

MULTIPLE ISOFORMS OF THYROID HORMONE RECEPTOR: AN ANALYSIS OF THEIR RELATIVE CONTRIBUTION IN MEDIATING THYROID HORMONE ACTION

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ABSTRACT

Thyroid hormone is essential for normal development and maintaining metabolic homeostasis. In mediating the thyroid hormone action, the thyroid hormone receptor (TR) plays a key role. Almost one decade ago, the cloning of TR was achieved, revealing the existence of at least two genes, TR α and TR β , which encode TR. From these genes several TR isoforms can be generated by alternative splicing. They are designated as TR α 1, TR α 2 (inactive form), TR β 1 and TR β 2. Since the discovery of these TR isoforms, many studies have attempted to demonstrate their relative contribution to mediate thyroid hormone in various tissues. The distinct tissue distribution and the ontogenic expression of the TR isoforms, and the fact that TR gene abnormalities associated with the syndrome of resistance to thyroid hormone (RTH) have been found only in the TR β gene, indicate that products of TR α and TR β have distinct roles. However, no direct evidence of the distinct roles of the TR isoforms has been shown. Gene knockouts of either TR isoform would provide important information to understanding their specific roles. In this review, the history of the TR isoform discovery and studies attempting to demonstrate the specific roles of TR isoforms are summarized, and recent reports dealing with knockouts of TR isoforms are comprehensively presented.

Key Words: thyroid hormone, receptor, syndrome of resistance to thyroid hormone, gene knockout

INTRODUCTION

Thyroid hormones, the only known iodine-containing compounds with biological activity, are important for normal development of animals including human beings.¹⁾ For example, a deficiency of the thyroid hormone in the early neonatal days causes severe mental and growth retardation as observed in cretinism.²⁾ Also, in amphibian metamorphosis, thyroid hormones are known to play a key role.³⁾ On the other hand, thyroid hormones act to maintain metabolic homeostasis in adults, affecting the function of virtually all organ systems. These functions can be understood from the signs and symptoms of adult patients with abnormal thyroid functions, such as hyper- or hypothyroidism.

Recent studies have uncovered the molecular and cellular events related to thyroid hormone action. As illustrated in Fig. 1, two active thyroid hormones, thyroxine (T₄) and 3,3',5-triiodothyronine (T₃), enter the cell by a mechanism yet to be defined. In the cytoplasm, T₄ is

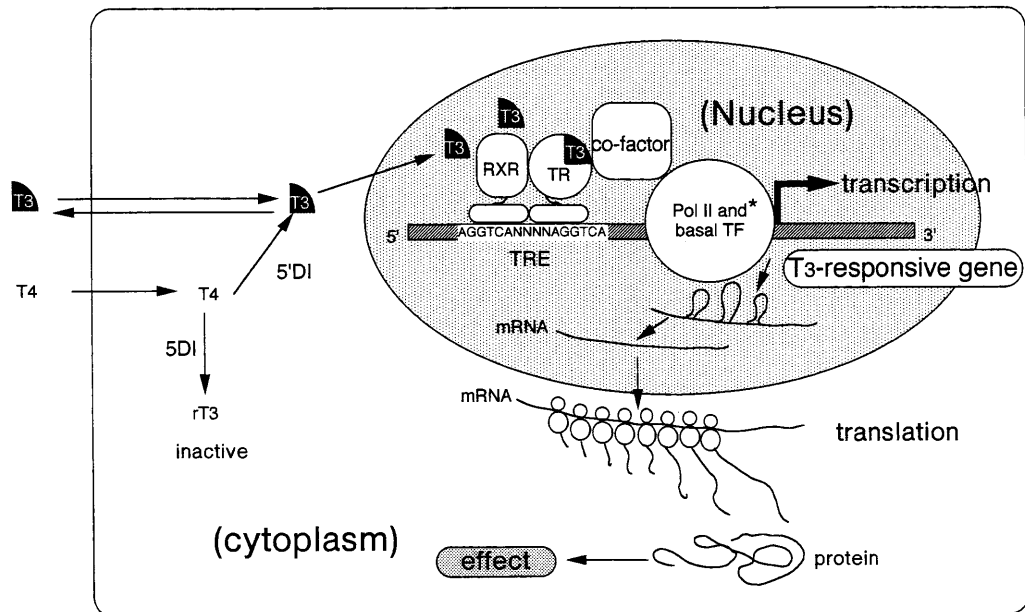


Fig. 1 Thyroid hormone action mechanism

converted to T₃ by the action of iodothyronine 5' deiodinase.⁴⁾ Alternatively T₄ is converted to biologically inactive 3,3',5'-triiodothyronine (reverse T₃, rT₃) by 5-deiodinase named type III iodothyronine deiodinase.⁵⁾ Only T₃ can be transported to the nucleus; it binds the T₃-receptor (TR), which usually heterodimerizes with retinoid-X receptor (RXR)⁶⁻⁹⁾ on the T₃-response element (TRE) of the regulatory region of a target gene for T₃. When T₃ binds to TR, co-factors are recruited and transmit signals to basal transcriptional machinery.¹⁰⁻¹³⁾ Accordingly, the transcriptions of the target genes are regulated by T₃ and, in this way, T₃ exerts its biological effects through the products of the transcripts. Although the thyroid hormone may have some important function through non-nuclear action,¹⁴⁾ those thyroid hormone actions mediated by nuclear TRs have been accepted as a major pathway. Therefore, TRs play a principal role in thyroid hormone action.

MOLECULAR CLONING OF THYROID HORMONE RECEPTORS

1986 remains a landmark year for researchers who are involved in the study of thyroid hormone action, because the first report of the molecular cloning of TRs was announced. In the same issue of *Nature*, two different research groups simultaneously reported that the previously isolated proto-oncogene, *c-erb-A*,^{15,16)} encodes TRs. Interestingly, they isolated two different genes encoding two distinct TRs. Sap et al.¹⁷⁾ cloned one TR from a chick embryo cDNA library, while the other was cloned from human placenta cDNA libraries by Weinberger et al.¹⁸⁾ One year later, when a rat TR with homology to the chick clone was isolated and its gene was mapped to human chromosome 17¹⁹⁾ and another type of TR was mapped to chromosome 3,¹⁸⁾ it became evident that the two different TR sequences did not reflect a species difference but indicated the existence of at least two TR isoforms. In addition, subsequent studies clearly

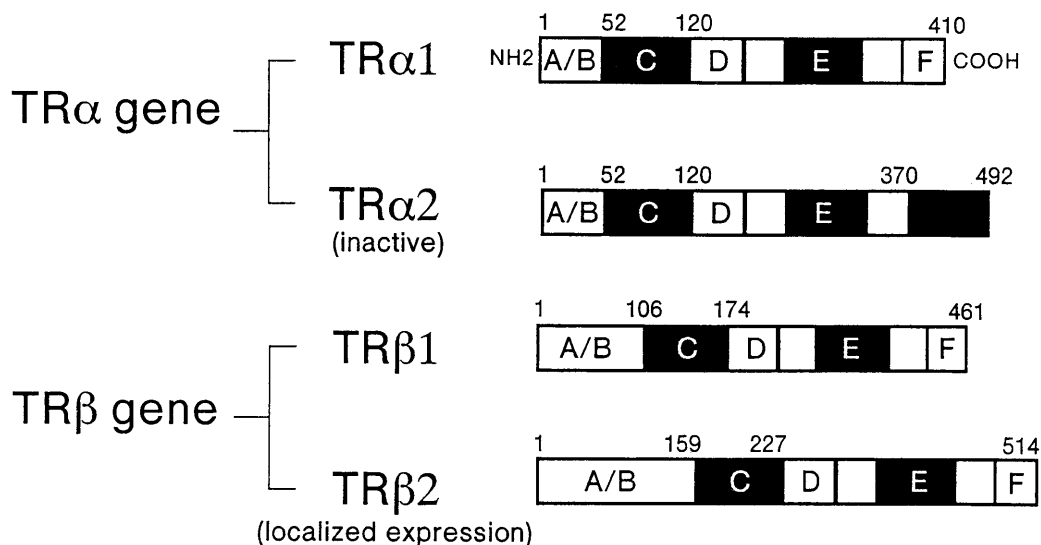
ROLES OF T₃ RECEPTOR ISOFORMS

Fig. 2 TR isoform structures

demonstrated that the existence of TR α and TR β extends across a variety of species. For reference, the TR isolated from chicken was designated alpha (TR α) because of its homology to previously isolated *erb-A* genes¹⁵⁾ while another TR originally from human placenta was designated beta (TR β).¹⁹⁾

The cloning of TR has also revealed that TR belongs to the steroid hormone receptor superfamily. Primary structures of TR and the steroid receptors are similar, consisting of 5 domains named as A/B, C, D, E, and F domain (Fig. 2). Among these domains, the C domain is a DNA-binding domain and is the most highly conserved region. The D-E-F domain is a hormone-binding domain.

As a matter of course, the cloning of TRs has resulted in great advances in the research field of thyroid hormone action. However, at the same time, a new question has been raised, namely whether TR α and TR β have distinct roles in the mediation of thyroid hormone action.

MULTIPLE TR ISOFORMS

It is now generally accepted that several TR isoforms are generated from each TR α and TR β gene by alternative splicing. Although various TR isoforms have been reported, these isoforms can be categorized into either TR α 1, TR α 2, TR β 1 or TR β 2. Schematic structures of these isoforms are illustrated in Fig. 2. TR α 1 and TR β 1 are the major products from TR α and TR β genes, respectively, and are widely distributed in the body. Alternative splicing of the 3'-most exon of TR α 1 results in the generation of TR α 2.^{20,21)} TR α 2 lacks the 40 amino acids of TR α 1 at its C-terminus but contains an additional 120 (human) or 122 (rat, mouse) amino acids with no homology to other known sequences.²²⁾ This C-terminal region is critical for T₃-binding, hence, TR α 2 is inactive for T₃-binding. TR α 2 is widely expressed, and in some tissues, the expression is more abundant than that of TR α 1. Although there was a report that TR α 2 has an inhibitory action against active TRs,²³⁾ this dominant negative effect has not

always been demonstrated. It thus remains to be clarified how TR α 2 is functioning in the body.

Alternative splicing of the N-terminus region of TR β 1 results in the generation of TR β 2.²⁴⁾ TR β 2 shows distinct tissue distribution. In the first report of TR β 2, the abundant expression of TR β 2 mRNA was demonstrated in rat anterior pituitary while the mRNA was not detected in liver, heart, cerebrum, or brown adipose tissue.²⁴⁾ However, in the following reports²⁵⁾ the use of reverse transcription coupled with polymerase chain reaction (RT-PCR) or immunohistochemistry, showed TR β 2 expression in other tissues as well, especially in the central nervous system. The existence of TR β 2 was originally reported in rat, but was also confirmed in mouse,²⁶⁾ chicken²⁷⁾ and human.²⁸⁾ TR β 2 can bind to T₃ and is thought to be functionally identical to TR β 1. However, the expression of TR β 2 is highly regulated by T₃ itself at least in the anterior pituitary. The expression was almost 85% suppressed by T₃ in the rat pituitary cell line (GH3).²⁴⁾ The exact physiological role of TR β 2 and its regulation by T₃ has not been clarified yet.

TISSUE DISTRIBUTION AND ONTOGENIC PROFILE OF THE EXPRESSION OF TR ISOFORMS

Up until now, no fundamental differences in function between TR α 1 and TR β 1 or TR β 2 has been demonstrated *in vitro*. Though differences in the affinity of *in vitro* synthesized TR α 1 and TR β 1 for the acetic acid analogue of T₃ was shown, their binding affinity for T₃ was similar.²⁹⁾ Also *in vitro* DNA-binding analyses and cell transfection studies have generally indicated that the TR α 1, TR β 1 and TR β 2 exhibit similar ligand dependent transcriptional activity.²²⁾

Despite these similarities *in vitro* function, there are several findings which imply differential *in vivo* roles between TR α 1, TR β 1, major isoforms generated from TR α and TR β genes, respectively. One is the unique tissue distribution of TR α 1 and TR β 1. Shown in Table 1 is a summary of the isoform distribution in rat tissue. In the brain, dominant expression TR α 1 is seen both at mRNA and protein levels whereas the expression of TR β 1 is dominant in the liver. In the heart and kidney, both isoforms are almost equally expressed. An ontogenic profile of TR expression in the rat brain suggests that TR α 1 and TR β 1 play specific roles in brain development. TR α 1 mRNA is widely distributed in the rat brain throughout the developmental period.³⁰⁾ The expression is detectable as early as embryonic day 11.5 (E11.5),³¹⁾ several days before the onset of fetal thyroid function. Wide distribution of TR α 1 expression is already seen on E14 and the expression increases throughout early fetal development. The mRNA reaches a peak during the neonatal period and decreases markedly to adult levels.³⁰⁾ On the other hand, the expression of TR β isoforms localize in specific brain regions. Both TR β 1 and TR β 2 are detectable as early as E12.5 in the portion of the ventral pole of the optic vesicle that gives rise to cochlea,³²⁾ but localization of the expression remains restricted to the area including cochlear cell lineage during the gestational days.^{31,32)} Another feature of the TR β 1 expression profile is a dramatic surge around the perinatal period. Strait et al.³³⁾ reported that a 40-fold increase in TR β 1 mRNA occurred in the transition between 19-day gestational fetus and the 10-day-old neonate. This increase corresponds well to the increase in T₃ contents in the brain.³³⁾ Since thyroid hormone effects on rat brain development appear to occur largely in the first 10–15 days of life, they hypothesized that TR β 1 may play a primary role in mediating T₃ effects in developing and adult animals. The validation of their hypothesis must await successful preparation of TR β 1 knockout mouse.

As described above, the tissue distribution and ontogenic profile of TR α 1 and TR β 1 imply distinct roles for these TR isoforms; however, TR abnormality in the syndrome of resistance to

ROLES OF T₃ RECEPTOR ISOFORMS

Table 1. Tissue distribution of TR isoforms in adult rats

	TR α 1		TR β 1		TR β 2	
	mRNA	Protein	mRNA	Protein	mRNA	Protein
Brain	++++	+++ (61%)	++	++ (29%)	-	+ (10%)
Pituitary	++	nd	+	nd	++	nd
Kidney	++	+ (45%)	+++	+ (41%)	nd	+ (15%)
Heart	+++	++ (41%)	+++	++ (41%)	-	+ (18%)
Liver	+	+ (13%)	+++	++++ (71%)	-	++ (17%)
Spleen	+	nd	-	nd	nd	nd

analysis:

mRNA by Northern blot

protein by immunoprecipitation

nd: not determined

references:

(mRNA): Murray, M.B. et al. J. Biol. Chem. 1988

(TR β 2): Hodin, R.A. et al. Science 1989

(protein): Schwartz, H.L. et al. J. Biol. Chem. 1994

thyroid hormone (RTH) would provide even stronger evidence for the distinct roles of TR α 1 and TR β 1.

THE SYNDROME OF RESISTANCE TO THYROID HORMONE (RTH)

RTH is characterized by reduced clinical and biochemical manifestations of thyroid hormone action relative to the circulating hormone levels.³⁴⁾ RTH was first reported by Refetoff et al. in 1967.³⁵⁾ Since the first report, an increasing number of cases has been reported, covering 347 patients as of 1993.³⁴⁾ Most patients have persistent elevation of serum free T₄ and free T₃ with inappropriately non-suppressed thyrotropin (TSH). Patients present goiter almost exclusively, and sometimes short stature, hyperactivity and learning disability in children or adolescents. Administration of a supraphysiological dose of thyroid hormone fails to produce the expected suppressive effect on the secretion of TSH and/or to induce metabolic responses in peripheral tissues.

Until the cloning of TR, the etiology and the pathogenesis of RTH had been left open to speculation. Defective transport, metabolism of thyroid hormones, or antagonism by another substance had been ruled out and the authenticity of thyroid hormones in patients with RTH was confirmed.³⁴⁾ An intracellular defect in thyroid hormone action was postulated soon after the discovery of the syndrome.³⁵⁾ Since the demonstration of a putative nuclear TR,³⁶⁾ several attempts were made to identify the TR abnormality in patients with RTH using circulating mononuclear cells and cultured skin fibroblasts. However, the results were inconsistent, although defective responsiveness to T₃ was clearly demonstrated in the fibroblasts from patients with RTH.^{37,38)} Therefore, until the cloning of TR, it had been presumed that RTH was caused by a

number of defects in T_3 action at various stages.

TR ABNORMALITIES IN RTH

Almost three years after the identification of TR genes, the first report showing a TR abnormality in the patient with RTH was published by Sakurai et al.³⁹⁾ They found a point mutation in the ligand binding domain of TR β in the patient. More importantly, they demonstrated that the mutation results in the loss of T_3 -binding activity in the mutant TR β . Since the first report about TR β mutation, a number of reports demonstrating similar mutations in the TR β gene have been released. Except for one case,⁴⁰⁾ the defect always involved one of the two TR β alleles. This fact is compatible with the dominant mode of inheritance in most RTH cases. An exceptional TR abnormality was found in one family with on RTH inherited recessive trait.⁴¹⁾ This family was the same family reported as the first case of RTH,³⁵⁾ and it was found that the coding sequence of both alleles of TR β gene was completely deleted in the affected members of this family. Heterozygotes of this family, expressing a single TR β gene, were clinically and biochemically normal. Thus, the inheritance patterns of RTH can be categorized in two ways; autosomal dominant and autosomal recessive.

As shown in Fig. 3, in a RTH case caused by a point mutation of a single TR β gene allele, there are three intact genes which encode functional TRs. As a matter of fact, it has been shown that a normal and the mutant TR β 1 genes are equally expressed in fibroblasts from patients with RTH.⁴²⁾ The expression of the TR α 1 gene is also intact. These results indicate that both intact TR α 1 and TR β 1 are expressed as well as mutant TR β 1 in patients with RTH, and raises a question as to why the existence of the mutant TR β 1 or TR β 2 causes clinical and biochemical manifestations of RTH. To answer this question, many studies has been carried out. It has recently been proposed that the mutant TR β 1 inhibits the normal TR function in a dominant negative manner.⁴³⁾ One subject with homozygous TR β mutation exhibited the most severe clinical manifestations. His resting heart rate was 190 beats/min and his T_3 , T_4 , and TSH serum levels were the highest ever seen. He exhibited the most sever delay in growth and central nervous system development among individuals with RTH. He died from cardiogenic shock complicating

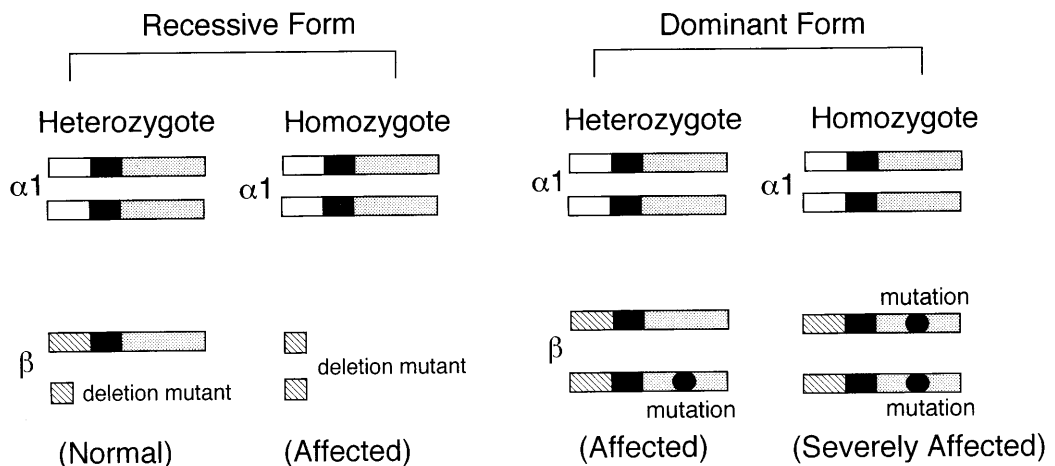


Fig. 3 TR gene anomalies in RTH and its mode of inheritance

ROLES OF T₃ RECEPTOR ISOFORMS

staphylococcal septicemia, and it has been believed that this is the only case in which RTH contributed to the patient's death.³⁴⁾ This case strengthens the case for a dominant negative effect by mutant TR β , because the clinical manifestation was much more severe than in the case of homozygous TR β deletion. Interestingly, there has been no reported case of RTH due to a TR α abnormality even though a case without any obvious TR α nor TR β abnormality was reported.⁴⁴⁾

The fact that the homozygous patients with TR β deletion also exhibited RTH indicates that TR β is necessary to maintain normal thyroid function and the peripheral effects of thyroid hormone. Then what about TR α ? Why have we not found a RTH case with defective TR α ? Two completely opposite hypotheses can be asserted. One is that a TR α mutation or deletion becomes fetal so that the subject with defective TR α cannot exist. Another is that a TR α abnormality does not exhibit any clinical manifestations. The preparation of TR α knockout mouse would be the only way to answer these questions. In addition, preparation of either a TR α or TR β knockout mouse would provide an ideal model to understand how TR α and TR β play their distinct roles in mediating thyroid hormone action.

TR β KNOCKOUT MOUSE

In 1996, Forrest et al.⁴⁵⁾ reported the gene knockout of TR β in a mouse. They targeted a part of the TR β gene encoding the first zinc finger of the DNA-binding domain. This gene targeting was predicted to disrupt the DNA-binding and T₃-binding domains. As a result of the homologous recombination with the targeting gene, a mRNA approximately 100 bp shorter than that of wild type was generated. The mutated mRNA had an aberrant open reading frame that terminated after 8 bp in exon 4. Thus, the protein product lacked the DNA-binding and T₃-binding domains of TR β , and as a matter of fact, the mutation precluded expression of functional TR β 1 or TR β 2 in all tissues examined. No gross compensatory increase in TR α expression was observed in the TR β deficient (TR β ^{-/-}) mouse.

As summarized in Table 2, the phenotype of TR β ^{-/-} was quite similar to that observed in the patient with RTH due to the deletion of TR β gene. High T₄ and T₃ without TSH

Table 2. Phenotype of TR β knockout mice (quoted from ref. 45)

1. Thyroid function

	Wild type (TR β ^{+/+})	TR β ^{-/-}
T ₄ (μ g/dl)	3.3-4.8	8.9-27.9
T ₃ (ng/dl)	93-125	155-387
TSH (%)	-40/0	+113/+391

2. Goiter

3. Hearing disturbance

4. No major anomaly, normal development, fertile

suppression and goiter were noted. These findings indicate that TR β is essential for the regulation of TSH secretion and TR α cannot compensate for such regulation. Interestingly, deaf mutism observed in patients with TR β gene deletion was reproduced in the TR $\beta^{-/-}$ mouse. The fact that a patient with TR β gene deletion had associated auditory dysfunction and that specific expression of TR β gene in cochlear cell lineage was observed during rat development, strongly suggested that TR β 1 and TR β 2 play an important role for the development of the auditory system. So the findings in a TR β deficient mouse has proven that TR β isoforms are essential for auditory development and indicate that distinct TR genes serve certain unique functions.⁴⁶⁾

A distinct profile of TR β gene expression during rat brain development implies that TR β isoforms play a key role in rat brain development. However, TR $\beta^{-/-}$ mice displayed no overt abnormality in neuroanatomy, behavior or in hippocampal long-term potentiation. Also in a human case, the deletion of the TR β gene did not cause mental and neurological disorders.³⁵⁾ Brain is the tissue where TR α 1 is predominantly expressed. Since levels T₃ and T₄ levels are high in TR $\beta^{-/-}$ mice, TR α 1 might be saturated with T₃ more than in the wild type and might compensate for the loss of TR β .

In contrast to the brain, TR β is predominantly expressed in the liver. In fact, by TR β knockout, the TR number was reduced to 24% in TR $\beta^{+/+}$ mice.⁴⁷⁾ As a result, several parameters showed that the liver became resistant to thyroid hormone. In TR $\beta^{-/-}$ mice the serum cholesterol level was significantly higher than that in wild type mice and it remained high even if a supraphysiological dose of T₃ was administered.⁴⁷⁾ The increase in serum alkaline phosphatase was also blunted in TR $\beta^{-/-}$ mice. Resistance to thyroid hormone in the liver of TR $\beta^{-/-}$ mice was also demonstrated in the expression of T₃-responsive genes. Spot 14⁴⁸⁾ and malic enzyme⁴⁹⁾ have been studied as hepatic T₃-responsive genes. The expression of these genes was greatly enhanced by the administration of T₃, however in TR $\beta^{-/-}$ mice, significant increases were missing.⁴⁷⁾ A T₃-dependent increase in another T₃-responsive genes, 5'DI,^{50,51)} was also blunted in TR $\beta^{-/-}$ mice, even though a slight but significant increase in T₃ was observed (unpublished data). On the other hand, heart rates and energy expenditure were not different between TR $\beta^{-/-}$ and wild type mice.⁴⁷⁾ These results suggest that TR β abnormalities are reflected mainly in the organ where TR β is predominantly expressed. The heterogeneity of refractoriness to T₃ among tissues in RTH may therefore be dependent to a variable degree on the presence of TR β .

TR α KNOCKOUT

1) Knockout of both TR α 1 and TR α 2

In a sense, researchers want a TR α knockout mouse more than a TR β knockout mouse, because the function of TR β is partially predicted from the phenotype of RTH patients with TR β gene deletion. Also, the question as to why a case of RTH with abnormal TR α has not been found could be answered by the inactivation of the TR α gene. By homologous recombination, the TR α gene was inactivated in mouse embryonic stem cells and it has been shown at the cellular level that neural differentiation induced by retinoic acid was inhibited.⁵²⁾ It has therefore been suggested that TR α influences neural differentiation by affecting retinoic acid action. However, we needed to wait another 3 years to know the consequence of a TR α gene knockout at the whole animal level. In 1997, the first report of TR α knockout mouse was made by Fraichard et al.⁵³⁾ They designed the targeting vector to eliminate the expression of both TR α 1 and TR α 2. By intercrossing heterozygous mice, approximately 25% of the resulting offspring were homozygous (TR $\alpha^{-/-}$). This report indicated that homozygous disruption of the TR α gene was not deleterious to embryonic development. However, the genetic disruption caused fatal changes in

ROLES OF T₃ RECEPTOR ISOFORMS

the phenotype of homozygous mice in the post-natal period. The growth of TR $\alpha^{-/-}$ offspring stopped completely 2 weeks after delivery, they lost 30–50% of their weight, and they died between postnatal days 20–35. Another peculiar phenotype of TR $\alpha^{-/-}$ mice was hypothyroidism, probably due to the reduced secretion of TSH. Their thyroid gland showed hypoplasia and serum concentrations of T₄ and T₃ were markedly reduced (Fig. 4B). TR $\alpha^{-/-}$ mice also exhibited delayed maturation of the small intestine and delayed bone development, findings that are compatible with hypothyroidism.⁵⁴⁾ These results strongly indicate that products of TR α gene positively regulate the production of thyroid hormones. Otherwise, there were no gross anatomical or behavioral anomalies. While wide distribution of TR α 1 in the early embryonic day and findings of TR α knockout at a cellular level suggest the importance of TR α 1 in neural development,⁵²⁾ TR $\alpha^{-/-}$ mice did not show any obvious cellular and morphological abnormalities in the brain except reduced size.

One interesting observation of TR $\alpha^{-/-}$ mice is that their lethal growth retardation was rescued by one week of T₃ administration (Fig. 4). Surprisingly, T₃ administration also rescued their thyroid function. The authors proposed two hypotheses to explain the T₃ rescue. One is that the injected T₃ can activate genes that are responsible for the production of thyroid hormone via a TR β dependent pathway. Alternatively, the injected T₃ may transiently cure

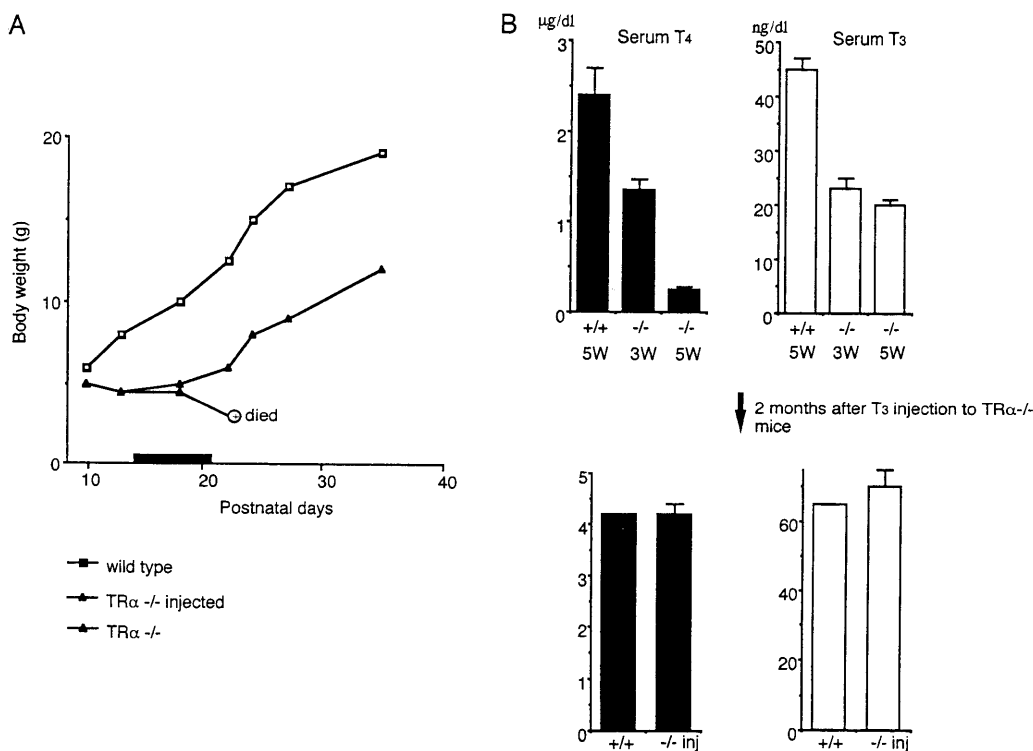


Fig. 4 TR $\alpha^{-/-}$ mice were rescued by T₃-injection (by Fraichard et al. EMBO J., 1997)

(A) Growth rate of animals: Three-week-old TR $\alpha^{-/-}$ mice were injected subcutaneously with 1 μ g of T₃ daily for 7 days.

(B) Impaired production of thyroid hormones in TR $\alpha^{-/-}$ mice was rescued by the T₃-injections for 1 week.

animals before the onset of delayed TR α -independent production of thyroid hormone. The inactivation of both TR α and TR β genes may provide the answer to these hypotheses.

This report seems to answer the question of why RTH with abnormal TR α has not been found, as TR α knockout causes lethal damage in mice. However, results from the specific inactivation of either TR α 1 or TR α 2 create another controversy.

2) Specific knockout of TR α 1, TR α 2, or rev-erb A α

Almost one year after the report by Fraichard et al., specific knockout of TR α 1 was reported by Wikstöm et al.⁵⁵⁾ A targeting vector was designed to inactivate TR α 1 specifically so that a functional TR α 1 was deleted but the splicing variant, TR α 2 and the related orphan receptor, rev-erb A α (transcribed on the opposite strand), were still expressed in homozygous mice (TR α 1^{-/-}). Surprisingly, the specific inactivation of TR α 1 did not cause any lethal abnormality. TR α 1^{-/-} mice were fertile and did not show any gross anatomical abnormalities with normal locomotor activity. The only abnormalities that they exhibited were bradycardia with prolonged QT duration, a very mild hypothyroidism and reduced body temperature. This report is truly surprising because TR α 1 is the only functional gene product of TR α in terms of mediating T₃ action. In addition, the report gave an alternative answer to why a case of RTH with a TR α abnormality has not been found, namely because a TR α abnormality does not show distinct clinical manifestations.

How then can we explain the difference in results between the TR α common knockout and the TR α 1 specific knockout? Does TR α 2 or rev-erb A α play a critical role in maintaining normal development in a mouse? As a matter of fact, a Swedish group which prepared the TR α 1 knockout have succeeded in specific knockout of TR α 2 (presented during 70th annual meeting of the American Thyroid Association in Colorado Springs, CO.). However, the results failed to explain the discrepancies. TR α 2^{-/-} mice showed mild hypothyroidism with reduced weight gain but their life span was normal. They also prepared rev-erb A α ^{-/-} mice but their life span was also normal. The question of why common knockout of TR α products resulted in lethal damage whereas the individual knockouts did not cause any serious abnormality remains to be answered.

3) Double knockout of TR α 1 and TR β

Finally, the Swedish group prepared TR α 1 and TR β double knockout mice by intercrossing TR α 1 and TR β deficient mice (also presented during 70th annual meeting of the American Thyroid Association). Since functional TRs are either TR α 1, TR β 1 and TR β 2, this double knockout would yield TR deficient mice. The phenotype of the TR deficient mice is even more surprising than that of TR α 1 knockout mice. The mice showed very high T₃ and T₄ levels and deaf mutism as observed in TR β deficient mice and shorter bones and reduced body weight, however, they could survive without any functional TR.

Clinical evidence, as well as animal experiments, clearly show that thyroid hormones are indispensable for the normal development of tissue, especially the brain. TR is supposed to play a key role in mediating thyroid hormone action. So what does the phenotype of TR deficient mice indicate? Are there any unidentified TR genes, such as TR γ ? Or, do other nuclear receptors substitute for the function of TR? Experiments with TR knockout mice have given us further important questions to answer.

REFERENCES

- 1) Farwell, A.P. and Braverman, L.E.: Thyroid and antithyroid drugs. In: *Goodman & Gilman's The pharmacological basis of therapeutics*, edited by Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W. and Gilman, A.G., pp.1383–1409 (1996), McGraw-Hill, New York.
- 2) Dussault, J.H. and Ruel, J.: Thyroid hormones and brain development. *Ann. Rev. Physiol.*, 49, 321–334 (1987).
- 3) Gudernatsch, J.F.: Feeding experiments on tadpoles. I. The influence of specific organs given as food on growth and differentiation. A contribution to the knowledge of organs with internal secretion. *Arch. Entwicklungsmech. Organ*, 35, 457 (1912).
- 4) Berry, M.J., Banu, L. and Larsen, P.R.: Type I iodothyronine deiodinase is a selenocystein-containing enzyme. *Nature*, 349, 438–440 (1991).
- 5) St. Germain, D.L., Schwartzman, R.A., Croteau, W., Kanamori, A., Wang, Z., Brown, D.D. and Galton, V.A.: A thyroid hormone-regulated gene in *Xenopus Laevis* encodes a type III iodothyronine 5-deiodinase. *Proc. Natl. Acad. Sci. USA*, 91, 7767–7771 (1994).
- 6) Yu, V.C., Delsert, C., Andersen, B., Holloway, J.M., Devary, O.V., Näär, A.M., Kim, S.Y., Boutin, J-M., Glass, C.K. and Rosenfeld, M.G.: RXR β : A Coregulator that enhances binding of retinoic acid, thyroid hormone, and vitamin D receptors to their cognate response elements. *Cell*, 67, 1251–1266 (1991).
- 7) Zhang, X-K., Hoffmann, B., Tran, P.B-V., Graupner, G. and Pfahl, M.: Retinoid X receptor is an auxiliary protein for thyroid hormone and retinoic acid receptors. *Nature*, 355, 441–446 (1992).
- 8) Kliewer, S.A., Umesono, K., Mangelsdorf, D.J. and Evans, R.M.: Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D3 signaling. *Nature*, 355, 446–449 (1992).
- 9) Leid, M., Kastner, P., Lyons, R., Nakshatri, H., Saunders, M., Zacharewski, T., Chen, J-Y., Staub, A., Garnier, J-M., Mader, S. and Chambon, P.: Purification, cloning, and RXR identity of the HeLa cell factor with which RAR or TR heterodimerizes to bind target sequences efficiently. *Cell*, 68, 377–395 (1992).
- 10) Horwitz, K.B., Jackson, T.A., Bain, D.L., Richer, J.K., Takimoto, G.S. and Tung, L.: Nuclear receptor coactivators and corepressors. *Mol. Endocrinol.*, 10, 1167–1177 (1996).
- 11) Onate, S.A., Tsai, S.Y., Tsai, M-J. and O'Malley, B.W.: Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science*, 270, 1354–1357 (1995).
- 12) Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S-C., Heyman, R.A., Rose, D.W., Glass, C.K. and Rosenfeld, M.G.: A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell*, 85, 403–414 (1996).
- 13) Ogrzko, V.V., Schiltz, R.L., Russanova, V., Howard, B.H. and Nakatani, Y.: The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell*, 87, 953–959 (1996).
- 14) Davis, P.J., Davis, F.B. and Lawrence, W.D.: Thyroid hormone regulation and membrane Ca²⁺-ATPase activity. *Endocr. Res.*, 15, 651–682 (1989).
- 15) Spurr, N.K., Solomon, E., Jansson, M., Sheer, D., Goodfellow, P.N., Bodmer, W.F. and Vennstrom, B.: Chromosomal localisation of the human homologues to the oncogenes *erbA* and B. *EMBO. J.*, 3, 159–163 (1984).
- 16) Dayton, A.I., Selden, J.R., Laws, G., Dorney, D.J., Finan, J., Tripputi, P., Emanuel, B., Rovera, G., Nowell, P.C. and Croce, C.M.: A human *c-erbA* oncogene homologue is closely proximal to the chromosome 17 breakpoint in acute promyelocytic leukemia. *Proc. Natl. Acad. Sci. USA*, 81, 4495–4499 (1984).
- 17) Sap, J., Munoz, A., Damm, K., Goldberg, Y., Ghysdael, J., Leutz, A., Beug, H. and Vennström, B.: The *c-erb-A* protein is a high-affinity receptor for thyroid hormone. *Nature*, 324, 635–640 (1986).
- 18) Weinberger, C., Thompson, C.C., Ong, E.S., Lebo, R., Gruol, D.J. and Evans, R.M.: The *c-erb-A* gene encodes a thyroid hormone receptor. *Nature*, 324, 641–646 (1986).
- 19) Thompson, C.C., Weinberger, C., Lebo, R. and Evans, R.M.: Identification of a novel thyroid hormone receptor expressed in the mammalian central nervous system. *Science*, 237, 1610–1614 (1987).
- 20) Mitsuhashi, T., Tennyson, G. and Nikodem, V.M.: Alternative splicing generates messages encoding rat *c-erbA* proteins that do not bind thyroid hormone. *Proc. Natl. Acad. Sci. USA*, 85, 5804–5808 (1988).
- 21) Nakai, A., Seino, S., Sakurai, A., Szilak, I., Bell, G.I. and DeGroot, L.J.: Characterization of a thyroid hormone receptor expressed in human kidney and other tissues. *Proc. Natl. Acad. Sci. USA*, 85, 2781–2785 (1988).
- 22) Lazar, M.A.: Thyroid hormone receptors: Multiple forms, multiple possibilities. *Endocr. Rev.*, 14, 184–193 (1993).
- 23) Koenig, R.J., Lazar, M.A., Hodin, R.A., Brent, G.A., Larsen, P.R., Chin, W.W. and Moor, D.D.: Inhibition of thyroid hormone action by a non-hormone binding *c-erbA* protein generated by alternative mRNA splicing. *Nature*, 337, 659–661 (1989).

- 24) Hodin, R.A., Lazar, M.A., Wintman, B.I., Darling, D.S., Koenig, R.J., Larsen, P.R., Moore, D.D. and Chin, W.W.: Identification of thyroid hormone receptor that is pituitary-specific. *Science*, 244, 76–79 (1989).
- 25) Schwartz, H.L., Lazar, M.A. and Oppenheimer, J.H.: Widespread distribution of immunoreactive thyroid hormone β 2 receptor (TR β 2) in the nuclei of extrapituitary rat tissue. *J. Biol. Chem.*, 269, 24777–24782 (1994).
- 26) Wood, W.M., Ocran, K.W., Gordon, D.F. and Ridgway, E.C.: Isolation and characterization of mouse complementary DNAs encoding α and β thyroid hormone receptors from thyrotrope cells: the mouse pituitary-specific β 2 isoform differs at the amino terminus from the corresponding species from rat pituitary tumor cells. *Mol. Endocrinol.*, 5, 1049–1061 (1991).
- 27) Sjöberg, M., Vennström, B. and Forrest, D.: Thyroid hormone receptors in chick retinal development: differential expression of mRNAs for α and N-terminal variant β receptors. *Development*, 114, 39–47 (1992).
- 28) Yen, P.M., Sunday, M.E., Darling, D.S. and Chin, W.W.: Isoform-specific thyroid hormone receptor antibodies detect multiple thyroid hormone receptors in rat and human pituitaries. *Endocrinology*, 130, 1539–1546 (1992).
- 29) Schueler, P.A., Schwartz, H.L., Strait, K.A., Mariash, C.N. and Oppenheimer, J.H.: Binding of 3,5,3'-triiodothyronine (T_3) and its analogs to the in vitro translational products of c-erbA protooncogenes: Differences in the affinity of the α - and β -forms for the acetic acid analog and failure of the human testis and kidney α -2 products to bind T_3 . *Mol. Endocrinol.*, 4, 227–234 (1990).
- 30) Mellström, B., Naranjo, J.R., Santos, A., Gonzales, A.M. and Bernal, J.: Independent expression of the α and β c-erb A genes in developing rat brain. *Mol. Endocrinol.*, 5, 1339–1350 (1991).
- 31) Bradley, D.J., Towle, H.C. and Young, W.S.,III: Spatial and temporal expression of α - and β -thyroid hormone receptor mRNAs, including the β 2-subtype, in the developing mammalian nervous system. *J. Neurosci.*, 12 (6), 2288–2302 (1992).
- 32) Bradley, D.J., Towle, H.C. and Young, W.S.,III: α and β thyroid hormone receptor (TR) gene expression during auditory neurogenesis: Evidence for TR isoform-specific transcriptional regulation in vivo. *Proc. Natl. Acad. Sci. USA*, 91, 439–443 (1994).
- 33) Strait, K.A., Schwartz, H.L., Perez-Castillo, A. and Oppenheimer, J.H.: Relationship of c-erb A mRNA to tissue triiodothyronine nuclear binding capacity and function in developing and adult rats. *J. Biol. Chem.*, 265, 10514–10521 (1990).
- 34) Refetoff, S., Weiss, R.E. and Usala, S.J.: The syndrome of resistance to thyroid hormone. *Endocr. Rev.*, 14, 348–399 (1993).
- 35) Refetoff, S., DeWind, L.T. and DeGroot, L.J.: Familial syndrome combining deaf-mutism, stippled epiphyses, goiter, and abnormally high PBI: possible target organ refractoriness to thyroid hormone. *J. Clin. Endocrinol. Metab.*, 27, 279–294 (1967).
- 36) Oppenheimer, J.H., Koerner, D., Schwartz, H.L. and Surks, M.I.: Specific-nuclear triiodothyronine binding sites in rat liver and kidney. *J. Clin. Endocrinol. Metab.*, 35, 330–333 (1972).
- 37) Murata, Y., Refetoff, S., Horwitz, A.L. and Smith, T.J.: Hormonal regulation of glycosaminoglycan accumulation in fibroblasts from patients with resistance to thyroid hormone. *J. Clin. Endocrinol. Metab.*, 57, 1233–1239 (1983).
- 38) Ceccarelli, P., Refetoff, S. and Murata, Y.: Resistance to thyroid hormone diagnosed by the reduced response of fibroblasts to the triiodothyronine-induced suppression of fibronectin synthesis. *J. Clin. Endocrinol. Metab.*, 65, 242–246 (1987).
- 39) Sakurai, A., Takeda, K., Ain, K., Ceccarelli, P., Nakai, A., Seino, S., Bell, G.I., Refetoff, S. and DeGroot, L.J.: Generalized resistance to thyroid hormone associated with a mutation in the ligand binding domain of human thyroid hormone receptor β . *Proc. Natl. Acad. Sci. USA*, 86, 8977–8981 (1989).
- 40) Ono, S., Schwartz, I.D., Mueller, O.T., Root, A.W., Usala, S.J. and Bercu, B.B.: Homozygosity for a “dominant negative” thyroid hormone receptor gene responsible for generalized resistance to thyroid hormone. *J. Clin. Endocrinol. Metab.*, 73, 990–994 (1991).
- 41) Takeda, K., Sakurai, A., DeGroot, L.J. and Refetoff, S.: Recessive inheritance of thyroid hormone resistance caused by complete deletion of the protein-coding region of the thyroid hormone receptor- β gene. *J. Clin. Endocrinol. Metab.*, 74, 49–55 (1992).
- 42) Hayashi, Y., Janssen, O.E., Weiss, R.E., Murata, Y., Seo, H. and Refetoff, S.: The relative expression of mutant and normal thyroid hormone receptor genes in patients with generalized resistance to thyroid hormone determined by estimation of their specific messenger ribonucleic acid products. *J. Clin. Endocrinol. Metab.*, 76, 64–69 (1993).
- 43) Nagaya, T., Madison, L.D. and Jameson, J.L.: Thyroid hormone receptor mutants that cause resistance to thyroid hormone—Evidence for receptor competition for DNA sequences in target genes—. *J. Biol. Chem.*, 267 (18), 13014–13019 (1992).

ROLES OF T₃ RECEPTOR ISOFORMS

- 44) Weiss, R.E., Hayashi, Y., Nagaya, T., Petty, K.J., Murata, Y., Tunca, H., Seo, H. and Refetoff, S.: Dominant inheritance of resistance to thyroid hormone not linked to defects in the thyroid hormone receptor α or β genes may be due to a defective cofactor. *J. Clin. Endocrinol. Metab.*, 81, 4196–4203 (1996).
- 45) Forrest, D., Hanebuth, E., Smeyne, R.J., Everds, N., Stewart, C.L., Wehner, J.M. and Curran, T.: Recessive resistance to thyroid hormone in mice lacking thyroid hormone receptor β : evidence for tissue-specific modulation of receptor function. *EMBO J.*, 15, 3006–3015 (1996).
- 46) Forrest, D., Erway, L.C., Ng, L., Altschuler, R. and Curran, T.: Thyroid hormone receptor β is essential for development of auditory function. *Nature genet.*, 13, 354–357 (1996).
- 47) Weiss, R.E., Murata, Y., Cua, K., Hayashi, Y., Seo, H. and Refetoff, S.: Thyroid hormone action on liver, heart and energy expenditure in thyroid hormone receptor β deficient mice. *Endocrinology* (in press).
- 48) Jump, D.B.: Rapid induction of rat liver S14 gene transcription by thyroid hormone. *J. Biol. Chem.*, 264, 4689–4703 (1989).
- 49) Song, M-K.H., Dozin, B., Grieco, D., Rall, J.E. and Nikodem, V.M.: Transcriptional activation and stabilization of malic enzyme mRNA precursor by thyroid hormone. *J. Biol. Chem.*, 263, 17970–17974 (1988).
- 50) Berry, M.J., Kates, A-L. and Larsen, P.L.: Thyroid hormone regulates type I deiodinase messenger RNA in rat liver. *Mol. Endocrinol.*, 4, 743–748 (1990).
- 51) Menjo, M., Murata, Y., Fujii, T., Nimura, Y. and Seo, H.: Effects of thyroid and glucocorticoid hormones on the level of messenger ribonucleic acid for iodothyronine type I 5'-deiodinase in rat primary hepatocyte cultures grown as spheroids. *Endocrinology*, 133, 2984–2990 (1993).
- 52) Lee, R.-U., Mortensen, R.M., Larson, C.A. and Brent, G.A.: Thyroid hormone receptor- α inhibits retinoic acid-responsive gene expression and modulates retinoic acid-stimulated neural differentiation in mouse embryonic stem cells. *Mol. Endocrinol.*, 8, 746–756 (1994).
- 53) Fraichard, A., Chassande, O., Plateroti, M., Roux, J.P., Trouillas, J., Dehay, C., Legrand, C., Gauthier, K., Kedinger, M., Malaval, L., Rousset, B. and Samarut, J.: The T₃R α gene encoding a thyroid hormone receptor is essential for post-natal development and thyroid hormone production. *EMBO J.*, 16, 4412–4420 (1997).
- 54) Legrand, J. Thyroid hormone effects on growth and development. In: *Thyroid hormone Metabolism*, edited by Hennemann, G., pp.503–534 (1986), Marcel Dekker, Rotterdam.
- 55) Wikström, L., Johansson, C., Salto, C., Barlow, C., Barros, A.C., Baas, F., Forrest, D., Thoren, P. and Vennström, B.: Abnormal heart rate and body temperature in mice lacking thyroid hormone receptor α 1. *EMBO J.*, 17, 455–461 (1998).