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Nucleosome Positioning and Gene Regulation

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Abstract Recent genetic and biochemical studies have revealed critical information concerning the role of nucleosomes in eukaryotic gene regulation. Nucleosomes package DNA into a dynamic chromatin structure, and by assuming defined positions in chromatin, influence gene regulation. Nucleosomes can serve as repressors, presumably by blocking access to regulatory elements; consequently, the positions of nucleosomes relative to the location of cis-acting elements are critical. Some genes have a chromatin structure that is "preset," ready for activation, while others require "remodeling" for activation. Nucleosome positioning may be determined by multiple factors, including histone–DNA interactions, boundaries defined by DNA structure or protein binding, and higher-order chromatin structure. © 1994 Wiley-Liss, Inc.

Key words: chromatin structure, preset/remodeling, gene repression/activation, nucleosome positioning, repressors

The mechanism of gene regulation in eukaryotic cells has been a major issue in modern biology. Extensive searches have identified many proteins that are involved in gene regulation through their interaction with DNA and the transcriptional apparatus. Recent results have shown that the histones play a general role in gene repression, and that many regulatory proteins act in conjunction with histones [reviewed by Grunstein, 1990]. Although the histones have been well characterized for over 20 years, it is only recently that their important role in regulating gene expression has been appreciated.

There are five different types of histones, histones H2A, H2B, H3, and H4 (the core histones) and histone H1 (the linker histone); all occur in modified forms and all but histone H4 have several variants [reviewed by van Holde, 1989]. While different eukaryotic organisms may not have all types of histones and their variants, all use the core histones in nucleosomal packaging, with possible exceptions among the dinoflagellates. The association of the eukaryotic DNA with histone octamers (two each of the core histones) leads to the 100 Å chromatin fiber of "string-wrapped-around beads;" further folding of the 100 Å chromatin fiber, utilizing histone H1 and other non-histone chromosomal proteins, results in higher order chromatin structures. This article first reviews the function of nucleosomes in gene regulation, followed by a brief description of the strategies and tools that are used for determining nucleosome positions. We will then summarize mechanisms that have been proposed for nucleosome positioning with an emphasis on in vivo studies. For additional information concerning nucleosome positioning see other recent reviews [Grunstein, 1990; Simpson, 1991; Thoma, 1992].

NUCLEOSOMES ARE INVOLVED IN EUKARYOTIC GENE REGULATION

Both in vivo and in vitro experiments have substantiated the idea that nucleosomes can serve as general repressors of transcriptional activity [reviewed by Grunstein, 1990; Workman and Buchman, 1993]. In *Saccharomyces cerevisiae*, a loss of nucleosome assembly caused by loss of histone H4 synthesis results in constitutive expression of a variety of genes that would otherwise be repressed without specific activation [Han and Grunstein, 1988; Han et al., 1988; Durrin et al., 1992]. In some cases, gene repression by a nucleosome has been shown to require specific interactions of histones with a nonhistone protein, such as the interaction between the $\alpha2$ repressor and the histone H4 N-terminal tail [Roth et al., 1992]. Analysis of specific mutations in the histone H4 gene indicates that di-

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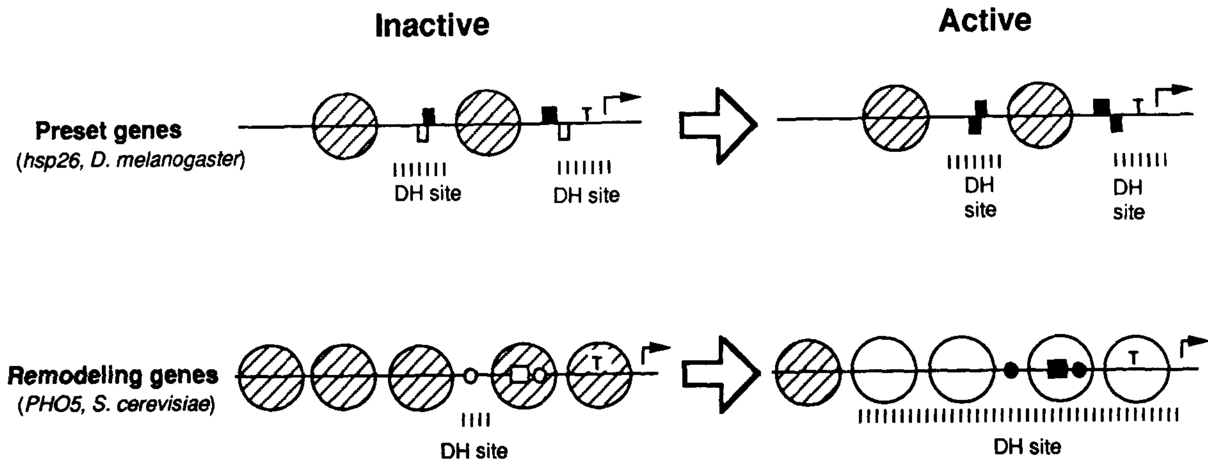

Fig. 1. Activation of a preset gene and of a remodeling gene. The sketch of a preset gene is based on the analysis of the *Drosophila melanogaster hsp26* gene [Cartwright and Elgin, 1986; Thomas and Elgin, 1988]; the sketch of a remodeling gene is based on the analysis of the yeast *PHO5* gene [Almer et al., 1986; Straka and Hörz, 1991]. Shaded circles indicate nucleosomes; "T" indicates the TATA box for each gene. Bent arrows indicate the transcriptional start site; vertical bars labeled "DH sites" indicate DNase I hypersensitive sites. For the *hsp26* gene, the boxes above the line indicate $(CT)_n \cdot (GA)_n$ sequences, and the boxes below the line indicate HSEs; filled boxes indicate occupation by the appropriate trans-acting factor, and unfilled boxes are not occupied by proteins. For the *PHO5* gene, small open circles and an open box on the line represent the binding sites for positive regulatory proteins PHO4 and PHO2, respectively; the filled circles and the box indicate occupation by the appropriate trans-acting factor. Large open circles indicate altered or displaced nucleosomes on the *PHO5* gene.

rect interactions occur between histone H4 and other chromosomal proteins to achieve the epigenetic regulation observed in silencing the HML and HMR mating type cassettes [Johnson et al., 1992; see references therein]. The involvement of nucleosomes is further substantiated by the observation that alleviation of repression for many genes also requires wild type histones; alteration of the lysine residues in the N-terminal tail of H4, which eliminates the potential for H4 acetylation, can reduce activation of inducible genes [Durrin et al., 1991]. In vitro, it has been shown that formation of a nucleosome can block utilization of a transcription start site, although nucleosomes do not appear to impede transcription elongation under these conditions [e.g., Lorch et al., 1987; reviewd by Grunstein, 1990].

The promoter region of a gene has a defined arrangement of regulatory elements. Since nucleosomes have the ability to block gene expression, promoters so packaged need to undergo "remodeling" during gene activation to allow access to the regulatory elements. These changes may include displacement or partial depletion of the histones [reviewed by Kornberg and Lorch, 1991; van Holde et al., 1992]. The mouse mammary tumor virus long terminal repeat (MMTV-LTR) promoter is packaged in an array of six positioned nucleosomes [Richard-Foy and Hager, 1987]. One of the nucleosomes (designated nucleosome B) incorporates sequences containing the binding sites for the glucocorticoid receptor and NF1-positive regulators [Perlmann and Wrange, 1988; Pina et al., 1990]. Upon dexamethasone induction, a DNase I hypersensitive site becomes apparent in this region, implying a perturbation of the histone–DNA interactions [Archer et al., 1991]. This change allows binding of NF1 and subsequent transcription. A similar situation has also been observed on induction of the yeast PHO5 gene [Fascher et al., 1990]. The promoter of the PHO5 gene is packaged in six positioned nucleosomes [Almer et al., 1986]. One of the nucleosomes (designated the −2 nucleosome) covers one of two binding sites for the PHO4 positive regulator and a binding site for the PHO2 positive regulator; another nucleosome (designated the −1 nucleosome) covers the TATA box. Upon phosphate deprivation, which causes an induction of PHO5, four of the nucleosomes are perturbed, including the two covering the positive regulatory elements [Fascher et al., 1990] (Fig. 1).

In contrast to the above promoters which undergo remodeling, there are promoters that are packaged in a nucleosome array such that

the important regulatory elements are accessible, even under noninducing conditions. The binding of transactivators does not affect the position of the nucleosomes. These promoters are designated "preset." The *Drosophila melanogaster hsp26* gene provides an excellent example of a preset promoter. Prior to activation, the *hsp26* promoter contains two prominent DNase I hypersensitive sites (DH sites) that map to the location of the two heat shock elements (HSEs) that are required for heat shock induction; a nucleosome is positioned precisely on sequences between the two DH sites [Cartwright and Elgin, 1986; Thomas and Elgin, 1988]. An RNA polymerase II molecule is transcriptionally engaged but paused at the promoter [Rougvie and Lis, 1990]. Upon heat shock, no major change occurs in the chromatin structure, except that the heat-shock factor (HSF) binds to the HSEs; the poised RNA polymerase II then continues transcription. The nucleosome between the two DH sites brings the proximal and distal HSEs into proximity; this could facilitate cooperative interactions between the heat shock factors that bind to the HSEs [Thomas and Elgin, 1988]. Alteration of the nucleosome array at this promoter results in gene repression [Lu Q, Wallrath LL, Elgin SCR; unpublished date]. The concept of preset genes and remodeling genes is schematically illustrated in Figure 1.

TOOLS AND STRATEGIES FOR MAPPING NUCLEOSOME POSITIONS IN ISOLATED NUCLEI

The positions of nucleosomes and pattern of protein/DNA interactions in a particular genomic region in nuclei can be mapped by detecting the pattern of DNA accessibility to various cleavage or modification reagents. The most commonly used cleavage reagents are micrococcal nuclease (MNase), DNase I and restriction enzymes. Since each cleavage reagent provides different information regarding protein-DNA contacts, more than one cleavage reagent is normally used to obtain the most complete picture [Lu et al., 1993a]. The most common methods of detection of cleavage or modification are indirect end-labeling [Wu, 1980; Nedospasov and Georgiev, 1980], primer extension [Axelrod and Majors, 1989], and ligation-mediated PCR (LMPCR) [Mueller and Wold, 1989; Pfeiffer et al., 1989].

MNase hydrolyzes 5′-phosphodiester bonds of DNA. It is the enzyme most commonly used to detect a nucleosome array, since cleavage of DNA by MNase is inhibited when DNA is associated with a histone octamer; the enzyme therefore cuts linker DNA preferentially [Kornberg, 1977]. Limited MNase digestion allows for detection of nucleosomal arrays in chromatin, whereas extensive MNase digestion generates mononucleosomes as the major product. MNase digestion products can be used to determine the boundaries of DNA incorporated into nucleosomes.

DNase I attacks the minor groove of DNA [Suck and Oefner, 1986]. At low levels of digestion, DNase I makes little distinction between core and linker DNA but does cut preferentially those regions along the chromatin fiber that are nucleosome free; these are the DH sites. At higher concentrations, DNase I is often used in footprinting experiments to detect specific protein binding. DNase I can also be used to determine whether a particular stretch of DNA is wrapped around the surface of a protein complex, showing which side of the DNA faces outward. When DNA is associated with a histone octamer, a 10–11-bp periodic cleavage pattern, spanning approximately 140 bp, can be detected by high resolution analysis [Noll, 1977; for an example, see Thomas and Elgin, 1988]. However, the production of such a cleavage pattern is insufficient by itself to infer the presence of a nucleosome, since the same cleavage pattern can also be generated by subnucleosomal components, even individual histones [Kerrigan and Kadonaga, 1992].

Restriction enzymes cleave DNA in a sequence-specific fashion, and can be used to quantitate the accessibility of a particular site within isolated nuclei [Jack et al., 1991; Lu et al., 1992, 1993a,b]. The degree of accessibility reflects features of the chromatin structure. Since the accessibility for restriction enzymes is high (50–80%) when the recognition sites are located in DH sites (nucleosome-free regions), and low (6–8%) when the recognition sites are associated with histone octamers, restriction enzymes are often used to map the borders of hypersensitive regions and the positions of nucleosomes [Jack et al., 1991; Straka and Hörz, 1991; Reik et al., 1991]. Restriction enzymes can also be used to compare the accessibility of the same site in wild-type and mutant transgenes [e.g., see Straka and Hörz, 1991, and Lu et al., 1992], or of a site in a given gene in different transcriptional states (repressed/induced) [e.g., see Jack et al., 1991].

PARAMETERS TO DESCRIBE NUCLEOSOME POSITIONS

Two interdependent structural features of DNA have been inferred to describe DNA sequences that can position a nucleosome: a "translational signal" and a "rotational setting" [Travers and Klug, 1987]. A translational signal is a DNA feature that determines the position of the histone octamer along the DNA sequence. Sequences that accommodate the DNA structural shifts seen near the dyad axis of the nucleosome may serve as a translational signal [Satchwell et al., 1986; Travers and Klug, 1987]. Alternatively, a translational signal may be provided by sequences in the linker region or on the edge of the nucleosome core. A rotational setting (signal), reflecting the curvature of the DNA, defines the side which faces the histone octamer. Alternating (A + T)-rich and (G + C)-rich sequences with a total 10 bp repeating periodicity provide a strong rotational setting for nucleosome positioning, with (A + T)-rich sequences at sites of minor-groove compression facing inside and (G + C)-rich sequences at sites with minor grooves facing outside on the histone octamer [see Travers and Klug, 1987].

HISTONE-DNA INTERACTIONS CAN PROVIDE BOTH ROTATIONAL AND TRANSLATIONAL SIGNALS FOR NUCLEOSOME POSITIONING

The interactions of the histone octamer with the associated DNA sequences in some cases appears to be the primary determinant of nucleosome positioning. Studies of the α-satellite DNA in African green monkey cells indicate that nucleosomes occupy one major and several minor positions on the satellite DNA [Zhang et al., 1983]. In vitro nucleosome assembly of the satellite DNA with purified histone octamers reveals a positioning pattern closely resembling that observed in vivo [Neubauer et al., 1986]. A similar observation has also been made on studying mouse satellite DNA [Linxweiler and Hörz, 1985]. The 5S rRNA genes of many organisms provide excellent examples of sequence-specific nucleosomal positioning [reviewed by Simpson, 1991]. Specific histone-DNA interactions that result in specific nucleosome positioning also occur with unique sequences. The position of a nucleosome in the promoter of the *Drosophila* Adh gene [Jackson and Benyajati, 1993], and those across the MMTV-LTR promoter region [Perlmann and Wrange, 1988; Pina et al., 1990], are similar both in vitro and in vivo, suggesting that the positions are determined by specific histone-DNA interactions. Note, however, that in many of the in vitro reconstitution experiments described above, short DNA fragments were used as templates, introducing a boundary that could influence the result.

DNA SEQUENCES WITH 10-bp PERIODICITY CAN ACT AS A TRANSLATIONAL SIGNAL

It has been demonstrated that (A + T) nucleotides periodically repeated at 10–11-bp intervals can confer a detectable curvature on the DNA double helix. This intrinsic bending has been detected by the anomalous migration of such DNA fragments in gels and has been visualized by electron microscopy [reviewed by Travers and Klug, 1987]. Analysis of mononucleosomal DNA sequences has indicated that short runs of (A + T) nucleotides, and short runs of (G + C) nucleotides occur preferentially with a 10–11-bp periodicity; these two modulations are in opposite phases [Satchwell et al., 1986]. These results indicate that nucleosomal sequences tend to be intrinsically bent, and that bent DNA may function as organizers of nucleosome positioning [Travers, 1987].

Guided by such results from mononucleosomal DNA sequence analysis, in an effort to make a perfect "nucleosome positioning" sequence, Shrader and Crothers [1989] designed a 20-bp oligonucleotide (TG) in which 5 bp of $(A + T)_3$ NN alternates with 5 bp of $(G + C)_3$NN. TG-5 is a pentamer of this sequence containing the $(A + T)_3$NN$(G + C)_3$NN motif repeated 10 times with 10.0 bp periodicity. Using purified histone octamers and short DNA fragments in in vitro reconstitution experiments, TG-5 appears to adopt a specific rotational orientation with the (A + T)-rich sequence at sites with the minor groove facing inside and the (G + C)-rich sequences at sites with the minor groove facing outside on the histone octamer [Shrader and Crothers, 1989, 1990]. However, by visual inspection, the TG-5 sequence does not appear to contain any obvious translational signals for nucleosome positioning, nor has the translational location of the bound histone octamer been determined experimentally [Shrader and Crothers, 1989, 1990].

To test the in vivo properties of TG-5, it has been introduced into different locations in a yeast minichromosome (within the region of a nucleosome, on the edge of a nucleosome, and in

a nuclease sensitive region), and the positions of the nucleosomes in the vicinity of the TG-5 sequence determined [Tanaka et al., 1992]. The results show that the TG-5 sequence is not associated with nucleosomes in vivo as evidenced by its sensitivity to MNase; rather, a nucleosome is positioned at the boundary of the sequence, with the nucleosome overlapping the end of the TG-5 sequence [Tanaka et al., 1992]. Similar observations were made when TG-5 was used to replace the nucleosomal region within the promoter of the *Drosophila hsp26* gene in a *hsp26/lacZ* transgene (Lu Q, Wallrath LL, Elgin SCR: unpublished data). In this case, the TG-5 sequence was found to be sensitive to MNase cleavage, indicating that the sequence is not associated with a nucleosome in vivo. In addition, the presence of the TG-5 sequence between the proximal and the distal regulatory elements of hsp26 resulted in a transgene that could not be activated to normal levels on heat shock. The results from yeast and *Drosophila* are consistent with each other and demonstrate that TG-5, a sequence with a strong rotational signal, does not form the center of a nucleosome in vivo [Tanaka et al., 1992]. These results suggest that the discontinuity between sequences with a strong rotational setting and those without, i.e., between the TG-5 sequence and the adjacent sequences, provides a translational signal for nucleosome positioning [Tanaka et al., 1992]. Sequences having the same organization as the TG-5 sequence have been successfully used to position nucleosomes on a nearby sequence in an in vitro experiment [Wollfe and Drew, 1989].

Nucleosome exclusion by sequences with periodic modulations has also been reported in native chromatin. In mapping the nucleosome distribution pattern in the SV40 minichromosome, Bina and colleagues found that while nucleosomes do not appear to occupy unique positions in the SV40 genome, there are several sites which are rarely incorporated into nucleosomes [Ambrose et al., 1990]. Some of the mapped nucleosome-free sites correspond to DNA sequences which have a periodic occurrence of an $(A)_{2-3}$ [or a $(T)_{2-3}$] motif at about 10 bp [Ambrose et al., 1990]; these SV40 sequences have been shown previously to be curved [Milton and Gesteland, 1988]. Taken together, the data suggest that sequences with a strong 10-bp periodicity can act as a translational signal for nucleosome positioning. The ability of other simple repetitive sequences that have been tested in vivo to form or to exclude nucleosomes is summarized in Table I.

LOCAL PROTEIN–DNA INTERACTIONS CAN PROVIDE A TRANSLATIONAL SIGNAL FOR NUCLEOSOME POSITIONING

Nucleosome positioning determined by local protein/DNA interactions was first inferred by studying nucleosome positioning in yeast minichromosomes [Thoma and Simpson, 1985]. The chromatin structure of a gene located on a minichromosome appears to be the same as it is in its native chromosomal location in its native nucleoplasm [reviewed by Simpson, 1991]. Thoma and his colleagues shuttled the *S. cerevisiae URA3* gene into *S. pombe* using minichromosome vectors to compare the nucleosome organization and expression of the gene in both systems. While the *S. cerevisiae URA3* gene in its own system is organized into an ordered nucleosome array and expressed normally, in *S. pombe* no ordered nucleosomal array can be detected on the gene nor is the gene correctly expressed [Bernardi et al., 1992]. Thus, the chromatin organization of the gene appears to be "species specific." Given that the histones are highly conserved, the differences observed are likely due to differences in the nonhistone chromosomal proteins, resulting in a lack of the gene-specific protein-DNA interactions required for the formation of an ordered nucleosome array.

Specific proteins have been shown to provide a translational signal for nucleosome positioning. Fedor et al. [1988] reported that binding of the protein GRF2 (factor Y) to the intergenic promoter region of the yeast *GAL1/GAL10* genes is essential for creating an ordered array of nucleosomes on both sides of the sequence when the genes are repressed. While the binding site for GRF2 overlaps a binding site for GAL4 protein, it is the GRF2 binding site (about 25 bp in length), and not the surrounding sequences (including the four GAL4 binding sites), that provides a translational signal for this ordered nucleosomal array. Purified GRF2 also binds to sequences in many other locations, including other upstream activator sequences, an enhancer for an rRNA gene, at centromeres and at telomeres [Chasman et al., 1990], suggesting that GRF2 may be used at many loci to generate boundaries for nucleosomes.

Chromatin structure analysis of the *Drosophila hsp26* gene indicates that GAGA factor is involved in organizing the nucleosomal array

TABLE I. Nucleosome-Forming Ability In Vivo of Repetitive DNA Sequences

Sequence	Source	*In vivo* system	Results	Reference
$[(A + T)_3 nn(G + C)_3nn]_{10}$	Shrader and Crothers [1989], synthetic	Yeast minichromosome	Excludes nucleosome; nucleosome overlapping end of the sequence	Tanaka et al. [1992]
$[(A + T)_3 nn(G + C)_3nn]_{10}$	Shrader and Crothers [1989], synthetic	*Drosophila hsp26/lacZ* transgene	Excludes nucleosomes	Q. Lu, L.L. Wallrath, S.C.R. Elgin, unpubl.
$[AAnnnTTnnn]_{10}$	Shrader and Crothers [1989], synthetic	*Drosophila hsp26/lacZ* transgene	Excludes nucleosomes	Q. Lu, L.L. Wallrath, S.C.R. Elgin, unpubl.
$(A)_{60}$ or $(CG)_5$	Synthetic	SV40 minichromosome	Excludes nucleosomes; nucleosome overlapping end of the sequence	Casasnovas and Azorin [1991]
$(CA)_{30}$	Synthetic	SV40 minichromosome	Incorporates into a nucleosome; causes misplacement of neighboring nucleosomes	Casasnovas and Azorin [1991]
$(AAGAG)_n$ or $(AATAT)_n$	*Drosophila* satellite 1.705 or 1.672	*Drosophila* chromatin	Incorporates into nucleosomes *in vivo*	Levinger [1985]
Sequences with ~ 10 bp periodic occurrence of $(A)_{2-3}$	SV40	SV40 minichromosome	Excludes nucleosomes *in vivo*	Ambrose et al. [1990]

at this promoter [Lu et al., 1992, 1993b]. In the *hsp26* promoter, stretches of $(CT)_n \cdot (GA)_n$ repeats are located on both sides of a precisely positioned nucleosome [Thomas and Elgin, 1988]. GAGA factor binds specifically to these $(CT)_n \cdot (GA)_n$ repeats [Gilmour et al., 1989; Lu et al., 1993b]. Mutation of these $(CT)_n \cdot (GA)_n$ repeats dramatically reduces gene expression [Glaser et al., 1990; Lu et al., 1992, 1993b]. Chromatin structure analysis of transgenes lacking the $(CT)_n \cdot (GA)_n$ repeats indicates that the DH sites flanking the wild-type nucleosome are largely lost; the accessibility for the restriction enzyme *XbaI* to its sites within the HSEs is drastically reduced [Lu et al., 1992, 1993b]. The data indicate that GAGA factor is critical in generating the DH sites at the *hsp26* gene promoter, presumably by organizing the nucleosomal array; deletion of the GAGA factor binding sites may result in randomization of the nucleosomes in this region.

A translational signal for nucleosome positioning generated by specific protein binding is also observed on studying α_2-mediated repression of a-cell-type specific gene expression in haploid α cells and in diploid a/α cells in yeast [reviewed by Herskowitz, 1989]. The α_2-repressor, in association with another promoter-specific protein MCM1, represses gene expression apparently by placing nucleosomes next to the α_2–MCM1 complex, resulting in nucleosome occupancy of the TATA box of the adjacent genes [Shimizu et al., 1991; Roth et al., 1992]. Five similar cases have been studied; in three of these, nucleosomes have been carefully mapped. The results indicate that in each case a nucleosome is placed with both rotational and translational specificity on DNA within 13–16 bp from the edge of the operator sequence, suggesting that the α_2–MCM1 complex may have achieved the positioning through an interaction with the core particle [Shimizu et al., 1991]. Studies of the *STE6* and *BAR1* genes in yeast strains with mutations of histone H4 have shown that deletion or point mutation of specific amino acids in histone H4 alters the location and/or the stability of the otherwise firmly and precisely positioned nucleosome [Roth et al., 1992]. These studies indicate that the binding of specific nonhistone proteins can provide a translational signal for nucleo-

some positioning in the immediate vicinity, and that the precise positioning involves interactions between a positioning protein (in this case, the α_2–MCM1 complex) and specific histones, including at least histone H4.

HIGHER-ORDER CHROMATIN STRUCTURE CAN INFLUENCE NUCLEOSOME POSITIONING

Since nucleosomes are the basic units of chromatin structure, the above discussion inevitably leads to questions concerning the roles of higher-order chromatin structure in nucleosome positioning and its impact on gene regulation. The contributions of the linker histone (histone H1 or histone H5) in chromatin condensation and of histone/histone interactions between nucleosomes have long been a subject of biochemical and biophysical studies [reviewed by van Holde, 1989 and Wolffe, 1992]; however, the contacts involved and the biological implications are not well understood.

Positioning of a nucleosome has been reported to have an effect on the positioning of neighboring nucleosomes [Satchwell and Travers, 1989; Costanzo et al., 1990]. Thoma and co-workers have studied the nucleosome distribution on minichromosomes of different sizes. Their results have led them to suggest that in the case studied, nucleosome positioning may be modulated by chromatin folding [Thoma and Zatchej, 1988]. Chromatin folding into domains may be directed by boundary elements such as the scs (special chromatin structure) elements of *Drosophila* [reviewed by Eissenberg and Elgin, 1991]. In addition, the B52 protein in *Drosophila* has been found to be associated with boundaries of transcriptionally active chromatin [Champlin et al., 1991]. It is speculated that this protein may be involved in the condensation or decondensation of chromatin at the 30-nm level, which could in turn affect nucleosome positioning.

NUCLEOSOME POSITIONING IS DETERMINED BY MULTIPLE FACTORS

So far, we have discussed the mechanisms proposed for nucleosome positioning in vivo, with several examples. It is obvious that there are many instances of nucleosome positioning that cannot be easily explained by any single one of the above mechanisms. The poly(dA) · poly(dT)-rich sequences found in the intergenic regions of the yeast *PET56/HIS3/DED1* genes map to the linker region, with nucleosomes overlapping the ends of the sequences, when the loci are cloned in yeast minichromosomes [Losa et al., 1990]. However, when DNA fragments containing the poly(dA) · poly(dT)-rich sequence (approx. 40 bp in length) between the *HIS3* and *DED1* genes were used in nucleosome assembly experiments with purified histone octamers from chicken erythocyte nuclei, different results were obtained when different size DNA fragments were used. The poly(dA) · poly(dT)-rich sequence was occupied by histone octamers when short DNA templates (approximately 140 bp) were used, but excluded from or towards the edge of the histone octamers when longer fragments (approximately 210 bp) were used [Losa et al., 1990].

Clearly nucleosome positioning over a specific DNA region is a dynamic process involving multiple factors, which sums up all the possible forces for positioning; the combined forces lead to a preferred location with minimum energy. This view may best be exemplified by the studies of van Holde and colleagues [Georgel et al., 1993] on the *Lytechinus variegatus* 5S rRNA genes. They have constructed a circular template (pPoll208.4) in which four repeats of the 5S rRNA gene are placed immediately downstream of an RNA polymerase I promoter from *Acanthamoeba castellanii*. When this circular template was reconstituted with purified histone octamers, the expected nucleosome positioning on the 5S rRNA genes was not observed. Correct nucleosome positioning on the rRNA genes was observed when the circular DNA template had been linearized at particular sites (but not at other sites) or when the reconstitution was performed in the presence of transcription factors TIF-IB, aUBF, and RNA polymerase I. While the reasons for this observation are not fully understood [see Georgel et al., 1993, for discussion], it is clear that the state of the template (circular or linearized), sequences in the vicinity of the 5S rRNA genes, and proteins present in the reconstitution mixture can all affect nucleosome positioning over the 5S rRNA genes. The above experiment may help us to explain some of the contradictory results observed concerning poly(dA) · poly(dT) sequences; a change in DNA and/or protein context could result in changes in the overall balance of the forces for positioning, resulting in the final preferred binding or displacement of the core histones.

For a gene in its native location in the genome (the DNA and its surrounding sequences being constant), nucleosome stability would most prob-

ably be influenced by changes in protein–DNA interactions due to the arrival of new proteins or modification of present ones (including histones), or by changes in torsional stress along the DNA resulting from transcriptional activity [e.g., see Lee and Garrard, 1991]. The former are likely to be critical in the remodeling of promoter regions (e.g., the MMTV-LTR promoter) [Archer et al., 1991], while the latter are likely to be pertinent to the shifts required for transcription elongation [Clark and Felsenfeld, 1991]. Further biochemical and genetic studies on the mechanisms of nucleosome formation and factors affecting nucleosome stability would greatly enhance our understanding of how genes are regulated in vivo.

CONCLUDING REMARKS

Clearly nucleosomes are not just "beads" required only to package DNA in the nucleus. Nucleosomes and their positioning play a dynamic role in the organization of chromatin. The importance of nucleosome positioning in vivo has become increasingly clear; nucleosomes are an integral part of the regulatory apparatus. Misplacement of nucleosomes may have serious consequences for regulated gene expression. The control of gene expression is regulated at multiple levels. Certainly, the involvement of chromatin structure adds further levels of regulation to that achieved by cis-acting elements and trans-acting factors; this may be essential for organisms with large genome.

In vitro reconstitution experiments using short DNA fragments have provided critical information on the mechanism of nucleosome positioning and critical information concerning binding and competition between different proteins for defined DNA sequences [reviewed by Hayes and Wollfe, 1992, and Workman and Buchman, 1993]. However, while the rotational orientation of nucleosomes is largely defined by interactions of DNA with histone octamers, the translational positioning of nucleosomes involves multiple factors. Caution should be taken in interpreting data obtained in vitro, particularly when only purified histone octamers have been used. A combination of such studies with results obtained using more complex assembly systems, and analysis of minichromosomes and transgenes in nuclei, will be required to gain full understanding of the organization of the chromatin fiber and the role of specific chromatin structure in gene regulation.

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