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REVIEW

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# Chromatin structure and the regulation of gene expression: remodeling at the MMTV promoter

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**Abstract** The role of the various levels of chromatin organization in the control of eukaryotic gene expression is discussed on the basis of recent advances in our understanding of chromatin structure in well-defined model systems. Particular attention is devoted to the precise structure and the possible functions of positioned nucleosomes and to the enzymatic mechanism of nucleosome remodeling. Some of the principles involved are illustrated with genomic footprinting results obtained with the mouse mammary tumor virus promoter, the nucleosomal organization of which is remodeled following transcriptional induction by steroid hormones. In this system a positioned nucleosome is responsible for transcriptional repression prior to hormone administration and participates in hormonal induction by facilitating the functional interaction among transcription factors on the promoter.

**Key words** Nucleosome positioning · Steroid hormone receptors · Core histone

**Abbreviations** *HRE* Hormone-response elements · *HRR* Hormone-responsive region · *LCRs* Locus control regions · *LTR* Long terminal repeat · *MARs* Matrix attachment regions · *MMTV* Mouse mammary tumor virus · *NFI* Nuclear factor I

## Introduction

The substantial expectations often placed on gene therapy have made scientists acutely aware of the limited knowledge which we now have of the mechanisms governing the stable and regulated expression of genes in particular cells. This knowledge stems mainly from established cell lines in culture, and often does not apply to the corresponding cell types in the intact animal. This information, however, is essential to the development of vectors for selective and sustained expression of transfected genes with therapeutic properties.

The principle concept is that cell-type restricted expression of genes depends on an adequate combination of *cis* elements for binding of ubiquitous and tissue-specific *trans*-acting factors in promoter and enhancer regions. The particular equipment of a defined cell type with transcription factors then determines the extent of expression of the gene in question. However, very often the information used to define *cis*-elements and *trans*-acting factors is collected from transient transfection assays and does not necessarily reflect the behavior of the same genes when stably integrated into chromosomes. The expression of stably transfected genes is affected by their organization in chromatin and by the particular position in which they integrate within the host genome. There are complex genetic elements, such as matrix attachment regions (MARs), locus control regions (LCRs), and insulators, which control the behavior of chromosomally integrated genes and can make their expression more reproducible or less dependent on the activity of adjacent genetic loci. The exact mechanism by which these elements work is not completely clear, but it seems to differ from the functioning of classical enhancers and very likely involves a contribution of the chromatin organization.

Apart from the distinction between heterochromatin and euchromatin, which seems to play a role in genetic imprinting and position effects, two different levels of chromatin organization have been implicated in regulating the expression of transfected genes. One involves the so-called domain structure of chromatin, and the other refers to the basic unit of chromatin, the nucleosome.

## Domain structure of chromatin

The idea that chromatin is organized in defined domains derives from microscopic images of lampbrush chromosomes, from biochemical studies of nuclease sensitivity,

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and from the phenomenon of position-effect variegation in *Drosophila*. [1]. Position-effect variegation means that the extent of expression of a gene is affected by its site of insertion into the chromosome. Insertion within 5–10 kb from a condensed heterochromatic region represses the transfected gene due to a process that spreads from the inactive chromatin region and involves changes in chromatin structure. Suppressor mutants of this effect identify genes which code either for structural components of chromatin or for proteins able to affect chromatin structure [2, 3].

Region-specific repression of gene expression similar to position-effect variegation is observed in *Saccharomyces cerevisiae* in the vicinity of the silent mating-type loci [4] and near the telomeres [5], and in *S. pombe* near the centromeres [6]. In *S. cerevsiae* transcriptional repression by telomeres requires the action of specific proteins, such as GAL11 [7], SIR3, SIR4 [8], and RAP1 [9, 10] and is associated with reduced acetylation of the core histones [11]. SIR3 and SIR4 interact directly with the N-terminal tails of histones H3 and H4 and with nuclear lamin [12]. These interactions have led to a model for heterochromatin formation at yeast telomeres in which the binding of RAP1 to $C_{1\text{-}3}A$ repeats is followed by recruitment of SIR3/SIR4, and nucleosomes are attached to the nuclear envelope via the SIR3/SIR4 complex.

One important property of the inactive chromatin state is the possibility of its being inherited from mother to daughter cells. This epigenetic mechanism is mediated by specific proteins, such as the members of the *Polycomb* group from *Drosophila*, which are able to maintain a repressed state of the homeotic genes [13]. The *Polycomb* group of proteins share a so-called chromo domain which is also found in the heterochromatin protein HP1 and their vertebrate homologues [14]. Point mutations in the chromo domain inactivate the silencing function of these proteins [15, 16]. The action of the *Polycomb* group of genes is counterbalanced by members of the *Trithorax* group [17]. In addition to the chromo domain, *Polycomb*, *Trithorax*, and *Brahma* (see below) share a PDH zinc finger-like motif, first detected in *Polycomblike*, as well as a bromo domain, a widespread motif [18–20] found in many proteins implicated in chromatin regulated transcription [21]. Intriguingly, these domains are also found in some coactivators for nuclear receptors [22] (see below), suggesting that one of the functions of these factors could be related to chromatin remodeling.

## Matrix attachment regions

Chromatin domains were originally thought to be punctuated by matrix or scaffold attachment regions [23–25]. Although these two concepts are not necessarily homologous, they are very often used as such. For simplicity, I refer here exclusively to MARs, even if in some cases the original literature does use a different nomenclature. MARs are often associated with enhancers [26], and the distinction is not always clear between enhancer function of genetic elements and their function as MAR. MARs often mark the limits of chromatin domains and have been used to define them [27]. Functional chromatin domains can also be defined biochemically as regions of generalized DNaseI sensitivity in particular cells [28]. In lymphocytes from transgenic mice, for instance, the appearance of general DNaseI sensitivity and transcription of the immunoglobulin μ whole locus depend on the presence of adjacent MAR regions [29].

The mechanism by which MARs fulfill their function in chromatin domains is not clear. MARs are unusually A/T-rich, and their tendency to unwind is correlated with their affinity for the nuclear matrix [30]. Histone H1 associates specifically with A/T-rich DNA regions in a highly cooperative manner [31], and due to their richness in As and Ts MARs may serve as nucleation centers for histone H1 assembly into the flanking DNA. Indeed oligo(dA)·oligo(dT) stretches of more than 130 bp can act as nucleators for histone H1 deposition. MAR regions are also enriched in topoisomerase II binding sites [32]. Treatment of intact cells or nuclei with distamycin, a minor groove binding reagent, enhances topoisomerase II and restriction nuclease cleavage at internucleosomal linkers of MARs [33], probably reflecting unfolding of the chromatin fiber due to distamycin-induced dissociation of histone H1 [34]. A similar situation may be generated in vivo by binding of factors, for instance, HMG-box containing proteins, which bind the minor groove of DNA and distort its structure. T7 RNA polymerase templates with MARs are inhibited selectively by added histone H1 due to nucleation of H1 at the A/T-rich regions of MARs [35]. Titration of these A/T-rich regions with HMGI/Y derepresses transcription by redistributing histone H1 to non-MAR DNA [35]. These findings may be physiologically relevant since HMGI/Y is enriched in chromatin fractions depleted of histone H1.

In addition to histone H1, HMG-like proteins, and topoisomerase II, other proteins binding to MARs in a sequence-specific manner have been reported. The MAR binding protein Satb1 interacts with the minor groove without contacting the bases [36]. The binding sites for Satb1 consist of mixed A's and T's with some C's interspersed, but no G's. This type of sequence is typically found as clusters in MARs. Mouse and human Satb1 are 98% homologous, suggesting that they fulfill an important function. A 150 amino acid region of Satb1 is sufficient for DNA binding and encompasses a novel motif with two hydrophilic ends [36]. A synthetic MAR affinity column has been used to purify a 100-kDa protein, which turned out to be nucleolin, a nucleolar protein with helicase activity essential for ribosomal RNA synthesis [37]. Nucleolin, as Satb1, binds specifically to the region of MARs prone to base unpairing. Nucleolin exhibits a curious strand selectivity: it binds to the T-rich strand of synthetic MARs 45-fold more efficiently than to the A-rich strand [37]. The function of Satb or nucleolin is unclear.

Other MAR binding proteins have been characterized. One of these binds to highly bent DNA [38] while another, the attachment region binding protein, is a major nuclear protein of 95 kDa that binds cooperatively to A/T-rich sequences in MAR fragments larger than 350 bp [39]. A small peptide of less than 15 kDa derived from the attachment region binding protein retains its MAR binding activity [40] and recognizes specific DNA sequences with high affinity [41]. Another MAR binding protein, SP120, localizes to the nuclear skeleton [42] and may be homologous to the human heterogeneous nuclear RNA binding protein [43]. Binding of this protein to large MAR-containing fragments is competed by poly(dG)·poly(dI), which forms four-stranded structures [43]. The physiological significance of these various MAR binding proteins remains to be established.

## Locus control regions

LCRs affect the activity of a whole genetic locus, often containing several related genes. Characteristically LCRs comprise a combination of a large number of elements, and their function may be mimicked by the clustering of multiple enhancer sites [44]. LCRs have been defined in stable transfection experiments and with transgenic mice techniques as complex elements containing multiple DNaseI hypersensitive sites and are able to confer copy number dependent and integration site independent expression to adjacent genes [45–48]. Whether these two functions can always be attributed to the same genetic element is unclear, and the function of LCRs may require additional elements. Whereas LCRs may be responsible for copy number dependent gene expression, insulators (see below) can also account for the independence of expression from the integration site in the chromosomes. MARs, on the other hand, stimulate but do not confer position-independent expression of transfected genes [49]. In the chicken β-globin locus the enhancer/LCR region and the promoter are required for the establishment of DNaseI hypersensitive sites and general DNaseI sensitivity of the locus, indicating that one of the functions of the LCR is to create a more open chromatin structure [50]. Constructs containing only the enhancer/LCR region lead to tissue-specific expression and DNaseI hypersensitivity only in a fraction of the transgenic mice [51]. It seems that cooperation between the enhancer/LCR and the promoter is essential for the proper functional behavior of the chromatin domain [51].

The mechanism by which LCRs affect the activity of various promoters within a given locus is unclear, but recent experiments are providing initial insights. In transgenic mice carrying β- and γ-globin genes in different arrays relative to the LCR, proximity to the LCR increases gene expression, but γ-gene expression is always higher, suggesting that promoter competition is the dominant feature [52]. Laser confocal microscopy has been used to detect time-resolved nascent RNA specific for the various genes of the β-globin locus in situ. In a particular cell nucleus, multiple genes appear to be transcribed alternatively rather than simultaneously. Therefore the LCR activation mechanism seems to be stochastic and monogene specific [53].

LCR regions have also gained clinical importance, as mutations affecting their function give rise to inherited defects. For instance, naturally occurring deletions that remove the LCR located 60 kb upstream of the human β-globin gene inactivate the whole locus and change the late replication properties and the sensitivity to DNaseI over a region of more than 100 kb, resulting in severe hemoglobinopathies [54].

## Insulators

MARs were initially considered responsible for marking the boundaries of chromatin domains, but it has since been shown that this function can also be assumed by insulator elements [55]. The first indication for such type of elements came from experiments on the 87A7 heat shock locus of *Drosophila*, where specialized chromosomal structures (scs and scs') of a few hundred base-pairs flank the heterochromatic regions. These elements, when inserted between enhancer and promoter, can block enhancer action, whereas MARs cannot [56–58]. Within the 900-bp corresponding to scs, DNA sequences associated with two DNaseI hypersensitive sites are essential for insulator function, while the central A/T-rich region is dispensable [59]. The boundary element seems to be assembled from a discrete number of functionally redundant sequences. Proteins that bind to the scs' in the hsp70 domain have been purified. The binding sequence is a palindrome, CGATA/TATCG, which abuts the DNaseI hypersensitive site. One of the proteins, BEAF-32, has been cloned. It binds to scs' but not to scs, indicating a polarity of the insulators, which is correlated with the fact that immunostaining with BEAF-32 antibodies decorates hundreds of interbands and many puff boundaries but only one end of the 87A7 puff [60]. Enhancer blocking assays attribute to the palindrome a boundary function, but the element does not work as an insulator in transient transfections, suggesting a role of the context or of chromatin structure [60].

Another structure which fulfills an insulator function in *Drosophila* is the complex of suppressor of Hairy wing [*su(Hw)*] and the transposable element *gypsy*. The *Su(Hw)* protein insulates expression of the *D. melanogaster white* gene from chromosomal position-effects [61]. *Su(Hw)* is a 100-kDa protein with 12 $C_2$-$H_2$ zinc fingers, a leucine zipper, and an acidic domain [62]. It binds to a 300-bp region of *gypsy* containing 12 repeats of a 10-bp A/T-rich sequence [63] and blocks enhancer activity when placed between enhancers and promoters [64] (Fig. 1). In wild-type flies *su(Hw)* does not prevent enhancer action on upstream promoters (promoter 2 in Fig. 1A), but mutations in modifier of mdg4

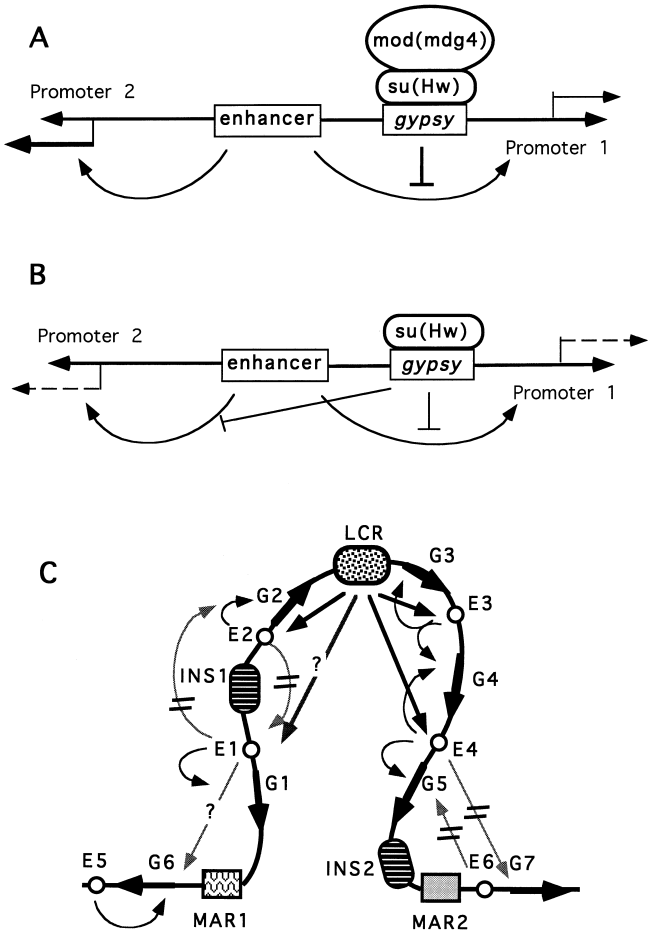


**Fig. 1A–C** Domain structure of chromatin. **A** In the presence of *mod(mdg4)*, *su(Hw)* bound to *gypsy* blocks the effect of an enhancer on promoter 1 but not on promoter 2. **B** In the absence of *mod(mdg4)*, promoter 2 is also inhibited, but the effect is variegated. **C** A hypothetical chromatin domain. MARs, insulators (*INS*), and LCR control the function of enhancers (*E*) and, indirectly, of genes (*G*)

[*mod(mdg4)*] eliminate this polarity and lead to variegated expression of reporter genes. The *mod(mdg4)* and *su(Hw)* interact directly. In the absence of *mod(mdg4)*, *su(Hw)* acts bidirectionally (Fig. 1B) whereas in its presence its effect is unidirectional [65]. The mechanism of action of *mod(mdg4)* remains to be established.

Insulators have also been found in vertebrates. A 5′ element of the chicken β-globin domain serves as an insulator in human erythroid cells and protects against position effect in *Drosophila* [66]. This insulator blocks the effect of a nearby LCR in erythroid cells in a directional manner but has no effect on the LCR within its own domain. In transgenic flies it protects the *white* gene from position effects. Its action involves a change in chromatin structure over the promoter [66]. The precise mechanism of this chromatin effects is unclear, but it could involve posttranslational modifications of the core histones or changes in the linker histones or HMG proteins which would affect the folding of the 10 nm chromatin fiber.

### A hypothetical chromatin domain

The emerging picture is tentatively represented in Fig. 1C. MAR1 and MAR2 are sites of attachment to the nuclear matrix and define a structural domain, often characterized by a differential sensitivity to DNaseI in various cell types. This structural domain, which may encompass a genetic locus, contains five genes, G1–G5, and four enhancers, E1–E4. The structural domain overlaps but does not coincide with the functional domain, which is delimited by the action of insulators, INS1 and INS2. The insulators specify the range of action of the enhancers. For instance, enhancer E1 can act on gene G1 but not on genes G2, G3, etc. Whether it can act on gene G6 depends on the nature of MAR1, which also determines whether enhancer E5 can act on gene G1. On the other hand, INS2 prevents the action of enhancer E4 on gene G7 and that of enhancer E6 on genes G5, G4, etc. It is less clear whether INS1 also blocks the action of the LCR on enhancer E1, but the MARs prevent LCR action on enhancers outside the structural domain, such as E5 and E6. Many of these statements are, however, based on very few examples, and their general validity awaits confirmation by the analysis of more chromatin domains. Nevertheless, it is obvious that an understanding of the functional organization of chromatin domains is an essential prerequisite for designing strategies of stable and cell-specific gene expression.

## Nucleosomal structure

The nucleosome, the basic repeating unit of chromatin, is composed of two copies each of the core histones H2A, H2B, H3, and H4, a linker histone, and approximately 180 bp DNA. The term nucleosome is very often used as a synonym for nucleosome core particle, which, more precisely, defines the complex of core histones and 145 bp DNA. Occasionally the term chromatosome is used to designate the core particle containing additional linker DNA and one linker histone. Because the length of DNA varies in different experiments, I use the term nucleosome below and specify the length of the DNA and the presence or absence of linker histones when appropriate.

After the discovery of the nucleosomal organization of chromatin the idea was favored that histones evolved as tools to pack DNA into the cell nucleus without interfering with DNA replication and transcription. The nucleosomes were thought to be randomly located along the DNA, and to pose no problems for proteins recognizing specific sequences or for enzymes involved in DNA metabolism. This view has been challenged by the accumulation of experimental evidence demonstrating a precise nucleosomal organization in relevant DNA regions of many genes. Many yeast genes, as well as a number of metazoan genes, have been shown to exhibit positioned nucleosomes in their promoter or enhancer regions, and it is becoming increasingly clear that the location of a

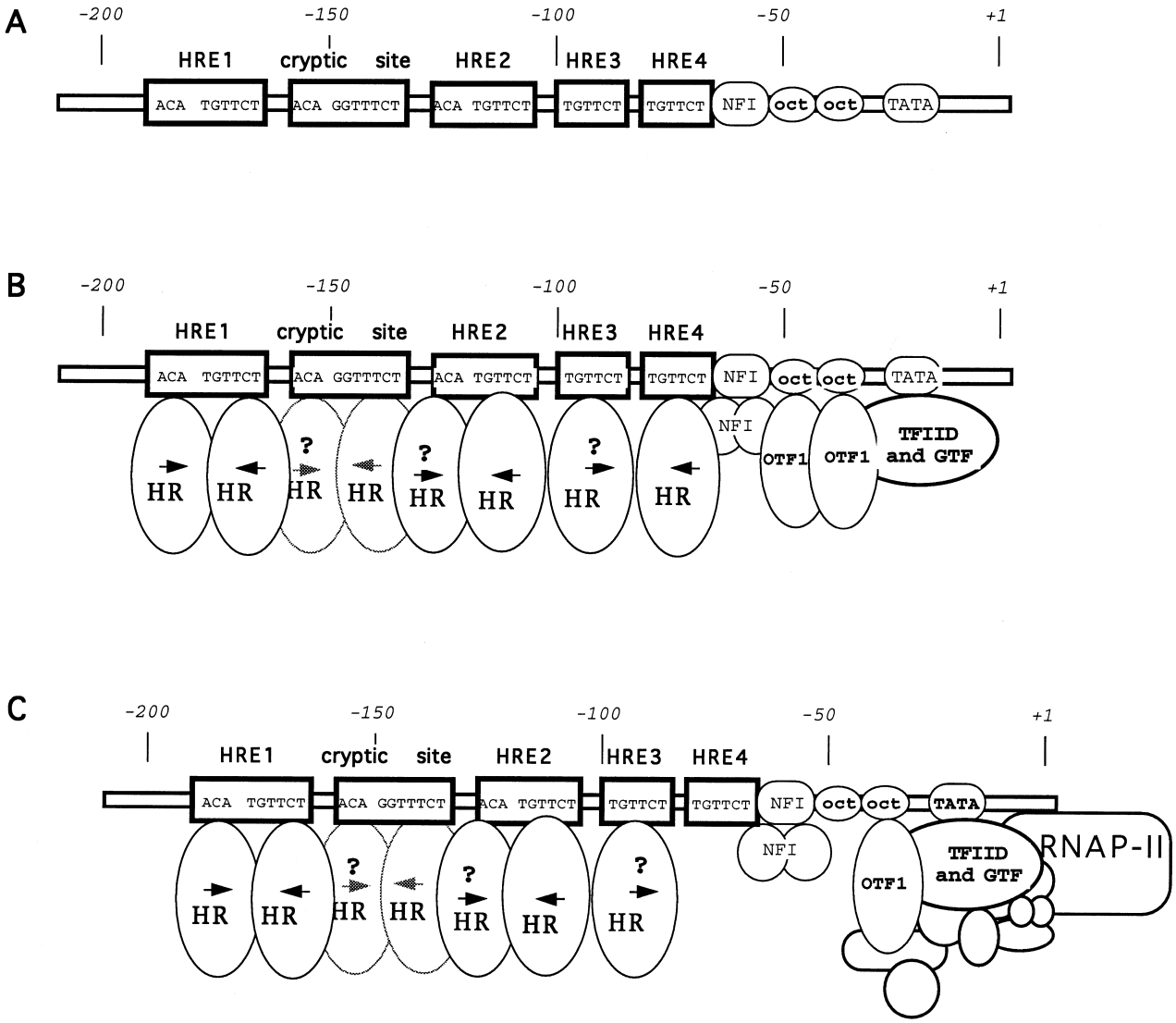


**Fig. 2A–C** The MMTV promoter. **A** *Cis*-acting elements on the MMTV promoter. **B** Hypothetical array of *trans*-acting factors on the MMTV promoter. **C** Current occupancy of the MMTV promoter as free DNA. *RNAP-II*, RNA polymerase II

DNA sequence relative to the histone octamer does affect its affinity for DNA binding proteins [67]. Experimental evidence also suggests that, along with the nucleosome packaging tools, eukaryotic cells have evolved a machinery able to handle DNA organized in chromatin. The genes and the molecular mechanisms involved in chromatin dynamics are only starting to be identified, but the claim seems justified that chromatin organization, in particular nucleosomal positioning, plays a general role in the regulated expression of the genetic information [68]. In the following I review briefly the role of nucleosomal organization in gene regulation based on the mouse mammary tumor virus (MMTV) promoter and summarize our present knowledge about nucleosome positioning and chromatin remodeling.

## The MMTV promoter

The MMTV proviral genome is transcribed from a promoter located in the long terminal repeat (LTR) region. Induction of MMTV transcription by glucocorticoids was established years ago as a classical system to study the mechanism of action of steroid hormones [69].

### *The hormone responsive elements*

The nucleotide sequences relevant for transcriptional activation of the proviral genome [70–73] are located between –200 and –50 upstream of the transcription start point in the LTR [74–76], a region which is preferentially bound by the glucocorticoid receptor [77–81].

Four short segments sharing the hexanucleotide motif TGTTCT were identified in the hormone-responsive region (HRR) of the MMTV LTR as being protected by the receptor against nuclease digestion [81]. The hexanucleotide motif in the hormone response element (HRE) 1 site is part of an imperfect palindrome with a spacing of

3 bp, while the HRE2 site is an imperfect palindrome with a spacing of 2 bp. The sites HRE3 and HRE4 are half palindromes (Fig. 2A). Mutation of any of these hexanucleotide motifs has a dramatic effect on hormonal induction [82]. In addition, a cryptic binding site was detected between positions –163 and –144 which exhibits a degenerated hexanucleotide sequence and a weak or no mutational phenotype in transient transfection assays [83]. These experiments demonstrated the existence of an HRR in the MMTV LTR composed of a complex array of binding sites for the hormone receptors which is able to mediate hormonal induction of the provirus. When the HRR was placed in front of a heterologous promoter, such as the promoter of the thymidine kinase gene of herpes simplex virus, it conferred to it the ability to respond to hormone administration with enhanced transcription [80]. This was the first demonstration of the existence of modular HREs.

MMTV induction was originally used as a classical example of glucocorticoid regulation, but the MMTV promoter has since been shown to respond to progestins [84], mineralocorticoids [85, 86], and androgens [87, 88]. The progesterone receptor from rabbit uterus protects a region against DNaseI very similar to that protected by the glucocorticoid receptor from rat liver [82, 89]. In general, steroid hormone receptors bind imperfect palindromes as homodimers in a highly cooperative fashion [90]. The elucidation of the three-dimensional structure of the DNA binding domain of glucocorticoid receptor complexed with various HREs suggests a strong interaction of one monomer with the best conserved half of the palindrome and a weak interaction of the other monomer with the more degenerated half [91]. Receptor dimers can also cooperate when bound to adjacent HREs [92–94].

In the MMTV promoter there is a functional interaction between the four main binding sites for the hormone receptors, which can be grouped into two blocks: a promoter distal block containing the strong palindromic site HRE1, and a promoter proximal cluster composed of the sites HRE2, HRE3, and HRE4. Although the exact stoichiometry of receptor binding to the promoter proximal block is not completely clear [95], a strong binding cooperativeness between the individual sites in this cluster and a functional synergism with the promoter distal HRE1 has been described [82, 96]. The functional synergism between the promoter distal and the promoter proximal block of receptor binding sites is demonstrated by the behavior of deletion mutants and by the effect of inserting oligonucleotides of different lengths between the two blocks [82]. These effects depend on the topology of the transfected DNA, suggesting that negatively supercoiled DNA favors the interaction between DNA bound receptor molecules [97]. Within the promoter proximal block of receptor binding sites there is also a strong binding cooperativeness among the three TGTTCT motifs [96], whereby the most promoter proximal motif appears to be less important for hormonal induction than the other two motifs [82]. The array of receptors on the HRR is not clear, but a possible scheme is shown in Fig. 2B, with the cryptic binding site occupied by an homodimer. It remains to be established exactly how the particular array of receptor binding sites affects the interaction with hormone receptors when the MMTV LTR is organized into chromatin (see below).

### *The NFI binding site*

In addition to the hormone receptors, other factors have been found to be involved in transcription from the MMTV promoter. Among these the ubiquitous transcription factor nuclear factor I (NFI) is probably the most important [98–102]. There are at least three different genes coding for proteins recognizing NFI sites [103], and differential splicing generates a large family of homologous proteins. Since these proteins can form homo- and heterodimers able to bind the palindromic NFI sites, the number of possible combinations is very large and could have implications in the regulation of specific genes [104]. The binding site for NFI in the MMTV promoter is located immediately downstream from the HRR (Fig. 2). The palindromic site binds a homodimer of NFI (Fig. 2), and mutations that inhibit NFI binding in vitro strongly reduce glucocorticoid [99, 100] or progesterone-induced transcription [102]. These experiments identify NFI as a transcription factor needed to obtain optimal hormonal induction of the MMTV promoter.

It has been shown previously that NFI and steroid hormone receptors can cooperate in transactivation of an artificial promoter carrying binding sites for the two proteins in the correct geometry [105, 106]. It was therefore thought that the NFI site in the MMTV promoter would participate in similar interactions. However, we found that purified hormone receptors do not cooperate but rather compete with NFI for binding to the MMTV promoter and vice versa [102]. The area protected against DNaseI by the hormone receptors overlaps by several bases with the footprint generated by NFI. Given the observed requirement of sequences flanking the HRE for efficient binding of the receptor [90], a steric hindrance in the interaction of the two proteins with the MMTV promoter would be expected [102]. Thus we are faced with the paradox that, although NFI acts as an essential transcription factor for the MMTV promoter and is required for optimal induction in vivo, there is no cooperation between steroid hormone receptors and NFI in terms of DNA binding or transcription of free DNA templates.

### *The octamer motifs*

A mutant MMTV promoter with disrupted NFI binding site is still able to respond to hormone administration, albeit with only one-tenth the efficiency of the wild-type promoter [102]. Thus there must be other factors able to mediate induction of the MMTV by steroid hormones. The MMTV promoter contains two octamer motifs be-

tween the NFI binding sites and the TATA box (Fig. 2) [107]. Mutations at these sites which interfere with OTF1 binding resulted in a significant reduction of the hormonal induction of the MMTV promoter in gene transfer experiments [107, 108]. The two sites are not functionally equivalent. The promoter distal site differs from the octamer consensus by a single mismatch and binds OTF1 with an affinity similar to that of the canonical octamer motif. Mutation of this site has a significant effect on MMTV transcription in hormone treated cells. This effects depends on the cell type and varies between three- and fourfold in HeLa cells treated with glucocorticoids and five- and sixfold in T47D cells treated with progestins. The promoter proximal site exhibits two mismatches, and mutations at this site are virtually silent in HeLa cells and show only a 50% reduction in activity in T47D cells [107]. That this site nevertheless binds OTF1 in the wild-type MMTV promoter is demonstrated by the presence of a dimer of OTF1 bound to DNA fragments containing both octamer motifs [107]. In fibroblasts stably transfected with MMTV constructs the octamer motifs also appear to be important for the basal expression of the promoter [109], and this may be due to an open chromatin conformation of the promoter (see below).

In vitro OTF1 binds weakly to the MMTV promoter in the absence of receptor. However, preincubation of either the progesterone or the glucocorticoid receptor with the MMTV DNA strongly enhances the binding of OTF1 [107]. Since these experiments were performed with highly purified preparations of receptor and OTF1, it is unlikely that the DNA binding cooperativeness is mediated by additional factors. Under cell-free conditions both OTF1 and OTF2 (Oct2) can cooperate with the progesterone or glucocorticoid receptor for transcription from the MMTV promoter, whereas their POU domains alone are inactive [110]. However, it has been reported that in transfected HeLa cells, but not in lymphoid cells, expression of the glucocorticoid receptor can inhibit transactivation by OTF2 [111], and therefore the outcome of the interaction may depend on additional factors or on the exact structure of the promoter.

In gel retardation experiments OTF1 and NFI do not cooperate for binding to the MMTV promoter; rather a simultaneous occupancy of the promoter distal octamer motif and the NFI site is not found in vitro [110] (Fig. 2C). Moreover, in cell-free transcription assays with naked DNA templates OTF1 and NFI do not synergize on the MMTV promoter, suggesting that they cannot cooperate in transcription under these conditions [110].

### *Other elements*

In addition to the hormone receptors NFI and OTF1, other factors are involved in the regulated transcription of the MMTV promoter. In vivo experiments have shown that mutations of the TATA box region diminish the activity of the MMTV promoter in response to glucocorticoids [108]. After hormone administration exonuclease III digestion of intact nuclei detects a stop at position +1 of the MMTV promoter, suggesting the presence of a protein bound to the region of the TATA box [112]. It has been shown that the general transcription factor TFIID, composed of the TATA box binding protein and its associated factors, is responsible for both binding to the TATA box region and its functional utilization in cell-free transcription [113]. Minimal promoters, containing only binding sites for the hormone receptors immediately upstream of a TATA box, respond to hormone treatment in gene transfer experiments [114, 115]. These findings suggest that under certain conditions the hormone receptors are able to interact directly or indirectly with TFIID. Whether this interaction plays a role in induction of the wild-type MMTV promoter, or whether the effect in this case is mediated exclusively by NFI and/or OTF1 remains to be studied. In any event, assembly of a transcription initiation complex on the promoter requires the additional recruitment of many general transcription factors and of RNA polymerase II, either sequentially or as a holoenzyme containing all relevant polypeptides (Fig. 3C) [116].

The existence of negative regulatory sites within the MMTV LTR has been repeatedly reported. Most reports localize the negative elements to regions upstream of the HRR. These elements are not discussed here since a short fragment of the promoter, lacking the sequences in question, exhibits complete regulation: repression in the absence of hormones and strong hormonal induction dependent on NFI and OTF1. However, other reports suggest the existence of negative regulatory elements in the region between the two blocks of HREs [117–119]. In genomic footprinting experiments we have not obtained evidence for the existence of such repressor binding sites in human mammary tumor cells lines (see below and [120]). It is intriguing, however, that thise region encompasses a cryptic binding site for the hormone receptors, which has been neglected in the past (see Fig. 2).

### *Organization in nucleosomes in vivo and in vitro*

The chromatin structure over the MMTV LTR has been reported to be highly ordered, with individual nucleosomes positioned precisely along the DNA sequence [121]. Both in cells carrying multicopy episomal vectors and in cells with chromosomally integrated single copies of the MMTV promoter, one nucleosome covers the HRR almost completely from position –190 to position –43, leaving only the octamer proximal motif within the linker DNA (Fig. 3) [120]. On the surface of this nucleosome the DNA double helix is rotationally phased in such a way that only the most distal and the most proximal HREs (HRE1 and HRE4) have their major grooves exposed for receptor binding, while the central HREs as well as the NFI binding site and the distal octamer motif are positioned with the major groove pointing toward the interior of the nucleosome, inaccessible for protein binding [120].

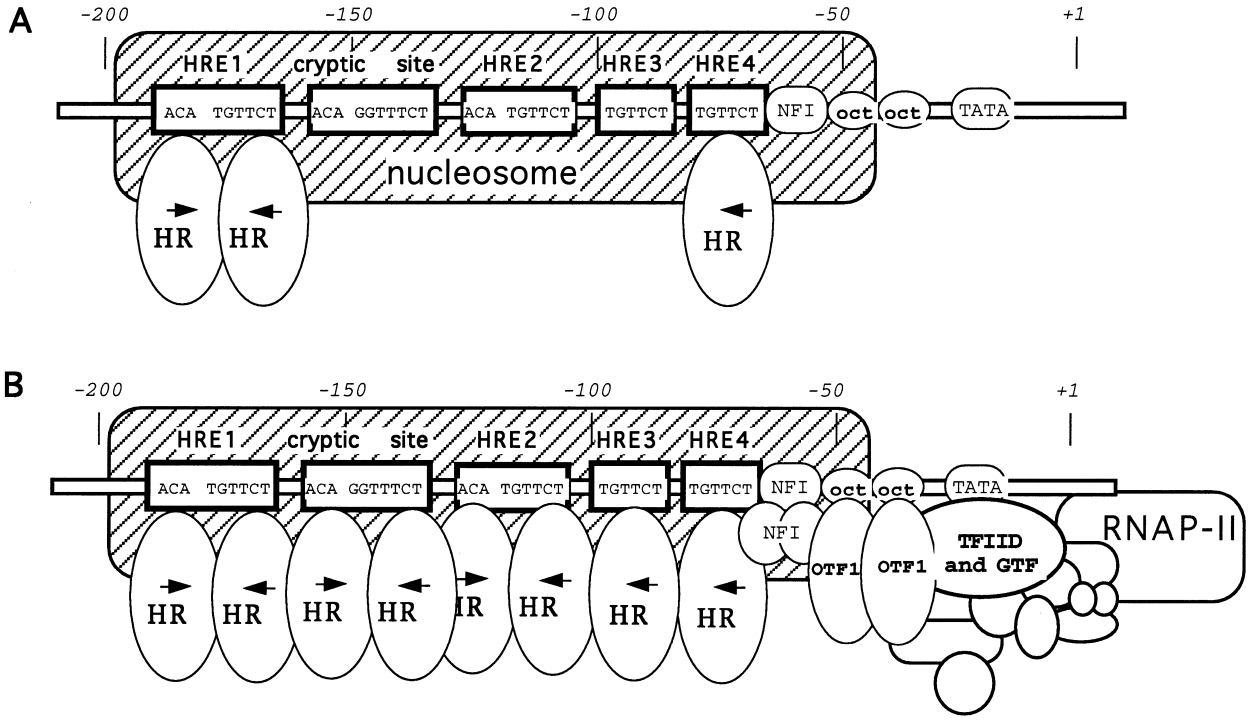


**Fig. 3A, B** Nucleosomal organization of the MMTV promoter. **A** Positioned nucleosome over the MMTV promoter and occupancy of *cis*-elements in vitro. **B** Actual occupancy of the MMTV promoter in intact cells, made possible by the organization of the promoter on he surface of a positioned nucleosome. *RNAP-II*, RNA polymerase II; *GTF*, general transcription factors

The same nucleosome positioning has been found in chromatin reconstitution experiments with histone octamers and linear or circular MMTV promoter fragments of various lengths [122–124]. In reconstituted MMTV nucleosomes the glucocorticoid and progesterone receptors can bind to the two external HREs, while the central HREs are much less accessible [122, 125, 126]. More importantly, no binding of purified or recombinant NFI to the NFI site is detected even at very high protein concentrations (Fig. 3A). These results support the concept that MMTV promoter sequences determine nucleosome positioning [123], and that the positioned nucleosome constitutively represses the MMTV promoter [127]. In agreement with this hypothesis, inhibition of chromatin assembly on DNA microinjected in *Xenopus* oocytes correlates with derepression of the MMTV promoter but not of the adenovirus major late promoter [128].

The general significance of the nucleosomal organization as a regulatory mechanism is suggested from experiments with yeast strains carrying mutated histone genes or an altered stoichiometry of core histones leading to altered nucleosome structure [129]. Although the mutated cells are still viable, they show alterations in several regulatory pathways, such as the mating type control and in the expression of some regulated promoters, including GAL4 and PHO5 [130]. In strains with low levels of histone H4 that exhibit nucleosome depletion, the chromatin structure of the PHO5 regulatory region is distorted, and this is accompanied by expression of the PHO5 gene, even under conditions of repression at a high phosphate concentration [130]. In this and other regulated genes, transcriptional stimulation by nucleosome depletion is observed even in the absence of upstream activating sequences, suggesting that one of the functions of this is to remove repression due to chromatin structure [129].

We have seen that the positioning of the MMTV DNA on the surface of the histone octamer is an intrinsic property of the nucleotide sequence since it is maintained under a variety of different conditions in vivo and in vitro [120, 123, 124]. Even in yeast, where the spacing of nucleosomes is usually 160 bp, the MMTV promoter is organized as in metazoan cells, suggesting that the primary sequence is dominant over the other parameters determining nucleosome positioning. Since this positioning has profound consequences on the accessibility and function of the promoter, we conclude that, as with proteins, DNA has a tertiary structure that determines part of its function. This implies that in addition to coding and regulatory information there is conformational or topological information in DNA which manifests itself in chromatin, modulates the accessibility of regulatory information, and is therefore critical for realization of the genetic program.

### *Chromatin may be required for optimal induction*

In cells carrying a single chromosomally integrated copy of the MMTV promoter no binding of sequence-specific factors can be detected prior to hormonal stimulation. This eliminates the possibility that the lack of MMTV transcription is due to the action of a sequence-

specific repressor [131]. After hormone induction a full complement of transcription factors binds to the MMTV HRR; all HREs, the NFI binding site, and the octamer motifs are occupied in the majority of MMTV promoters, as shown in Fig. 3B [120]. These findings are in apparent contradiction to the results obtained on free DNA (Fig. 2C, steric hindrance between receptor and NFI and between NFI and OTF1) and with the results obtained on reconstituted nucleosomes (Fig. 3A, receptor binding only to the two external HREs, no binding of NFI). Most unexpectedly, the nucleosome covering the MMTV HRR is not displaced or removed after induction but appears to remain in place, as determined by low- and high-resolution micrococcal nuclease digestion data [120]. We therefore postulate that the assembly of a full complement of transcription factors may be facilitated by their binding to the surface of a positioned nucleosome.

This idea is compatible with preliminary results obtained in yeast strains carrying the MMTV reporter system (see above) and a histone H4 gene driven by the GAL promoter and upstream activating sequences [129]. In the presence of galactose these yeast strains respond to hormone administration with an NFI-dependent induction of transcription from the MMTV promoter. However, when the cells are grown in the presence of glucose and are therefore partly depleted of nucleosomes, the response to hormone is not better than in wild-type strains, as would be expected if the nucleosome fulfilled an exclusively negative function. On the contrary, nucleosome depletion leads to an impaired hormone response, in agreement with a positive role of nucleosomes in induction (S. Chávez, unpublished). The idea that transcription factors may bind to DNA sequences on the surface of a nucleosomes is supported not only by results from the MMTV system. A similar situation has been reported for the enhancer of the albumin gene in rat liver [132] and for the heat shock factor in yeast [133].

*How is the nucleosome rearranged upon induction?*

The statement that factor binding takes place on a positioned nucleosome presupposes that the organization of the DNA helix on the surface of the histones is altered during hormone induction to permit factor binding to the major groove of sites originally masked. It was reported several years ago that hormone induction is accompanied by structural changes of chromatin, as indicated by the appearance of a DNaseI hypersensitive region over the HREs [134], which is also hypersensitive against DNA cleaving reagents [121]. These findings have been interpreted as reflecting removal or disruption of the positioned nucleosome covering the HRR, an interpretation that contradicts our genomic footprinting results. In cells carrying a single integrated copy of the MMTV promoter, even after full loading with transcription factors, there is no indication for displacement of a nucleosome [120]. However, hormone induction generates a very narrow zone of nuclease hypersensitivity coinciding with the pseudo-dyad axis (the approximate center) of the positioned nucleosome over the HRR, indicating a change in conformation of the nucleosomal DNA [120]. This novel DNaseI hypersensitive site can be detected only when the cells are treated with nuclease under very gentle conditions, and it is lost when the cell nuclei are purified prior to nuclease digestion. Under these conventional conditions a broader DNaseI hypersensitive region covering the HRR is detected, as previously reported [134]. Therefore the hormone induced conformational change of chromatin is not stable and generates a nucleosome, which does not resist manipulation of cell nuclei.

The nature of this conformational change remains obscure, but experiments with inhibitors of histone deacetylases and genetic results in *S. cerevisiae* suggest a role for modification of the core histones. Among the changes in nucleosomes that have been associated with transcriptionally active chromatin are hyperacetylation of lysine residues in the N-terminal tails of all four core histones [135]. In the case of MMTV the inhibition of histone deacetylase with 5–10 mM sodium butyrate blocks hormone induction and nucleosome remodeling [136]. However, we find that lower concentrations of butyrate do indeed activate hormone-independent transcription from single-copy integrated MMTV reporters. A similar response is observed with a more selective inhibitor of histone deacetylase activity, trichostatin A, which acts at nanomolar concentrations [137]. Moreover, inducing concentrations of butyrate or trichostatin A generate the same type of DNaseI hypersensitivity over the pseudo-dyad axis of the regulatory nucleosome that we have observed following hormone induction (M. Truss, J. Bartsch, J. Bode, and M., Beato, unpublished). These results suggest that the nucleosome remodeling induced by receptor binding could involve changes in the behavior of the core histone tails. Recently it has been found that restriction of GAL4 binding to reconstituted nucleosomes containing GAL4 binding sites can be alleviated by proteolytic digestion of the histone tails, suggesting a general repressive role for these highly charged domains of the core histones [67, 138]. Similarly, binding of the transcription factor TFIIIA to a reconstituted nucleosome carrying a 5S RNA gene is enhanced by acetylation of the histone tails [139]. However, alternative changes in nucleosome structure, such as removal of histone H2A/H2B [140] or even depletion of linker histones [141], must be considered as additional possibilities for facilitating factor binding to nucleosomally organized DNA sequences.

Other mechanisms may also modulate the binding of factors to nucleosomally organized DNA. One possibility is a direct interaction with core histones, as has recently been reported for SIR3 and SIR4 and the N-terminal regions of histones H3 and H4 in the context of telomere silencing in yeast [12]. An involvement of the histone tails in gene regulation has been already postulated in studies with yeast strains carrying various mutations in these histone domains [142].

*Two Classes of DNA binding proteins*

The nucleosome structure described above suggests the existence of two types of DNA-binding regulatory proteins: those that are able to interact with nucleosomally organized DNA and those that are not. Provided that the DNA remains in the B form, if a protein needs to contact more than five consecutive basepairs through the major groove, it is unable to see its target sequences organized in a nucleosome, independently of their precise rotational positioning. Regardless of the current phase of the double helix a part of the recognized major groove is masked. The same is true if the protein contacts two sets of short sequences that are not on the same face of the double helix. For this kind of protein the essential determinant for DNA binding would be the translational phasing, namely, whether their binding sites are within the boundaries of a nucleosome. In this case the rotational phasing of the DNA double helix may be relatively irrelevant, but linker histones may affect access to the DNA sites in regions connecting nucleosome cores. On the other hand, a protein that contacts one or several short stretches of less than five basepairs located on one side of the double helix is able to recognize its cognate sequence on nucleosomes, provided that the major grooves are properly oriented. For these proteins the rotational phasing of the double helix on the surface of histone octamer would be the determinant feature for binding site recognition. To this latter class belong the steroid hormone receptors [122, 143]. This type of protein would take a higher position in the hierarchy of regulatory proteins, as they would be able to initiate a chromatin remodeling event allowing access of other factors to nucleosomally organized regulatory elements.

*A machinery for chromatin remodeling*

It is intriguing that the hormone-induced structural alteration in chromatin structure takes place at the nucleosome pseudo-dyad axis, as this region has been shown to be crucial for control of chromatin-mediated gene expression involving the SWI/SNF complex in yeast [144, 145]. The SWI/SNF complex encompasses a set of pleiotropic transactivators which are important for transcription of inducible genes and counteract repressing functions of chromatin [145]. The participation of members of the SWI/SNF complex in glucocorticoid gene induction has been reported in yeast and in animal cells, suggesting a direct protein-protein interaction with the hormone receptor [146, 147]. Recently a soluble complex of all SWI/SNF gene products has been identified which may play an important role in facilitating chromatin transcription [148, 149]. This complex enhances binding of GAL4 derivatives to nucleosomally organized GAL 4 binding sites in an ATP-dependent manner [150]. Mutations in histones H3 and H4 located near the nucleosome dyad axis are able to suppress the phenotype of *swi/snf* mutations [145], suggesting that the architecture of this region of the nucleosome is an important determinant of transcriptional activity.

Other ATP-dependent chromatin remodeling activities have been recently identified [151]. In particular, the *Drosophila* nucleosome remodeling factor (NURF) complex contains four polypeptides, each of which differs from those found in the SWI/SNF complex [152]. One of these, ISW1, exhibits an ATPase activity which is activated by nucleosomes and is involved in the remodeling of the nucleosomal organization of the hsp70 promoter following heat shock [153].

A comprehensive description of the induction process requires knowledge of the changes in chromatin organization of the MMTV promoter at the nucleotide level during the different phases of hormonal induction in vivo. This kind of analysis may depend on the development of new, gentle, and efficient methods for stabilizing transient interactions between macromolecules in intact cells. Ultimately, however, a precise understanding of the transactivation mechanism will only be possible only when correctly reconstituted chromatin templates are successfully transcribed in vitro using purified receptors, NFI, OTF1/Oct1, all general transcription factors with accessory proteins and RNA polymerase II.

## Conclusions

Although based on just a few well characterized systems, the above description should provide an impression of the multiple levels at which the chromatin organization of DNA may affect the regulated expression of genes. Though we are only beginning to understand the complexity of the regulatory mechanisms involved, it is clear that the simple identification of regulatory elements and the study of their function in transfection experiments is not sufficient to evaluate the function of eukaryotic enhancers and promoters. It is important to study these elements in their natural context, not only in terms of the precise nucleotide sequence found in natural promoters/enhancers but also in respect to their organization in nucleosomes and in chromatin domains in intact cells. As for the nucleotide sequence, it would be naive to assume that only the sequences directly involved in factor binding are relevant for regulation. It is very likely that the particular array of sites and the topological properties of the sequences between sites are essential for the interactions among factors bound to these sites in vivo. This may be achieved by intrinsic bending properties of DNA sequences or indirectly by the action of so-called architectural factors [154], whose function is to bend DNA and to generate a structural organization adequate for other factors to interact synergistically or in other meaningful ways. In most cases, however, this structural function is assumed by the chromatin organization of the DNA, which represents the starting point of the regulatory process.

In the near future we will have to analyze in detail the in situ chromatin structure of other well-characterized

regulatory regions of DNA, using techniques such as genomic footprinting and UV laser cross-linking, to evaluate the general validity of the principles illustrated above. Yeast genetic techniques will probably help to identify and understand the enzymology involved in chromatin remodeling, but ultimately we will need appropriate cell-free systems to study and manipulate the biochemistry of these processes. Eventually this knowledge should lead to a better understanding of the requirements for achieving controlled expression of stably transfected genes.

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## References

1. Henikoff S (1990) Position-effect variegation after 60 years. Trends Genet 6:422–426
2. Eissenberg JC, James TC, Foster-Hartnett DM, Hartnett T, Ngan V, Elgin SCR (1990) Mutation in a heterochromatin-specific chromosomal protein is associated with suppression of position-effec variegation in *Drosophila melanogaster*. Proc Natl Acad Sci USA 87:9923–9927
3. Reuter G, Spierer P (1992) Position-effect variegation and chromatin proteins. Bioessays 14:605–612
4. Brand AH, Breeden L, Abraham J, Sternglanz R, Nasmyth K (1985) Characterization of a "silencer" in yeast: a DNA sequence with properties opposite to those of a transcriptional enhancer. Cell 41:41–48
5. Gottschling DE, Aparicio OM, Billington BL, Zakian VA (1990) Position effect at *S. cerevisiae* telomeres: reversible repression of pol II transcription. Cell 63:751–762
6. Allshire RC, Javerzat JP, Redhead NJ, Cranston G (1994) Position effect variegation at fission yeast centromeres. Cell 76:157–169
7. Suzuki Y, Nishizawa M (1994) The yeast GAL11 protein is involved in regulation of the structure and the position effect of telomeres. Mol Cell Biol 14:3791- 3799
8. Moretti P, Freeman K, Coodly L, Shore D (1994) Evidence that a complex of SIR proteins interacts with the silencer and telomere binding protein RAP1. Gene Dev 8:2257–2269
9. Thompson JS, Johnson LM, Grunstein M (1994) Specific repression of the yeast silent mating locus hmr by an adjacent telomere. Mol Cell Biol 14:446–455
10. Lustig AJ, Kurtz S, Shore D (1990) Involvement of the silencer and UAS binding protein RAP1 in regulation of telomere length. Science 250:549–553
11. Braunstein M, Rose AB, Holmes SG, Allis CD, Broach JR (1993) Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. Gene Dev 7:592–604
12. Hecht A, Laroche T, Strahl-Bolsinger S, Gasser SM, Grunstein M (1995) Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: a molecular model for the formation of heterochromatin in yeast. Cell 80:583–592
13. Paro R (1990) Imprinting a determined state into the chromatin of *Drosophila*. Trends Genet 6:416–421
14. Singh PB, Miller JR, Pearce J, Kothary R, Burton RD, Paro R, James TC, Gaunt SJ (1991) A sequence motif found in a *Drosophila* heterochromatin protein is conserved in animals and plants. Nucl Acids Res 19:789–794
15. Messmer S, Franke A, Paro R (1992) Analysis of the functional role of the polycomb chromo domain in *Drosophila melanogaster*. Genes Dev 6:1241–1254
16. Platero JS, Hartnett T, Eissenberg JC (1995) Functional analysis of the chromo domain of HP1. EMBO J 14:3977–3986
17. Kuzin B, Tillib S, Sedkov Y, Mizrokhi L, Mazo A (1994) The *Drosophila trithorax* gene encodes a chromosomal protein and directly regulates the region-specific homeotic gene fork head. Gene Dev 8:2478–2490
18. Haynes SR, Dollard C, Winston F, Beck S, Trowsdale J, David IB (1992) The bromodomain: a conserved sequence found in human, *Drosophila* and yeast proteins. Nucleic Acids Res 20:2603
19. Delmas V, Stokes DG, Perry RP (1993) A mammalian DNA-binding protein that contains a chromodomain and an SNF2 SWI2-like helicase domain. Proc Natl Acad Sci USA 90: 2414–2418
20. Koonin EV, Zhou SB, Lucchesi JC (1995) The chromo superfamily: new members, duplication of the chromo domain and possible role in delivering transcription regulators to chromatin. Nucleic Acids Res 23:4229–4233
21. Aasland R, Gibson TJ, Stewart AF (1995) The PHD finger: implications for chromatin-mediated transcriptional regulation. Trends Biochem Sci 20:56–59
22. LeDouarin B, Zechel C, Garnier JM, Lutz Y, Tora L, Pierrat B, Heery D, Gronemeyer H, Chambon P, Losson R (1995) The N-terminal part of TIF1, a putative mediator of the ligand-dependent activation function (AF-2) of nuclear receptors, is fused to B-*raf* in the oncogenic protein T18. EMBO J 14:2020–2033
23. Gasser SM, Laemmli UK (1986) The organisation of chromatin loops: characterization of a scaffold attachment sites. EMBO J 5:511–518
24. Phi-Van L, Strätling WH (1988) The matrix attachment regions of the chicken lysozyme gene co-map with the boundaries of the chromatin domain. EMBO J 7:655- 664
25. Stief A, Winter DM, Strätling WH, Sippel AE (1989) A nuclear DNA attachment element mediates elevated and position-independent gene activity. Nature 341:343–345
26. Gasser SM, Laemmli UK (1986) Cohabitation of scaffold binding regions with upstream/enhancer elements of three developmentally regulated genes of *D. melanogaster*. Cell 46:521–530
27. Targa FR, Razin SV, Gallo CVD, Scherrer K (1994) Excision close to matrix attachment regions of the entire chicken α-globin gene domain by nuclease S1 and characterization of the framing structures. Proc Natl Acad Sci USA 91:4422–4426
28. Weintraub H, Groudine M (1976) Chromosomal subunits in active genes have an altered conformation. Science 193:848–856
29. Forrester WC, van Genderen C, Jenuwein T, Grosschedl R (1994) Dependence of enhancer-mediated transcription of the immunoglobulin m gene on nuclear matrix attachment regions. Science 265:1221–1225
30. Bode J, Kohwi Y, Dickinson L, Joh T, Klehr D, Mielke C, Kohwi-Shigematsu T (1992) Biological significance of unwinding capability of nuclear matrix-associating DNAs. Science 255:195–197
31. Izaurralde E, Kass E, Laemmli UK (1989) Highly preferential nucleation of histone H1 assembly on scaffold-associated regions. J Mol Biol 210:573–585
32. Adachi Y, Kas E, Laemmli UK (1989) Preferential, cooperative binding of topoisomerase II to scaffold associated regions. EMBO J 8:3997–4006
33. Kas E, Poljak L, Adachi Y, Laemmli UK (1993) A model for chromatin opening: stimulation of topoisomerase-II and restriction enzyme cleavage of chromatin by distamycin. EMBO J 12:115–126
34. Yaneva J, Schroth GP, van Holde KE, Zlatanova J (1995) High-affinity binding sites for histone H1 in plasmid DNA. Proc Natl Acad Sci USA 92:7060–7064
35. Zhao K, Kas E, Gonzalez E, Laemmli UK (1993) SAR-dependent mobilization of histone H1 by HMG-I/Y in vitro. HMG-I/Y is enriched in H1- depleted chromatin. EMBO J 12:3237–3247
36. Nakagomi K, Kohwi Y, Dickinson LA, Kohwi-Shigematsu T (1994) A novel DNA-binding motif in the nuclear matrix attachment DNA-binding protein Satb1. Mol Cell Biol 14: 1852–1860

37. Dickinson LA, Kohwi-Shigematsu T (1995) Nucleolin is a matrix attachment region DNA-binding protein that specifically recognizes a region with high base- unpairing potential. Mol Cell Biol 15:456–465
38. Hibino Y, Nakamura K, Tsukada S, Sugano N (1993) Purification and characterization of nuclear scaffold proteins which bind to a highly repetitive bent from rat liver. Biochim Biophys Acta 1174:162–170
39. von Kries JP, Buhrmester H, Strätling WH (1991) A matrix/scaffold attachment region binding protein: identification, purification and mode of binding. Cell 64:123–135
40. von Kries JP, Rosorius O, Buhrmester H, Strätling WH (1994) Biochemical properties of attachment region binding protein ARBP. FEBS Lett 342:185–188
41. Buhrmester H, Von Kries JP, Strätling WH (1995) Nuclear matrix protein ARBP recognizes a novel DNA sequence motif with high affinity. Biochemistry 34:4108–4117
42. Tsutsui K, Tsutsui K, Okada S, Watarai S, Seki S, Yasuda T, Shohmori T (1993) Identification and characterization of a nuclear scaffold protein that binds the matrix attachment region DNA. J Biol Chem 268:12886–12894
43. von Kries JP, Buck F, Strätling WH (1994) Chicken MAR binding protein p120 is identical to human heterogeneous nuclear ribonucleoprotein (hnRNP) U. Nucleic Acids Res 22: 1215–1220
44. Ellis J, Talbot D, Dillon N, Grosveld F (1993) Synthetic human β-globin 5′HS2 constructs function as locus control regions only in multicopy transgene concatamers. EMBO J 12:127–134
45. Caterina JJ, Ryan TM, Pawlik KM, Palmiter RD, Brinster RL, Rehringer RR, Townes TM (1991) Human β-globin locus control region: Analysis of the 5′ DNaseI hypersensitive site HS 2 in transgenic mice. Proc Natl Acad Sci USA 88:1626–1630
46. Bonifer C, Yannoutsos N, Kruger G, Grosveld F, Sippel AE (1994) Dissection of the locus control function located on the chicken lysozyme gene domain in transgenic mice. Nucl Acids Res 22:4202–4210
47. Reitman M, Lee E, Westphal H, Felsenfeld GF (1990) Site-independent expression of the chicken βA-globin gene in transgenic mice. Nature 348:749–752
48. Bonifer C, Vidal M, Grosveld F, Sippel AE (1990) Tissue specific and position independent expression of the complete gene domain for chicken lysozyme in transgenic mice. EMBO J 9:2843–2848
49. Poljak L, Seum C, Mattioni T, Laemmli UK (1994) SARs stimulate but do not confer position independent gene expression. Nucl Acids Res 22:4386–4394
50. Bresnick EH, Felsenfeld G (1994) Dual promoter activation by the human β- globin locus control region. Proc Natl Acad Sci USA 91:1314–1317
51. Reitman M, Lee E, Westphal H, Felsenfeld G (1993) An enhancer/locus control region is not sufficient to open chromatin. Mol Cell Biol 13:3990–3998
52. Peterson KR, Stamatoyannopoulos G (1993) Role of gene order in developmental control of human γ-globin and β-globin gene expression. Mol Cell Biol 13:4836–4843
53. Wijgerde M, Grosveld F, Fraser P (1995) Transcription complex stability and chromatin dynamics in vivo. Nature 377:209–213
54. Forrester WC, Epner E, Driscoll MC, Enver T, Brice M, Papayannopoulou T, Groudine M (1990) A deletion of the human β-globin locus activation region causes a major alteration in chromatin structure and replication across the entire β-globin locus. Genes Dev 4:1637–1649
55. Kalos M, Fournier REK (1995) Position-independent transgene expression mediated by boundary elements from the apolipoprotein B chromatin domain. Mol Cell Biol 15:198–207
56. Udvardy A, Schedl P (1984) Chromatin organization of the 87A7 heat shock locus of *Drosophila melanogaster*. J Mol Biol 172:385–403
57. Udvardy A, Maine E, Schedl P (1985) The 87A7 chromomere: identification of novel chromatin structure flanking the heat shock locus that may define the boundaries of higher order domains. J Mol Biol 185:341–368
58. Kellum R, Schedl P (1992) A group of scs elements function as domain boundaries in an enhancer-blocking assay. Mol Cell Biol 12:2424–2431
59. Vazquez J, Schedl P (1994) Sequences required for enhancer blocking activity of scs are located within two nuclease-hypersensitive regions. EMBO J 13:5984–5993
60. Zhao K, Hart CM, Laemmli UK (1995) Visualization of chromosomal domains with boundary element-associated factor BEAF-32. Cell 81:879–889
61. Roseman RR, Pirrotta V, Geyer PK (1993) The su(Hw) protein insulates expression of the *Drosophila melanogaster* white gene from chromosomal position- effects. EMBO J 12:435–442
62. Parkhurst SM, Harrison DA, Remington MP, Spana C, Kelley RL, Coyne RS, Corces VG (1988) The *Drosophila su(Hw)* gene, which controls the phenotypic effects of the *gypsy* transposable element, encodes a putative DNA binding protein. Genes Dev 2:1205–1215
63. Spana C, Harrison D, Corces VG (1988) The *Drosophila melanogaster suppressor of Hairy wing protein* binds to specific sequences of the *gypsy* retrotransposon. Genes Dev 2:1414–1423
64. Geyer PK, Corces VG (1992) DNA position-specific repression of transcription by a *Drosophila* zinc finger protein. Gene Dev 6:1865–1873
65. Gerasimova TI, Gdula DA, Gerasimov DV, Simonova O, Corces VG (1995) A *Drosophila* protein that imparts directionality on a chromatin insulator is an enhancer of position-effect variegation. Cell 82:587–597
66. Chung JH, Whiteley M, Felsenfeld G (1993) A 5′ element of the chicken β- globin domain serves as an insulator in human erythroid cells and protects against position effect in *Drosophila*. Cell 74:505–514
67. Vettese-Dadey M, Walter P, Chen H, Juan L-J, Workman JL (1994) Role of the histone amino termini in facilitated binding of a transcription factor, GAL4-AH, to nulceosomal cores. Mol Cell Biol 14:970–981
68. Wolffe AP (1994) Nucleosome positioning and modification: chromatin structures that potentiate transcription. Trends Biochem Sci 19:240–244
69. Ringold GM, Yamamoto KR, Tomkins GM, Bishop JM, Varmus HE (1975) Dexamethasone-mediated induction of mouse mammary tumor virus RNA: a system for studying glucocorticoid action. Cell 6:299–305
70. Buetti E, Diggelmann H (1981) Cloned MMTV virus DNA is biologically active in transfected mouse cells and its expression is stimulated by glucocorticoid hormones. Cell 23:335–345
71. Huang AL, Ostrowski MC, Berard D, Hager GL (1981) Glucocorticoid regulation of the Ha-MuSV p21 gene conferred by sequences from mouse mammary tumor virus. Cell 27:245–255
72. Hynes NE, Kennedy N, Rahmsdorf U, Groner B (1981) Hormone responsive expression of an endogenous proviral gene of MMTV after molecular cloning and gene transfer into cultured cells. Proc Natl Acad Sci USA 78:2038–2042
73. Lee F, Mulligan R, Berg P, Ringold G (1981) Glucocorticoid regulates expression of dihydrofolate reductase cDNA in mouse mammary tumor virus chimeric plasmids. Nature 294:228–232
74. Buetti E, Diggelmann H (1983) Glucocortiocid regulation of MMTV: identification of a short essential region. EMBO J 2:1423–1429
75. Hynes NE, van Ooyen A, Kennedy N, Herrlich P, Ponta H, Groner B (1983) Subfragments of the large terminal repeat cause glucocorticoid responsive expression of mouse mammary tumor virus and of an adjacent gene. Proc Natl Acad Sci USA 80:3637–3641

76. Majors J, Varmus HE (1983) A small region of mouse mammary tumor virus long terminal repeat confers glucocorticoid hormone regulation on a linked heterologous gene. Proc Natl Acad Sci USA 80:5866–5870
77. Payvar F, Wrange Ö, Carlstedt-Duke J, Okret S, Gustafsson JA, Yamamoto KR (1981) Purified glucocortiocid receptors bind selectively in vitro to a cloned DNA fragment whose transcription is regulated by glucocorticoids in vivo. Proc Natl Acad Sci USA 78:6628–6633
78. Payvar F, DeFranco D, Firestone GL, Edgar B, Wrange Ö, Okret S, Gustafsson JA, Yamamoto KR (1983) Sequence-specific binding of glucocorticoid receptor to MTV DNA at sites within and upstream of the transcribed region. Cell 35:381–392
79. Geisse S, Scheidereit C, Westphal HM, Hynes NE, Groner B, Beato M (1982) Glucocorticoid receptors recognize DNA sequences in and around murine mammary tumour virus DNA. EMBO J 1:1613–1619
80. Chandler VL, Maler BA, Yamamoto KR (1983) DNA sequences bound specifically by glucocorticoid receptor in vitro render a heterologous promoter hormone responsive in vivo. Cell 33:489–499
81. Scheidereit C, Geisse S, Westphal HM, Beato M (1983) The glucocorticoid receptor binds to defined nucleotide sequences near the promoter of mouse mammary tumour. Nature 304:749–752
82. Chalepakis G, Arnemann J, Slater EP, Brüller H, Gross B, Beato M (1988) Differential gene activation by glucocorticoids and progestins through them hormone regulatory element of mouse mammary tumor virus. Cell 53:371–382
83. Scheidereit C, Beato M (1984) Contacts between receptor and DNA double helix within a glucocorticoid regulatory element of mouse mammary tumor. Proc Natl Acda Sci USA 81:3029–3033
84. Cato ACB, Miksicek R, Schütz G, Arnemann J, Beato M (1986) The hormone regulatory element of mouse mammary tumour virus mediates progesterone induction. EMBO J 5: 2237–2240
85. Arriza JL, Weinberger C, Cerelli G, Glaser TM, Handelin BL, Housmann DE, Evans RM (1987) Cloning of human mineralocorticoid receptor cDNA: structural and functional kinship with the glucocorticoid receptor. Science 237:268–275
86. Cato ACB, Weinmann J (1988) Mineralocorticoid regulation of transfected mouse mammary tumour virus DNA in cultured kidney cells. J Cell Biol 106:2119- 2125
87. Darbre P, Page M, King RJB (1986) Androgen regulation by the long terminal repeat of mouse mammary tumor virus. Mol Cell Biol 6:2847–2854
88. Cato ACB, Henderson D, Ponta H (1987) The hormone response element of the mouse mammary tumour virus DNA mediates the progestin and androgen induction of transcription in the proviral long terminal repeat region. EMBO J 6:363–368
89. Ahe D, Janich S, Scheidereit C, Renkawitz R, Schütz G, Beato M (1985) Glucocorticoid and progesterone receptors bind to the same sites in two hormonally regulated promoters. Nature 313:706–709
90. Chalepakis G, Schauer M, Cao X, Beato M (1990) Efficient binding of glucocorticoid receptor to its responsive element requires a dimer and DNA flanking sequences. DNA Cell Biol 9:355–368
91. Luisi BF, Xu WX, Otwinowski Z, Freedman LP, Yamamoto KR, Siegler PB (1991) Crystallographic analysis of the interaction of the glucocorticoid receptor with DNA. Nature 352:497–505
92. Jantzen HM, Strähle U, Gloss B, Stewart F, Schmid W, Boshart M, Miksicek R, Schütz G (1987) Cooperativity of glucocorticoid response elements located far upstream of the tyrosine aminotransferase gene. Cell 49:29–38
93. Schmid W, Strähle U, Schütz G, Schmitt J, Stunnenberg H (1989) Glucocorticoid receptor binds cooperatively to adjacent recognition sites. EMBO J 8:2257–2263
94. Martinez E, Wahli W (1989) Cooperative binding of estrogen receptor to imperfect estrogen-responsive DNA elements correlates with their synergistic hormone- dependent enhancer activity. EMBO J 8:3781–3791
95. Truss M, Chalepakis G, Beato M (1992) Interplay of steroid hormone receptors and transcription factors on the mouse mammary tumor virus promoter. J Steroid Biochem Molec Biol 43:365–378
96. Perlmann T, Erikson P, Wrange Ö (1990) Quantitative analysis of the glucocortiocid receptor-DNA interaction at the mouse mammary tumor virus glucocorticoid response element. J Biol Chem 265:17222–17229
97. Piña B, Haché RJG, Arnemann J, Chalepakis G, Slater EP, Beato M (1990) Hormonal induction of transfected genes depends on DNA topology. Mol Cell Biol 10:625–633
98. Nowock J, Borgmeyer U, Päschel A, Rupp AW, Sippel AE (1985) The TGGCA protein binds to the MMTV-LTR, the adenovirus origin of replication, and BK virus. Nucl Acid Res 13:2045–2062
99. Miksicek R, Borgmeyer U, Nowock J (1987) Interaction of the TGGCA- binding protein with upstream sequences is required for efficient transcription of mouse mammary tumor virus. EMBO J 6:1355–1360
100. Buetti E, Kühnel B, Diggelmann H (1989) Dual function of a nuclear factor I binding site in MMTV transcription regulation. Nucl Acids Res 17:3065–3078
101. Gowland PL, Buetti E (1989) Mutation in the hormone regulatory element of mouse mammary tumor virus differently affect the response to progestins, androgens, and glucocorticoids. Mol Cell Biol 9:3999–4008
102. Brüggemeier U, Rogge L, Winnacker EL, Beato M (1990) Nuclear factor I acts as a transcription factor on the MMTV promoter but competes with steroid hormone receptors for DNA binding. EMBO J 9:2233–2239
103. Rupp RAW, Kruse U, Multhaupt G, Göbel U, Beyreuther K, Sippel AE (1990) Chicken NFI/TGGCA proteins are encoded by at least three independent genes: NFI-A, NFI-B and NFI-C with homologous in mammalian genomes. Nucleic Acids Res 18:2607–2616
104. Kruse U, Sippel AE (1994) Transcription factor nuclear factor I proteins form stable homo- and heterodimers. FEBS Lett 348:46–50
105. Schüle R, Muller M, Otsuka-Murakami H, Renkawitz R (1988) Many transcription factors interact synergistically with steroid receptors. Science 242:1418- 1420
106. Strähle U, Schmid W, Schütz G (1988) Synergistic action of the glucocorticoid receptor with transcription factors. EMBO J 7:3389–3395
107. Brüggemeier U, Kalff M, Franke S, Scheidereit C, Beato M (1991) Ubiquitous transcription factor OTF-1 mediates induction of the mouse mammary tumour virus promoter through synergistic interaction with hormone receptors. Cell 64:565–572
108. Toohey MG, Lee JW, Huang M, Peterson DO (1990) Functional elements of the steroid hormone-responsive promoter of mouse mammary tumor virus. J Virol 64:4477–4488
109. Buetti E (1994) Stably integrated mouse mammary tumor virus long terminal repeat DNA requires the octamer motifs for basal promoter activity. Mol Cell Biol 14:1191–1203
110. Mäws C, Preiss T, Slater EP, Cao X, Verrijzer CP, van der Vliet P, Beato M (1994) Two independent pathways for transcription from the MMTV promoter. J Steroid Biochem Mol Biol 51:21–32
111. Wieland S, Dšbbeling U, Rusconi S (1991) Interference and synergism of glucocorticoid receptor and octamer factors. EMBO J 10:2513–2522
112. Cordingley MG, Riegel AT, Hager GL (1987) Steroid-dependent interaction of transcription factors with the inducible promoter of mouse mammary tumor virus in vivo. Cell 48:261–270
113. Horikoshi M, Wang CK, Fujii H, Cromlish JA, Weil PA, Roeder RG (1989) Cloning and structure of a yeast gene encoding a general transcription initiation factor TFIID that binds to the TATA box. Nature 341:299–303

114. Strähle U, Klock G, Schütz G (1987) A DNA sequence of 15 base pairs is sufficient to mediate both glucocorticoid and progesterone induction of gene expression. Proc Natl Acad Sci USA 84:7871–7875
115. Schatt MD, Rusconi S, Schaffner W (1990) A single DNA-binding transcription factor is sufficient for activation from a distant enhancer and/or from a promoter position. EMBO J 9:481–487
116. Beato M, Herrlich P, Schütz G (1995) Steroid hormone receptors: many actors in search of a plot. Cell 83:851–857
117. Langer SJ, Ostrowski MC (1988) Negative regulation of transcription in vitro by a glucocorticoid reponse element is mediated by a *trans*-acting factor. Mol Cell Biol 8:3872–3881
118. Tanaka H, Dong Y, Li Q, Okret S, Gustafsson J-A (1991) Identification and characterization of a *cis*-acting element that interferes with glucocorticoid-inducible activation of the mouse mammary tumor virus promoter. Proc Natl Acad Sci USA 88:5393–5397
119. Härtig E, Nierlich B, Mink S, Nebl G, Cato ACB (1993) Regulation of expression of mouse mammary tumor virus through sequences located in the hormone response element. Involvement of cell-cell contact and a negative regulatory factor. J Virol 67:813–821
120. Truss M, Bartsch J, Schelbert A, Haché RJG, Beato M (1995) Hormone induces binding of receptors and transcription factors to a rearranged nucleosome on the MMTV promoter in vivo. EMBO J 14:1737–1751
121. Richard-Foy H, Hager GL (1987) Sequence-specific positioning of nucleosomes over the steroid-inducible MMTV promoter. EMBO J 6:2321–2328
122. Piña B, Brüggemeier U, Beato M (1990) Nucleosome positionining modulates accessibility of regulatory proteins to the mouse mammary tumor virus promoter. Cell 60:719–731
123. Piña B, Truss M, Ohlenbusch H, Postma J, Beato M (1990) DNA rotational positioning in a regulatory nucleosome is determined by base sequence. An algorithm to model the preferred superhelix. Nucleic Acids Res 18:6981–8987
124. Piña B, Barettino D, Truss M, Beato M (1990) Structural features of a regulatory nucleosome. J Mol Biol 216:975–990
125. Perlmann T, Wrange Ö (1988) Specific glucocorticoid receptor binding to DNA reconstituted in a nucleosome. EMBO J 7:3073–3079
126. Perlmann T (1992) Glucocorticoid receptor DNA-binding specificity is increased by the organization of DNA in nucleosomes. Proc Natl Acad Sci USA 89:3884–3888
127. Piña B, Barettino D, Beato M (1991) Nucleosome positioning and regulated gene expression. In: N Maclean (eds) Oxford Surveys on Eukaryotic Genes. Oxford University Press, Oxford, pp 83-117
128. Perlmann T, Wrange O (1991) Inhibition of chromatin assembly in *Xenopus* oocytes correlates with derepression of the mouse mammary tumor virus promoter. Mol Cell Biol 11:5259–5265
129. Han M, Grunstein M (1988) Nucleosome loss activates yeast downstream promoters in vivo. Cell 55:1137–1145
130. Han M, Kim UJ, Kayne P, Grunstein M (1988) Depletion of histone H4 and nucleosomes activates the PHO5 gene in *Saccharomyces cerevisiae*. EMBO J 7:2221- 2228
131. Truss M, Bartsch J, Beato M (1994) Antiprogestins prevent progesterone receptor binding to hormone responsive elements in vivo. Proc Natl Acad Sci USA 91:11333–11337
132. McPherson CE, Shim EY, Friedman DS, Zaret KS (1993) An active tissue- specific enhancer and bound transcription factors existing in a precisely positioned nucleosomal array. Cell 75:387–398
133. Pederson DS, Fidrych T (1994) Heat shock factor can activate transcription while bound to nucleosomal DNA in Saccharomyces cerevisiae. Mol Cell Biol 14:189- 199
134. Zaret KS, Yamamoto KR (1984) Reversible and persistent changes in chromatin structure accompanying activation of a glucocorticoid-dependent enhancer element. Cell 38:29–38
135. Hebbes TR, Thorne AW, Crane-Robinson C (1988) A direct link between core histone acetylation and transcriptionally active chromatin. EMBO J 7:1395–1402
136. Bresnick EH, John S, Berard DS, LeFebvre P, Hager GL (1990) Glucocorticoid receptor-dependent disruption of a specific nucleosome on the mouse mammary tumor virus promoter is prevented by sodium butyrate. Proc Natl Acad Sci USA 87:3977–3981
137. Yoshida M, Kijima M, Akita M, Beppu T (1990) Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. J Biol Chem 265:17174–17179
138. Juan L-J, Utley RT, Adams CC, Vettese-Dadey M, Workman JL (1994) Differential repression of transcription factor binding by histone H1 is regulated by the core histone amino termini. EMBO J 13:6031–6040
139. Lee DY, Hayes JJ, Pruss D, Wolffe AP (1993) A positive role for histone acetylation in transcription factor access to nucleosomal DNA. Cell 72:73–84
140. Hayes JJ, Wolffe AP (1992) Histones H2A/H2B inhibit the interaction of transcription factor-IIIA with the *Xenopus borealis* somatic 5S RNA gene in a nucleosome. Proc Natl Acad Sci USA 89:1229–1233
141. Bresnick EH, Bustin M, Marsaud V, Richard-Foy H, Hager GL (1992) The transcriptionally-active MMTV promoter is depeleted of histone H1. Nucl Acids Res 20:273–278
142. Johnson L, Kayne P, Kahn E, Grunstein M (1990) Genetic evidence for an interaction between SIR3 and histone H4 in the repression of the silent mating loci in Saccharomyces cerevisiae. Proc Natl Acad Sci USA 87:6285–6290
143. Li Q, Wrange O (1995) Accessibility of a glucocorticoid response element in a nucleosome depends on its rotational positioning. Mol Cell Biol 15:4375–4384
144. Peterson CL, Herskowitz I (1992) Characterization of the yeast SWI1, SWI2, and SWI3 genes, which encode a global activator of transcription. Cell 68:573–583
145. Winston F, Carlson M (1992) Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. Trends Genet 8:387–391
146. Yoshinaga SK, Peterson CL, Herskowitz I, Yamamoto KR (1992) Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. Science 258:1598–1604
147. Muchardt C, Yaniv M (1993) A human homologue of *Saccharomyces cerevisiae* SNF2/SWI2 and *Drosophila-brm* genes potentiates transcriptional activation by the glucocorticoid receptor. EMBO J 12:4279–4290
148. Cairns BR, Kim YJ, Sayre MH, Laurent BC, Kornberg RD (1994) A multisubunit complex containing the SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 gene products isolated from yeast. Proc Natl Acad Sci USA 91:1950–1954
149. Peterson CL, Dingwal A, Scott MP (1994) Five SWI/SNF gene products are components of a large multisubunit complex required for transcriptional enhancement. Proc Natl Acad Sci USA 91:2905–2908
150. Côte J, Quinn J, Workmann JL, Peterson CL (1994) Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. Science 265:53–59
151. Tsukiyama T, Becker PB, Wu C (1994) ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor. Nature 367:525–532
152. Tsukiyama T, Wu C (1995) Purification and properties of an ATP-dependent nucleosome remodelling factor. Cell 83: 1011–1020
153. Tsukiyama T, Daniel C, Tamkun J, Wu C (1995) ISWI, a member of the SWI2/SNF2 ATPase family, encodes the 140 kD subunit of the nucleosome remodelling factor. Cell 83:1021–1026
154. Grosschedl R, Giese K, Pagel J (1994) HMG domain proteins: architectural elements in the assembly of nucleoprotein structures. Trends Genet 10:94–100