, in principle, serve as the mechanism for recruitment of HMGB1 or 2 to particular DNA sites. In some cases transfection experiments indicate functional interactions in vivo. Direct interactions between Nhp6p and the Gal4p and Tup1p transcription factors have also been inferred in vivo by a split-ubiquitin screen and confirmed by a pull-down assay. Although the demonstrated interactions in vitro so far involve an HMGB protein and a single transcription factor, it is entirely possible that in vivo, in a natural regulatory context, the bending of DNA by HMGB1 and 2 could potentially allow the recruitment of a second transcription factor to the complex, in an analogous manner to the action of sequence-specific HMG-box transcription factors such as LEF-1 in the enhanceosome at the T cell receptor alpha (TCRα). HMGB1 may play a catalytic, chaperone role, since it does not appear to be stably incorporated into the final complex. Although a role for HMGB1 and HMGB2 induced DNA bending in the facilitated binding of transcription factors, while being entirely plausible, has not been directly established, it is strongly suggested by the ability of HU to substitute for the HMGB1-stimulated binding of the Epstein Barr virus transactivator Rta to its cognate binding sites. A possible role for an HMGB1-induced change in DNA conformation in facilitation of transcription factor binding is also suggested by the observation that HMGB1 promotes binding of p53 to linear DNA but not to 60 bp DNA circles. However, in this case the data do not distinguish between possible effects of DNA bending or untwisting.

The biological roles of the HMGB proteins have been studied using gene knock-outs. In mice the loss of HMGB1 but not of HMGB2 is lethal although in the former knock-out there are pleiotropic effects on glucose metabolism while in the latter spermatogenesis is impaired. This suggests a functional redundancy between members of the HMGB1 and 2 family. A similar situation occurs with Nhp6ap and Nhp6bp in yeast. However, the different phenotypes of the HMGB1 and HMGB2 null mice probably reflect specific roles for the two proteins in different tissues. In *S. cerevisiae* the transcriptional effects of *NHP6* are not general but gene-specific. At the *CHAl* locus, loss of *NHP6* results both in an increase in the basal level of transcription and in a substantial decrease in the induced level. This suggests an effect at the level of chromatin. The *CHAl* regulatory region contains a positioned nucleosome which occludes the TATA box under non-inducing conditions. On induction the TATA region becomes accessible. However in the mutant strain, consistent with the increased basal level transcription, the chromatin structure of the TATA region in the uninduced state is similar to that in the induced wild-type strain. *NHP6* thus appears to be required for establishment of the organised chromatin structure characteristic of the uninduced state. The RSC remodelling complex is also required for this process suggesting that RSC and Nhp6p may cooperate to remodel chromatin.

Further insights into how Nhp6ap and Nhp6bp function were provided by studies on the *HO* gene. Loss of *NHP6* function can be suppressed by mutations that increase nucleosome accessibility and mobility, and enhanced by those with the opposite effect. Mutations both in the *SIN3* and *RPD3* genes, encoding components of a histone deacetylase complex, and in *SIN4*, partially restore wild-type function in cells lacking both Nhp6ap and Nhp6bp, while loss of the histone acetylase Gcn5p (also a component of the SAGA histone acetylase complex) in the same cells results in a more severe phenotype. Rpd3p and Gcn5p contribute to the dynamic balance between histone acetylation and deacetylation. Both histone acetylation and the sin (SWI/SNF independence) phenotype are correlated with chromatin unfolding and/or enhanced nucleosome accessibility while histone deacetylation would be expected to
favour folding. On this argument one role of the Nhp6p proteins would be to antagonise folding and possibly promote nucleosome accessibility.

The ability of the HMGB proteins to promote both transcription factor binding to their cognate sites and also chromatin remodelling implies that these activities could be coordinated to alter chromatin structure in the vicinity of a factor binding site. Like the HMGA proteins the abundant HMGB proteins bind to nucleosomes at sites close to the DNA exit and entry points. An insight into how HMGB proteins might alter the accessibility of nucleosomal DNA was provided by the observation that HMGB1 could facilitate the binding and subsequent remodelling function of the ACF remodelling complex in vitro. Further observations showed that HMGB-D, a Drosophila HMGB protein, when bound to nucleosome core particles increased the accessibility of nucleosomal DNA to restriction endonucleases at particular sites. These sites were asymmetrically distributed, one site being located at one end of the bound DNA and the other in the vicinity of the nucleosome dyad. This effect required the acidic tail of the HMGB protein: without it the HMG-D reduced accessibility at all sites tested on the nucleosome. This result argues that certain HMGB proteins can alter the structure of nucleosomes and to do so presumably by interacting with an available basic region of the histone octamer. From the distribution of the sites with increased accessibility a prime candidate would be one (but not both) of the N-terminal tails of histone H3 or one of the C-terminal tails of histone H2A. It is important to note that the yeast Nhp6 proteins lack an acidic region and so could not interact with histones directly in this way. However they can associate with two other proteins, Pob3p and Spt16p, to form a complex, SPN, involved in chromatin remodelling.

Both these proteins contain extensive acidic regions and so, in principle, could substitute for the lack of an acidic region in Nhp6p.

**Concluding Remarks**

The abundant HMGA and HMGB chromosomal proteins share several common features. Both interact with nucleosomes, both can also bind a set of transcription factors, both are involved in enhanceosome formation and both can facilitate the recruitment of chromatin remodelling complexes. Interestingly the HMGN class of HMG proteins shares with the HMGA and HMGB classes the ability to interact with nucleosomes and also possesses a C-terminal region with a net negative charge.

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