The Interaction of Transcription Factors with Nucleosomal DNA

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Summary

Nucleosome positioning is proposed to have an essential role in facilitating the regulated transcription of eukaryotic genes. Some transcription factors can bind to DNA when it is appropriately wrapped around the histone core, others cannot bind due to the severe deformation of DNA structure. The staged assembly of nucleosomes and positioning of histone-DNA contacts away from promoter elements can facilitate the access of transcription factors to DNA. Positioned nucleosomes can also facilitate transcription through providing the appropriate scaffolding to bring regulatory factors bound at dispersed sites into juxtaposition.

Introduction

Within the eukaryotic nucleus, complex metabolic processes involving DNA occur on a chromatin template. The formation and maintenance of chromatin structure is essential for the compaction of DNA within the nucleus. However, trans-acting factors must still gain access to regulatory DNA sequences within chromatin in order to initiate such fundamental processes as transcription and replication. How this contrasting requirement for compaction and accessibility may be resolved is the focus of this article.

We will briefly review certain aspects of chromatin structure and its relevance to gene expression. Then we will compare and contrast three systems in which the interplay of chromatin structure and transcription factor binding are different. In the TFIIIA system, the association of regulatory DNA sequences with histones prevents recognition by trans-acting factors. The glucocorticoid receptor, on the other hand, is able to activate transcription from a chromatin template. In the third type of interaction, transcription factors such as PHO4 bind to DNA between two positioned nucleosomes.

The Structure of DNA in a Nucleosome

The fundamental repeating unit of chromatin is the nucleosome. Approximately 160bp of DNA is coiled around a protein core consisting of two molecules each of the four core histones (H2A, H2B, H3 and H4). A single molecule of a linker histone (generally H1) is found to be associated with the exterior of this complex and with the stretch of DNA that links neighboring nucleosomes together. Many structural studies requiring homogenous samples have made use of nucleosome core particles which contain only the core histones and the 146bp of DNA that make the strongest histone-DNA contacts. In these preparations, the more loosely bound linker DNA has been removed along with the linker histone protein. Current models for the structure of DNA in a nucleosome build on the analysis of crystals of nucleosome core particles by Klug and colleagues[1]. The core particle has a disc-like shape, 11 nm in diameter and 5.6 nm in height. The DNA (146bp) is wrapped in 1.75 turns of a left-handed super helix around the histone core (Fig. 1). The structure has a pseudodyad axis of symmetry that passes through the center of the nucleosomal DNA. However, the winding of DNA around the core histones is not uniform. The crystal structure shows that the bending of the DNA is most severe at positions one and four helical turns (about 10 and 40bp) to either side of the dyad axis (center of the DNA) (Fig. 1B). The rise of the DNA superhelix is also not uniform, being gently inclined at either end and very steep in the center of the nucleosome (Fig. 1A). These studies and others attest to the large deformations in the path of DNA necessary to wrap it up into a nucleosome[2-4]. However, other important details of DNA structure such as twist (number of base pairs/turn) are not defined by these studies.

Information about these other details of DNA structure in the nucleosome is available from solution studies with chemical cleavage reagents. Singlet oxygen is a sensitive probe for structural deformations in DNA which are associated with an increase in the roll angle between adjacent base pairs. This reagent preferentially reacts with nucleosomal DNA about 1.5 turns to either side of the nucleosomal dyad[3]. The sensitivity of this site is consistent with the presence of a junction between two regions with different DNA structures (Fig. 1B). Indeed, hydroxyl radical cleavage of DNA in nucleosomal core particles indicates that distinct local regions with different helical twist do exist within the nucleosome. Quantitative analysis of these data show that the central two to three turns of DNA in the nucleosome have a different number of base pairs per turn (~ 10.7) than in the remainder of the structure (~ 10.0). The junction between these regions may be sufficiently distorted so as to be sensitive to singlet oxygen. These results also imply that the structure of DNA changes dramatically on association with a histone core away from the B-form normally found in solution (10.5bp/turn)[5-6]. A variety of other methodological approaches yield results that are in substantial agreement with these conclusions[4,7,8].

Trans-acting factors recognizing defined sequences of B-form DNA in solution will encounter several problems following the incorporation of this DNA into a nucleosome. One face of the DNA helix will be occluded by association with the histone core, access to even more of the helix will be hindered due to the adjacent turn of DNA wrapped around the core (Fig. 1C). As stated above, dependent on the position of DNA in the nucleosome, the path of the double helix will be more or less severely distorted. On average, the DNA will be bent such that the width of the major groove would change from 11 Å facing the core to 20 Å on the outside, and that of

the minor groove from 7 Å facing the core to 13 Å on the outside. Moreover, the helical twist of DNA will change from 10.5bp/turn to 10bp/turn, altering the precise separation of the edges of adjacent base pairs in the major groove.

The problem of access is further complicated by the presence of linker histones such as histone H1, their interaction with linker DNA, and the consequent stabilization of higher-order chromatin structures such as the 30nm fibre. A single molecule of histone H1 binds across the dyad axis of the nucleosome and stabilizes the association of the peripheral 20bp of DNA as it enters and exits the nucleosome[9]. Once within the nucleosome, the highly basic tails of histone H1 neutralize the negatively charged phosphodiester backbone of linker DNA allowing arrays of nucleosomes to be folded into the 30nm fibre[10]. Following the formation of the 30nm fibre the DNA within such a structure is likely to be 'invisible' to DNA binding proteins. Molecular mechanisms that unravel the fibre, at least around key regulatory regions, are likely to have an important role in enhancing nuclear events.

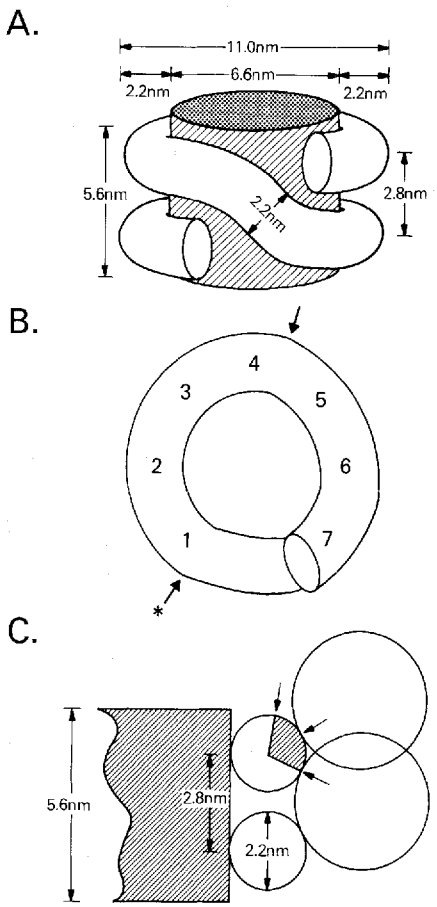

Fig. 1. The organization of DNA in the nucleosome. A. The dimensions of the nucleosome: the histone core is represented by the hatched cylinder, DNA by the open tube. This view overlooks the center of nucleosomal DNA, at this point the DNA superhelix rises very steeply. B. The path of one turn of DNA. Numbers refer to turns of the DNA helix away from the dyad axis. The arrows represent the positions of more severe bending in DNA seen in the crystal structure[1]. The asterisk marks the approximate position of a structural discontinuity detected by singlet oxygen or hydroxyl radical cleavage[3,5]. C. The problem of transcription factor access to DNA in the nucleosome. A cross-section of one side of the nucleosome is shown. Transcription factor (large circle) access to DNA (small circle) is restricted by the histone core (hatched box) and the adjacent turn of DNA. The only freely accessible region of the DNA helix is the hatched segment - marked by arrows (see ref 48).

Contributions of Protein Domains of the Histone Core to the Structure of DNA in the Nucleosome

The utilization of DNA for cellular processes is thus impeded at several levels by chromatin structure. At the primary level of chromatin organization, the conformation of nucleosomal DNA is sufficiently altered from that found in isolation so as to make 'normal' recognition of a cognate binding site by a protein rather unlikely. Further, nucleosomal DNA is sterically occluded by the histone proteins and by the adjacent turn of the DNA superhelix (see above). Certain modifications of the histone proteins have been correlated with processes such as transcription[11]. The effect of these modifications is likely to be to alter the nucleosomal DNA structure in some way in order to circumvent these restrictions. Each of the core histones consists of a N-terminal basic domain (tail) and a globular domain. The most highly modified domains of the histone proteins are the 'tail' regions. Surprisingly, the tails have been shown to play no role in nucleosome positioning or in defining the precise structure of DNA in the nucleosome[6].

The histone core has a tripartite structure in which a centrally located $(H3/H4)_2$ tetramer is flanked by two H2A/H2B dimers[12]. It has been suggested that nucleosomes deficient in H2A/H2B are more accessible to transcription factors[13]. It is possible to reconstitute just the $(H3/H4)_2$ tetramer with DNA. Hydroxyl radical cleavage patterns of DNA in such particles demonstrate that the tetramer organizes the central 120bp of DNA identically to that within a nucleosome core particle[6]. This type of footprinting analysis with sub-nucleosomal particles agrees well with chemical cross-linking experiments which physically map histone-DNA contacts along nucleosomal DNA[14]. Particularly strong protein-DNA contacts occur where histone H3 appears to distort the central turns of DNA in the nucleosome, perhaps accounting for the change in helical twist in this region.

Nucleosome Positioning

In light of the many impediments to the interaction of trans-acting factors in chromatin, it is clear that the cell must make use of multiple mechanisms to render important regulatory elements accessible. These include both the exclusion of histones from key DNA sequences and the specific arrangement of the relevant surface of the DNA helix on the histone core such that it is exposed to trans-acting factors capable of recognizing bent, non B-form DNA. These possibilities introduce the idea that the precise positioning of nucleosomes around eukaryotic promoters is important for gene regulation.

There are now many *in vivo* examples of positioned nucleosomes around DNA regulatory elements[15]. Evidence exists for the directed positioning of nucleosomes through interaction with trans-acting factors, both acting as passive boundaries and through specific contacts between histones and non-histone proteins[16,17]. However, most of our information on the molecular basis of nucleosome positioning is derived from the analysis of that directed by specific DNA sequences[2].

Considerable evidence exists in support of sequence-directed positioning of nucleosomes on DNA. For example, chicken, frog, and human core histones *in vitro* and yeast histones *in vivo* all recognize the same structural features of a 5S ribosomal RNA gene which direct nucleosome position[15]. In fact, removal of the basic tail domains or histones H2A/H2B does not alter the recognition of positioning sequences[6,18]. Mutagenesis experiments in which DNA sequences in and around the 5S RNA gene are perturbed, indicate that a region comprising 20-30bp to either side of the dyad axis contains the elements necessary for positioning[19]. In addition, hydroxyl radical cleavage analysis of the nucleosome positioning sequence of 5S DNA in solution reveals a DNA structure with a modulation in minor groove width every turn of the helix. Such modulations are indicative of an intrinsic curvature or bendability in 5S DNA[5]. It is energetically favorable to incorporate a DNA sequence that is already curved into a nucleosome, as DNA has to be bent around the histone core[2,8]. Other experiments indicate that inflexible DNA tends to be excluded from nucleosomes[7,8]. Thus, the sequence directed positioning of nucleosomes is determined by adjustment of histone-DNA interactions such that the smallest amount of energy is expended in wrapping a given DNA sequence around the histone core[2].

Interaction of Trans-acting Factors with Nucleosomal DNA

A large number of experiments have suggested that the association of regulatory elements with histones will prevent their recognition by trans-acting factors[20]. In a few instances the position of a nucleosome relative to a particular DNA sequence has been manipulated *in vitro* and *in vivo*. These experiments have established that the precise position of a regulatory element in the nucleosome can have important consequences for interaction with a trans-acting factor[15,21,22].

Among the best studied examples of trans-acting factors known to interact with chromatin are the association of transcription factor (TF) IIIA with a 5S ribosomal RNA gene assembled into a nucleosome or sub nucleosomal particle, and of the glucocorticoid receptor with the mouse mammary tumor virus (MMTV) long terminal repeat (LTR) as chromatin.

TFIIIA

Early studies demonstrated that the prior association of histones with naked 5S RNA genes prevented transcription *in vivo* and *in vitro*[23]. More recently it has been established that the assembly of *Xenopus borealis* 5S RNA genes into positioned nucleosomes inhibits transcription[24-26]. Importantly it was found that the assembly of $(H3/H4)_2$ tetramers onto 5S RNA genes did not completely inhibit transcription[13,25,27]. Thus it was of interest to examine the structural differences between the *X. borealis* 5S RNA gene associated with a tetramer or octamer of histones and the consequences for the binding of TFIIIA.

Substantial information exists concerning the essential sequences recognized by the zinc-finger protein TFIIIA within the 5S RNA gene (see for example, ref 28). More recently the crystallization of a zinc finger protein complexed with DNA[29] and missing nucleoside experiments[30] have led to significant insights into how TFIIIA binds to 5S DNA. These latter results demonstrate that two 10bp segments from +51 to +61, and from +81 to +91 (relative to the start of transcription at +1) are continuously contacted by TFIIIA on both strands. However, between these elements, TFIIIA makes contact with only one side of the DNA helix (position +74 and +75 on the coding strand and +69 on the non-coding strand) (Fig. 2A). These results support models in which TFIIIA is envisioned to wrap around the DNA helix, following the major groove at either end of the complex and then lying on one face of the DNA helix between these contacts (see also, ref 31). TFIIIA has nine zinc fingers, of which three fingers are believed to contact DNA over the 10bp regions at either end of the complex and three fingers are believed to act as a linker associated with one side of the DNA helix. Such an extensive interaction with DNA would appear incompatible with the simultaneous wrapping of this DNA around the histone core.

Pioneering experiments suggested that it was possible to form a complex in which both histones and TFIIIA were bound to a *Xenopus borealis* 5S RNA gene[32]. However, a large number of functional studies[13,23,24-27], demonstrated that prior association of the 5S RNA gene with a histone octamer prevented transcription. These functional studies were in agreement with the observation that the prior assembly of a *Xenopus laevis* 5S RNA gene with a complete octamer of core histones prevented the subsequent binding of TFIIIA[33]. This important issue of transcription factor access to histone associated DNA has recently been reexamined using both the *Xenopus borealis* 5S RNA gene, purified TFIIIA, nucleosomal and subnucleosomal particles.

Hydroxyl radical footprinting of the a *X. borealis* 5S RNA gene associated with a histone octamer revealed that key contacts that should be made with TFIIIA in these nucleosomes were bound by histone. TFIIIA would not bind to the 5S RNA gene under these conditions (Fig. 2B). In contrast, the key contacts between +81 and +91 are exposed when the *X. borealis* gene is associated with a histone tetramer (Fig. 2C). TFIIIA interacts quite readily with the 5S RNA gene with equivalent affinity whether or not it is associated with a histone tetramer[34].

Thus the efficiency of TFIIIA binding depends on the type and stoichiometry of histones associated with the 5S RNA gene, and the specific histone-DNA contacts with the promoter. The biological relevance of these observations is that both chromatin structure and transcription complexes are disrupted by DNA replication[11]. Each cell division event

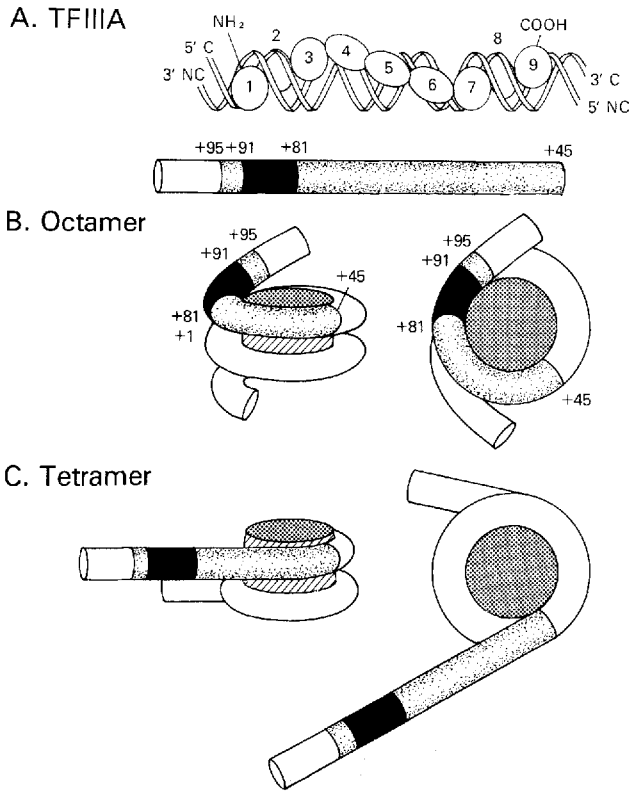

Fig. 2. Histone and TFIIIA binding to a 5S RNA gene. A. A representation of TFIIIA bound to a 5S RNA gene is shown. TFIIIA is believed to consist of nine domains, or zinc fingers (see text for details). The binding site for TFIIIA is also represented as a cylinder, the speckled region is protected from DNase I cleavage. Missing contact analysis (ref 30, data not shown) reveals the region from +81 to +91 to be an essential contact for TFIIIA (solid cylinder). B. When associated with a complete octamer of core histones $(H2A/H2B/H3/H4)_2$ the key contacts from +81 to 91 are in contact with the histone core, and TFIIIA cannot bind to the gene[13,23,24-27,34]. C. When associated with a tetramer of histones $(H3/H4)_2$ the key contacts from +81 to +91 are accessible to TFIIIA which forms a triple complex with the gene[34].

leads to competition between chromatin structural proteins and transcription factors for binding to DNA and a new opportunity for reestablishing or altering the state of gene expression. *In vivo* and *in vitro*, chromatin assembly on replicating DNA has been found to occur in stages, with a tetramer of histones H3 and H4 first rapidly associating with the newly replicated DNA[13,35]. Next, histones H2A and H2B are assembled into the nascent chromatin to form ordered arrays of nucleosomes. Thus the staging of chromatin assembly, coupled with the positioning of nucleosomes on the 5S RNA gene promoter will present a window of opportunity for TFIIIA to bind to the gene, and thus the eventual assembly of a functional transcription complex[36].

The Glucocorticoid Receptor

The glucocorticoid receptor is also a zinc finger protein, however, the DNA binding domain of this protein only has two zinc-binding domains. The NMR solution structure of the DNA binding domain reveals the zinc fingers to be arranged very differently from those of TFIIIA[37]. Each DNA binding domain uses an α-helix in one of the two fingers to interact with a short 6bp region in the major groove, the other finger is involved in protein-protein interaction. The glucocorticoid receptor associates with DNA as a dimer. The second molecule of the receptor has similar interactions on the same side of the DNA helix, one helical turn away (or 3.4nm).

One well studied model system in which the glucocorticoid receptor activates transcription from a chromatin template is the regulated transcription of the mouse mammary tumor virus long terminal repeat (MMTV LTR). Hager and colleagues established that the MMTV LTR is incorporated into six positioned nucleosomes, both in episomes and within a mouse chromosome[38]. Induction of transcription by glucocorticoids requires binding of the glucocorticoid receptor (GR) to the LTR, disruption of the local chromatin structure, and the assembly of a transcription complex over the TATA box[39].

Remarkably the GR binds to the nucleosomal MMTV LTR with only a slight reduction in affinity relative to naked DNA[40-42]. This interaction appears independent of the precise translational position of the nucleosome, GR binding occurs when the nucleosome is at −188 to −45[41] and at −219 to −76[40] or −221 to −78[42] relative to the start of transcription (+1). In both instances the rotational orientation of the GR binding sites will be similar since the separation of nucleosome boundaries is by almost exactly three helical turns of DNA. The latter *in vitro* nucleosome positions compare favorably with those determined *in vivo*[38].

Within the nucleosome, the MMTV LTR has several recognition elements for the GR receptor occurring at −175, −119, −98 and −83. The two elements at −119 and −98 face towards the core and remain unbound. The two elements at −175 and −83, which are oriented away from the histone core, are footprinted by the GR *in vitro*[40-42]. The GR, in fact seems well-suited to interact specifically with nucleosomal DNA. The short recognition elements (6bp) within the GRE are separated by one helical turn of DNA causing both to be aligned on the same face of the DNA helix. Consequently, the GR/GRE interaction occurs on only one side of the DNA, much like a prokaryotic repressor, thus possibly circumventing steric interference by the presence of the histone core. Furthermore, the GR homodimer can bind specifically to DNA containing only one GRE half-site, presumably by making both specific and non-specific contacts to DNA in each half of the dimer, respectively[43]. Thus highly bent nucleosomal DNA might still provide enough precisely aligned contacts for at least one specific half-site interaction which could then be supplemented by non-specific contacts. It is also possible that the wrapping of DNA around the histone core brings the two accessible recognition elements close enough for DNA binding of the GR to be stabilized by protein-protein interactions. In any event the GR represents one of the few examples in which a transcription factor can interact with exposed DNA recognition elements in spite of the large structural distortion of DNA in the nucleosome.

Surprisingly association of the GR with the nucleosome containing its binding sites, appears to have no effect on the integrity of the nucleosome itself *in vitro*, unlike the apparent disruption of the nucleosome that occurs *in vivo*[39]. The binding of other promoter specific transcription factors (eg. NF1) which is facilitated by the GR *in vivo*, does not occur *in vitro*[42]. Like TFIIIA, NF1 does not recognize DNA actually in contact with histones. The discrepancy between the events believed to occur *in vivo* and the *in vitro* results might be explained by the absence of certain nuclear components that presumably facilitate chromatin structural changes from the *in vitro* system. Nevertheless, it is clearly possible to reconstitute the first step in the process of transcription factor mediated activation of gene expression.

Transcription Factors that Bind Between Nucleosomes

TFIIIA represents an example of protein association with DNA at the edge of a nucleosome, whereas the glucocorticoid receptor represents a sequence-specific interaction with DNA actually within a nucleosome. There are also several examples of trans-acting factors binding to recognition elements between positioned nucleosomes. These elements are consequently in linker DNA and will normally be bound by linker histones such as histone H1. However, there is substantial evidence that under physiological conditions histone H1 exchanges quite readily out of chromatin[44]. The promoters of several genes are deficient in histone H1 compared to bulk chromatin[45].

In *Saccharomyces cerevisiae*, linker histones may not exist at all. The first step in the induction of the *Saccharomyces* PHO5 (acid phosphatase) gene is the binding of the trans-acting factor PHO4 between two positioned nucleosomes. A second binding site for PHO4 and a site for another trans-acting factor PHO2 are incorporated into one of these nucleosomes. PHO4 binding leads by unknown mechanisms to the disruption of both nucleosomes, although PHO2 is also known to be required. Directed changes in the DNA sequences adjacent to the PHO4 binding site, disrupt nucleosome position and prevent efficient transcriptional activation[22]. Therefore, once again the precise placement of regulatory elements between or within nucleosomes is clearly important for regulation of this promoter.

Nucleosome positioning is established as having a passive role in allowing trans-acting factor access to promoters that are otherwise compacted into chromatin. It is also possible that the formation of positioned nucleosomes over promoters serves to repress transcription in general unless the appropriate trans-acting factors are available to unlock gene activity. However, it is also conceivable that positioned nucleosomes could have a role in actually potentiating gene activity. Elgin and colleagues have mapped nucleosome positions around the *Drosophila* hsp26 promoter *in vivo* (see ref 46). Transcription of this gene is regulated through the association of heat shock transcription factor (HSTF) with recognition elements at −51, −170, −269, −348 and −369 relative to the start of transcription (+1). *In vivo* mapping reveals a nucleosome to be positioned from −300 to −140. Although the intermediate sites for HSTF binding are thought to be occluded by nucleosome formation, Elgin has proposed that the wrapping of DNA around the histone core might bring HSTF bound to

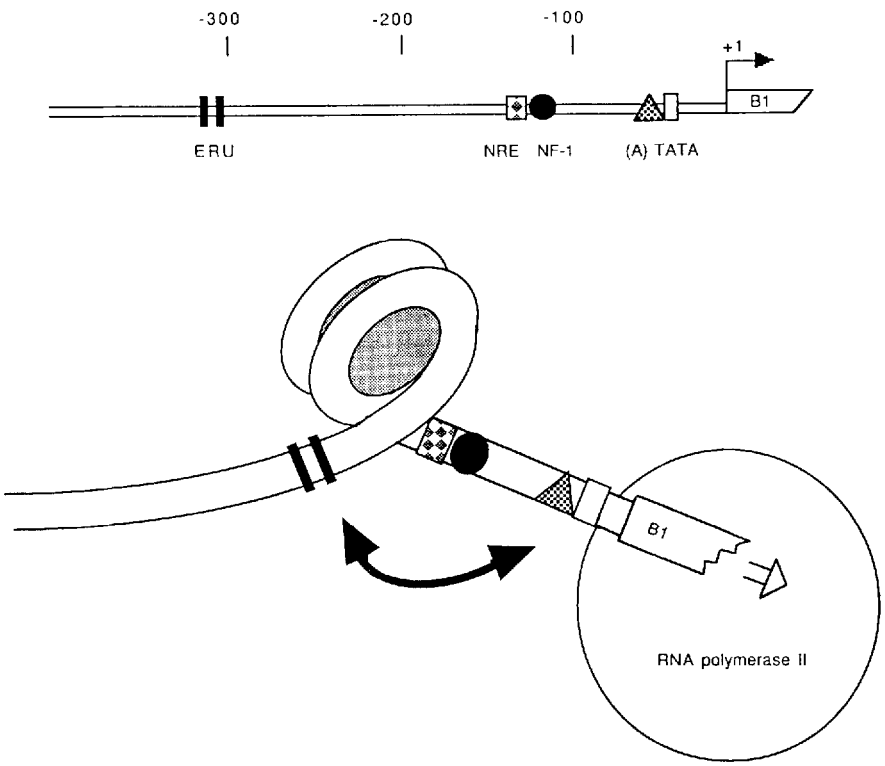

Fig. 3. Stimulation of transcription from the vitellogenin B1 promoter by a positioned nucleosome. The position of the binding sites for the estrogen receptor (ERU), a repressor binding site (NRE), transcription factors (NF1 and factor A) and the TATA box are shown (see ref 49), together with the start of transcription (+1). The folding of DNA around the core histones creates a static loop that is proposed to facilitate the interaction of the estrogen receptor complex with the transcription factors present at the proximal promoter elements (black arrow).

the sites at −348 and −369 into close proximity to HSTF associated with the site at −51 and thereby potentiate transcriptional activation[46]. This might occur through clustering of activation domains. A similar situation to the hsp26 promoter exists with the *Xenopus* vitellogenin genes. Here the recognition elements for the estrogen receptor are at −300, and the proximal promoter element binding a NF1-like activity, is at −120. A nucleosome is positioned between these sites *in vitro*. Formation of this nucleosome potentiates estrogen-receptor mediated transcription approximately 5-10 fold *in vitro* (C. Schild, W. Wahli and A.P.W. unpublished). Thus, incorporation of a promoter into a nucleosome can provide a scaffold that will maximize function (Fig. 3). There are many precedents for this type of auxiliary function being mediated by scaffolding proteins such as HU and IHF in prokaryotic systems[47].

Conclusions

The assembly of DNA into chromatin potentially imposes many constraints on the interaction of trans-acting factors with their recognition elements. The deformation of the DNA helix on wrapping around the core histones, and the occlusion of helix surfaces clearly can serve a general function of repressing transcription by preventing access of trans-acting factors. In many cases such repression serves an important function in the regulated expression of genes.

Trans-acting factors that can bind to DNA when incorporated into a nucleosome, such as the glucocorticoid receptor, probably mediate the first steps in the disruption of repressive chromatin structures. How this disruption might be accomplished is unknown[20]. Unlike other factors such as TFIIIA with extended DNA-protein contacts, the GR recognizes only a short stretch of sequence (6bp) in the major groove of the DNA helix. Such an interaction can clearly still occur even if the spacing and orientation of these base pairs changes as long as the major groove is orientated away from the histone core. The rotational positioning of the DNA helix in the nucleosome is essential for this to occur.

Trans-acting factors that wrap around the DNA helix with extensive contacts such as TFIIIA cannot interact efficiently with DNA wrapped around the histone core. One possible solution to facilitate transcription factor access to genes in such cases is to have a nucleosome positioning element direct the first deposition of histones away from the TFIIIA binding site. The staged assembly of chromatin then provides a window of opportunity for productive transcription factor binding. Thus the function of a nucleosome positioning element in this case is only transitory. Subsequent binding of other transcription factors might result in removal of the residual tetramer from the DNA. Substantial evidence exists for trans-acting factors mediating nucleosome disruption in some way. GR binding disrupts nucleosomes *in vivo*, likewise the binding of the yeast factor PHO4 initiates a chain of events resulting in nucleosome displacement. A problem yet to be resolved is how these additional steps for chromatin disruption occur.

Nucleosome assembly need not necessarily be repressive, since histones can provide the scaffolding to actually promote transcription. Thus, nucleosome positioning has a compelling role in allowing the compaction of DNA while still permitting the requisite accessibility for the initiation of highly regulated processes.

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