Bachelor’s thesis

Regulation of gene expression by thyroid hormone receptors

Jana Oltová

Advisor: RNDr. Petr Bartůněk, CSc.

Institute of Molecular Genetics AS CR
Department of Cell Differentiation
I would like to acknowledge my advisor, RNDr. Petr Bartůněk, CSc., for his excellent guidance. Special thanks belong to Mgr. David Sedlák for his enormous patience and advice. Furthermore, I would like to thank all the lab members for their useful help during my laboratory work.

I swear that I wrote this thesis myself and that all sources of information used are properly cited in the References.

Prague 2010

Jana Oltová
# Table of contents

Abstract..................................................................................................................4  
Abstrakt..................................................................................................................5  
List of Abbreviations..............................................................................................6  
1 Introduction .......................................................................................................8  
   1.1 Hormones and their receptors .................................................................8  
   1.2 Thyroid hormones .................................................................................9  
      1.2.1 Thyroid hormone synthesis ............................................................9  
      1.2.2 Neurodevelopmental and neurophysiological aspects ................10  
2 Regulation of eukaryotic transcription .........................................................11  
   2.1 Transcription factors and RNA polymerase ........................................11  
   2.2 Regulatory elements ............................................................................12  
      2.2.1 Core promoters ...........................................................................12  
      2.2.2 Enhancers ....................................................................................13  
   2.3 Chromatin ...............................................................................................13  
      2.3.1 Chromatin modifications ...............................................................14  
3 Nuclear receptors ............................................................................................18  
   3.1 Structure of nuclear receptors ...............................................................18  
   3.2 Interaction with target genes .................................................................20  
   3.3 Transcriptional regulation by nuclear receptors ....................................21  
4 Thyroid hormone receptors ..........................................................................23  
   4.1 Multiple TR isoforms ...........................................................................23  
   4.2 TH-response elements .........................................................................24  
   4.3 TR functional domains ..........................................................................24  
      4.3.1 DNA-binding domain ...................................................................24  
      4.3.2 Ligand-binding domain ...............................................................25  
      4.3.3 Hinge region ................................................................................26  
      4.3.4 Amino-terminal domain ...............................................................26  
   4.4 Transcriptional regulation by TRs ..........................................................26  
      4.4.1 Transcriptional activation .............................................................28  
      4.4.2 Negative regulation by TRs ...........................................................29  
   4.5 Disp3 – a TH target gene ........................................................................29  
   4.6 Non-genomic effects of TH .................................................................30  
5 Conclusion .......................................................................................................31  
6 References ........................................................................................................32
Abstract

Hormones coordinate various processes in living organisms and their action is mediated either by cell membrane receptors or nuclear receptors. Thyroid hormones (THs) have prominent effects on the growth, development, and many aspects of metabolism, embryogenesis and early life. Their receptors (TRs) belong to the nuclear receptor superfamily, whose members function as ligand-activated transcription factors, and depending on the context, they can act both as activators or as inhibitors of transcription. Thyroid hormone receptors are encoded by two genes, TRα and TRβ, and by alternative splicing multiple isoforms are generated. The major form of TR binds to T₃-response element as a heterodimer with retinoid X receptor (RXR). TRs are able to bind T₃-response elements (TREs) independently of ligand occupancy. TREs contain two or more subsequent half-site sequences of AGGTCA, which are usually arranged as direct repeats with four nucleotide spacing (DR4). TH-responsive target genes are involved in a wide range of cellular pathways. Example of such gene is Disp3, which might link TH and cholesterol metabolism in certain cell types.

Keywords: thyroid hormone (TH), thyroid hormone receptor (TR), T₃-response element (TRE), DR4, Disp3
**Abstrakt**

Hormony řídí v živých organismech nejrůznější procesy a jejich činnost je zajišťována buď membránovými, nebo jadernými receptory. Tyroidální hormony (THs) významně ovlivňují růst, vývoj a mnoho aspektů metabolismu, embryogeneze a raných fáz života. Jejich receptory paří mezi jaderné, tzn. fungují jako ligandem aktivované transkripční faktory a podle kontextu mohou působit jako aktivátory nebo inhibitory transkripce. Receptory thyroidálního hormonu (TRs) jsou kódovány dvěma geny, TRα and TRβ, a pomocí alternativního sestřihu vzniká několik isoform. Nejzastoupenější forma thyroidálního receptoru se váže na T3-responsive elementy (TREs) jako heterodimer s retinoidním receptorem X (RXR). Thyroidální receptory jsou schopné vazby na TREs nezávisle na přítomnosti ligandu. TREs se skládají ze dvou nebo více následných sekvencí AGGTCA, které jsou obvykle uspořádány jako dvě přímé repetice oddělené čtyřmi nukleotidy (DR4). TH-responsive cílové geny jsou součástí mnoha různých buněčných drah. Příkladem takového genu je Disp3, který by mohl v určitých buněčných typech propojovat thyroidální hormony a metabolismus cholesterolu.

**Klíčová slova:** thyroidální hormon (TH), receptor thyroidálního hormonu (TR), DR4, T3-responsive element (TRE), Disp3
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF-1</td>
<td>activation function-1</td>
</tr>
<tr>
<td>AF-2</td>
<td>activation function-2</td>
</tr>
<tr>
<td>bp</td>
<td>base pair</td>
</tr>
<tr>
<td>CBP</td>
<td>CREB-binding protein</td>
</tr>
<tr>
<td>CpG</td>
<td>linear sequence (not a base pair) of cytosine and guanine</td>
</tr>
<tr>
<td>CREB</td>
<td>cAMP-response-element-binding protein</td>
</tr>
<tr>
<td>DBD</td>
<td>DNA binding domain</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DNA-PK</td>
<td>DNA-protein kinase</td>
</tr>
<tr>
<td>DRIP/TRAP</td>
<td>vitamin D receptor-interacting protein/TR associated protein</td>
</tr>
<tr>
<td>DR</td>
<td>direct repeat</td>
</tr>
<tr>
<td>GTFs</td>
<td>general transcription factors</td>
</tr>
<tr>
<td>HAT</td>
<td>histone acetyltransferase</td>
</tr>
<tr>
<td>HDAC</td>
<td>histone deacetylase</td>
</tr>
<tr>
<td>HPT</td>
<td>hypothalamic/pituitary/thyroid</td>
</tr>
<tr>
<td>HRE</td>
<td>hormone response element</td>
</tr>
<tr>
<td>Hsp90</td>
<td>heat shock protein 90</td>
</tr>
<tr>
<td>Inr</td>
<td>initiator</td>
</tr>
<tr>
<td>kbp</td>
<td>kilo-base pair</td>
</tr>
<tr>
<td>LBD</td>
<td>ligand binding domain</td>
</tr>
<tr>
<td>MAR</td>
<td>matrix attachment region</td>
</tr>
<tr>
<td>MBD</td>
<td>methyl-CpG-binding domain</td>
</tr>
<tr>
<td>NCoR</td>
<td>nuclear receptor co-repressor</td>
</tr>
<tr>
<td>NIS</td>
<td>Na⁺/I⁻ symporter</td>
</tr>
<tr>
<td>NRs</td>
<td>nuclear receptors</td>
</tr>
<tr>
<td>PCAF</td>
<td>p300/CBP-associated factor</td>
</tr>
<tr>
<td>PPAR</td>
<td>peroxisome proliferator-activated receptor</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RXR</td>
<td>retinoid X receptor</td>
</tr>
<tr>
<td>SAR</td>
<td>scaffold attachment region</td>
</tr>
<tr>
<td>SMRT</td>
<td>silencing mediator for RAR and TR</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>SRC</td>
<td>steroid receptor co-activator</td>
</tr>
<tr>
<td>T₃</td>
<td>L-triiodothyronine</td>
</tr>
<tr>
<td>T₄</td>
<td>L-tetraiodothyronine, thyroxine</td>
</tr>
<tr>
<td>TAFs</td>
<td>TBP-associated factor</td>
</tr>
<tr>
<td>TATA box</td>
<td>promoter motif of a -31 TATAWAAR consensus</td>
</tr>
<tr>
<td>TBP</td>
<td>TATA-binding protein</td>
</tr>
<tr>
<td>TFIIB</td>
<td>general transcription factor</td>
</tr>
<tr>
<td>TFIID</td>
<td>general transcription factor</td>
</tr>
<tr>
<td>TFs</td>
<td>transcription factors</td>
</tr>
<tr>
<td>Tg</td>
<td>thyroglobulin</td>
</tr>
<tr>
<td>THs</td>
<td>thyroid hormones</td>
</tr>
<tr>
<td>TPO</td>
<td>thyroid peroxidase</td>
</tr>
<tr>
<td>TR</td>
<td>thyroid hormone receptor</td>
</tr>
<tr>
<td>TRBP</td>
<td>TR-binding protein</td>
</tr>
<tr>
<td>TRD</td>
<td>transcriptional repression domain</td>
</tr>
<tr>
<td>TRE</td>
<td>T₃ response element</td>
</tr>
<tr>
<td>TRH</td>
<td>thyrotropin releasing hormone</td>
</tr>
<tr>
<td>TRs</td>
<td>thyroid hormone receptors</td>
</tr>
<tr>
<td>TSH</td>
<td>thyroid stimulating hormone</td>
</tr>
<tr>
<td>UAS</td>
<td>upstream activating sequences</td>
</tr>
<tr>
<td>VDR</td>
<td>vitamin D receptor</td>
</tr>
</tbody>
</table>
1 Introduction

1.1 Hormones and their receptors

The word “hormone” has already been used for over one hundred years in a wide range of research fields. Role of hormones in organism is fundamental as they coordinate and integrate activities of various processes in diverse target cells in response to environmental signals. Basically, any extracellular substance, which is after binding to its receptor able to activate a signal transduction mechanism evocating specific responses in target cells, can be considered a hormone. Understanding the nature and actions of these molecules has led to enormous benefits to human health and social progress, such as contraception or in vitro fertilization.

Primarily, hormones have been named after the gland or organ, from which they are secreted, such as thyroid or adrenal. However, this terminology is not accurate because distinct hormones can be secreted by the same gland. Later on, increasing knowledge led to hormones being named according to their physiological actions, for instance growth hormone or prolactin. But even this nomenclature does not consider the fact that various hormones exert different actions in different target tissues.

It has been known since 1960’s that hormonal signals regulate gene expression on the transcriptional level. Nevertheless, mechanism of this regulation would remain unknown without understanding hormone receptors. They are commonly divided into two main groups: cell membrane receptors and nuclear receptors (NRs). First group includes mainly receptors for protein hormones, growth factors, and various neurotransmitters. Many of them are derived from the cellular homologues of the oncogenes v-erbB, v-ros and v-mpl. A lot of membrane receptors are linked to adenylyl cyclase and G proteins, and through these enzymes to cytoplasmic phosphokinases that initiate signal transduction pathway [reviewed in 1]. The superfamily of nuclear receptors includes for instance receptors for steroid and thyroid hormones. They function as ligand-activated transcription factors and many of them are products of the cellular homologue of the oncogene v-erbA [reviewed in 2].

Hormone receptors have a crucial role in current concepts of signalling mechanisms. Nuclear receptors control central pathways that are critical to pathophysiology of many diseases. Furthermore, their ligands are small molecules, which can be easily modified. These attributes make them good pharmacological targets, and therefore attractive for further studies.
1.2 Thyroid hormones

Proper function and metabolism of thyroid hormones (THs) – L-triiodothyronine (T₃) and L-tetraiodothyronine or thyroxine (T₄) – are of a huge biological, medical, and social importance. They have prominent effects on the growth, development, and many aspects of metabolism, and also a crucial role in embryogenesis and early life.

![Structure of thyroid hormones](image)

Figure 1: Structure of thyroid hormones

1.2.1 Thyroid hormone synthesis

Both thyroid hormones are synthesized by the thyroid gland. T₄ is the major secreted hormone, whereas T₃ binds to TH receptors (TRs) with 10-fold higher affinity, therefore is more potent within the cell. Production of circulating T₃ requires 5’ deiodination of the outer ring of T₄ by special selenoproteins – deiodinases. There are three types of deiodinases, differing in T₄ affinity and tissue specificity. Type I deiodinase converts circulating T₄ to T₃ and is found in kidney, liver, and other peripheral tissues. Type II deiodinase has high affinity for T₄ and is found primarily in the pituitary gland, brain, and brown fat, and it contributes not only to peripheral but also to intracellular conversion to T₃. Finally, type III deiodinase is found mainly in placenta, brain, and skin, and converts T₄ and T₃ to an inactive TH metabolites [reviewed in 3].

Regulation of TH synthesis and secretion is ensured by hypothalamic/pituitary/thyroid (HPT) axis. Thyrotropin releasing hormone (TRH) is synthesized in the paraventricular nucleus of the hypothalamus, then transported via axons to the median eminence, and then via the portal capillary plexus to the anterior pituitary. There TRH binds to its receptors in pituitary thyrotropes – a subpopulation of pituitary cells that can secrete thyroid stimulating hormone (TSH). Both TRH and TSH secretion are negatively regulated by THs. TSH binds to its receptor (G-protein coupled) inducing stimulation of many thyroid genes, for instance Na⁺/I⁻ symporter (NIS), thyroglobulin (Tg) and thyroid peroxidase (TPO). NIS ensures active iodide transport to the thyroid. Iodide is oxidized by TPO and incorporated into tyrosine residues of Tg. After that, monoiiodinated and diiodinated residues are enzymatically coupled to form T₄ and T₃. The iiodinated Tg is then stored as a polypeptide within the lumen of
thyroid follicular cells. Genetic defects in this pathway are major causes of congenital hypothyroidism in iodine-replete areas.

The secretion requires endocytosis of the stored iodinated Ig from the apical surface of the thyroid follicular cells. Tg then undergoes proteolytic digestion and release of T\(_4\) (majority) and T\(_3\) into the circulation follows. Absolute majority of the total T\(_4\)/T\(_3\) serum is protein-bound but it is the free TH that enters target cells [reviewed in 4].

1.2.2 Neurodevelopmental and neurophysiological aspects

Low maternal T\(_4\) level causes neurological hypothyroidism in the foetus, which results in endemic neurological cretinism. Even though it can be easily prevented by iodine supplementation, iodine deficiency remains the most common endocrine disorder worldwide. On the contrary, consequences of neonatal hypothyroidism are less severe.

Although thyroid hormones have no influence on very early development events, they regulate later processes, such as neurogenesis, myelination, dendrite proliferation and synapse formation. The timing of the onset of thyroid hormone action in the developing brain is crucial. There are three stages of action of thyroid hormones in the developing brain. Firstly, thyroid hormone exposure comes only from maternally synthesized hormone and during this period neuronal proliferation and migration are influenced. After the onset of foetal thyroid function, brain is supplied both with thyroid hormones from the foetus and the mother. At that point, thyroid hormone-dependent processes include neurogenesis, neuron migration, axonal outgrowth, dendritic branching and synaptogenesis. Moreover, initiation of glial cell differentiation and migration and the onset of myelination is in progress. During the third stage, i.e., in the neonatal and postnatal period, thyroid hormone supplies to the brain are entirely derived from the child. Migration of granule cells, pyramidal cells and Purkinje cells, and gliogenesis and myelination during this period, are sensitive to thyroid hormones and essential for continuing maturation [reviewed in 5].
2 Regulation of eukaryotic transcription

The basic concept of eukaryotic transcription is well-known. The transcription machinery consists of protein-binding regulatory DNA sequences and specific proteins, functioning either as activators or as repressors that bind to these sequences. Inactive genes are assembled into condensed chromatin, which inhibits the binding of RNA polymerases and general transcription factors (GTFs) required for initiation of transcription. Activators generally cause chromatin decondensation, and thus enable RNA polymerase to bind to the promoter. On the other hand, repressors cause chromatin condensation resulting in inhibition of polymerase binding. There are three different eukaryotic RNA polymerases. All protein-coding genes are transcribed by RNA polymerase II, which initiates transcription at DNA sequences corresponding to the 5’ cap of mRNAs. RNA polymerases I and III transcribe genes for ribosomal and transfer RNA.

2.1 Transcription factors and RNA polymerase

Transcription is controlled by DNA-binding proteins called transcription factors (TFs). Their function usually depends on their association with other proteins and they are generally divided into three classes. First contains sequence-specific mediators of gene-selective transcriptional activation or repression. These cofactor complexes facilitate the binding and function of RNA pol II at the core promoter. They are recruited to the DNA template via interactions with transcriptional activators, including nuclear receptors, such as the thyroid hormone receptor or vitamin D receptor, and they may serve as bridges between distal activators and the core promoter. Moreover, they can be induced to undergo conformational changes that might be essential for activating or repressing transcription. Secondly, there are components of multi-protein RNA polymerase required for promoter recognition and catalysis of RNA synthesis. Third group includes chromatin remodelling and modification complexes that help navigate the transcriptional apparatus through the chromatin. There may be as many as 3,000 transcription factors in human genome [reviewed in 6].

A rather high degree of specificity needs to be achieved by transcription factors to recruit the transcription machinery. Eukaryotic transcription factors are grouped in families according to the structure of their DNA-binding domains. More than 80% of TFs in higher eukaryotes contain a helix-turn-helix, a helix-loop-helix, a zinc finger, or a leucine zipper. Helix-loop-helix and leucine zipper domains allow dimerization of factors with N-terminal α-helix that ensures interactions with DNA. Interestingly, eukaryotic TFs recognize shorter
sequences than the prokaryotic ones. Specificity can be increased by homo- or heterodimerization of TFs, or by transcriptional synergy [reviewed in 7].

The initiation of transcription proceeds in two stages. At first, repression by chromatin is relieved, which is triggered by activators recruiting chromatin-modifying complexes (more on this topic in chapter 2.3.1). Then the exposed promoter assembles with RNA polymerase II, general TFs, and mediator in a transcription initiation complex. The polymerase is a large multiprotein complex consisting of 12 subunits, which requires general transcription factors to recognize the promoter and initiate transcription. For RNA polymerase II, these factors are TFIIB, -D, -E, -F and –H. The TFIID complex recognizes the promoter – the TBP (TATA-binding protein) binds to the TATA box and bends it, while the remaining subunits called TBP-associated factors (TAFs) interact with other neighbouring sequences. Bended DNA is suitable for binding of TFIIB, which positions the polymerase on the promoter. TFIIH serves as an ATP-dependent helicase, i.e., unwinds the promoter around the start site [reviewed in 8]. The mediator transduces regulatory information from activator and repressor proteins to RNA polymerase. Mediator is unique in eukaryotes and interacts with RNA polymerase II forming “holoenzyme” complex [reviewed in 8, 9].

2.2 Regulatory elements

Regulatory elements in eukaryotic DNA can be found both close to the start site (proximal) and many kilobases away, either upstream or downstream (distal).

2.2.1 Core promoters

Transcription usually depends on DNA sequences located immediately 5’ of the transcription start site. Core promoter is a DNA sequence that specifies, where RNA polymerase binds and initiates transcription. There are two major types of promoters – focused and dispersed. Focused promoters contain either a single transcription site or a cluster of start sites over several nucleotides. On the contrary, dispersed promoters consist of several start sites over 50 – 100 nucleotides and are typically found in CpG islands in vertebrates. Although most eukaryotic promoters are focused, the vast majority of vertebrates’ genes contain dispersed promoters. Multiple promoters can be used for a single gene to achieve diversity in gene regulation. Promoters may contain many different sequence motifs such as TATA box and Inr that specify transcription mechanism and responses to enhancers. These are typically found in focused promoters. Little is known about the sequences and factors that participate in transcription of dispersed promoters.
The initiator (Inr) motif contains the transcription start site and is a recognition site for TFIID. It has a consensus of YYANWYY in humans with the A nucleotide being often the +1 start site. The TATA box, which is the most ancient and most widely used promoter motif, has a consensus of TATAWAAR, where the upstream T nucleotide is commonly at -31 or -30 position. TATA box is recognized by TBP (TATA box-binding protein), which is a part of TFIID eukaryotic complex. The regulation of TBP binding depends on upstream activating sequences (UAS) composed of 2 or 3 closely linked binding sites for one or two different sequence-specific transcription factors. Vast majority of UAS is located within few hundred base pairs of the TATA box [reviewed in 6, 10].

2.2.2 Enhancers

A typical animal gene usually contains several enhancers located in 5′ or 3′ tissue specific regions, as well as within introns. Enhancers usually mediate expression within specific cell type. A typical enhancer is about 500 bp long and consists of about ten binding sites for minimally three different transcription factors, usually two different activators and one repressor. Enhancers integrate different regulatory outputs produced by multiple signalling pathways to specific patterns of gene expression. Different enhancers can work independently of one another.

There are three mechanisms for ensuring that the right enhancer interacts with the right promoter: insulator DNAs, gene competition and promoter-proximal tethering elements. So-called insulators prevent enhancers associated with one gene from inappropriately regulating neighbouring genes. They are typically 300 bp to 2 kbp long and contain clustered binding sites for large zinc finger proteins. Gene competition occurs when a shared enhancer preferentially interacts with just one of two linked promoters. Selectivity depends on cis-elements within the core promoter, especially the TATA box and Inr. Many genes contain binding sites for proximal regulatory factors located just 5′ of the core promoter. These factors might serve as mediators that recruit distal enhancers to the core promoter or as classical activators/repressors.

Enhancers, silencers and insulators are scattered over long distances, roughly 100 kb in mammals [reviewed in 6].

2.3 Chromatin

Chromosomal DNA does not occur as naked DNA in cells but as protein-DNA complexes known as chromatin. DNA in chromatin is closely associated with proteins called
histones that enable DNA to fold into several higher levels of condensation. The lowest level consists of 147 bp of DNA wrapped one and two-thirds turns around an octameric histone core. The core is composed of two molecules each of histones H2A, H2B, H3, and H4. This complex of the histone octamer and DNA is known as the nucleosome core particle. In vivo, each nucleosome core particle is associated with one “linker histone” H1. Nucleosomes serve as fundamental units of chromatin structure, that are repeated millions of times along the entire DNA molecule with short “linker” DNA stretches between them. Moreover, the chromatin forms condensed 30 nm fibre and higher-ordered folded structures, unless it is being transcribed or replicated [reviewed in 11].

In addition, eukaryotic chromatin is organized as independent loops anchored to nuclear matrix or chromosomal scaffold. The loop is essential for DNA replication, transcriptional regulation and chromosomal packaging. The formation of each loop is dependent on anchoring sequences, termed MARs (matrix \(^1\) attachment region) and SARs (scaffold attachment regions) [12]. The length of DNA loops ranges from 5 to 200 kb in size. Two types of S/MARs have been reported. Permanent S/MARs contain regulatory non-transcribed DNA, transients S/MARs contain transcribing and replicating DNA. S/MARs are located more often in non-coding regions of DNA with regulatory elements and binding sites of DNA topoisomerase II. In general, S/MAR regions have AT-rich sequences. S/MARs have a role in chromatin organization, as well as in replication and regulation of gene expression, and they might mediate the opening of chromatin in the downstream promoter region, which subsequently enhances transcription. The nuclear matrix is probably an attachment region for replication in eukaryotes and might serve as a hot spot region of chromosomal recombination. S/MARs coincide with non-B-DNA structures, like DNA curvature. DNA curvature can be involved in the formation of a DNA-protein complex in the nuclear matrix and in processes of DNA recognition by various transcription factors. DNA curvature can be also a structural feature of the S/MARs present in gene upstream regions, and thus might participate in the transcriptional regulation [reviewed in 13].

2.3.1 Chromatin modifications

The core histones are predominantly globular except for their N-terminal tails. These tails (but not only them) possess a large number of modified residues. There are at least eight distinct types of modifications found on histones [reviewed in 14].

\(^1\) Refers to the nuclear matrix, i.e., interphase nuclear substructure.
Table 1: An overview of different classes of modifications identified on histones
(Adapted from [14])

<table>
<thead>
<tr>
<th>Chromatin modifications</th>
<th>Residues modified</th>
<th>Function regulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylation</td>
<td>K-ac</td>
<td>Transcription, Repair, Replication, Condensation</td>
</tr>
<tr>
<td>Methylation (lysines)</td>
<td>K-me1 K-me2 K-me3</td>
<td>Transcription, Repair</td>
</tr>
<tr>
<td>Methylation (arginines)</td>
<td>R-me1 R-me2a R-me2s</td>
<td>Transcription</td>
</tr>
<tr>
<td>Phosphorylation</td>
<td>S-ph T-ph</td>
<td>Transcription, Repair, Condensation</td>
</tr>
<tr>
<td>Ubiquitylation</td>
<td>K-ub</td>
<td>Transcription, Repair</td>
</tr>
<tr>
<td>Sumoylation</td>
<td>K-su</td>
<td>Transcription</td>
</tr>
<tr>
<td>ADP ribosylation</td>
<td>E-ar</td>
<td>Transcription</td>
</tr>
<tr>
<td>Deimination</td>
<td>R &gt; Cit</td>
<td>Transcription</td>
</tr>
<tr>
<td>Proline isomerization</td>
<td>P-cis &gt;P-trans</td>
<td>Transcription</td>
</tr>
</tbody>
</table>

Modifications on histones are changing rapidly and not all of these modifications are on one histone at the same time. Nevertheless, each individual modification leads to a biological consequence. There are two mechanisms of action of these modifications. The first is based on disruption of contacts between nucleosomes in order to unfold chromatin, the second on recruitment of non-histone proteins. Acetylation has the greatest potential to unfold chromatin since it neutralizes the positive charge of the lysine.

Basically, functions of histone modifications fall into two categories. First includes the establishment of global chromatin environment, where modifications help partition genome into distinct domains known as euchromatin, where DNA is accessible for transcription, and heterochromatin, where DNA is inaccessible for transcription. Second comprises the orchestration of DNA-based biological tasks, such as transcription, DNA repair, replication, and chromosome condensation. All these functions are based on recruitment of the machinery to unravel DNA, manipulating it and then putting it back to the default chromatin state [reviewed in 14]. Complexes known to bring about changes in the state of chromatin are called chromatin-modifying and -remodelling complexes. Chromatin-remodelling complexes alter the DNA packaging in an ATP-dependent manner. On the other hand, chromatin-modifying complexes can cause phosphorylation, ubiquitination, methylation, ADP-ribosylation, and the best characterized mechanism — acetylation [reviewed in 15].

2.3.1.1 Acetylation

Acetylation is known to play a major role in eukaryotic transcription regulation. Enzymes that are responsible for acetylation of histones are known as histone
acetyltransferases (HATs). Those, which act on particular lysine side chains of histones and other proteins, are involved in transcriptional activation. Nucleosomal DNA is generally repressive to transcription, because nucleosome structure and DNA-histone interactions make the DNA inaccessible for binding of transcription factors.

HATs are able to transfer an acetyl group from acetyl-coenzyme A to the ε-amino group of certain lysine side chains within a histone’s basic N-terminal tail. Lysine acetylation neutralizes part of a tail region’s positive charge, and thus destabilizes nucleosome structure. Histone acetylation is a reversible process. Acetylation is associated with activation, whereas the lack of acetylation correlates with repression that is ensured by histone deacetylases [reviewed in 15].

2.3.1.2 Methylation

Methylation is a covalent modification that commonly occurs on carboxyl groups of glutamate, leucine, and isoprenylated cysteine, or on the side-chain nitrogen atoms of lysine, arginine, and histidine residues. Histones are methylated on arginine and lysine residues only. Arginine can be either mono- or dimethylated, whereas lysine can be mono-, di-, or trimethylated. Histones H3 and H4 are preferred for methylation [reviewed in 16].

Methylation plays an important role in transcriptional repression and its major biological consequence is gene silencing. Most mammalian TFs have GC-rich binding sites and a lot of them have CpGs in their DNA recognition elements. Methylation prevents binding of factors that require contact with cytosine in the major groove of the double helix. Binding of many TFs is impeded or abolished by CpG methylation. Alternatively, CpG methylation can inactivate genes by direct exclusion of the transcriptional machinery from methylated promoter DNA.

The connection between DNA methylation and transcriptional silencing is mediated by methyl-CpG-binding proteins. They consist of a single polypeptide and contain both methyl-CpG-binding (MBD) domain and transcriptional repression domain (TRD). The TRD interacts with Sin3A, which further interacts with histone deacetylase. Histone deacetylases are known to establish a repressive chromatin environment [reviewed in 17].

The highest density of non-methylated CpGs is found in so-called CpG islands in vertebrates. CpG island is a stretch of DNA, where the frequency of CpGs is approximately 10 times higher than in the rest of the genome. They usually contain promoter or other regulatory DNA that is required for transcription of a gene. CpG island chromatin is enriched
in hyperacetylated histones H3 and H4 and deficient in linker histone H1, which makes it “active” or “open”. Approximately 60% of all human genes are associated with CpG islands, including all the housekeeping genes and about half of the tissue-specific genes (e.g., significant proportion of the brain or neurally expressed genes) [reviewed in 17, 18].
3 Nuclear receptors

Nuclear receptors (NRs) are a superfamily of transcription factors that bind steroids and other lipophilic molecules, such as retinoids, thyroid hormone and vitamin D [reviewed in 19]. Moreover, this family encodes molecular sensors for other cholesterol-related and diet-derived hormones and even for some phospholipids and heme [20, 21]. But there is still a group of NRs that remain “orphan”, i.e., lacking a defined endogenous ligand.

Nuclear receptors are involved in many biological events, such as cell growth and differentiation, development and cellular homeostasis. This family comprises 48 members in human, 49 in mice, and 47 in rats.

Moreover, nuclear receptors can be divided into subfamilies and groups [22]. Following list is an overview of the most important NRs organized into subfamilies:

**Subfamily 1 – Thyroid hormone receptor-like**
A. Thyroid hormone receptors (TRα, TRβ)
B. Retinoic acid receptors (RARα, RARβ, RARγ)
C. Peroxisome proliferator-activated receptors (PPARα, PPARβ/δ, PPARγ)
D. Rev-ErbA (Rev-ErbAα, Rev-ErbAβ)
F. RAR-related orphan receptors (RORα, RORβ, RORγ)
H. Liver X receptor-like (LXRα, LXRβ, FXR)
I. Vitamin D receptor-like (VDR, PXR, CAR)

**Subfamily 2 – Retinoid X receptor-like**
A. Hepatocyte nuclear factor-4 (HNF4α, HNF4β)
B. Retinoid X receptor (RXRα, RXRβ, RXRγ)
C. Testicular receptors (TR2, TR4)
E. TLX/PNR
F. COUP/EAR (COUP-TFI, COUP-TFII, EAR-2)

**Subfamily 3 – Oestrogen receptor-like**
A. Oestrogen receptors (ERα, ERβ)
B. Oestrogen related receptors (ERRα, ERRβ, ERRγ)
C. 3-ketosteroid receptors (GR, MR, PR, AR)

**Subfamily 4 – Nerve growth factor IB-like** (NGFIB, NURR1, NOR1)

**Subfamily 5 – Steroidogenic factor-like** (SF1, LRH1)

**Subfamily 6 – Germ cell nuclear factor-like** (GCNF)

**Subfamily 0 – Miscellaneous** (DAX1, SHP)

3.1 Structure of nuclear receptors

All nuclear receptors function as ligand-activated zinc-finger transcription factors with a modular structure [2]. Almost all NRs contain a DNA binding domain (DBD), which is the
most conserved, and a ligand-binding domain (LBD), which is, on the contrary, the most variable. DBD is positioned along the centre of the polypeptide and LBD along the C-terminal. The N-terminal regions (or A/B domains) are highly variable and in some cases have so-called activation function-1 (AF-1), which is independent on the LBD-ligand interaction. The LBD and DBD are linked via a variable hinge region, that often contains DNA minor groove binding residues [23]. LBD can form homo- and heterodimerization surface [24] and for most NRs it contains activation function-2 (AF-2), which refers to the recruitment of transcriptional activators in a ligand-dependent manner. Some receptors can interact with co-repressors that are ligand dependent too. Co-regulators act as scaffolds for histone-modifying factors, and thus link them with NRs [25, 26].

**Figure 2: Structure of nuclear receptors**

Nuclear receptors can be categorized according to their ability to form cytoplasmic complexes with Hsp90 and are active as a monomer or a homodimer, or as heterodimer (see Table 2). NRs were found to exist as multiple isoforms, which might account for the tissue, target-gene and developmental specificity of the hormone action [27-29].

**Table 2: Classification of some members of the nuclear receptor family (Adapted from [2])**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Natural ligand</th>
<th>Complex with Hsp</th>
<th>Active as monomer or homodimer</th>
<th>Active as heterodimer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oestrogen</td>
<td>Oestrogens</td>
<td>+</td>
<td>+</td>
<td>–/-/+</td>
</tr>
<tr>
<td>Glucocorticoid</td>
<td>Glucocorticoids</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Androgen</td>
<td>Androgens</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Progesterone</td>
<td>Progesterone</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Mineralocorticoid</td>
<td>Mineralocorticoids</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><strong>Group II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid hormone</td>
<td>Thyroid hormone</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>Retinoic acid</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Retinoic X rec.</td>
<td>9-cis-retinoic acid</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ecdysteroid</td>
<td>Ecdysteroids</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Vitamin D₃</td>
<td>Vitamin D₃</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>COUP-TF</td>
<td>“Orphan”</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>PPAR</td>
<td>Fatty acids</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>
Group I receptors bind to palindromic repeats in a homodimeric head-to-head arrangement only in the presence of the ligand. Conversely, Group II receptors are able to bind constitutively to response elements containing direct repeats in the absence of the ligand. They often exert repressive effect and heterodimerize with RXR [30].

3.2 Interaction with target genes

The DBD contains a core of two helices, one of which contacts the half-site in the major groove of the hormone response element (HRE). Specificity in target genes is attained by very precise spacing of nucleotide repeats in HRE of the promoter of the target gene, which is recognized by the DBD of the receptor. The most common feature of all HREs is a consensus of hexanucleotide sequence, which is usually present as a pair. However, the sequence of each hexad and their relative position provide necessary variability of the HRE, and thus a high degree of specificity of the receptor-gene interaction.

HREs recognized by steroid receptors are characterized by a 15-nucleotide motif of two hexads forming a palindrome that is separated by three nucleotides. Variability is ensured by diverse hexad sequences [2]. On the other hand, non-steroid receptors of the Group II in Table 2 (TR, RAR, RXR, VDR, PPAR) and the ecdysteroid receptor have the same AGGTCA hexad, but they are organised as direct repeats separated by various number of nucleotides ranging from one to five. This arrangement of HREs is termed the “1-2-3-4-5 rule” and it explains how the heterodimers formed with RXR can discriminate between the target genes [reviewed in 31, 32] (Figure 3).

Figure 3: The “1-2-3-4-5 rule”
Non-steroidal nuclear receptors that function as heterodimers with the RXR recognize the direct repeat of the hexad AGGTCA, which is separated by one to five nucleotides (n). (Source [2])
3.3 Transcriptional regulation by nuclear receptors

Nuclear hormone receptors act as ligand-activated transcription factors. Many non-receptor TFs have been found to interact with nuclear receptors, functioning either in synergistic or antagonistic manner [33]. Protein molecules such as CREB-binding protein (CBP) and p300 form bridges between NRs and other transcription factors. Unliganded nuclear receptors seem to interfere with the activity of the non-receptors transcription factor AP-1 by competing for the limited amounts of CBP or p300, and thus to inhibit transcription [34]. Other important elements of the complex are the p160 nuclear receptor co-activator and the nuclear receptor co-repressor NCoR [31]. Nuclear receptors can, depending on the context, function both as activators or inhibitors of transcription [2].

![Diagram of transcriptional regulation by nuclear receptors]

Figure 4: A simplified model of a complex for RNA polymerase II-catalysed transcription

A bridging protein such as CREB-binding protein (CBP)/p300 would closely contact sequence-specific transcription factors, TATA box-binding protein, and TFIIIB. CBP/p300 would form complexes with several other TFs without the involvement of DNA, such as nuclear receptors or cyclic AMP response element-binding protein (CREB). (Source [2])

In general, unliganded NRs preferentially interact with co-repressors, whereas liganded NRs serve as activators due to their ability to bind co-activators. However, a few exceptions have been revealed [35]. Moreover, there are co-regulatory factors, such as the ATP-dependent chromatin remodelling complexes, which are able to regulate both gene activation and repression [36, 37].

Direct protein-protein interactions have been reported between receptors and general transcription factors (GTFs), especially with TBP and several TAFs, which consequently serve as nuclear receptor co-activators [reviewed in 30].
Figure 5: Model for transactivation by a nuclear receptor (Source [30])

Conversely, Group II nuclear receptors are able to lower the basal promoter activity in the absence of ligand by competing either for DNA binding or for dimerization partners. That results in blocking access of activators or transcription factors to the promoter [38].
4 Thyroid hormone receptors

Thyroid hormone receptors (TRs) mediate actions of T3 and they can bind T3 with high affinity. They exist in multiple isoforms and like other nuclear receptors, TRs have a modular structure [reviewed in 39, 40]. They serve as transcription factors, which regulate target gene expression directly through DNA response elements. The major form of TR binds to T3-response element (TRE) as a heterodimer with retinoid X receptor (RXR). However, some TRs can bind to TRE as monomers or homodimers [40-42]. TRs are able to bind TREs independently of ligand occupancy [reviewed in 31, 39, 43]. In general, unliganded TR represses basal transcription, while ligand binding results in activation of target gene transcription [40].

4.1 Multiple TR isoforms

TR is encoded by two separate genes, known as TRα and TRβ, located in human chromosomes 17 and 3, respectively. Due to alternative splicing, multiple TR isoforms are generated, including TRα-1 and c-erbAα-2 (TRα-2) from the TRα gene and TRβ-1 and TRβ-2 from the TRβ gene [39].

The variant form of TRα, c-erbAα-2, has a unique carboxyl terminus and therefore cannot bind T3. Additionally, c-erbAα-2 binds TREs weakly but cannot transactivate TH-response genes, therefore is not an authentic TR. However, it may act as an inhibitor of TH action by competing for binding to TREs [44]. Other TRα variant, c-erbAα-2V, was described by Mitsuhashi et al., but its functions remain unknown [45]. Another interesting feature of the TRα gene is the employment of the opposite strand to encode a gene product, rev-erbA. This protein also is a member of NR superfamily and is considered to be an orphan [46, 47].

On the other hand, TRβ gene contains two promoter regions serving as alternate promoter regions [48]. The resultant isoforms have identical DBD, LBD and hinge region sequences, but the amino-terminal regions bear no homology [49]. Both of them are authentic receptors. The expression of the two isoforms might be regulated by pituitary specific TFs [48].

Both TRα-1 and TRβ-1 are expressed in almost all tissues. In rats, TRα-1 mRNA is most expressed in skeletal muscle and brown fat and TRβ-1 mRNA in brain, liver, and kidney. c-erbAα-2 mRNA expression is highest in the testis and brain. On the other hand, TRβ-2 is expressed restrictively in the anterior pituitary gland, hypothalamus, and in the
developing brain and inner ear [50-53]. Moreover, TRβ-2 mRNA is expressed in the developing retina in the chick and mouse [54].

4.2 TH-response elements

TH-response elements (TREs) are typically located in the upstream promoter regions of target genes. In positively regulated target genes, TREs contain two or more subsequent hexamer half-site sequences of AGGTCA. In general, half-sites of TREs can be arranged as direct repeats (DR), everted repeats (ER), and palindromes that contain optimal spacing of four, six, or zero nucleotides between half-sites, respectively. However, the DR4 (direct repeat spaced by four base pairs) is the most usual configuration [55-57].

4.3 TR functional domains

All thyroid hormone receptors have a domain organization similar to that of all nuclear receptors: an amino terminal A/B domain, a DNA binding domain (DBD) containing two zinc fingers, a hinge region containing the nuclear localization signal, a carboxy-terminal LBD, and a C-terminal region [39]. Each of these regions might serve multiple functions, although their names reflect only the first function that was ascribed to them.

![Diagram of TR domains and functional subregions](Source [3])

**Figure 6: General organization of major TR domains and functional subregions**

4.3.1 DNA-binding domain

The DBD is a central domain of TR and it contains two zinc fingers, each composed of four cysteines coordinated with a zinc ion. The integrity of these zinc fingers is crucial for DNA-binding and transcriptional activity of TRs [58, 59]. Sequence recognition and conferring the spacing specificity is mediated by DNA recognition helix (P box) in the C-terminus of the first zinc finger, which directly contacts the major groove nucleotides. It is important that the P box can distinguish a single base change in the half-site used by two subfamilies of the NRs (AGGACA for the glucocorticoid receptor versus AGGTCA for the oestrogen and thyroid receptors) [60]. It was shown in the crystal structures of TR/RXR
bound to DR4 that additional minor groove contact is made through the carboxyl-terminal extension positioned downstream of the second zinc-containing module [61]. This extension recognizes two additional nucleotides T, (A/G) at the 5′ of the hexamer, and thus enhances the monomer binding affinity to the octamer [62]. Mechanism of spacing recognition is based on a specific, weak interaction between the D box of the RXR DNA-binding domain (5′ position) and the T box of the DNA-binding domain of the thyroid receptor at 3′ position [56]. There is a strict binding polarity of TR/RXR heterodimer on DR4 such that RXR occupies the upstream half site and TR occupies the downstream one [61, 63]

4.3.2 Ligand-binding domain

LBD is necessary for TH binding, dimerization, transactivation, and basal repression by unliganded TR. Ligand binds deep into a hydrophobic pocket in the LBD formed by discontinuous stretches spanning almost the entire LBD. Hydrophobic surface of the most carboxy-terminal region serves as a part of the ligand-binding cavity. Crystal structure has shown, that helix 12 of the receptor projects into the solvent and therefore is likely to undergo conformational changes upon ligand binding, which resembles to a “mouse trap” mechanism [64, 65].

![Figure 7: Conformational changes upon ligand and co-activator binding on the RXR model](Source [19])

There are discontinuous heptad repeats scattered through the LBD that have been proposed to form hydrophobic interfaces for homo- and heterodimerization [66]. Mutations of the ninth heptad repeat region have selectively decreased TR homo- and heterodimer formation [58, 67, 68]. According to the LBD crystal structure of TRα-1, there is a hydrophobic surface in the ninth heptad repeat region that could serve as a surface for
The relative contributions to dimerization by the LBD and DBD interfaces depend on the receptor isoform. Several studies suggest that the ninth heptad region might be important for TRα-1 heterodimerization, whereas DBD may contain dominant dimerization surfaces of c-erb-Aα-2 because it lacks the ninth heptad region due to an alternative splicing [69].

### 4.3.3 Hinge region

The hinge region between DBD and LBD most likely contains the nuclear localization sequence [43]. It is a lysine-rich sequence, which is highly conserved among NRs. TRs are imported into the nucleus shortly after synthesis, but unlike some steroid hormone receptors, TRs do not associate with cytoplasmic heat shock proteins [70]. Additionally, it has been suggested, that the hinge region of unliganded TRs may serve as a contact surface with co-repressors or have allosteric effects on their interaction [71, 72].

### 4.3.4 Amino-terminal domain

The amino-terminal regions are variable in length and sequence among the TR isoforms and among species [73]. The role of these regions in transcription is still controversial. Some studies suggest that the amino-terminal domain of TRβ-1 does not contain a major activation domain AF-1 [58, 74]. On the contrary, studies of TRα-1 and TRβ-1 from several species have shown that the amino-terminal region might be important for transcriptional activation and interactions with TFIIB [75, 76].

### 4.4 Transcriptional regulation by TRs

TH-responsive target genes are involved in a wide range of cellular pathways and functions, such as gluconeogenesis, lipogenesis, insulin signalling, adenylate cyclase signalling, cell proliferation, and apoptosis. Except for the directly regulated genes, there are TH-responsive genes that are regulated indirectly through intermediary genes. TRs can also regulate transcription through protein-protein interactions with other transcription factors. Moreover, THs might regulate mRNA stability of some target genes. However, positively regulated target genes that contain TREs are the best-characterized class of TH-regulated genes [reviewed in 3].

It was shown, that in the absence of TH, TRs bind to TREs and even repress basal transcription [40]. There are several TR-interacting proteins that play an important role in mediating basal repression including nuclear receptor co-repressor (NCoR) and silencing mediator for RAR and TR (SMRT) [71, 72]. These 270 kDa proteins bind preferentially with
unliganded TR and enable formation of repressive transcription complexes. They have three repression domains and two carboxy-terminal α-helical interaction domains. These interaction domains contain a consensus LXX(I/H)XXX(I/L), where X stands for any amino acid. On the other hand, sequence enabling co-activators to interact with NRs is LXXLL. These sequences allow both co-repressors and co-activators to interact with similar amino acid residues on certain helices of the TR LBD. Differences in the length and specific sequences of the interaction sites and ligand-induced conformational changes in the AF-2 region determine whether a co-repressor or a co-activator binds to TR [reviewed in 77]. Moreover, co-repressors can bind to RXR as its co-repressor binding site becomes available after heterodimerization with TR [78].

Recently, it has been shown that co-repressors can form a larger complex with other repressors (Sin3, histone deacetylase 3) [4, 40, 77]. Histone deacetylation of chromatin near the TREs might help shut down the basal transcription. In addition, DNA-methylation probably plays a role in basal repression as methyl-CpG-binding proteins can associate with a co-repressor complex containing Sin3 and histone deacetylase [79]. Finally, unliganded TR can interact directly with the general transcription factor TFIIB, which can also promote silencing [75, 76].

![Figure 8: Molecular mechanism for basal repression in the absence of T₃ and transcriptional activation in the presence of T₃ in a positively-regulated target gene](Source [3])
TR alters the level of gene transcription both in the absence and presence of T₃, which has important implications. At low hormone concentrations, such as hypothyroidism, the unliganded TR represses expression, which makes the clinical features of such mice more severe than by TRα and TRβ knock-out mice [80, 81]. This might be due to the fact, that elimination of the receptor also eliminates its ability to function as a repressor of target genes and therefore causes a milder phenotype.

4.4.1 Transcriptional activation

An increasing number of co-factors have been shown to interact with liganded NRs and enhance transcriptional activation. At least two major complexes appear to be involved in ligand-dependent transcriptional activation of NRs: the steroid receptor co-activator (SRC) complex and vitamin D receptor-interacting protein/TR associated protein (DRIP/TRAP) complex (see Figure 7) [reviewed in 4, 30]. The first member of SRC family, SRC-1, was identified by O’Malley and co-workers. This protein interacts directly with NRs, including TRs, and enhances their ligand-dependent transcription [82]. Subsequent work has shown that at least two other related members of the SRC family – SRC-2 and SRC-3 – can also enhance transcription by liganded NRs [30].

The SRCs contain multiple NRs interaction sites, which contain LXXLL sequence (X represents any amino acid) that is important for binding to the TR LBD. Moreover, SRCs interact with the CBP (the co-activator for cAMP-stimulated transcription) and related p300 [30]. CBP/p300 can serve as co-activators for CREB, p53, AP-1, and NF-κB, and thus might integrate multiple cell signalling pathways [83].

Furthermore, CBP/p300 interacts with PCAF (p300/CBP-associated factor) [30, 83], which has histone acetyltransferase (HAT) activity directed primarily towards histones H3 and H4. PCAF itself is a part of a preformed complex containing TAFs, which can interact with SRC-1 and SRC-3. In addition, CBP is a part of a stable complex with RNA polymerase II [84].

The DRIP/TRAP complex comprises approximately 15 subunits that interact either directly or indirectly with liganded vitamin D receptors (VDRs) and TRs. One of its subunits contains an LXXLL motif and appears to anchor the rest of the proteins in the complex to the NR. In addition, the DRIP/TRAP complex probably does not have an intrinsic HAT activity. However, it has been suggested that TR recruitment of this complex might help stabilize the RNA pol II holoenzyme [85, 86].
Recently, another co-activator, TR-binding protein (TRBP) has been shown to interact with TR via an LXXLL motif [87]. It can also interact with both CBP/p300 and one DRIP subunit, as well as with a DNA-protein kinase (DNA-PK) [88]. DNA-PK can also interact with the NCoR/SMRT co-repressor complexes and might enhance HDAC activity by phosphorylation of HDAC3 [89]. However, the precise interplay between TRBP, DNA-PK, and the other co-factor complexes is not known yet.

Recently, it has been shown that some chromatin remodelling proteins with ATPase activity can associate with NRs in vitro and activate transcription [90]. It has also been suggested that there might be a cyclical recruitment of receptor and co-activator complexes to HREs [91-93].

4.4.2 Negative regulation by TRs

Transcriptional activity of negatively regulated genes can be activated in the absence of THs. The negatively regulated TRH and TSHα- and TSHβ-subunit genes are critical for feedback control of the HPT axis. Their TREs have been localized to the proximal promoter regions [94]. However, the interaction is weak, and thus is not known whether TR-dependent regulation occurs by direct TR binding or by protein-protein interaction with other co-factors.

The precise changes in histone acetylation and resultant alterations in chromatin structure during negative regulation by TRs are not well characterized yet.

4.5 Disp3 – a TH target gene

Disp3 gene was identified by screening for T₃-regulated genes. It has been discovered that Disp3 mRNA is positively or negatively regulated by T₃ in vitro depending on the cell type analyzed. This gene encodes a sterol-sensing domain-containing protein DISP3 that is related to the Dispatched family of proteins. It is a multispan transmembrane protein predominantly expressed in specific cell types of brain, retina, and testis. It can be also referred to as TRUP1, KIAA1337 or PTCHD2. DISP3 is localized within the endoplasmic reticulum and was found to co-localize with cholesterol. Furthermore, its overexpression leads to accumulation of cholesterol and lipid droplets formation. It has been proposed that DISP3 represents a new molecular link between TH and cholesterol metabolism [95].

Interestingly, tissues identified as expressing DISP3 are known to be affected in a number of neurodegenerative disorders. For instance, level of cholesterol in certain areas of the hippocampus is significantly altered in patients with Alzheimer’s disease. It has been reported that cholesterol metabolism in the brain is critical to Alzheimer’s disease
pathophysiology [96]. DISP3 might thus play a role in the development or progression of neurodegenerative diseases.

Moreover, Disp3 gene has been identified as a candidate cancer-causing gene in murine brain tumours as it appears to be a common site for insertional mutagenesis by retroviruses [97].

**Figure 9: Schematic representation of the 5´ part of murine Disp3 locus**
Abbreviations: P - promoter, E – exon, MAR – matrix attachment region, DR4 – direct repeat 4/TH-response element, CpG – CpG island
(Kajlich, A., Bartunek, P., Oltova, J., unpublished data)

### 4.6 Non-genomic effects of TH

Although this thesis is aimed at regulation of gene expression, it is important to mention that also some non-transcriptional pathways are regulated by THs. These pathways include for instance stimulation of phosphatidylinositol 3-kinase at the plasma membrane and direct effects of THs on mitochondria [reviewed in 3]. That is possible because although TRs are primarily nuclear receptors, up to 10% of them are located in the cytoplasm [98]. Moreover, a number of actions of THs have been ascribed to pathways that do not involve TH binding to TRs [3].
5 Conclusion

Regulation of gene expression by thyroid hormone receptors is a complex process and its exact molecular mechanisms still remain unclear. Although several TH-response genes are known, many others are yet to be identified.

Initially, it has been proposed, that liganded TRs act generally as activators. However, this concept was disproved as TRs can function both as activators and repressors according to cellular and developmental context.

![Diagram of TR activation and repression](image)

**Figure 10: Model for regulation of gene expression by TRs (Source [99])**

Disp3 is an example of a gene, which is regulated both in TH-dependent and tissue-specific manner. It has been shown, that in the presence of T3 it can be either up-regulated or down-regulated depending on the cell type [95]. During the characterization of the Disp3 gene structure, two DR4 elements have been discovered (see picture 8) but their physiological importance remains unknown. I would like to further explore molecular mechanisms of the TH-dependent regulation of Disp3 gene as a subject of my diploma thesis.
References


70. Dalman, F.C., et al., In contrast to the glucocorticoid receptor, the thyroid hormone receptor is translated in the DNA binding state and is not associated with hsp90. J Biol Chem, 1990. 265(7): p. 3615-8.


