Biochimica et Biophysica Acta 1830 (2013) 3900–3907

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

Thyroid hormone receptors: The challenge of elucidating isotype-specific functions and cell-specific response☆

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ARTICLE INFO

Article history:
Received 13 March 2012
Received in revised form 4 June 2012
Accepted 5 June 2012
Available online 13 June 2012

Keywords:
Thyroid hormone
Nuclear receptor
Mouse genetics

ABSTRACT

Background: Thyroid hormone receptors TRα1, TRβ1 and TRβ2 are broadly expressed and exert a pleiotropic influence on many developmental and homeostatic processes. Extensive genetic studies in mice precisely defined their respective function.
Scope of review: The purpose of the review is to discuss two puzzling issues:

- The isoform specificity problem: the different functions of TRα1, TRβ1 and TRβ2 might reflect either their different distribution in tissues or differences in the receptor intrinsic properties.
- The cell-specificity problem: one would expect that different cell types share a common repertoire of TR target genes, but current knowledge does not support this assumption. How TR function is affected by the cellular context is an unsolved question.

Major conclusions: Mouse genetics support a balanced contribution of expression pattern and receptor intrinsic properties in defining the receptor respective functions. The molecular mechanisms sustaining cell specific response remain hypothetical and based on studies performed with other nuclear receptors.
General significance: The isoform-specificity and cell-specificity questions have many implications for clinical research, drug development, and endocrine disruptor studies. This article is part of a Special Issue entitled Thyroid hormone signalling.

1. Introduction

Thyroid hormone (tri-iodo-thyronine or T3) directly activates transcription by binding to the thyroid hormone receptors (TRs). TRs are thought to act mainly as heterodimers with RXRs, and to bind DNA, at so-called positive T3 response elements (TRE) both in the presence and absence of ligand. In the absence of ligand, they repress transcription. T3 binding changes the conformation of the N-terminal domain. This favors the recruitment of coactivators at the expense of corepressors, and results in transcription activation [1]. This canonical transactivation mechanism is completed by alternate pathways which are poorly understood and often controversial: for example, on some TRE, the binding of T3 to TR induces a paradoxical switch from transactivation to transrepression [2]. Transcriptome analyses indicate that T3-induced negative regulation is widespread, but do not ascertain that it is directly mediated by liganded TR. TR have also been proposed to act without binding to DNA, by interacting with cytoplasmic signaling molecules. T3 might even exert some influence without binding to TR [3] or by binding to a mitochondrial isotype [4]. As their physiological relevance is not fully appreciated, and would require a deeper discussion of controversial issues, we will not consider these alternative pathways, which might nevertheless explain some of the puzzling results presented below.

The three main isotypes of thyroid hormone receptors (TRα1, TRβ1, TRβ2 collectively called TRs) are encoded by the two *TRα* and *TRβ* genes (*THRA* and *THRB* for human genes in the NCBI nomenclature, *Thra* and *Thrb* for mice genes, alias NR1A1 and NR1A2). They fulfill a number of important functions in many different cell types, both during development and in adults. *TRα1* mRNA is nearly ubiquitous, being expressed from the very beginning of development [5], but its expression is higher in neural tissues and lower in hepatocytes [6,7]. Although *TRβ1* mRNA is also present in many tissues [8], variations between cell types are more pronounced. Major expression sites are liver, heart, inner ear, retina and several brain areas, including hypothalamus. *TRβ2* mRNA has a much more restricted distribution, limited to pituitary, retina, inner ear and hypothalamus. TRs are protein with very low abundance, which are hardly detectable by western-blotting or immunocytochemistry in many cell types. It is thus often assumed that protein levels parallel mRNA levels, as these are much easier to detect and quantify. However, early investigations already noticed discrepancies between mRNA and

☆ This article is part of a Special Issue entitled Thyroid hormone signalling.

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doi:10.1016/j.bbagen.2012.06.003

protein levels, indicative of translation regulation of TRβ1 in hepatocytes [9]. More recently, miR27a microRNA was found to regulate TRβ1 protein level in cardiomyocytes [10].

Although a combination of *in vitro* and *in vivo* investigations clarified the mechanisms of T3 signaling, they also highlight gaps in our current knowledge. The purpose of the present review is to discuss two difficult issues:

- The isotype specificity problem: although TR isotypes display very similar properties in most *in vitro* assays, their respective *in vivo* functions are very different. It is usually assumed that these contrasting functions reflect differences in expression patterns, but recent data strongly suggest that subtle differences in the isotype intrinsic properties are also involved.
- The cell-specificity problem: T3 is thought to promote the differentiation of many cell types during development and possesses the ability to stimulate energy expenditure and mitochondrial function in several adult tissues. One would thus expect that different cell types share a common repertoire of TR target genes, but current knowledge does not support this assumption. How TR function is affected by the cellular context is an unsolved question.

2. Investigating the repertoire of TR target genes

These two important questions can only be addressed in a general manner if the repertoire of TR target genes is defined. However, the inventory is far from completed. The traditional approach consists in screening for genes for which mRNA level is changed after T3 stimulation, either *in vivo* or in cultured cells. Promoter fragments of the putative target genes are then cloned and their ability to confer T3 regulation in transfected cells is then tested in transient expression assays. Such assays identify direct target genes, for which transcription can be directly transactivated by TRs. Indirect targets are genes for which sensitivity to T3 is a downstream consequence of early changes in gene expression induced by T3, which can for example modify the cellular content in transcription factors and cofactors. It is now recognized that transient expression assays are inadequate, or at least insufficient, because DNA is poorly chromatinized in transfected cells [11], while TRs are thought to act mainly by recruiting enzymes that modify histone tails. A full characterization implies the definition of TR occupancy sites by chromatin immunoprecipitation, at a genome wide scale, which is currently only possible for a few cell types. Therefore, although transcriptome analyses identified many putative TR target genes, only few have been confirmed as direct targets to date.

3. Human genetic data outline different function for *THRA* and *THRB*

The recent discovery of the first human patients with a *THRA* mutation [12,13] and the consequences of which that are very different from all *THRB* mutations [14] is the best illustration that the two loci fulfill very different functions *in vivo*. The human *THRA* patients share typical clinical signs of hypothyroidism. Their mental and motor development is mildly affected, and their skeletal growth is retarded. Both suffer from constipation, but T3 level is clearly elevated only in one case. These clinical features contrast with the *THRB* associated genetic disease which causes a complex syndrome known as resistance to thyroid hormone (RTH). In RTH, T3 level is elevated and T3 signaling affected to a variable extent in the different tissues: patients associate hypothyroid-like traits in tissues under TRβ1/2 control, and hyperthyroidism-like traits, in tissues in which TRα1 function is preeminent.

4. Recombinant mouse alleles to analyze TR *in vivo* functions

The development of transgenic mice to analyze TR functions and model human diseases is made difficult by the complex structure of the *Thra* and *Thrb* loci. *Thrb* encodes two receptors and *Thra* generates, by alternate promoter usage and alternate splicing, a number of proteins with unclear functions, that lack the ligand binding domain (TRα2, TRα3) and/or the DNA binding domain (TRΔα1, TRΔα2, TRαΔE6) [15–17]. Use of downstream translation initiation sites of the TRα1 mRNA also leads to p43 protein synthesis. A fraction of p43 is found in mitochondria and has been proposed to regulate mitochondrial transcription [4]. 22 mouse strains carrying knock-out or knock-in mutations have been produced either by deleting one or more TR isotypes, or introducing point mutations in their reading frames (Table 1). Point mutations introduced in the C-terminal domain alter the ability of the receptors to transactivate, either by reducing ligand binding or by preventing coactivator recruitment, but maintain their ability to bind DNA. These knock-in mutations therefore transform the receptors in constitutive transcription repressors for the genes that are normally transactivated by T3. As a consequence, they behave like unliganded receptors and are expected to reproduce symptoms of hypothyroidism. Accordingly these knock-in mutations lead to more severe phenotypes than knock-outs, in which negative regulation is also lost [18–20].

Surprisingly, different point mutations expected to have similar consequences on transactivation have very different phenotypic outcomes [21,22]. For example the $TR\alpha1^{P398H}$ knock-in mutation provokes obesity, while other similar *Thra* mutants are lean even when challenged with high fat diet. This difference has been attributed to the specific ability of the $TR\alpha1^{P398H}$ mutant receptor to interfere with PPARα signaling in the liver [23]. Therefore the design of the recombinant locus deeply influences the phenotypic outcome, which can range from lethality to full viability and fertility. Nevertheless, combining all the observations made on these models allows to identify the respective function of TRα1, TRβ1 and TRβ2 isotype and the mitochondrial p43 isotype [24]. The results are ambiguous for the TRα2 isotype, which does not bind T3, because the modest consequences of the selective knock-out of this isotype could be interpreted as resulting from the concomitant overexpression of TRα1 [25]. Although genetic ablation of the short TRΔα1 and TRΔα2 isotypes makes the intestinal epithelium hypersensitive to T3, the underlying mechanism and the physiological function of these non-receptor isotypes remain unclear [26,27]. In the following we will focus on TRα1, TRβ1 and TRβ2 isotypes, the only true T3 receptors in mice (the TRβ3 isotype apparently exists only in rat).

5. Isotype specific functions revealed by TR mutations in mice

Mutant mice phenotypes indicate that TRα1 function is important for early post-natal development, before weaning, a stage marked by a peak in the circulating level of T3, rapid skeletal growth, intestinal epithelium remodeling, change in red blood cell populations and brain maturation. All these events are affected by T3 deficiency, TRα1 knock-in mutations, and, in a milder way by TRα1 knock-outs [28,29,26,19,30,31]. In the adult, TRα1 function is important to maintain heart rate [32], muscle strength [33], body temperature and energy expenditure [34]. Therefore lack of TRα1 function mimics many features of congenital and adult hypothyroidism, without changing T3 level.

Most mutations introduced in the *Thrb* locus concomitantly affect TRβ1 and TRβ2 making the identification of TRβ1 specific function problematic. T3 exerts a negative feedback regulation on the secretion of TRH in hypothalamus and of TSH in pituitary. TRH stimulates TSH secretion which in turn stimulates the production of thyroxine (T4, the inactive precursor of T3) by the thyroid gland. The feedback regulation therefore ensures the stability of TSH, T4 and T3 concentrations in serum. Like human RTH patients, mice with *Thrb* mutations have elevated circulating level of TSH, T4, and T3, indicating that the feedback regulation is mainly a TRβ1 and TRβ2 function. In retina cones of *Thrb* mutant mice, M opsin expression is lost and all cones switch to S opsin expression. Due to a cochlear defect, these