Introduction

The initial identification of thyroid hormone receptors (TRs\(^1\)) was based on binding studies (Oppenheimer et al., 1972). The TR main ligand is 3,5,3′-triiodo-l-thyronine (T3). T3 production primarily results from deiodination of thyroxine (T4), which is secreted by the thyroid gland. Most metabolites of T4 and T3 are poor TR ligands except for 3,3′,5-triiodothyroacetic acid (TRIAC), which is present at very low levels. TRs are encoded by the THRA \((NR1A1)\) and THRB \((NR1A2)\) genes. The THRA gene was originally identified in chicken as the cellular homolog of the \(v\)-erbA oncogene (Sap et al., 1986). THRB was also cloned by low-stringency screening with the same probe of human and rat cDNA libraries (Weinberger et al., 1986; Thompson et al., 1987).

Although mRNA and protein abundance is variable, THRA is ubiquitously expressed. THRB expression pattern is more restricted and is developmentally regulated. Its main expression sites are the liver, pituitary, inner ear, retina, and several brain areas. The THRA promoter possesses a response element for the estrogen receptor-related-\(\alpha\) (NR3B1) orphan receptor (Vanacker et al., 1998), and the 3′-end overlaps at its \(RevErbA_\alpha\) \((NR1D1)\)-encoding gene, which is transcribed from the antisense DNA strand. The consequences of these features on THRA regulation are unclear, although the 3′-end overlap might explain the moderate diurnal variations of TRa2 isoform protein level observed in the liver (Zandieh-Doulabi et al., 2003). THRA and THRB encode the major receptor isoforms TRa1, TRb1, and TRb2 [the TRb3 receptor (Williams, 2000) is apparently rat-specific], as well as several isoforms unable to bind any ligand (TRa2, TRa3, TR\(\Delta a1\), TR\(\Delta a2\), and the rat-specific TRb3). TRa2 and TRa3 mRNA results from alternate splicing and differs from TRa1 at their C terminus. TR\(\Delta a1\) and TR\(\Delta a2\) are truncated versions of TRa1 and TRa2, respectively, and are translated from mRNA initiated from an internal promoter present in intron 7. In transfected cells, all of these isoforms prevent the T3-induced transcriptional activation mediated by the T3-binding isoforms, but the underlying mechanisms are poorly understood. Alternative translation initiations on the TRa1 mRNA still provide other isoforms (Bigler et al., 1992). One of these isoforms, p43, has been proposed to be a mitochondrial receptor that regulates mitochondrial transcription (Casas et al., 1999). In vitro data indicate that TR acts mainly as heterodimers with RXR, although TRb1 homodimers and TR/retinoic acid receptor heterodimers can form (Forman et al., 1992; Lee and Privalsky, 2005). DNA binding of TR/RXR heterodimers is not ligand-dependent and is efficient on DR-4 elements (5′-AGGTCANNNAGGTC-3′) and inverted palindromes. Although it has been thought that RXR in the TR/RXR heterodimer could not bind its cognate ligand, more recent studies indicate that at least in some cases the RXR ligand 9-cis retinoic acid can influence the activity of the TR/RXR heterodimer (Li et al., 2002) (Castillo et al., 2004; Li et al., 2004).

Structure

X-ray crystallography revealed the structure of the TR ligand-binding domain bound to agonist (Wagner et al., 2001; Borngraeber et al., 2003; Nunes et al., 2004). These data suggest that upon T3 binding the C-terminal helix 12 folds into the scaffold formed by helix 3,4,5, creating a surface with a hydrophobic cleft suitable for
coactivator interaction (Feng et al., 1998; Ribeiro et al., 1998) and preventing corepressor interaction (Marmuthu et al., 2002). The structure of TR/RXR DNA-binding domains bound to a DR-4 element also demonstrate that this spacing between the two binding sites is suitable for optimal RXR/TR dimerization (Rastinejad et al., 1995).

**Target Genes**

The probably large repertoire of TR target genes remains to be clearly defined. The demonstration that a given gene is directly regulated by TR requires the convergent accumulation of several experimental evidences. Increasing or decreasing T3 levels in cultured cells or living animals should change the mRNA steady-state level T3 regulation and should also be observed in a transient expression assay using an artificial construct, where a fragment of the putative target gene is introduced. The T3 response element(s) present in this DNA fragment should be precisely mapped by deletion analysis. In vitro protein interaction studies should identify the TR-binding site present on this fragment. Finally, it will soon be requested to demonstrate by chromatin immunoprecipitation the actual occupancy by TR of the region containing the T3 response element in a chromosomal context. Very few genes fulfill all the criteria to be considered direct TR targets. The best-known TR target genes encode type 1 deiodinase in liver (Koenig, 2005) and the basic transcription element-binding protein (Morita et al., 2003), Hairless corepressor (Thompson, 1996), and neurogranin (Guadano-Ferraz et al., 1997; Morte et al., 1997) in brain. Recent microarray analyses identified many new putative candidates in this matrix (Morita et al., 2003). Other ligands discovered after high throughput screening or in silico virtual screening are currently being evaluated. O-Alkyl derivatives of T3 have been synthesized with the aim of stabilizing a nonproductive conformation of key residues in the ligand-binding pocket and thus disfavoring the equilibrium to the agonist conformation of helix-12. Some induce a stabilization of an inactive conformation and lead to an “indirect antagonism” (Hedfors et al., 2005). It has been shown recently that the deamination of some β-aminoketones produce reactive unsaturated ketones that covalently bind to TR, inhibiting TR-coactivator interaction and suppressing its transcriptional activity (Arnold et al., 2005).

**Pharmacology**

T4 and T3 treatment has several potentially beneficial effects, including the lowering of body weight and plasma cholesterol level; however, an excess of T3 provokes bone and muscle loss and a dangerous tachycardia and can lead to atrial arrhythmia. These adverse effects largely counterbalance the possible benefits. One would expect that TR agonists or antagonists would be of great interest if they could act in an isotype isoform or tissue-specific manner. Because T3 is unrelated to all known ligands for other nuclear receptors, T3 analogs might also act on TR without interfering with the ligand domain of other nuclear receptors. It should be kept in mind, however, that at least in cultured cells T3 and related compounds display a TR-independent activity called nongenomic activity (Davis et al., 2005). GC-1 and KB-141 are the most promising available compounds because they are almost specific for TRβ. In animal models, they were found to decrease plasma cholesterol and triglycerides levels and induce fat loss without a visible effect on heart and muscle (Grover et al., 2003; Baxter et al., 2004). Clinical trials are underway for other ligands from the KB series designed by KaroBio with similar properties. Finally, compounds that can rescue functionally impaired TRβ receptors may provide new strategies for the treatment of resistance to thyroid hormone (RTH; see “Pathology”) (Koh and Biggins, 2005).

Currently, there is no true high-affinity antagonist available for TR (Schapira et al., 2003). The mode of action of the widely used antiarrhythmic drug amiodarone is unclear. Its main metabolite desethylamiodarone is thought to be a weak competitive ligand of T3 for TRα1 but not for TRβ1. A noncompetitive binding site is postulated to be on the outside surface of the TRβ1 receptor, overlapping the regions where coactivator and corepressor bind. Amiodarone treatment would act by preventing the recruitment of coactivators by TRβ1 (van Beeren et al., 1995, 1996, 2000, 2003). The NH3 compound acts as a relatively specific antagonist; its binding places the TRα1 receptor in a neutral conformation that does not permit either coactivator or corepressor recruitment (Nguyen et al., 2002, 2005). Other ligands discovered after high throughput screening or in silico virtual screening are currently being evaluated. O-Alkyl derivatives of T3 have been synthesized with the aim of stabilizing a nonproductive conformation of key residues in the ligand-binding pocket and thus disfavoring the equilibrium to the agonist conformation of helix-12. Some induce a stabilization of an inactive conformation and lead to an “indirect antagonism” (Hedfors et al., 2005). It has been shown recently that the deamination of some β-aminoketones produce reactive unsaturated ketones that covalently bind to TR, inhibiting TR-coactivator interaction and suppressing its transcriptional activity (Arnold et al., 2005).

**Pathology**

T3 exerts a pleiotropic effect on development and homeostasis (Yen, 2001). Circulating levels of T4 and T3 in adults are usually very stable. Hyperthyroidism, often a consequence of Graves' disease, can result in goiter, periorbital edema, weight loss, tachycardia, palpitations, muscle weakness, osteoporosis, (especially in postmenopausal women), and mood disorders. Common signs of hypothyroidism are goiter, myxedema, fatigue, cold-intolerance, thinning hair, depression, dry skin, constipation, and bradycardia. If untreated, fetal and neonatal hypothyroidism also limit bone growth and are
responsible for deafness and an irreversible mental retardation called cretinism. Thyroid-related pathology is largely avoided in developed countries with established neonatal screening for abnormalities in thyroid hormone levels. However, there is growing concern that chemical substances present in food and in the environment might act as thyroid hormone disruptors that alter the circulating levels of T3 and TSH. The permanent exposure to such substances would favor the onset of a number of pathologies and would be harmful for pre- and postnatal brain development (Aoki, 2001; Zoeller et al., 2002). These substances include polychlorinated biphenyls (Zoeller et al., 2000; Gauger et al., 2004), bisphenol A and related compounds (Zoeller et al., 2005), and dioxin and dioxin-like compounds (Nishimura et al., 2003; Viluksela et al., 2004; Yamada-Okabe et al., 2004). The underlying mechanisms are very complex and poorly understood. It seems that some of these molecules act as weak TR ligands (Moriyama et al., 2002; Kita- mura et al., 2005).

Human germline mutations are known for THRB but not for THRα, suggesting that THRα mutations might be either lethal or related to unexpected clinical features. THRβ mutations cause a dominant and polymorphic genetic disease known as RTH (Weiss and Refetoff, 2000; Yen, 2003). Many mutations have been reported that fall into three clusters located in the ligand-binding domain: AA234–282, 310–353, and 429–461 (Collingwood et al., 1998). Almost all of these mutations compromise ligand-binding coactivator recruitment or corepressor release. High levels of T4 and T3 without TSH suppression are typically observed. Inheritance of RTH is dominant since mutant THRβ1 and THRβ2 interfere in a dominant-negative fashion, with the function of wild-type THRβ receptors altering feedback regulation on pituitary TSH secretion. Elevated circulating levels of T4 and T3 can create a condition that resembles hyperthyroidism in tissues that mainly express THRα. For example, tachycardia may be due to hyperthyroidism in the heart, where cardiomyocytes mainly express THRα. The condition is closer to hypothyroidism in tissues that express the mutated THRβ allele, such as the liver.

**Mouse Genetics**

A collection of seven mutant alleles for THRα and nine mutant alleles for THRβ that carry either knockout or knockin mutations have been generated over the last 10 years (Forrest and Vennstrom, 2000; Flamant and Samarut, 2003; Wondisford, 2003), and the collection is still growing. Although the diversity of phenotypes is confusing, at first glance the analysis provides a deeper view on TR function in vivo. The following summary conclusions can be drawn.

THRα1 is a main regulator of development in some tissues during the first weeks of postnatal preweaning development. These 3 weeks are characterized by a peak of circulating T3 and present some analogies with amphibian metamorphosis. As this point, T3 regulates intestinal remodeling (Plateroti et al., 2001), cerebral development (Morte et al., 2002), spleen erythropoiesis (Angelin-Duclos et al., 2005), and bone growth (Bassett and Williams, 2003), mainly by activating THRα1. THRα1 also has a major role in setting cardiac function and thermogenesis (Wikstrom et al., 1998).

THRβ1 is the main isoform that regulates liver function and the development of hearing, and together with THRβ2, it has a major role in the feedback regulation of the hypothalamic-pituitary-thyroid axis (Forrest et al., 1996a,b). THRβ2 has a specific role in the differentiation of retinal cone photoreceptors required for color vision. THRβ2 also cooperates with THRβ1 in the feedback regulation for the hypothalamic-pituitary-thyroid axis. It may also be involved in the auditory system, but this role can be substituted by THRβ1, which is coexpressed with THRβ2 in the cochlea (Abel et al., 2001; Ng et al., 2001).

Unliganded THRα1 can regulate gene expression. This function is mainly evidenced by the fact that knocking out THRα or both THRα/THRβ is less detrimental to development than either hypothyroidism (Flamant et al., 2002) or dominant-negative knockin THRα mutations (Tinnikov et al., 2002; Liu et al., 2003). Although the possibility for a nongenomic action of T3 should also be considered, these data support the idea that recruitment of corepressor on T3 target genes by unliganded THRα1 is detrimental to the development of hypothroid animals. Due to uneven T3 distribution (Quignodon et al., 2004), unliganded THRα1 might be present in some tissues—even in nonpathological situations. It has been shown to repress cardiac gene expression in fetuses in euthyroid situation (Mai et al., 2004).

The contributions of THRα1, THRβ1, and THRβ2 to T3 action on a given tissue usually parallels their respective abundance. For example, the liver mainly expresses THRβ1, and microarray data indicate that the THRβ knockout has a much more visible effect than THRα knockout on liver response to T3 (Yen et al., 2003). This difference suggests that, at least at first sight, TR functions are equal and redundant in tissues where they are simultaneously present.

Noncoding isoforms seem to modulate TR function. As discussed previously (Flamant and Samarut, 2003), this function is not clear for THRα2 but very likely for TRΔα1 and/or TRΔα2. The underlying mechanisms remain poorly understood (Gauthier et al., 2001).

Phenotypic analyses have been performed extensively, and a complete description would go beyond the scope of this review since it seems that every aspect of physiology and postnatal development can be influenced by T3 and TR. The main difficulty of these analyses is to unravel the cell-autonomous consequences of mutations from indirect effects. For example, THRβ is expressed in the cerebellum only in Purkinje cells, but a THRβ
knockin mutation affects the proliferation of the neighboring granular cells, suggesting that T3 exerts part of its effect on granular cells indirectly by activating the secretion of trophic factors by Purkinje cells (Hashimoto et al., 2001). The CRE/loxP recombination strategy will certainly provide a new impetus to these studies by allowing for a spatial and temporal control of gene mutations. Some discrepancies also suggest that we are far from a complete understanding of TR action in vivo. For example, two knockin mutations of THRA have been made that are a priori-equivalent, but only one of these leads to obesity (Timminkov et al., 2002; Liu et al., 2003). All of these observations suggest that THRA- and THRB-somatic and -germline mutations might be involved in a much larger number of human pathological conditions, including cancer (Cheng, 2003), than is usually assumed and that new TR ligands will find many applications.

Tables 1 and 2 describe the major molecular, physiological, and pharmacological properties of TRα and TRβ, respectively.

Acknowledgments. F.F. and J.S. are supported by the French Ministry of Research (Action Concertée Initiative Biologique Cellulaire Moléculaire et Structurale), Ligue Contre le Cancer (Équipe Labelée), and the CASCADE European Network of Excellence (European Ministry of Research (Action Concertée Incitative Biologie Cellulaire). Many applications.


TABLE 1

**TRα**

<table>
<thead>
<tr>
<th>Receptor nomenclature</th>
<th>NR1A1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor code</td>
<td>4.10.1:TH-A1</td>
</tr>
<tr>
<td>Other names</td>
<td>THRα, c-erbαα</td>
</tr>
</tbody>
</table>
| Molecular information  | Hs: 410aa, P10827, chr. 17q11.2<sup>1</sup>  
Rn: 410aa, P63059, chr. 10q31<sup>2</sup>  
Mm: 410aa, q542U8, chr. 11 D-E<sup>3</sup> |
| DNA binding            | Monomer, heterodimer, RXR partner |
| HRE core sequence      | AGGTCA (DR-4, palindromic) |
| Agonists               | TRIAC (154), T₄ (14), reverse T₃ (0.11)<sup>4</sup>, T₃<sup>8</sup> (58 pM), GC-1 (440 pM) [Kₐ]<sup>5,6</sup> |
| Antagonists            | NH<sub>3</sub> (20 nM) [Kₐ]<sup>7</sup> |
| Conoactivators         | NCOA1, NCOA2, NCOA3, PPARBP<sup>2-10</sup> |
| Corepressors           | NCOR1, NCOR2<sup>11,12</sup> |
| Biologically important isoforms | TRα1(Hs, Mm, Rn): main isoform; TRα2(Hs, Mm, Rn): splice variant, DNA binding but no T₃ binding, acts as antagonist<sup>13,14</sup>, TRβα1(Hs, Mm, Rn): truncated, no DNA or T₃ binding, acts as antagonist<sup>15</sup>, TRβα2(Hs, Mm, Rn): truncated, no DNA binding, acts as antagonist<sup>15</sup>; TRβα3(Mm): splice variant, DNA binding but no T₃ binding<sup>16</sup> |
| Tissue distribution    | Ubiquitous [Hs, Mm, Rn] [in situ hybridization]<sup>17</sup> |
| Functional assays      | Heart response [Mm]<sup>18</sup> |
| Main target genes      | Activated: Hr [Mm], Hen2 [Mm]<sup>19,20</sup> |
| Mutant phenotype       | Pleiotropic; usually viable and fertile [Mm] [knockout]<sup>18,21,22</sup>; knockin mutation-changing AF2 domain results in dwarffism and obesity [Mm] [knockin]<sup>23,24</sup> |

<sup>aa</sup> amino acids; <sup>chr.</sup> chromosome; <sup>PPARBP</sup> peroxisome proliferator-activated receptor binding protein.

<sup>1</sup> Radioligand.


Receptor nomenclature: NR1A2
Receptor code: 4.10.1:TH:B1
Other names: THRβ, c-erbAβ
Molecular information: 
- Hs: 461aa, P10828, chr. 3p24.3
- Rn: 461aa, P18113, chr. 15p16
- Mm: 461aa, P37242, chr. 14 A3

DNA binding
Structure: Homodimer, heterodimer, RXR partner
HRE core sequence: ACGTCA (DR-4, palindrome)
Agonists: TRIAC (20 pm), GC-1* (67 pm), T3 (81 pm), T4 (3 nM), reverse T3 (46 nM) [Kd]1-6
Antagonists: NH3 (93 nM) [Kd]10
Corepressors: NCOA1, NCOA2, NCOA3, PPARβ/δ-10
Biologically important isoforms: Trβ1 (Hs, Mm, Rn): main isoform in most cases; Trβ2 (Hs, Mm, Rn): alternative promoter usage, N-terminal variant; Trβ3 (Rn): alternative promoter usage and splicing

Tissue distribution: Liver, heart, several brain areas (Hs, Mm, Rn) [Northern blot, Q-PCR]15

Mutant phenotype: Deafness, color perception, elevated T3 level [Mm]16

Human disease: Resistance to thyroid hormones

aa, amino acids; chr., chromosome; Q-PCR, quantitative polymerase chain reaction; PPARβ/δ, peroxisome proliferator-activated receptor binding protein.