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Review

Hormone action and chromatin remodelling

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Abstract. Current attention in transcriptional regulation is focused on the properties of coactivators and corepressors that mediate communication between sequence-specific transcription factors, the basal transcriptional machinery and the chromatin environment. Nuclear and steroid hormone receptors represent the best-understood transcription factors that utilize coactivators and corepressors. This review considers the access of these receptors to chromatin, the modifications of chromatin structure that the receptors instigate and the implications for transcriptional control. Nucleosome positioning and targeted histone modification emerge as central controlling elements for gene expression.

Key words. Steroid receptor; nuclear receptor; coactivator; corepressor; transcriptional activation; nucleosome positioning; histone modification; transcriptional repression.

Introduction

Research on nuclear and steroid hormone action continues to provide many novel insights into gene regulation at the transcriptional level. A focus of much past and current attention is the functional role of chromatin structural transitions directed by nuclear and steroid hormone receptors. Several well-characterized model systems have been effectively exploited to define how receptors gain access to nucleosomal DNA and then target the remodelling of chromatin structure. Remodelling of chromatin involves the receptor-mediated recruitment of transcription intermediary factors, coactivators and corepressors that stabilize or destabilize histone–DNA interactions. These large multicomponent protein machines also interact directly with the basal transcriptional machinery. The combination of direct effects on the assembly and stability of the preinitiation complex and manipulation of the chromatin environment within which it functions controls transcription. The purpose of this review is to summarize past observations on chromatin and hormone receptors, and to integrate these achievements with current knowledge. We also discuss unresolved issues and problems for future investigation.

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Nuclease accessibility to DNA in chromatin is dependent on hormone receptors: the examples of the ovalbumin and vitellogenin genes

The earliest studies on chromatin accessibility utilized prokaryotic RNA polymerases as probes with the clear result that genes such as ovalbumin that were actively transcribed in chicken oviduct were preferred sites for association with RNA polymerase [1]. These studies were subsequently extended using another nonspecific DNA-binding protein, the nuclease DNase I. The ovalbumin gene was found to be two- to threefold more readily digested by DNase I than bulk chromatin in nuclei isolated from chicken oviduct [2–4]. DNase I sensitivity was independent of ongoing transcription and extended over a 100-kb domain of DNA [5, 6]. The molecular basis for this general increase in accessibility of chromatin

domains to nucleases and RNA polymerase is still not understood. The existence of DNase I hypersensitive sites (DHSs) in the chromosomal domain is one potential reason for increased access; other reasons include the targeted post-translational modification of histones (see later). DHSs are chromosomal sites where DNA is very selectively cleaved across both strands by DNase I. Detailed structural analysis reveals that these sites can be between nucleosomes or even on the surface of a nucleosome [7, 8]. DHSs invariably require a transcriptional regulator or other nonhistone protein to be bound to DNA. They are a universal feature of transcriptionally competent genes in chromatin. Classic studies on the chicken vitellogenin gene (VTGII) by Burch and Weintraub demonstrated that a series of DHSs were present in the promoter of the VTGII gene in liver [9]. One of these sites was specifically inducible by oestrogen [9]; this DHS includes the binding sites for oestrogen receptor [10]. The other DHSs in the VTGII promoter correspond to sites occupied by liver-specific transcription factors and the basal transcriptional machinery [11]. Interestingly, high-resolution genomic footprinting results are consistent with specific nucleosomal structures existing between sites of transcription factor association.

Wahli and colleagues have explored the functional roles of such specific chromatin structures on the *Xenopus* vitellogenin B1 gene promoter in some detail (fig. 1). These investigators first developed a liver-specific, oestrogen-responsive in vitro transcription system [12, 13]. Next they established that nucleosome assembly and the transcription factor NF1 acted synergistically to facilitate transcription from the vitellogenin B1 promoter in vitro [14]. In vitro experiments demonstrated for the first time that nucleosome assembly could stimulate transcription [14, 15]. A positioned nucleosome is assembled due in part to the selective association of the histone octamer with intrinsically curved DNA between the oestrogen response element at −300 and proximal promoter elements at −120 [15]. The formation of a specific chromatin structure on this promoter potentiates transcription over 100-fold relative to the assembly of nonspecific structures (fig. 1). DNA sequence alone is insufficient to accurately translationally position this stimulatory nucleosome with respect to DNA sequence, and NF1 makes specific contacts with histone H3 to help mediate the exact positioning of the histone octamer with respect to regulatory DNA [16].

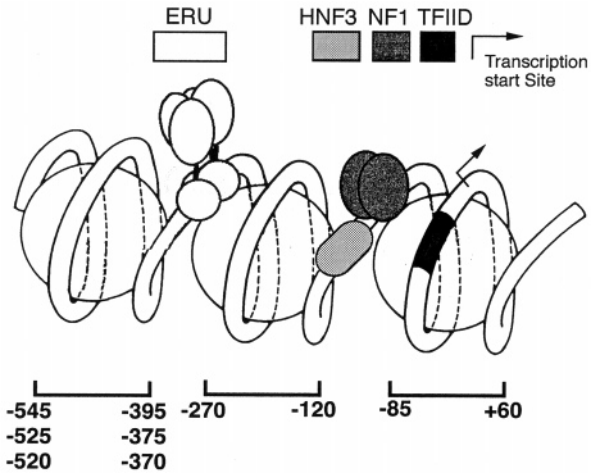

Figure 1. Specific regulatory nucleoprotein architecture assembled on the vitellogenin B1 promoter. The positions of three nucleosomes are illustrated together with their exact boundaries relative to the start site of transcription, +1 (A. Gegonne, D. Robyr, W. Wahli and A. P. Wolffe unpublished). The sites of interaction of sequence specific transcription factors in linker DNAs is shown (ERU, estrogen response unit; HNF3, hepatocyte nuclear factor 3; NF1, nuclear factor 1; TFIID, transcription factor IID).

The vitellogenin genes provide a useful system for studying changes in chromatin structure following both de novo and repeated induction of gene expression on multiple exposures to hormone. This is because the vitellogenin genes are not transcribed in male liver until hepatocytes are exposed to oestrogen [17]. Oestrogen is rapidly metabolized in the male, leading to only the transient activation of vitellogenin gene transcription following a single exposure to hormone [18, 19]. The de novo transcription of the vitellogenin genes in male liver following addition of oestrogen correlates with an increase in general DNase I sensitivity, as well as the appearance of DHSs [18]. Subsequent addition of oestrogen to male liver leads to a more vigorous and sustained transcription of the vitellogenin genes [19]. However, chromatin structural transitions do not appear to be important for this 'memory' of prior hormonal stimulation; instead, autoregulatory activation of oestrogen receptor gene expression has the major role [20]. Cell division events correlate with the progressive loss of oestrogen-induced chromatin structural transitions [21].

Several investigators have begun to address the molecular mechanisms responsible for the remodelling of chromatin targeted by the oestrogen receptor. The receptor itself in the absence of functional activation domains has been shown to gain access to chromatin in *Saccharomyces cerev*isiae [22, 23]. How this is accomplished is not known; however, sequence-specific DNA-binding proteins (other than the basal transcriptional machinery) appear able to recognize their sites in yeast chromatin without difficulty [24]. This may reflect unique properties of yeast chromatin, such as constitutive hyperacetylation of the core histones and the presence of a linker histone of unusual structure [25, 26]. The presence of an activation domain on the chromatin-bound oestrogen receptor can lead to more dramatic rearrangements of chromatin structure [27, 28]. The proteins mediating these rearrangements might include

the mammalian homologues of the yeast SWI/SNF complex [29] or the coactivator/histone acetyltransferase p300 [30]. Exactly how these molecular machines function to regulate transcription is a topic of an intense current research effort (see later).

The experimental examples that we have presented using the ovalbumin and vitellogenin genes were recapitulated for many other acutely regulated genes for which recombinant DNA probes were available [31]. The important discoveries were (1) that specific chromatin structures in responsive genes existed prior to the addition of hormone; (2) that specific structural transitions occurred following hormone addition both in the promoter and over the chromatin domain containing responsive genes; and (3) that these transitions were reversible. Recent progress has substantiated and further defined the functional role of these chromatin-remodelling events.

Nucleosome positioning, reversible nucleosome disruption and transcriptional control: the example of the MMTV LTR and the glucocorticoid receptor

The promoters of acutely inducible genes such as those under hormonal control are known to be organized into specific regulatory nucleoprotein architectures [32, 33]. These regulatory nucleoprotein complexes are not static, but dynamic with respect to both protein–protein and protein–DNA interactions. The paradigm for considering hormone action in the context of a positioned nucleosomal array is the regulation of transcription by the glucocorticoid receptor on the mouse mammary tumour virus (MMTV) long terminal repeat (LTR).

Hager and colleagues established that the MMTV LTR is incorporated into six positioned nucleosomes in both episomes and within a mouse chromosome. The positioned nucleosomes serve to prevent the basal transcriptional machinery associating with the promoter under normal circumstances, that is in the absence of glucocorticoids [34]. Induction of transcription by glucocorticoids requires binding of the glucocorticoid receptor (GR) to the LTR, disruption of the local chromatin structure initiated by the GR binding to recognition elements within nucleosomes (GREs) and the assembly of a transcription complex over the TATA box [34, 35]. Thus, the generation of a DHS [31] involves the regulated association of the glucocorticoid receptor, chromatin structural rearrangements and the final assembly of a functional transcription complex.

Vigorous attempts have been made to reconstruct the transcriptional regulation and concomitant chromatin structural changes of the MMTV LTR in vitro. The GR, which is a zinc-finger protein, appears to bind nucleosomal DNA with only a slight reduction in affinity relative to naked DNA. This interaction is dependent on the precise position of a key regulatory nucleosome that contains the GREs and hence the translational position of the GR-binding site within the nucleosome. GR binding is reported to occur when this regulatory nucleosome is at -188 to -45 [36] and at -219 to -76 [37] or -221 to -78 [38] relative to the start site of transcription ($+1$). In these instances the rotational orientation of the individual GREs on the surface of the histone octamer will be similar due to the separation of nucleosome boundaries by almost exactly three helical turns of DNA. The latter in vitro nucleosome positions compare favourably with those determined in vivo [39]. Recent work suggests that, although a predominant in vivo translational position exists for this particular nucleosome, a number of distinct translational positions can be detected [40]. Detailed in vitro analysis suggests that these variant positions are dependent on the exact boundaries of the DNA sequences used for reconstitution [41]. This heterogeneity in translational position appears to reflect nucleosome mobility [42]. The instability of histone–DNA contacts leading to nucleosome mobility is reflected in the histone octamer sampling multiple translational positions along the double helix. Nucleosome mobility can be an important contributory factor in facilitating transcription [43]. The DNA sequence containing the GREs has regions of intrinsic flexibility and curvature that direct the histones to bind it in a particular way [41, 44]. Four GREs are within this DNA segment at -175, -119, -98 and -83, and have different rotational and translational positions within the nucleosome. The two elements at -119 and -98 face toward the core histones and remain unbound in the presence of GR, whereas the elements at -175 and -83, which face toward solution, are bound by the GR. These sites are separated by 92 bp, which places them together on one side of the nucleosome. This proximity might facilitate both the binding and subsequent activity of GR [45]. The display of DNA-binding sites on the surface of the nucleosome might actually promote the formation of a functional transcription complex [46, 47].

Experiments that compare the affinity of the GR for a recognition element as free DNA, compared with one facing toward solution in the nucleosome at different translational positions, show that binding affinity is reduced 3- to 11-fold in the nucleosome [48]. This is a remarkably small reduction compared with the complete absence of binding when the recognition element is facing toward the histones [49]. This suggests that the key variable determining the accessibility of the GR to its recognition elements within the nucleosome is not the translational position of the recognition element, but the rotational position of the DNA sequence with respect to the surface of the histones (see also refs 8 and 50).

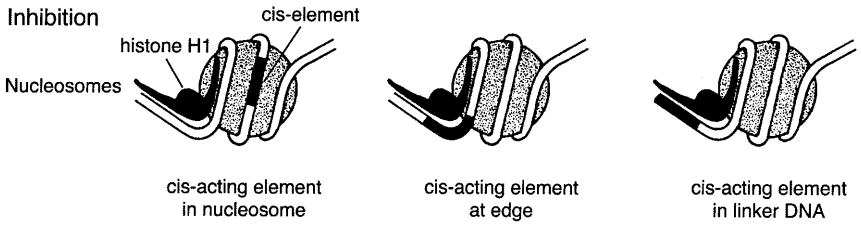

Figure 2. Transcription factor binding might be inhibited by placement of a *cis*-acting sequence in contact with histones H3/H4 inside the nucleosome, with histones H2A/H2B at the edge or with histone H1 in linker DNA. The more internal sites of placement of the *cis*-acting element inside the nucleosome require a more complete disruption of histone-DNA interactions.

The GR is well suited to interact specifically with nucleosomal DNA. GR binds to DNA using a domain containing two zinc fingers: an α helix in one of the two fingers interacts with a short 6-bp region in the major groove of the double helix, while the other finger is involved in protein-protein interaction [51]. The GR associates with DNA as a dimer. The second molecule of the receptor has similar interactions on the same side of the double helix, one helical turn away. Thus GR can bind to DNA on the one side exposed toward solution in the nucleosome, thereby circumventing steric interference by the histone core. Moreover, the GR dimer can bind specifically to DNA containing only one GRE half site, presumably by making both specific and nonspecific contacts to DNA in each half of the dimer. Thus, highly bent nucleosomal DNA might still provide enough precisely aligned contacts for at least one specific half-site interaction which could then be supplemented by nonspecific contacts. This type of nonspecific interaction could account in part for the reduction in affinity of the GR for nucleosomal recognition elements [48]. Surprisingly, association of the GR with the nucleosome containing its binding site appears to have no effect on the integrity of the structure in vitro, unlike the apparent consequence in vivo. Binding of the promoter-specific transcription factor NF1 is facilitated by the GR in vivo; however, this does not occur in vitro on nucleosomal templates [52, 53]. Certain nuclear components that presumably facilitate chromatin structural changes in vivo have so far been lacking in the in vitro system.

What molecular process causes chromatin structure to be disrupted? Some of the possible ways in which a *cis*-acting element could be incorporated into a nucleosome are shown in figure 2. Potential mechanisms for disruption of the nucleosome and transcription factor access are shown in figure 3. DNA replication is the one event certain to disrupt chromatin and provide access of transcription factors to their cognate sequences. However, DNA replication is not required for chromatin disruption and transcriptional activation of the MMTV LTR [3, 5]. Schutz, Richard-Foy and colleagues have extended these studies by examining not only the initial disruption of chromatin structure over the enhancer of the rat tyrosine amino transferase gene (TAT) following induction of GR binding but also the reformation of normal chromatin structure on hormone withdrawal. Both of these changes occur within a few hours, implying that neither disruption nor reassembly of nucleosomes is dependent on DNA replication [54, 55] (see also ref. 56).

If replication is not involved in chromatin rearrangement, nucleosomes must be disrupted in some alternative way. *Trans*-acting factors might displace histones from nucleosomes directly. Alternatively, they might wait either for histones to exchange passively out of chromatin or for DNA to spontaneously unravel from association with the histones before binding to their recognition sequences [57, 58]. The characterization of chromatin-remodelling enzymes and machines at the genetic and biochemical level would make these passive mechanisms appear unlikely, even though they clearly explain some in vitro results [59]. In most assays, nucleases are used to examine the incorporation of specific promoter elements into nucleosomes. It is possible that nucleosomes that have altered their position or composition would lose sharp boundaries to nuclease protection even though DNA could remain associated with histones [60]. Protein–DNA cross-linking reagents and antibodies against histones are being used to explore this possibility.

Histone H1 and histones H2A/H2B are known to exchange readily in and out of chromatin under physiological conditions [61, 62]. A partial disruption of the nucleosome in addition to histone acetylation (see later) seems by far the most likely mechanism by which *trans*-acting factors might gain access to DNA in chromatin. Removal of histone H1 from the nucleosome might facilitate some mobility of the contacts made by the histone octamer with DNA [63]. This local nucleosome sliding could facilitate transcription factor access to recognition sites otherwise constrained within the nu-

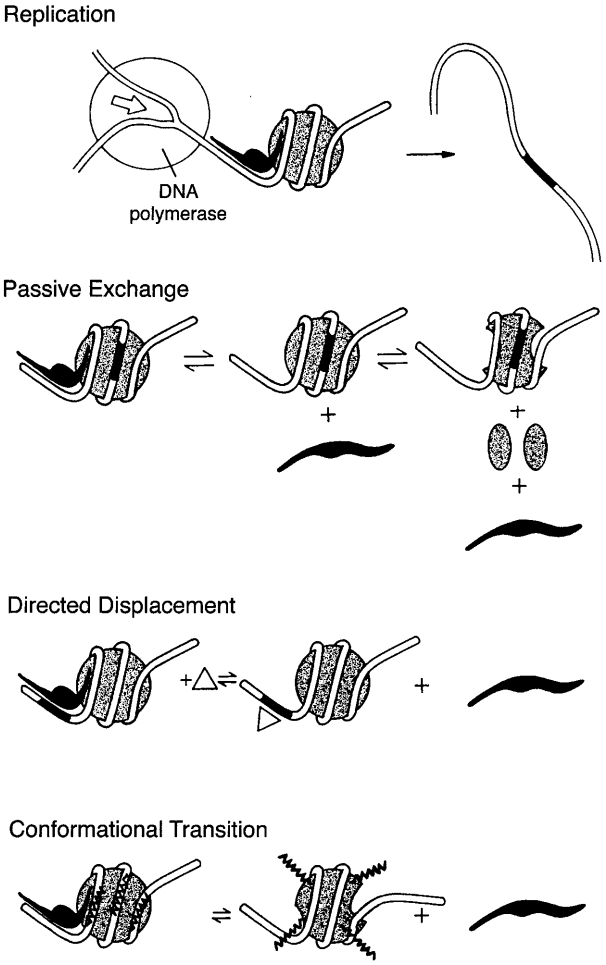

Figure 3. Mechanisms of chromatin disruption. Potential means of chromatin disruption are illustrated, including DNA replication, passive exchange of histones, directed displacement and conformational transitions. The last two possibilities might be facilitated by molecular machines such as SWI/SNF or targeted enzymatic modifications such as core histone acetylation.

cleosome [43, 64–66]. A certain amount of DNA folding would occur even in the absence of these proteins. The capacity to reassemble a complete nucleosome would also remain in the residual interaction of histones H3/H4 with DNA [67]. The integrity of the nucleosome is almost entirely due to the stability of the interaction of the arginine-rich histones H3/H4 with DNA. Histones H1, H2A and H2B are not able to bind correctly in chromatin unless histones H3/H4 are already sequestered. It is also possible that complete histone octamers might exchange from DNA in vivo. However, this seems unlikely, as solutions of high ionic strength (>1 M NaCl) or high temperatures (>80 °C) are required to completely disrupt a nucleosome in vitro. Only very basic arginine-rich proteins, which are unlike known transcription factors, are known to effectively compete histones for association with DNA in a natural context.

The proteins that GR recruits to the MMTV chromatin include the mammalian homologues of the SWI (Switch) 1, 2, 3 proteins of *S. cerevisiae* [68, 69]. The exact mechanism whereby they disrupt chromatin is unknown, but they initiate a chain of events that causes the removal of histone H1 from the linker DNA in MMTV chromatin [70] and a substantial increase in the accessibility of the DNA that is within the positioned nucleosomes to nucleases [31]. The positioning of the nucleosomes is likely to have an important role in the displacement of histone H1, since the linker region between the nucleosomes contains the binding sites for histone H1, NF1 and the octamer factor. It is possible that histone H1 and the transcription factors might compete for binding to this linker region. In any event, the transcription factors NF1, the octamer factor and TFIID that lie in the linker DNA and at the periphery of the positioned nucleosomes are recruited to their binding sites in this disrupted chromatin [34] and assemble an active transcription complex. Transcriptional activation by GR is only transient and after a few hours the basal transcription complex, NF1, octamer factor and GR are displaced from the MMTV LTR, and their binding sites are reincorporated into the positioned nucleosomes and the promoter is repressed [71]. The molecular mechanisms responsible for displacement of the transcription factors have not been determined; however, these results indicate that both transcription factor and histone complexes with promoters are likely to be dynamic.

Although targeted histone acetylation is yet to be documented for MMTV chromatin in response to the presence of hormone-bound GR, it provides an attractive mechanism to locally disrupt chromatin and facilitate the function of the basal transcriptional machinery (fig. 3, conformational transition). Consistent with such a mechanism, Beato and colleagues have found that moderate increases in histone acetylation activate the mouse mammary tumour virus promoter and remodel its chromatin structure [72].

The MMTV LTR provides a powerful example of the remarkable hierarchy of nucleoprotein complexes and their restructuring during transcriptional activation. The rotational positioning of regulatory DNA on the histone octamer surface, the translational positioning of the histone octamer with respect to DNA sequence and the histones present in chromatin all contribute to the regulation of glucocorticoid receptor-mediated gene activation. However, it is not only the activation of transcription that illustrates the dynamic nature of the MMTV LTR nucleoprotein complex but also the subsequent inactivation of the transcription process in the presence of ligand-bound receptor. Inactivating genes is as important in the regulation of transcription as activation itself. The dual contribution of gene activation and repression to regulatory phenomena is best illustrated by the thyroid hormone receptor.

Ncleosome positioning, reversible histone modification, transcriptional repression and activation: the example of the thyroid hormone receptor

The thyroid hormone receptor was established as an integral component of chromatin from pioneering studies in the lab of Herb Samuels [73, 74]. Recent evidence suggests that the thyroid hormone receptor regulates transcription through interaction with a variety of macromolecular complexes including those that remodel chromatin. Thyroid hormone receptor–binding proteins include heterodimeric partners [75–77], the basal transcriptional machinery [78, 79], transcriptional intermediary factors (TIFs)/coactivators [80–83] and corepressors [84–86]. Transcriptional coactivators include p300 and associated proteins such as PCAF which possess histone acetyltransferase activity [82, 87–90]. Transcriptional corepressors include NCoR (Nuclear receptor Compressor) [84] and SMRT (Silencing Mediator for Retinoid and Thyroid receptors) [85] and associated proteins such as SIN3 and histone deacetylase [91–93]. The observations that transcriptional activation is associated with recruitment of histone acetyltransferase, and repression with recruitment of histone deacetylase leads to the hypothesis that the modification of chromatin structure targeted by the thyroid hormone receptor might contribute to transcriptional control.

Acetylation of the core histones correlates with gene activity [94–97]. Nucleosomes containing acetylated core histones are accessible to the transcriptional machinery and much less repressive both to transcription initiation and elongation [98, 99]. The discovery that the *S. cerevisiae* coactivator GCN5p has histone acetyltransferase activity led to the recognition of that targeted acetylation of histones might occur in metazoans [100]. A human homologue of GCN5p known as p300/CBP associated factor (PCAF) acetylates histones [88], as does p300/CBP itself [89]. p300/CBP serves as an integrator to mediate regulation by a wide variety of sequence-specific transcription factors [82], including steroid and nuclear hormone receptors, c-Jun/vJun, cMyb/v-Myb, c-Fos and MyoD [101]. To strengthen the analogy with the GCN5p/ADA2p/ADA3p complex, p300/CBP has a domain highly similar to part of ADA2p and associates with PCAF, the homologue of GCN5p [88]. Most recently, a component of the DNA-binding basal transcription factor TFIID has also been shown to have histone acetyltransferase activity [102]. $TAF_{II}250$ serves as an architectural core protein for TFIID, interacting with the other TAFs (TBP associated factors) as well as with TBP. $TAF_{II}250$ is required for the activation of particular genes indicative of coactivator function, and associates with components of the basal transcriptional machinery such as TFIIA, TFIIE and TFIIF [103]. In addition, $TAF_{II}250$ functions as both a kinase and a histone acetyltransferase [102–103]. p300/CBP, PCAF and $TAF_{II}250$ also have the capacity to acetylate components of the basal transcriptional machinery such as TFIIE and TFIIF [104]. Although GCN5p and PCAF are related proteins, there is no significant sequence identity or known structural similarity with p300/CBP or $TAF_{II}250$. Thus, diverse proteins in metazoans (and potentially in *S. cerevisiae*) possess histone acetyltransferase activity. In an interesting link between the mammalian SWI/SNF (Sucrose Non-Fermentor) activator complex, monoclonal antibodies against p300 immunoprecipitate a complex of p300/CBP together with at least seven other cellular proteins [105]. Within this complex are TBP, $TAF_{II}250$ and hSNF2β (BRG1), suggesting that functions of histone acetyltransferases might be linked to those of other activators that contend with chromatin. The capacity of TR/RXR to bind to p300/CBP suggests that targeted acetylation of the core histones and potentially of TFIIE and F might contribute to gene activation. This hypothesis is made even more attractive by the definition of histone deacetylase as a targeted transcriptional repressor.

The purification of the mammalian histone deacetylase and the recognition of the similarities to *S. cerevisiae* RPD3p [106] has provided considerable insight into transcriptional repression in metazoans. The first direct evidence for mammalian homologues of RPD3p being involved in transcriptional repression came from two hybrid screens, indicating that the transcriptional regulatory factor YY1 interacted with mouse and human RPD3p [107]. The fusion of mammalian RPD3p to a targeted DNA-binding domain directed transcriptional repression by more than 10-fold. Mutations in a glycine-rich domain of YY1 that directs binding to RPD3p could abolish transcriptional repression by YY1, suggesting that YY1 negatively regulates transcription by tethering RPD3p. YY1 is a mammalian zinc-finger transcription factor [108] that is proposed to regulate cell growth and differentiation [109].

A second well-defined protein complex that influences cell growth and differentiation in mammalian cells is the Mad-Max heterodimer [110–112]. Max is a widely expressed sequence-specific transcriptional regulator of the basic region-helix-loop-helix-leucine zipper family (bHLH-ZIP). Max heterodimerizes with the Myc family of bHLH-ZIP proteins including Myc, Mad and Mxi-1 [113, 114]. While the Myc-Max complex activates transcription and transformation, the Mad-Max complex represses these events. Eisenman and colleagues identified two mammalian proteins, mSin3A and mSin3B, that interact with Mad and that have striking homology to *S. cerevisiae* Sin3p, including the four-paired amphipathic helix (PAH) domains. The association between Mad-Max and mSin3A and B requires the second PAH domain. Mutations in this domain eliminate the interac-

tion with mSin3A and prevent the Mad-Max complex from repressing transcription [115]. The next step was to establish that the mSIN3 proteins interact with the mammalian histone deacetylases. Mad, mSIN3 and the mammalian histone deacetylases coimmunoprecipitate [91–116]. The third PAH domain of mSIN3 interacts with the mammalian RPD3p homologues and can confer transcriptional repression when attached to a DNA-binding domain. More subtle mutational analysis suggests that the cell transformation and transcriptional repression suppressed by the Mad-Max complex depend on distinct domains of the mSIN3 proteins [91]. However, an active role for histone deacetylation in transcriptional control is demonstrated by the use of deacetylase inhibitors such as trichostatin A [117] that abolish Mad's ability to repress transcription. The existence of a conserved transcriptional repression mechanism that utilizes SIN3p and histone deacetylase emphasizes the significance of the chromatin environment for transcriptional control. Histone deacetylation directs the assembly of a stable repressive chromatin structure.

A role for chromatin had already been established in the control of transcription by the thyroid hormone receptor [8, 118, 119]. These studies provide a useful example of how the histones can contribute to gene regulation. The assembly of minichromosomes within the *Xenopus* oocyte nucleus has been used to examine the role of chromatin in both transcriptional silencing and activation of the *Xenopus* TRβA promoter. Transcription from this promoter is under the control of thyroid hormone and the thyroid hormone receptor [120], which exists as a heterodimer of TR and RXR. Microinjection of either single-stranded or double-stranded DNA templates into the *Xenopus* oocyte nucleus offers the opportunity for examination of the influence on gene regulation of chromatin assembly pathways that are either coupled or uncoupled to DNA synthesis [121]. The staged injection of mRNA-encoding transcriptional regulatory proteins and of template DNA offers the potential for examining the mechanisms of transcription factor-mediated transcriptional activation of promoters within a chromatin environment. In particular, it is possible to discriminate between pre-emptive mechanisms in which transcription factors bind during chormatin assembly to activate transcription, and postreplicative mechanisms in which transcription factors gain access to their recognition elements after they have been assembled into mature chromatin structures. TR/RXR heterodimers bind constitutively within the minichromosome, independently of whether the receptor is synthesized before or after chromatin assembly. Rotational positioning of the TRE on the surface of the histone octamer allows the specific association of the TR/RXR heterodimer in vitro [8]. The coupling of chromatin assembly to the replication process augments transcriptional repression by unliganded TR/RXR without influencing the final level of transcriptional activity in the presence of thyroid hormone. The molecular mechanisms by which the unliganded thyroid hormone receptor makes use of chromatin in order to augment transcriptional repression also involve mSin3 and histone deacetylase [91, 92]. The unliganded thyroid hormone receptor and retinoic acid receptor bind a corepressor, NCoR [84]. NCoR interacts with Sin3 and recruits the histone deacetylase [91, 92]. All of the transcriptional repression conferred by the unliganded thyroid hormone receptor in *Xenopus* oocytes [118, 119] can be alleviated by the inhibition of histone deacetylase using trichostatin A, indicative of an essential role for deacetylation in establishing transcriptional repression in a chromatin environment.

The addition of thyroid hormone to the chromatin-bound receptor leads to the disruption of chromatin structure [118, 119]. Chromatin disruption is not restricted to the receptor binding site and involves the reorganization of chromatin structure in which targeted histone acetylation by the PCAF and p300/CBP activators may have a contributory role [88, 89]. The exact coactivators that function in *Xenopus* are being defined. It is possible to separate chromatin disruption from productive recruitment of the basal transcription machinery in vivo by deletion of regulatory elements essential for transcription initiation at the start site and by the use of transcriptional inhibitors [118, 119]. Therefore, chromatin disruption is an independent hormone-regulated function targetted by DNA-bound thyroid hormone receptor. It is remarkable just how effectively the various functions of the thyroid hormone receptor are mediated through the recruitment of enzyme complexes that modify chromatin. These results provide compelling evidence for the productive utilization of structural transitions in chromatin as a regulatory principle in gene control [122].

The genetic, biochemical and cell biological evidence that we have outlined provides a substantial rationale for considering chromatin structural proteins as integral components of the transcriptional machinery. It is important to recognize that chromatin structure is not necessarily static and obstructive to transcription but provides a means of display for the DNA template that determines function. Variation in the quality of histone–DNA interactions and in the three-dimensional path of the double helix can directly influence transcription [15, 99]. Conformation is a well-known determinant of enzymatic activity; alterations in chromatin conformation may well determine transcriptional activity.

The facts that (i) core histone acetylation greatly facilitates the access of transcription factors to DNA in a nucleosome, (ii) that transcriptional repressors recruit histone deacetylases and (iii) that transcriptional coactivators are histone acetyltransferases lead to a model for transcriptional regulation in which the recruitment of repressors could direct the local stabilization of repressive histone–DNA interactions and where the recruitment of

activators could destabilize these interactions. Repressive nucleosomes might prevent either the association or function of the basal transcriptional machinery on a particular promoter. However, it is important to note that certain transcriptional regulators such as the thyroid hormone receptor can bind to their recognition elements in a nucleosome even when the histones are deacetylated [118, 119], and that nucleosome assembly is not always repressive [15].

The recruitment of histone deacetylase by chromatin-bound repressors will potentially eliminate basal levels of histone acetylation and impede the recruitment or function of the basal transcriptional machinery. Targeted acetylation provides a means of allowing the basal machinery to displace nucleosomes, assemble a functional transcription complex and never have to deal with chromatin again. For example, we can propose three steps in the regulation of transcription by thyroid hormone receptor: (i) thyroid hormone receptor binds to chromatin on the surface of a positioned nucleosome and facilitates the assembly of a repressive chromatin structure; (ii) in response to hormone, the receptor recruits molecular machines or enzymes that disrupt local chromatin structure; (iii) the hormone-bound receptor and associated activators facilitate the recruitment and activity of the basal transcriptional machinery to further activate transcription. Additional interesting possibilities include the regulated association and activity of histone acetyltransferases and deacetylases within a common complex. In this way transcriptional activity could be continually modulated through variation in chromatin conformation.

Nuclear compartmentalization

A relatively unexamined aspect of steroid and nuclear receptor function concerns their compartmentalization in the nucleus. These receptors are now generally thought to be found predominantly in the nucleus independent of the presence or absence of hormone [123–129]. However, there are several potential compartments that receptors can occupy. These have been examined in most detail for the glucocorticoid receptor. The glucocorticoid receptor is unusual in having a fraction of the total cellular receptor present in the cytoplasm anchored by various members of the heat shock and immunophilin families [130–132] that bind the C-terminal ligand-binding domain [133–134]. On addition of ligand this cytoplasmic glucocorticoid receptor enters the nucleus but is not randomly distributed. A substantial fraction of the receptor asociates with the nuclear matrix, which represents the residual nuclear fraction that remains after most chromatin, has been extracted [135, 136]. Cytologically, the receptor is visualized as present in discrete foci within the nucleus [137]. The function of either the foci or nuclear matrix association is as yet unresolved. The foci might represent sites of glucocorticoid receptor-mediated transcription, association with coactivators or corepressors. Alternatively, they might represent storage sites, sites of degradation or nonspecific aggregation. This latter possibility of artefactual association is, however, rendered unlikely by the careful mutagenesis and cell biological experiments of De Franco and colleagues [135, 136, 138].

Conclusions

Nuclear and steroid hormone receptors function effectively within a chromatin environment. In many ways the function of these receptors depends upon their ability to manipulate chromatin structure toward instability or stability. These events occur dynamically within a structure that allows receptor association and that can reversibly accommodate the conformational and compositional transitions induced by the receptor. Most attention in this research field is currently focused on the chromatin remodelling machines that activate or repress transcription. It is nevertheless essential to understand the structures that exist before remodelling, the remodelled state and those that are present when the gene is inactivated. Moreover, attention should be focused not only on histone–DNA interactions but also on the activities of the basal transcriptional machinery in the context of chromatin. The principal function of receptors may well be to facilitate the conversation between the basal transcriptional machinery and the chromatin environment in which it functions.

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