REVIEW

Nuclear receptors: coactivators, corepressors and chromatin remodeling in the control of transcription

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ABSTRACT

A contemporary view of hormone action at the transcriptional level requires knowledge of the transcription factors including the hormone receptor that may bind to promoters or enhancers, together with the chromosomal context within which these regulatory proteins function. Nuclear receptors provide the best examples of transcriptional control through the targeted recruitment of large protein complexes that modify chromosomal components and reversibly stabilize or destabilize chromatin. Ligand-dependent recruitment of transcriptional coactivators destabilizes chromatin by

mechanisms including histone acetylation and contacts with the basal transcriptional machinery. In contrast, the recruitment of corepressors in the absence of ligand or in the presence of hormone antagonists serves to stabilize chromatin by the targeting of histone deacetylases. Both activation and repression require the action of other chromatin remodeling engines of the switch 2/sucrose non-fermentable 2 (SWI2/SNF2) class. Here we summarize this information and integrate hormone action into a chromatin context.

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INTRODUCTION

The nuclear hormone receptors provide transcription research with a clear example of how the reversible modification of chromatin structure can contribute to the control of gene expression. Remarkable progress in the definition of intermediary protein complexes that activate or repress transcription has allowed common themes in transcriptional control by a wide variety of receptors to emerge. The roles of these coactivators and corepressors in mediating the activities of nuclear receptors within chromatin is a key element of contemporary molecular endocrinology. The best-studied receptors in this regard are those for the glucocorticoid and thyroid hormones.

The glucocorticoid receptor recognizes response elements within nucleosomes as a first step towards a finely orchestrated rearrangement of histone-DNA contacts concomitant with the assembly of a functional transcription complex. The determinants of chromatin remodeling and the molecular

machines that carry it out are now understood in considerable detail. Likewise, the thyroid hormone receptor recognizes nucleosomal DNA and utilizes chromatin to regulate transcription. However, in this case the receptor exerts a dual function. In the absence of ligand, the thyroid hormone receptor recruits a corepressor complex that stabilizes chromatin structure. Upon addition of hormone the receptor releases this repressive complex, and recruits coactivators that destabilize chromatin and promote transcription.

This review illustrates the role of chromatin, coactivators and corepressors in gene control as orchestrated by the glucocorticoid and thyroid hormone receptors.

COACTIVATORS AND COREPRESSORS

Coactivators

The interplay of distinct nuclear receptor responsive transcription pathways has been recognized for

TABLE 1. Yeast SWI/SNF complexes and mammalian BRG1/BAF complexes: structural and functional similarities (Kennison & Tamkun 1988, Tamkun *et al.* 1992, Elfring *et al.* 1998)

Components		Structure-function
SWI/SNF	BRG1/BAF	
SWI 1	BAF 250	Contain LXXLL motifs – may be targeted by nuclear receptors
SWI 2	BRG1	DNA-dependent ATPase engine for chromatin disruption
SWI 3	BAF 155/170	Similar sequence – no known function
SWP 73	BAF 60	Similar sequence – no known function
SWP 61	BAF 53	Actin-like protein – important for association with the nucleus
SWP 59	Actin	Actin or actin-like protein – important for association with the nucleus

SWP, switch associated protein.

some time (Bocquel *et al.* 1989, Meyer *et al.* 1989). The capacity for the activation of transcription by one nuclear receptor to compromise the transcriptional response dependent on a second receptor implied that shared components of transcriptional machinery were involved. An explanation for this transcriptional interference became apparent with the discovery of shared coactivator proteins that facilitated communication between nuclear receptors, the basal transcriptional machinery and the chromatin environment (reviewed by Torchia *et al.* 1998).

Coactivators can be divided into two general classes: members of the switch/sucrose non-fermentable (SWI/SNF) family of proteins and members of the histone acetyltransferase family. Both classes of coactivators have the capacity to modify the chromatin environment facilitating transcription indirectly by alleviating the repressive effects of histone–DNA contacts. They can also potentially influence the activity of the basal transcriptional machinery directly through protein–protein contacts. The SWI/SNF family of proteins were first characterized as regulators of gene expression in the yeast *Saccharomyces cerevisiae* (Neigeborn & Carlson 1984, Stern *et al.* 1984). Ligand-dependent transcriptional activation by the glucocorticoid receptor (GR) in yeast was facilitated by several SWI gene products such as SwI1p, SwI2p and SwI3p (Yoshinaga *et al.* 1992). These are all components of a large SWI/SNF chromatin remodeling complex (Peterson *et al.* 1994). The targeting of the SWI/SNF complex to the GR might be explained by the presence of receptor binding motifs (LXXLL) in SWI1p (Heery *et al.* 1997). The *Drosophila* gene *Brahma* (Brm) encodes a protein highly similar in sequence to SwI2p.

Brahma is required for the developmental control of several of the homeotic genes that define segmental identity in the *Drosophila* embryo. It remains to be determined whether *Brahma* is required for transcriptional regulation by the several members of the nuclear receptor superfamily found in *Drosophila*. The *Brahma* protein is assembled into a large complex that includes several other proteins that are homologous to the yeast SWI/SNF complex (Papoulas *et al.* 1998). The equivalent mammalian complex has a similar subunit composition in which the SWI2p homologue is represented by the protein encoded by *Brahma*-related gene 1 (BRG1) (Table 1; Zhao *et al.* 1998). Human BRG1 and related proteins interact with nuclear receptors in mammalian cells (Muchardt & Yaniv 1993, Chiba *et al.* 1994). These interactions have functional consequences for the remodeling of chromatin targeted by the glucocorticoid receptor (Fryer & Archer 1998).

Histone acetyltransferase coactivators (reviewed by Torchia *et al.* 1998) were identified initially on the basis of their interaction with the ligand binding domain of a variety of nuclear receptors in the presence of cognate receptor ligands, with subsequent studies revealing their histone acetyltransferase (HAT) activity (Spencer *et al.* 1997). Perhaps the best characterized group of HAT coactivators is the p160 family, in which multiple variations have been isolated which share a striking homology, the structural features of which are typically represented by steroid receptor coactivator-1a (SRC-1a) in Fig. 1a. To date, three distinct members of the p160 family have been isolated that exist as multiple splice variants including SRC-1/NCoA-1, TIF2/GRIP1/NCoA-2, and p/CIP/ACTR/RAC3/AIB1/TRAM-1 (Onate *et al.* 1995, Hong *et al.* 1996,

FIGURE 1. Structural features of nuclear receptor coactivators and corepressors. Conserved motifs or regions important for enzymatic activity are shown in bold text. Factors known to interact with these proteins are shown in plain text. Homology is represented as identity. (a) The structure of SRC-1a is representative of a class of highly homologous coactivators. These are divided into three subgroups (i, ii, iii) based on more stringent homology. Asterisks denote LXXLL motifs. bHLH, basic helix-loop-helix; PAS, period/aryl hydrocarbon receptor/single minded; S/T, serine/threonine rich; AB, acidic basic; HAT, histone acetyltransferase activity; QQQ, glutamine rich. (b) p300 vs CBP. C/H, cysteine rich; Bromo, bromodomain. (c) SMRT vs NCoR. RD, repression domain; NR, nuclear receptor interacting domain; SG, serine/glycine rich; H, α -helical. (d) TIF1 α vs TIF1 β . B, B box fingers.

TABLE 2. Nuclear receptor cofactors

Protein	Nuclear receptor target <i>in vitro</i>	Function
<i>Coactivators</i>		
SRC-1, NCoA-1	ER, GR, PR, TR, RXR	Acetyltransferase Interacts with CBP
TIF2, GRIP1, NCoA2	ER, AR, GR, PR	Interacts with CBP
P/CIP, ACTR, RAC3	ER, PR, TR, RAR,	Acetyltransferase
AIB1, TRAM1	RXR, VDR	Interacts with CBP, PCAF
PCAF	RAR, RXR	Acetyltransferase Interacts with p300/CBP
P300/CBP	ER, TR, RAR, RXR	Acetyltransferase Interacts with SRC-1, PCAF
<i>Corepressors</i>		
NCoR, SMRT	TR, RAR, RXR ER+hormone antagonist PR+hormone antagonist	Interacts with SIN3 to recruit histone deacetylase
TIF1α, TIF1β	RAR	Interacts with HP1 and histone deacetylase

Kamei *et al.* 1996, Voegel *et al.* 1996, Anzick *et al.* 1997, Chen *et al.* 1997, Li *et al.* 1997, Takeshita *et al.* 1997, Torchia *et al.* 1997, summarized in Table 2). SRC-1 also recruits other acetyltransferases including p300, cyclic AMP response element (CREB) binding protein (CBP) and p300 CBP associated factor (PCAF) (Hanstein *et al.* 1996, Kamei *et al.* 1996, Yao *et al.* 1996, Voegel *et al.* 1998, summarized in Table 2). Interestingly, studies with retinoic acid receptor (RAR) have found that acetyltransferase domain of SRC-1 is not essential for transcriptional activation but that the corresponding domain of PCAF is required (Korzus *et al.* 1998).

p300 and CBP are highly related histone acetyltransferases (Fig. 1b) that interact with SRC-1, but also bind independently to nuclear hormone receptors in a ligand-dependent manner (Chakravarti *et al.* 1996). p300 and CBP can enhance ligand-dependent transcriptional activation in synergy with SRC-1 (Smith *et al.* 1996). p300, CBP and SRC-1 also interact independently with PCAF (Yang *et al.* 1996, Torchia *et al.* 1997, Blanco *et al.* 1998) leading to the concept that a very large histone acetyltransferase factory might be assembled in the vicinity of a ligand-bound receptor (Wade *et al.* 1997). Biochemical fractionation indicates that diverse acetyltransferases exist as distinct subcomplexes in the cell (McKenna *et al.*

1998). Analysis of nuclear receptor coactivators has revealed the existence of multiple highly conserved amphipathic 'LXXLL' helical motifs (Heery *et al.* 1997). These motifs play an important role in mediating the interaction between coactivators and receptors by associating with critical residues in a coactivator interface region of the receptor ligand binding domain. Mutation of these residues abrogates both coactivator recruitment and trans-activation (Collingwood *et al.* 1997, 1998, Feng *et al.* 1998, Nolte *et al.* 1998). Nolte and colleagues also showed that a single peptide from SRC-1 could associate with a liganded PPARγ (peroxisome proliferator activated receptor) homodimer and that each of two adjacent LXXLL motifs made identical contacts with both subunits of the homodimer (Nolte *et al.* 1998). These motifs are central to determining the specificity of the receptor-coactivator interaction since studies on the three core LXXLL motifs in SRC-1 showed that whereas a single motif was necessary for activation of the estrogen receptor (ER), different combinations of two appropriately spaced motifs are required for activation of thyroid hormone (TR), retinoic acid (RAR), progesterone (PR) and peroxisome proliferator activated (PPARγ) receptors (McInerney *et al.* 1998). Residues adjacent to the motifs are also important for determining the receptor specificity of interaction and it is suggested that the common

structural elements facilitate regulation through multiple coactivators, while side chains at the interface impart specificity (Darimont *et al.* 1998). In addition, LXXLL motifs have been shown to be important in mediating the interaction between SRC-1 and CBP/p300 (McInerney *et al.* 1998), as well as between a 270 kDa protein related to CBP/p300 and components of the human SWI/SNF complex (Dallas *et al.* 1998). This combinatorial flexibility might contribute both to the specificity of transcriptional activation by particular receptors in certain cell types and to the interdependence of regulatory pathways where different receptors require a distinct subcomplex of acetyltransferases. This indicates that the two general classes of coactivator might act in concert to regulate transcription. Exactly how this might be achieved will be discussed later.

Corepressors

Just as coactivators facilitate the activation of transcription by ligand-bound receptors, corepressors can help repress transcription dependent on the presence of certain nuclear receptors such as RAR and TR in the absence of ligand or for ER in the presence of antagonists. Since many components of chromatin can non-specifically repress transcription, an important component in the activity of corepressors is their targeting to the receptor. Early experiments that suggested the existence of corepressors came from 'repression-interference' assays where TR-mediated repression was reduced in the presence of excess unliganded RAR or v-ErbA (Baniahmad *et al.* 1992). Subsequent experiments have identified two potential classes of corepressors: the nuclear hormone receptor corepressor (NCoR) and silencing mediator of retinoid and thyroid receptors (SMRT) proteins for the major family (Fig. 1c; Chen & Evans 1995, Horlein *et al.* 1995, Kurokawa *et al.* 1995, Zamir *et al.* 1997, Ordentlich *et al.* 1999) and the transcription intermediary factor-1 (TIF1) proteins as the other family (Le Douarin *et al.* 1995, 1996, Vom Baur *et al.* 1996). Like the coactivators, the corepressors appears to exert significant functions through the modification of chromatin. Both classes of corepressors function to recruit histone deacetylase to the vicinity of the receptor.

The NCoR and SMRT corepressors interact with the unliganded TR or RAR when associated with their retinoid X receptor (RXR) heterodimeric partner (Chen & Evans 1995, Horlein *et al.* 1995, Kurokawa *et al.* 1995, Lee *et al.* 1995, Jackson *et al.* 1997, Smith *et al.* 1997, Lavinsky *et al.* 1998).

Studies have defined a region, termed the CoR box, in the hinge between the receptor DNA binding domain (DBD) and ligand binding domain (LBD) that is critical for association with corepressors (Horlein *et al.* 1995). Subsequent work indicates that the extreme carboxy-terminus is also important, particularly in facilitating corepressor release as a result of hormone-induced allosteric changes (Baniahmad *et al.* 1995, Lin *et al.* 1997). A role for hormone in effecting this structural transition is highlighted by the observation that a highly mutated form of the chicken TR, the oncoprotein v-ErbA, fails to bind hormone, is continuously associated with SMRT (Chen & Evans 1995) or NCoR (Horlein *et al.* 1995) and is thought to act as a constitutive transcriptional repressor. The ER can also bind the NCoR and SMRT corepressors in the presence of antagonists such as tamoxifen and RU486 (Laherty *et al.* 1998, Lavinsky *et al.* 1998). NCoR and SMRT are components of a complex that contains SIN3 and histone deacetylase (Alland *et al.* 1997, Heinzel *et al.* 1997, Nagy *et al.* 1997). SIN3, a protein containing four paired amphipathic helical domains, interacts with a diverse collection of nuclear proteins. The main function of SIN3 appears to be to serve as an intermediary between the receptor and histone deacetylase itself (Wong & Privalsky 1998).

The TIF1 α and β family of corepressors (Fig. 1d) were initially identified by the capacity of TIF1 α to interact with the ligand-binding domain of the RAR in the presence of hormone (Le Douarin *et al.* 1995, 1996). This would initially suggest that they should activate transcription, however subsequent work has established that the highly related TIF1 β corepressor binds to the KRAB repression domain found in many zinc-finger proteins and with heterochromatin protein (HP1). These observations led to the proposal that nuclear receptors might target the assembly of a microdomain of heterochromatin (Le Douarin *et al.* 1995). Alternative proposals from these workers include the possibility that the TIF1 proteins might be involved in ligand-dependent transcriptional repression or in promoting the conversion of an inactive heterochromatin-like structure to an active chromatin structure (Le Douarin *et al.* 1998). How this latter possibility might be achieved is so far unclear. Subsequent work has indicated that KRAB and TIF β repression also depends on the recruitment of deacetylase (R Kimmel & A P Wolffe, unpublished observations). Thus both classes of corepressors will recruit histone deacetylase. The consequences of the local concentration of deacetylase enzyme will be to stabilize chromatin structure and repress transcription.

TABLE 3. Glossary of chromatin terms

Term	Explanation
Nucleosome	Nucleoprotein complex of two molecules of each of the four core histones H2A, H2B, H3 and H4, one molecule of linker histone H1 and 166–200 bp of DNA.
Histone	Small basic protein with a C-terminal domain involved in histone–histone interaction and an N-terminal ‘tail’ domain that projects outside the nucleosome.
Chromatin fiber	Compact structure about 30 nm in diameter that is assembled from nucleosome arrays.
Superhelix	A helix formed by a strand that is itself a helix, as in DNA.
Dyad axis	The point in the nucleosome that forms an axis of symmetry.
Open chromatin	Chromatin that contains DNA that is accessible to nucleases and the transcription machinery.
Closed chromatin	Chromatin that is inaccessible to nucleases and the transcriptional machinery and is generally transcriptionally silent.

CHROMATIN AND THE TRANSCRIPTIONAL MACHINERY

Chromatin modification and disruption

Chromatin is composed of a simple repetitive structural element known as the nucleosome, which has the capacity to self-associate into a higher-order structure described as the chromatin fiber (see Table 3 for a glossary of terms). The individual chromatin fibers can also self-associate into the basic matrix of chromosomes (Fig. 2, Wolffe 1998). These self-association events are controlled by the modification state of the histones (Tse *et al.* 1998). Acetylation of the core histones destabilizes chromatin folding at all levels. A major consequence of this destabilization is that histone acetylation facilitates transcription factor access to DNA and transcription itself (Lee *et al.* 1993, Lefebvre *et al.* 1998, Ura *et al.* 1997, Nightingale *et al.* 1998, Tse *et al.* 1998). An effect of acetylating the basic N-terminal tail domains of the core histones is to reduce the stability of electrostatic interactions between the tail domains and both the acidic phosphodiester backbone of DNA and the acidic domains of other proteins present in nucleosomes and other chromatin fibers (Hong *et al.* 1993, Luger *et al.* 1997, Tse *et al.* 1998). There may also be consequences for both histone secondary structure and interactions with non-histone proteins following acetylation (Hansen *et al.* 1998).

Every nucleosome in a typical animal cell consists of two molecules of each of the four core histones, H2A, H2B, H3 and H4, about 180 bp of DNA and a single molecule of a linker histone H1. Each histone has two domains, an N-terminal tail that faces solution at the outer surface of the nucleosome and a C-terminal histone fold domain that is

involved in wrapping DNA (Arents *et al.* 1991, Luger *et al.* 1997). In *S. cerevisiae*, mutations of the N-terminal tails that reduce basicity eliminate the requirement for histone acetyltransferases to activate transcription (Zhang *et al.* 1998). In contrast, mutations in the histone fold domains eliminate requirements for the SWI/SNF coactivators (Kruger *et al.* 1995). These mutations that influence

FIGURE 2. Folding of chromatin as visualized in the electron microscope and assayed using the analytical ultracentrifuge. As ionic strength increases chromatin changes from a beads-on-a-string configuration to a array in which nucleosomes make direct physical contacts, to a flat fiber, 30 nm in diameter which can self-associate to form higher-order structures. These structural transitions are impeded by increases in the acetylation level of the core histones (Tse *et al.* 1998).

the need for the SWI/SNF complex destabilize the nucleosome (Kurumizaka & Wolffe 1997, Weschser *et al.* 1997). DNA is still wrapped around the core histones in a destabilized nucleosome, but the double helix is much less constrained in terms of which surface faces solution compared with being in contact with the histones. Thus genetic and structural data combine to implicate chromatin modification and disruption as major functions for both the histone acetyltransferase and the SWI/SNF coactivators.

Linker histones are also targets for post-translational modification, in this case by phosphorylation. Like acetylation of the core histones, phosphorylation of H1 weakens interaction of the protein with DNA. Histone H1 consists of a globular central domain flanked by lysine-rich highly basic amino terminal and carboxyl terminal tails (Wolffe *et al.* 1997). The globular domain interacts with DNA in contact with the core histones (Pruss *et al.* 1996), whereas the tails bind to linker DNA. Phosphorylation of the histone H1 tails occurs on serine residues predominantly at conserved SPKK motifs present in the charged tail regions. In addition to weakening the electrostatic interactions with DNA, phosphorylation of H1 will also destabilize α -helical structures present in the linker histone tail domains (Clark *et al.* 1988, Szilak *et al.* 1997) further reducing the stability of association with chromatin (Hill *et al.* 1991).

We will see that core histone acetylation, linker histone phosphorylation and the SWI/SNF complex mediated disruption of chromatin can all contribute to gene control by coactivators and corepressors. However, to understand the significance of these structural transitions we first have to describe why chromatin modification and disruption are important for transcription control.

Chromatin and transcription factor access to DNA

Wrapping DNA around the core histones in a nucleosome creates severe steric impediments for transcription factors that need to gain access to specific recognition sequences. One side of the double helix is in association with the histones with the adjacent turn of DNA in the nucleosome also limiting accessibility. In addition, DNA is severely distorted into two shallow superhelical turns of 80 bp each, this will require a protein that binds to DNA on the outer face of the double helix in the continued presence of the core histones to distort itself around this surface (Fig. 3; Wolffe 1998). For the vast majority of transcription factors, the assembly of their recognition elements into

FIGURE 3. Impediments to transcription factor access in the nucleosome. The nucleosome is shown with the histones as a hatched cylinder and DNA as the open tube. The top panel shows an image overlooking the center of nucleosomal DNA, at which point the DNA superhelix rises very steeply. The middle panel shows the path of a single turn of DNA in the nucleosome. Numbers refer to turns of the double helix away from the dyad axis. The arrows represent the positions of more severe DNA distortion. The asterisk marks the approximate position of base pair unstacking detected by singlet oxygen and hydroxyl radical cleavage. The lower panel illustrates the limited access of transcription factors to DNA in the nucleosome. A cross-section of one side of the nucleosome is shown. Transcription factor (large circle) access to DNA (small circle) is restricted by the histone core (hatched box) and the adjacent turn of DNA. The only freely accessible region of the double helix is the hatched segment marked by arrows.

FIGURE 4. A model for the nucleoprotein organization of the *Xenopus* vitellogenin B1 promoter. The organization of three nucleosomes relative to the start site of transcription (+1) is shown (Schild *et al.* 1993, D Robyr, A Gegonne, A P Wolffe & W Wahli, unpublished) together with the binding sites for ER, hepatocyte nuclear factor 3 (HNF3), NF1 and TFIID as illustrated.

nucleosomes reduces their binding affinity by two to three orders of magnitude. The basal transcriptional machinery has special problems in gaining access to nucleosomal DNA because of the severe distortion in the path of DNA that occurs on association of TBP (TATA-box binding proteins). The assembly of a TATA box into a nucleosome prevents TBP binding independent of the position of the sequence relative to the histone surface (Imbalzano *et al.* 1994, Godde *et al.* 1995). *In vivo* the assembly of a TATA box into a nucleosome is a very efficient mechanism to repress transcription (Patterson & Simpson 1994, Li *et al.* 1998). Thus nucleosomes must be disrupted over the TATA box in order for the basal transcriptional machinery to function effectively. Even once the basal transcriptional machinery has gained access to DNA and recruited RNA polymerase, the elongation of the polymerase can be impeded by chromatin (Morse 1989, Hansen & Wolffe 1992, 1994). Both histone acetylation and the activity of SWI/SNF chromatin remodeling complexes can facilitate the processivity of RNA polymerase through chromatin (Brown *et al.* 1996, Ura *et al.* 1997).

A limited number of specialized transcriptional regulators retain the capacity to bind to their recognition elements even when they are wrapped up into nucleosomes. These include the glucocorticoid receptor (GR) and the thyroid hormone

receptor (TR). The association of these regulatory proteins within chromatin positions them ideally to control transcription through the targeted recruitment of enzyme complexes that modify histone–DNA interactions and remodel nucleosomes. The GR and TR are examples of regulatory factors that can bind to DNA when it is wrapped on the nucleosome surface. There are other examples of nuclear receptors binding to the linker DNA between nucleosomes. The estrogen receptor is one such case which binds to linker DNA in the *Xenopus* vitellogenin B1 promoter adjacent to a positioned nucleosome that activates transcription (Fig. 4; Schild *et al.* 1993). Thus chromatin assembly should not always be considered as invariably repressive.

THE MOUSE MAMMARY TUMOR VIRUS LONG TERMINAL REPEAT AND TRANSCRIPTIONAL CONTROL BY GLUCOCORTICOID RECEPTOR TARGETED CHROMATIN REMODELING

Architectural considerations

Pre-eminent among the systems exploited to understand the interrelationships between chromatin structure and transcription as determined by nuclear receptors is the regulation by glucocorticoids of transcription of the mouse mammary tumor virus (MMTV) long terminal repeat (LTR) (Zaret & Yamamoto 1984). 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 (Archer *et al.* 1992). 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 sequences within nucleosomes, and the assembly of a transcription complex over the TATA box (Archer *et al.* 1989, 1992). Thus the following sequences of events occur on this promoter: an inducible transcription factor binds to the chromatin template, chromatin structure is rearranged facilitating access of other regulatory factors to sequences within the promoter, a transcription complex is assembled and transcription is activated.

Vigorous attempts have been made to reconstruct the transcriptional regulation and concomitant chromatin structural changes of the MMTV LTR *in vitro*. The GR appears to bind nucleosomal DNA

FIGURE 5. The organization of the MMTV LTR into a specific chromatin structure. Nucleosome positions and key recognition elements for *trans*-acting factors are indicated. The hooked arrow indicates the start site of transcription. Numbers indicate base pair positions of DNA incorporated into nucleosomes relative to the start site of transcription (+1). OCT, octamer factor.

with only a slight reduction in affinity relative to naked DNA. This interaction is dependent on the precise position of the nucleosome and hence the translational position of the GR binding site within the nucleosome (Perlmann & Wrangé 1988, Pina *et al.* 1990a,b, Archer *et al.* 1991). *In vitro* nucleosome positions compare favorably with those determined *in vivo* (Richard-Foy & Hager 1987). However, although a predominant *in vivo* translational position exists for this nucleosome, a number of distinct translational positions can be detected (Fragoso *et al.* 1995). Detailed *in vitro* analysis suggests that these variant positions are dependent on the DNA sequence (Roberts *et al.* 1995). This heterogeneity in translational position appears to reflect nucleosome mobility (Meersseman *et al.* 1992, Ura *et al.* 1995). The DNA sequence containing the GR-binding sites has regions of intrinsic flexibility and curvature that direct the histones to bind it in a particular way (Pina *et al.* 1990a,b, Roberts *et al.* 1995). Two GR recognition elements (GREs) 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 (Perlmann & Wrangé 1991). The display of DNA binding sites on the surface of the nucleosome might actually promote the formation of a functional transcription complex (Fig. 5; Truss *et al.* 1995, Chavez & Beato 1997).

Experiments that compare the affinity of the GR for a recognition element as free DNA compared with one facing towards solution in the nucleosome at different translational positions, show that binding affinity is reduced 3- to 11-fold in the nucleosome (Li & Wrangé 1993). This is a remarkably small reduction compared with the complete absence of binding when the recognition element is facing towards the histones (Li & Wrangé 1995). This suggests that the key variable determining the accessibility of the GR to its recognition element 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.

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 (Luisi *et al.* 1991). 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 towards solution in the nucleosome, thereby circumventing steric interference by the histone core. 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 other promoter-specific transcription factors (nuclear factor 1 - NF1), which is facilitated by the GR *in vivo*, does not occur *in vitro* on nucleosomal templates (Blomquist *et al.* 1996, Candau *et al.* 1996). Importantly, the GR is now positioned within chromatin such that it can effectively recruit the molecular machines necessary to remodel the MMTV chromatin in order to facilitate transcription.

Chromatin remodeling

The key molecular machine that GR recruits to the MMTV LTR is the mammalian BRG1/BRG1 associated factor (BAF) chromatin remodeling complex (Fryer & Archer 1998). The BRG1/BAF complex had been known for a while to facilitate transcriptional activation by the ligand-bound glucocorticoid receptor (Murchardt & Yaniv 1993), but the mechanism of targeting had been obscure. Fryer and Archer (1998) found that recruitment of the BRG1/BAF complex to the glucocorticoid receptor *in vivo* is dependent on the presence of ligand. The physical interaction between the

receptor and the BRG1/BAF complex in the presence of endogenous levels of GR and progesterone receptor (PR) was eliminated by addition of an antiprogesterin drug, which also blocked chromatin remodeling and transcriptional activation directed by the glucocorticoid receptor on the MMTV LTR. Interestingly, the interaction of the GR with the histone acetyltransferase family of coactivators as represented by p300 and SRC-1 was unaffected by the antiprogesterin drug. Thus Fryer and Archer suggest that the recruitment of the BRG1/BAF complex is the key regulatory event at the MMTV LTR and that the histone acetyltransferases have a subsidiary role. However, Bartsch and colleagues have also found that moderate increases in histone acetylation dependent on addition of the deacetylase inhibitor Trichostatin A will activate the MMTV LTR and remodel its chromatin structure (Bartsch *et al.* 1996). It is interesting to note that while GR binding will modestly facilitate BRG1/BAF disruption of nucleosomal DNA *in vitro* (Ostlund-Farrants *et al.* 1997), histone acetylation alone does not induce a major unfolding of the chromatin infrastructure *in vitro* or *in vivo* (Bresnick *et al.* 1990, 1991). Thus these *in vitro* observations are largely consistent with *in vivo* data.

The remodeling of the MMTV LTR chromatin involves a number of physical changes including the removal of histone H1 from the linker DNA of nucleosomes including regulatory DNA (Bresnick *et al.* 1992) and a substantial increase in the accessibility of the DNA that is within the positioned nucleosomes to nucleases (Zaret & Yamamoto 1984). The phosphorylation of histone H1 in response to ligand-bound GR is an essential component of transcriptional activation. Thus it is probable that the GR facilitates the activation and recruitment of a protein kinase to the MMTV LTR (Lee & Archer 1998). The positioning of the nucleosomes is also 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 transcription factor IID (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 (Archer *et al.* 1992) 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 repressed (Lee & Archer 1994). Histone H1 dephosphorylation might also have a role in the silencing of the MMTV LTR since prolonged glucocorticoid exposure leads to both events occurring simultaneously (Lee & Archer 1998). Although the exact molecular mechanisms responsible for displacement of the transcription factors have not been determined, these results indicate that both transcription factor and histone complexes with promoters are likely to be dynamic.

THE *XENOPUS* THYROID HORMONE RECEPTOR β A PROMOTER AND TRANSCRIPTIONAL CONTROL BY THYROID HORMONE RECEPTOR TARGETED CHROMATIN REMODELING

One of the best studied examples of hormone action during vertebrate development, the involvement of thyroid hormone in amphibian metamorphosis (Brown *et al.* 1995, Tata 1996), also provides a useful paradigm for the utility of targeted chromatin modification in effecting transcriptional regulation by nuclear hormone receptors (Wong *et al.* 1995). We have focused on the gene for the β isoform of the thyroid hormone receptor in the African clawed frog, *Xenopus laevis* (Shi *et al.* 1993). During *Xenopus* ontogeny, TR β levels correlate positively with the titer of its cognate ligand (Yaoita & Brown 1990). A simple mechanism to effect such coupling could be based on the direct regulation of the TR β gene by TR, and would exploit the transcription silencing properties of unliganded TR as well as its robust function as a transcriptional activator when agonist-bound. Work from this laboratory described in the following section elaborates on the details of this mechanism and highlights the efficiency with which the receptor exploits the assembly of its target gene into chromatin to effect changes in TR β mRNA levels.

Architectural considerations

The promoter of the TR β gene contains several thyroid hormone receptor response elements (TREs), and one of which, located at ca +270, is sufficient to confer the regulation by triiodothyronine (T3) on plasmid templates microinjected into oocytes (Ranjan *et al.* 1994, Wong *et al.* 1995), while the remaining TREs are likely involved in fine-tuning hormone responsiveness. We find that the DNA sequences within the first 500 bp of the TR β A gene have the capacity to direct the translational positioning of histone octamers

(Wong *et al.* 1997a, Guschin *et al.* 1998). The type of thyroid receptor recognition element present in the TR β A promoter is a direct repeat separated by four base pairs (Glass 1994). The rotational position adopted by the TRE on the surface of the nucleosome assembled on the wild-type TR β A gene appears optimal for continued access of the TR–RXR within chromatin. The key contacts made by the receptor with the TRE through the major groove are predicted to remain accessible from solution. The TR–RXR heterodimer is able to bind to the TRE present in the TR β A gene in a nucleosome without significant impediment. The importance of rotational positioning for the continued capacity of TR–RXR to bind to nucleosomal DNA is shown by introducing a small 3 bp alteration in placement of the TRE, leading to an approximate 102° change in orientation with respect to the histone surface. This rotation leads to a reduction in the capacity of TR–RXR to bind to nucleosomal DNA *in vitro*, to a reduction in the capacity of TR–RXR to bind to polynucleosomal arrays *in vivo* and to a reduction in the capacity of TR–RXR to activate transcription (Wong *et al.* 1997a). In contrast, the manipulation of translational position of nucleosomes over the TR β A promoter is without major consequences for TR–RXR association. Thus considerable flexibility might exist in terms of exactly where in the nucleosome the TRE is positioned for transcriptional regulation as long as the TRE is presented on the histone surface with the appropriate rotational organization.

The *Xenopus* TR β A promoter is influenced by an upstream enhancer containing three additional TREs, as suggested by transient transfection (Machuca *et al.* 1995), as well as by genomic footprinting and plasmid microinjection assays in oocytes (F D Urnov & A P Wolffe, unpublished). Two of these TREs are spaced by 180 bp, which is sufficient to assemble a nucleosome. This type of architecture would cluster the bound thyroid hormone receptors and is likely to potentiate their activity (Fig. 6; F D Urnov, unpublished observations).

Functional tests of the role of chromatin in TR-regulated transcription

In order to understand the role of chromatin assembly in gene control by TR it is important to use an experimentally manipulatable system. The microinjection of DNA into *Xenopus* oocyte nuclei leads to the assembly of chromatin which can be alternately transcriptionally repressed or active (Almouzni & Wolffe 1993, Landsberger *et al.* 1995,

FIGURE 6. The organization of the *Xenopus* TR β A promoter into a specific chromatin structure. Nucleosome positions and key recognition elements for *trans*-acting factors are indicated, including the upstream promoter element (UPE, Wong *et al.* 1997a) and binding sites for the TR–RXR heterodimer (indicated by spheres). The hooked arrow indicates the start site of transcription. Numbers indicate nucleosome positions 5' (–) or 3' (+) of the transcription start site.

Landsberger & Wolffe 1995, Wong *et al.* 1995). These active or repressed minichromosomes provide a powerful tool to dissect transcriptional regulation in an *in vivo* context.

In vivo chromatin assembly normally occurs during S phase, when DNA and the histones are synthesized and when the molecular chaperones that direct the deposition of histones onto nascent DNA are maximally effective (Kaufman & Botchan 1994, Marheineke & Krude 1998, Wolffe 1998, Shibahar & Stillman 1999). Microinjection of single-stranded DNA into a *Xenopus* oocyte nucleus leads to rapid second strand synthesis, an event to which chromatin assembly is coupled (Almouzni & Wolffe 1993). This rapid and efficient chromatin assembly pathway leads to the repression of basal transcription in the fully assembled minichromosome. Repression occurs because the molecular mechanisms driving histone deposition outcompete those that direct the association of the basal transcriptional machinery with the promoter (Almouzni *et al.* 1991). The second pathway of chromatin assembly occurs when double-stranded DNA is microinjected into oocyte nuclei. This

assembly pathway is not coupled to replication and resembles the consequence of transiently transfecting or microinjecting plasmid DNA molecules into mammalian tissue culture cells. Under these conditions chromatin assembly will still occur on the fraction of DNA that is taken up into the nucleus, but does so with much slower kinetics in a process occupying at least 4 h in the oocyte (which has large stores of histones) and as long as 24 h in a mammalian tissue culture cell (Reeves *et al.* 1985, Almouzni & Wolffe 1993, Wong *et al.* 1998). This provides plenty of time for the basal transcriptional machinery to gain access to promoter elements and to assemble an active minichromosome.

The *Xenopus laevis* TR β A promoter is transcriptionally repressed when assembled into chromatin in a replicative pathway following injection of single-stranded templates into oocyte nuclei, whereas it is active when double-stranded templates are injected. In both instances the final density of nucleosomes is equivalent, only the pathway of chromatin assembly differs (Wong *et al.* 1995). This illustrates the potential importance of chromatin context and assembly pathway on transcriptional control. The *Xenopus* oocyte lacks adequate TR to regulate transcription in a T3 responsive manner. However, microinjection of TR mRNA into the oocyte cytoplasm leads to TR synthesis and the acquisition of T3 responsive transcription. Hormonal responsiveness is greatly enhanced in the presence of the retinoid X receptor RXR, which indicates that the primary functional form of TR in the oocyte is as a heterodimer with RXR (Wong & Shi 1995). In the absence of T3, TR–RXR will repress transcription from the active minichromosome and will further reduce transcription from the already repressed minichromosome assembled during replication (Wong *et al.* 1995). Consistent with these functional studies, *in vivo* DNase I footprinting indicates that TR–RXR will bind to a thyroid response element (TRE) in the TR β A gene independent of the prior assembly of this element into chromatin and independent of the presence of T3 (Wong *et al.* 1995).

There are several unanticipated consequences of the TR–RXR binding to chromatin within the minichromosomal context. In the absence of T3 the underlying nucleosomal array with which TR–RXR is associated remains very regular upon binding of the receptors with no increase in accessibility to micrococcal nuclease (Wong *et al.* 1997a). This regularity is surprising because the TR–RXR generates a major site of preferential cleavage by DNase I (Wong *et al.* 1997a,b). We interpret these results to indicate that the unliganded TR–RXR binds to the minichromosome and functions to

repress transcription within a regular nucleosomal infrastructure. With respect to the DNase I hypersensitive site, it is clear that neither disruption of a nucleosomal array nor transcriptional activity are necessary to generate such a site. We speculate that TR–RXR may recruit DNase I through protein–protein interactions much as transcriptional coactivators and corepressors might be recruited. DNase I hypersensitive sites are therefore useful markers for chromatin-bound TR and also potentially for v-ErbA bound in chromatin. Indeed, several transcriptional silencers that contain v-ErbA sites are hypersensitive to DNase I *in vivo* (Banahmad *et al.* 1990) consistent with stable association of the v-erbA protein, once again raising the issue of the importance of targeted chromatin remodeling for the establishment of a DNase I hypersensitive site.

On the addition of T3, transcriptional repression is relieved on a minichromosome generated under replication-coupled chromatin assembly conditions (injection of single-stranded template). This relief of repression leads to an apparent activation of transcription because the final transcriptional levels are more than 200 times those measured under repressed conditions (Wong *et al.* 1995). However, for the TR β A gene little true activation occurs because the level of transcriptional activity in the presence of TR–RXR and T3 only approximates to that seen on chromatin templates assembled in a non-replicative pathway (injection of double-stranded template), the extended dynamic range of regulation being due to a lower level of repressed transcription. When chromatin assembly occurs through a non-replicative pathway the basal transcriptional machinery functions as if associated with naked DNA in the absence of TR–RXR. Thus the TR–RXR might regulate gene activity entirely through the establishment of a repressed transcriptional state.

It is useful to consider the physiological rationale behind unliganded receptor occupancy of target genes and concomitant repression. Unliganded TR has been implicated in maintenance of the proliferative state during vertebrate ontogeny (Bauer *et al.* 1998), while an important consequence of induction by T3 is the initiation of cell cycle arrest and differentiation (Brown *et al.* 1995, Tata 1996, Bauer *et al.* 1998). The transient immaturity of chromatin following passage of the DNA replication fork (Seale 1975, 1978) offers the opportunity for spurious activation of genes by the basal transcription machinery. Thus, in rapidly dividing cells the persistent interaction of unliganded TR with target genes required for differentiation and cell cycle arrest may represent a mechanism by

which inappropriate gene activation during post-replicative chromatin maturation is suppressed. In this context, the ability of TR to repress transcription on partially chromatinized templates (Wong *et al.* 1998) may represent a useful functional feature of the receptor.

The TR–RXR remains bound to the TRE in chromatin following transcriptional activation in the presence of hormone but the underlying nucleosomal infrastructure becomes much more accessible to micrococcal nuclease (Wong *et al.* 1997*a,b*). The TRE itself is hypersensitive to DNase I digestion and additional sites of DNase I cleavage are generated in the vicinity of the promoter on addition of hormone. The increased accessibility of chromatin to nucleases in the presence of ligand-bound TR is consistent with the earlier observations of Samuels *et al.* (1982). The important point is that the TRE remains occupied with TR–RXR when chromatin structure is disrupted and the TR β A gene becomes active (Wong *et al.* 1995). The continual presence of TR–RXR offers the opportunity to down-regulate transcription when hormone concentrations become limiting.

The role of histone deacetylase in transcriptional repression by TR

As we have discussed, biochemical approaches using the ligand binding domains of nuclear receptors to screen for interacting factors have identified a number of interesting proteins. The thyroid hormone receptor has the capacity to interact with diverse coactivators and corepressors in a manner which is dependent on the presence or absence of T3. In the absence of ligand, TR has been shown to interact with the corepressors NCoR (Horlein *et al.* 1995) and SMRT (Chen & Evans 1995), while in the presence of ligand the receptor recruits a number of transcriptional coactivators. In addition to their recruitment by TR, NCoR and SMRT also interact with the mammalian proteins mSin3A and mSin3B that have striking homology to *S. cerevisiae* Sin3p (Alland *et al.* 1997, Heinzl *et al.* 1997, Laherty *et al.* 1997, 1998, Nagy *et al.* 1997). Mammalian Sin3 contains four paired amphipathic helices (PAH1–4). NCoR associates with the most amino terminal of these domains, PAH1. *Saccharomyces cerevisiae* Sin3p interacts with the histone deacetylase RPD3 (Kadosh & Struhl 1997) and likewise the mammalian Sin3 proteins associate with the RPD3 homologue using contacts between the PAH3 and 4 domains (Alland *et al.* 1997). These interconnections suggested that TR–RXR might target transcriptional repression by recruiting histone deacetylase to modify the local chromatin

environment (Wolffe 1997). However, it remained possible that components of the recruited corepressor complex such as NCoR, SMRT or Sin3 might exert repressive activities on components of the basal machinery or organize repressive chromatin structures directly without the requirement for histone deacetylase (for example, Fondell *et al.* 1993, Le Douarin *et al.* 1995). We were able to discriminate between these possibilities using the microinjection of double-stranded DNA templates into *Xenopus* oocyte nuclei. The slow kinetics of chromatin assembly on double-stranded DNA were especially advantageous for following the role of nucleosomes in transcriptional silencing. We found that histone deacetylase alone could repress transcription of the *Xenopus* TR β A gene dependent on the assembly of a nucleosomal template (Wong *et al.* 1998) and that this repression could be overcome either by hormone-bound TR–RXR or by inhibition of the histone deacetylase using trichostatin A (TSA). Interestingly, there are distinct requirements for chromatin assembly in the establishment of transcriptional repression by the histone deacetylase alone compared with those needed in the presence of unliganded TR–RXR. The receptor can direct the repression of transcription under conditions where low levels of chromatin assembly have occurred on the template, while the deacetylase alone requires much higher levels of chromatin assembly to repress transcription. However, it is important to note that even when unliganded TR–RXR represses transcription during chromatin assembly, inhibition of histone deacetylase using Trichostatin A blocks this repression. Possible explanations for the repression of transcription by unliganded TR–RXR occurring under conditions of incomplete chromatin assembly, yet still requiring histone deacetylase include the following. (i) The active recruitment of histones to the transcriptional corepressor complex so that they are used as part of the regulatory nucleoprotein architecture to repress transcription after deacetylation (Roth *et al.* 1990, Edmondson *et al.* 1996, Wolffe & Pruss 1996). (ii) Deacetylase may act to modify basal transcription factors and not histones (Imhof *et al.* 1997), generating acetylated components of the basal transcriptional machinery with enhanced functional properties. (iii) The initial process of establishing transcriptional repression might involve interactions between TR–RXR and/or the corepressor complex with the basal transcriptional machinery that might interfere with transcription initiation (Baniahmad *et al.* 1993, Fondell *et al.* 1993). This state of repression would be subsequently stabilized by other events requiring deacetylase activity, potentially including nucleosome assembly.

FIGURE 7. Transcriptional regulation in chromatin. Hormone-bound thyroid hormone receptor recruits a coactivator complex (p300 (CBP, P/CAF)) that retains chromatin in an 'open' configuration and a functional transcriptional machinery associated with the promoter. This complex counteracts the continued activity of the histone deacetylase (HD1). TAF_{II}250, TATA binding protein associated factor _{II}250.

Exactly how the unliganded TR–RXR uses histone deacetylase to repress transcription remains unclear, nevertheless the major enzymatic consequence is the targeting of deacetylase activity to the vicinity of the promoter. In the presence of ligand this situation is reversed, and histone acetyltransferases are recruited.

Chromatin remodeling for transcriptional activation by TR

A unifying feature of several of the diverse coactivator proteins described earlier is their capacity to function as histone acetyltransferases (Ogryzko *et al.* 1996, Yang *et al.* 1996, Chen *et al.* 1997). Thus a common theme for transcriptional activation by ligand-bound nuclear receptors is the recruitment of histone acetyltransferase activity. This recruitment might be expected to counteract the repressive influence of histone deacetylase. Consistent with this hypothesis, the addition of histone deacetylase inhibitors can relieve the requirement for transcriptional coactivators in chromatin assembled on the TRβA promoter in *Xenopus* oocyte nuclei (Li *et al.* 1998, 1999, Wong *et al.* 1998). Histone acetylation states are dynamic, with the acetylated lysines of hyperacetylated histones turning over rapidly with half-lives of minutes within transcriptionally active chromatin, but much less rapidly for the hypoacetylated histones of transcriptionally silent regions (Covault & Chalkley 1980, Zhang & Nelson 1988). This observation implies that transcription coactivators must continually exert their acetyltransferase functions in the vicinity of a promoter (Fig. 7). The

dynamics of histone acetylation provides an attractive mechanistic foundation for the reversible activation and repression of transcription.

We have discussed how the TR–RXR can bind to nucleosomal DNA to target both transcriptional repression and activation. The TR–RXR silences transcription effectively in the context of a positioned nucleosomal array (Wong *et al.* 1995, 1997a). On the addition of ligand this regular nucleosomal array is disrupted. The requirements for this disruption offer additional insight into the potential roles for histone acetylation and chromatin modification in gene control.

The TR–RXR alone and its recognition element are sufficient to initiate a process of chromatin disruption over an extended segment of DNA sequence as assayed by micrococcal nuclease cleavage (Wong *et al.* 1997a). Each TRE can target the loss of topological constraint found in three to four nucleosomes, provided the TREs are separated by at least 400 bp of DNA. Transcription is not required for chromatin disruption and additional proximal promoter elements are necessary to facilitate hormone-dependent activation of transcription by the TR–RXR. The T3-dependent chromatin disruption depends on the AF-2 activity of the LBD. Thus we can dissect the process of gene activation by TR–RXR into at least three steps: (i) binding of the receptor to chromatin and the targeted assembly of a repressive chromatin structure; (ii) the disruption of chromatin on the addition of ligand; (iii) subsequent transcriptional activation (Wong *et al.* 1997b). The separation of chromatin disruption from transcription would be consistent with the opening up of a promoter from a

repressive chromatin environment in order to facilitate the subsequent activity of the basal transcription machinery (Wolffe & Pruss 1996). An important, yet unresolved question concerns the role of histone acetyltransferases in this disruption process. At this time, the role of transcriptional coactivators such as PCAF, p300 and SRC-1 in chromatin disruption itself has not been assayed, even though the structural and functional consequences of histone acetylation have been intensively investigated.

Chromatin disruption targeted by ligand-bound TR-RXR leads to major topological changes in minichromosomes consistent with a loss of wrapping of DNA around the histones (Bauer *et al.* 1994, Germond *et al.* 1975, Wong *et al.* 1997a). Although histone acetyltransferases are recruited to the ligand-bound TR-RXR, it is difficult to account for the large topological change observed through acetylation alone (Norton *et al.* 1989, Bauer *et al.* 1994). In fact, transcription can be activated without significant topological change from a TR β A promoter complexed in chromatin with unliganded TR-RXR simply by the addition of the deacetylase inhibitor TSA (Wong *et al.* 1998). Thus, histone hyperacetylation in isolation seems unlikely to account for chromatin disruption. Comparable conclusions were obtained with the GR-dependent regulation of MMTV chromatin. More importantly, the chromatin disruption phenomenon in a minichromosomal context as assayed by topological change and increases in micrococcal nuclease digestion is not necessary for transcriptional activation. Whether this holds true in the case of GR-mediated transcriptional activation on the MMTV LTR remains to be determined.

Chromatin disruption as assayed by topological change and increases in micrococcal nuclease cleavage has generally been interpreted as essential for transcriptional activation (Hirschhorn *et al.* 1992, Imbalzano *et al.* 1994, Tsukiyama *et al.* 1994). However, transcriptional activation can occur with minimal changes in DNA topology (Pederson & Morse 1990, Drabik *et al.* 1997). This would be anticipated if histone acetylation was the only alteration to chromatin structure necessary for transcriptional activation. It is possible that the pathways directing chromatin disruption in the oocyte nucleus in response to ligand-bound TR-RXR do not involve histone acetylation and that alternative means of disrupting histone-DNA contacts are employed leading to similar transcriptional consequences. Candidate mechanisms include recruitment of molecular machines such as SWI/SNF (Côté *et al.* 1994, Imbalzano *et al.* 1994, Wang *et al.* 1996a,b; Fryer & Archer 1998) or RNA

polymerase itself (Wilson *et al.* 1996, Gaudreau *et al.* 1997). Chromatin disruption directed by TR-RXR might serve other purposes aside from the activation of transcription on minichromosomes. It might be necessary to destabilize higher-order chromatin structures (Hansen *et al.* 1991, Schwarz & Hansen 1994). Such substantial alterations in chromatin structure, including loss of DNA wrapping in the nucleosome, might serve as an epigenetic mark for the propagation of gene activity states through replication and chromosomal duplication (Weintraub 1985), or for the recruitment of the promoter to a nuclear site competent for transcription in a true chromosomal environment (Cook 1994).

CONCLUSIONS

Nuclear receptors make very effective use of the chromatin environment to regulate transcription. As we have illustrated with the thyroid hormone and glucocorticoid receptors, their function depends upon their ability to reversibly manipulate chromatin structure towards stability or instability. These events occur dynamically within regulatory nucleoprotein architectures that can accommodate the conformational and compositional transitions induced by the receptors. Most attention in this research field is currently focused on the chromatin remodeling machines that activate or repress transcription. It is nevertheless essential to understand the structures that exist before remodeling, the remodeled 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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