The thyroid hormone receptor (TR) controls a wide variety of biological responses involved in growth, development and differentiation [1]. TR is a member of the steroid/nuclear receptor superfamily [2]; these proteins associate with DNA as homo- or hetero-dimers and contain ligand-inducible transcription activation domains. Although TR can regulate gene expression as a homodimer, in vivo TR readily forms heterodimers with other members of the nuclear receptor superfamily, especially the receptors for 9-cis-retinoic acid (retinoid X receptors, RXRs) [3].

The activity of TR is influenced by the presence or absence of thyroid hormone [4], by heterodimerization and by the exact DNA recognition sequence [5,6]. TR has both the capacity to activate transcription in the presence of thyroid hormone and the capacity to repress transcription in the absence of hormone [7].

---

**Figure 1**

Transcriptional regulation by TR/RXR from chromatin templates via replication-independent and replication-coupled chromatin assembly pathways

(A) TR/RXR represses basal transcription from TrβA promoter microinjected as double-stranded DNA in the absence of T₃; the addition of T₃ relieves the repression. The scheme shown at the top of the panel illustrates the features of chromatin assembly from injected double-stranded DNA. Groups of 20 oocytes were first injected with an increasing amount of TR/RXR mRNA species (0.4, 1.2, 3.7, 11.1, 33.3 and 100 ng/ml from lane 2 to 7 and from lane 9 to 14 in a volume of 27 nl per oocyte) and injected with pTRDA double-stranded DNA (100 ng/ml, 23 nl per oocyte) 2-3 h later. The oocytes were incubated at 18°C overnight without (−) or with (+) T₃ as indicated. Transcription was analysed by primer extension. To make sure that the injection of DNA was equivalent for each group of samples, DNA was recovered from each group and analysed by slot-blot hybridization with a probe from the TrβA promoter (from +218 to +314). (B) Liganded TR/RXR activates transcription from the repressive chromatin template assembled via replication-coupled chromatin assembly pathway. The injection, transcription analysis and DNA recovery were as described above except that pTRβA single-stranded DNA (50 ng/ml, 23 nl per oocyte) was used.
Modulators of Nuclear Receptor Function

Repression of transcription might operate by either passive or active mechanisms. TR might passively occlude binding sites for transcriptional activators or basal factors; it might also passively repress transcription through the formation of inactive heterodimers [8]. Active repressive mechanisms could require TR to act directly through inhibitory interactions with the general initiation factors, with activators or with cofactors that communicate between general initiation factors and activators [9]. Alternatively TR could actively repress transcription through indirect mechanisms such as recruiting repressive factors or directing the assembly of a repressive chromatin structure. Roeder and co-workers [10] have demonstrated that unliganded TR alone can function as an active transcriptional repressor in vitro. In their system in vitro, TR inhibits transcription at an early step in pre-initiation complex assembly, because pre-assembled complexes are refractory to inhibition.

Whatever the mechanism by which TR activates or represses transcription, a prerequisite is that this nuclear factor needs to be bound to thyroid response elements (TREs) in the absence or presence of thyroid hormone. This requires TR to gain access to TREs within chromatin. Early studies by Samuels and colleagues established that the TR is constitutively associated with chromatin in vivo [11]. Chromatin structure has the capacity to prevent trans-acting factors from gaining access to their recognition elements [12]. Molecular mechanisms that allow transcription factors to bind to DNA in spite of nucleosome assembly include translational positioning of the histone octamer with respect to the DNA sequence. Such positioning can direct repressive histone–DNA interactions away from transcription factor-binding sites [13].

Figure 2

TR/RXR heterodimer binds to the TRE but not the mutated TRE reconstituted into a nucleosome in vitro

A 150 bp end-labelled DNA fragment from the TRβA promoter (from +163 to +322) containing either the wild-type TRE (TRE) or the mutated TRE (mTRE) was generated by PCR amplification with one of the two primers end-labelled with ^32P (position +322), purified and reconstituted into a nucleosome in vitro with histone octamers purified from chicken erythrocytes. The reconstituted nucleosome was then incubated with extract from oocytes with or without (control) over-production of TRβ/RXRa. For a comparison, the binding experiment was also conducted with the end-labelled naked DNA (lanes 7–12). In a 20 µl binding reaction, about 0.5 ng of free probe or 1 ng of end-labelled probe reconstituted into nucleosome plus 200 ng of carrier nucleosomes plus 1 or 2 µl of control oocyte extract or oocyte extract with TR/RXR were incubated at room temperature for 20 min and then resolved by a 4% (w/v) native polyacrylamide gel in 0.5 x Tris/borate/EDTA buffer. NS is a TR/RXR-independent non-specific complex.

Reconstituted Nucleosome

Free Probe

Extract

Control TRβ RXRa

Control TRβ RXRa

TR-RXR-
Nucleosome

TR-RXR-DNA

Nucleosome

Free DNA

1 2 3 4 5 6 7 8 9 10 11 12

NS

TR-RXR-DNA

Free DNA
tively rotational positioning of DNA can occur on the surface of the histone octamer such that recognition sites for transcription factors are exposed towards solution [14]. It is also possible that molecular machines might exist that disrupt local chromatin structure [15].

We have made use of the assembly of mini-chromosomes within the Xenopus oocyte nucleus 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 [16], 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 [17] (Figure 1). 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 chromatin assembly to activate transcription, and post-replicative 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 (Figure 2). The coupling of transcription factors gain access to their recognition elements after they have been assembled into mature chromatin structures.

**Figure 3**
Chromatin disruption in vivo by the binding of TR/RXR heterodimer occurs only in the presence of T₃.

Oocytes were injected with (+) or without (−) TRβ/RXRA mRNA species 6 h before injection of single-stranded pTRβA and treated with (+) or without (−) T₃ as indicated. After incubation overnight they were processed for micrococcal nuclease (MNase) digestion with increasing amounts of MNase (60, 20, 6.7, 2.3, 0.8 and 0 units/ml respectively). After digestion with MNase, DNA was purified and analysed on a 1.5% (w/v) agarose gel in 1× Tris/borate/EDTA buffer before transfer to a filter. The filter was hybridized first with a random primed radiolabelled DNA fragment of the TRPA promoter region that contained the TRE (from +218 to +314) (A). After boiling off the probe, the filter was hybridized again with a 270 bp fragment of the chloramphenicol acetyltransferase gene (+318 to +588) (B). Note that strong nucleosome disruption was observed in the TRE region between mono- and tri-nucleosomes (lanes 13–15 in A). The disruption was also apparent in the further downstream region, although somewhat weaker (lanes 13–15 in B). The positions of mono-, di-, and tri-nucleosomes are indicated on the right and the size markers (in bp) are shown on the left. Lanes D, a and b, show the digestion of naked DNA with micrococcal nuclease.

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRβ/RXRA (mRNAs)</td>
<td></td>
</tr>
<tr>
<td>T3 (50nM)</td>
<td></td>
</tr>
<tr>
<td>MNase</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 addition of thyroid hormone to the chromatin bound receptor leads to the disruption of chromatin structure [18] (Figure 3).

Chromatin disruption is not restricted to the receptor-binding site; instead it extends over a region encompassing several contiguous nucleosomes. We define a domain of the receptor necessary for directing this chromatin disruption. Mutant TR proteins able to bind DNA and 3,3',5-tri-iodothyronine (T₃), but deficient in this domain, neither disrupt chromatin nor activate transcription. We separate chromatin disruption from productive recruitment of the basal transcriptional machinery in vivo by the deletion of regulatory elements essential for transcription initiation at the transcription start site and by the use of transcriptional inhibitors. Only TR/RXR and its binding site are necessary to target the disruption process. Thus chromatin disruption is an independent hormone-regulated function targeted by the DNA-bound TR.

Our results lead us to propose three steps in the regulation of transcription by TR:

1. TR binds to chromatin on the surface of a positioned nucleosome and facilitates the assembly of a repressive chromatin structure.
2. In response to hormone, the receptor recruits molecular machines or enzymes that disrupt local chromatin structure.
3. The hormone-bound receptor facilitates the activity of the basal transcriptional machinery to further activate transcription.


Received 24 October 1996