REVIEW

Insight into the physiological actions of thyroid hormone receptors from genetically modified mice

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Introduction

Thyroid hormones exert a range of developmental and physiological actions in all vertebrates. Serum concentrations of L-thyroxine (T4) and 3,5,3′-L-tri-iodothyronine (T3) are maintained by a negative feedback loop involving T3 inhibition of hypothalamic thyrotrophin-releasing hormone (TRH) and pituitary thyroid-stimulating hormone (TSH) secretion, and by tissue-specific and hormone-regulated expression of the three iodothyronine deiodinase enzymes that activate or metabolise thyroid hormones (Bianco *et al.* 2002). T3 actions are mediated by two T3 receptors, TRα and TRβ, which act as hormone-inducible transcription factors. The TRα (NR1A1) and TRβ (NR1A2) genes encode mRNAs that are alternatively spliced to generate nine mRNA isoforms (TRα1, α2, α3, Δα1, Δα2, β1, β2, β3 and Δβ3), of which four (TRα1, α2, β1 and β2) are known to be expressed at the protein level *in vivo* (Lazar 1993, Chassande *et al.* 1997, Williams 2000) (Fig. 1). The numerous TR mRNAs are expressed widely in tissue- and developmental stage-specific patterns, although it is important to note that levels of mRNA expression may not correlate with receptor protein concentrations in individual tissues (Forrest *et al.* 1990, Schwartz *et al.* 1994). The TRα2, α3, Δα1 and Δα2 transcripts encode proteins that fail to bind T3 *in vitro*. These 'non-binding' isoforms, in addition to TRΔβ3 which does bind hormone, may act as dominant negative antagonists of the true T3-binding receptors *in vitro*, but their physiological functions and those of the TRβ3 isoform have not been determined. In order to obtain a new understanding of the complexities of T3 action *in vivo* and the role of TRs during development, many mouse models of disrupted or augmented thyroid hormone signalling have been generated. The aim of this review is to provide a picture of the physiological actions of thyroid hormones by considering the phenotypes of these genetically modified mice.

Endocrine system

TRα$^{0/0}$ knockout

TRα$^{0/0}$ mice lack expression of all products of the *TRα* locus (Gauthier *et al.* 2001) (Table 1). Animals survive normally and are fertile with normal basal thyroid status and pituitary growth hormone (GH) mRNA concentrations (Gauthier *et al.* 2001). Nevertheless, compared with wild-type littermates, TRα$^{0/0}$ mice display increased sensitivity to thyroid hormones in the pituitary and liver following provocative testing with increasing doses of T3 to suppress TSH and T4 secretion (Macchia *et al.* 2001).

TRα$^{-/-}$ knockout

Disruption of exon 2, the first coding exon within the TRα locus, prevents transcription of both TRα1 and TRα2 mRNAs, but does not interfere with expression of TRΔα1 and Δα2 from an internal promoter in intron 7 (Chassande *et al.* 1997, Fraichard *et al.* 1997) (Table 1). TRα$^{-/-}$ pups die shortly after weaning unless treated with T3 (Fraichard *et al.* 1997). The thyroid gland develops abnormally and homozygous mutants are profoundly hypothyroid with T3 and T4 concentrations reduced to 40% and <10% of wild-type concentrations by 5 weeks of age. The greater reduction in T4 compared with T3 suggests that progressive hypothyroidism results from defective hormone production rather than an abnormality in peripheral T3 production (Fraichard *et al.* 1997). Accordingly, pituitary TSHβ mRNA expression was decreased more than 3-fold in TRα$^{-/-}$ animals although GH mRNA levels were unaffected. Homozygous mutants die within 5 weeks, although they can be rescued by hormone replacement for only 1 week (1 μg T3 subcutaneous injection) during the third week of life at the time of weaning. Thereafter, animals gain weight and hormone levels of 2·5-month-old rescued animals are normal

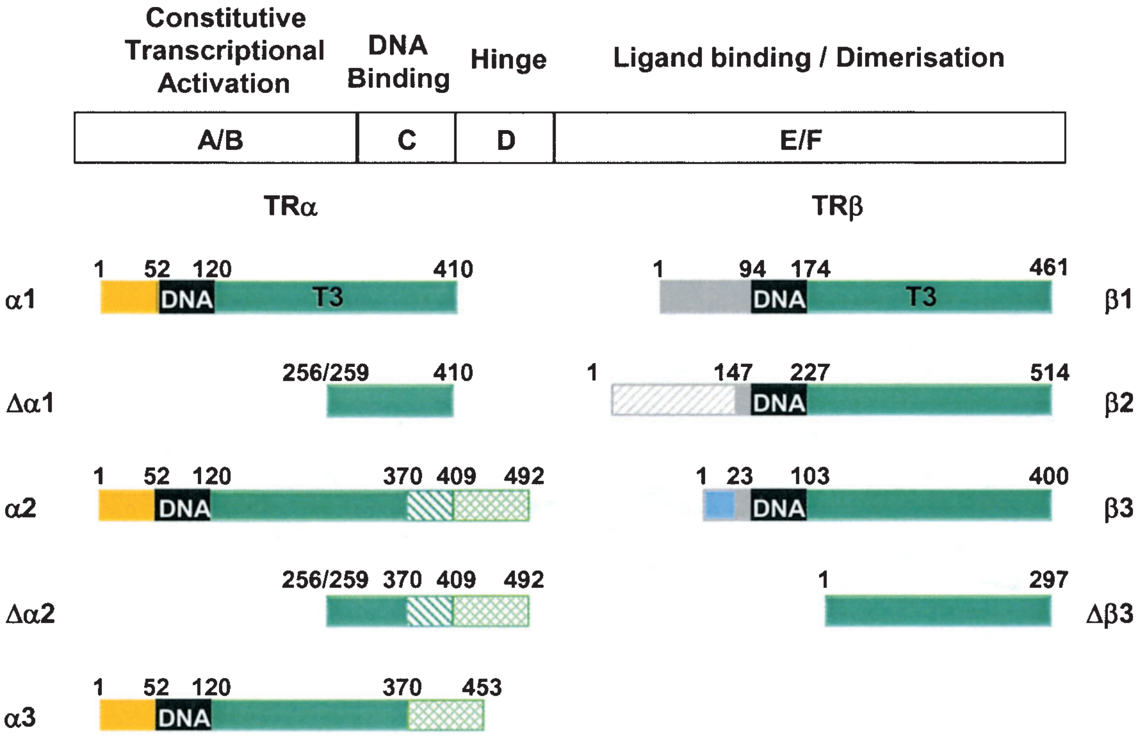

Figure 1 Thyroid hormone receptor isoforms. Protein products arising from the TRα and TRβ genes are displayed below a schematic diagram of the functional domains present within proteins of the nuclear receptor superfamily. Numbering represents the amino acid positions within the rat TR proteins. Shading indicates identical shared regions and differences between TR variants that are translated from alternately spliced mRNA isoforms.

Table 1 Genotypes and thyroid status of TR null and related knockout mice

	Reference	Deleted TR mRNAs	Expressed TR mRNAs	Thyroid status
Knockout				
TRα$^{-/-}$	Fraichard *et al.* (1997)	α1, α2	Δα1, Δα2, all β isoforms	Progressive to severely hypothyroid
TRα1$^{-/-}$	Wikstrom *et al.* (1998)	α1, Δα1	α2, Δα2, all β isoforms	Mildly hypothyroid
TRα2$^{-/-}$	Salto *et al.* (2001)	α2, Δα2	α1 (over-espressed), Δα1, all β isoforms	Mixed hyper/ hypothyroid
TRα$^{0/0}$	Gauthier *et al.* (2001)	All α isoforms	All β isoforms	Euthyroid
TRβ$^{-/-}$	Forrest *et al.* (1996*b*)	All β isoforms	All α isoforms	RTH
TRβ2$^{-/-}$	Abel *et al.* (1999a), Ng *et al.* (2001)	β2	β1, β3, Δβ3, all α isoforms	RTH
TRα$^{0/0}$β$^{-/-}$	Gauthier *et al.* (2001)	All α isoforms, all β isoforms	—	Profound RTH
Pax8$^{-/-}$	Mansouri *et al.* (1998)	Deficiency of thyroid follicular cells following thyroid gland agenesis		Severely hypothyroid

RTH indicates mice with resistance to thyroid hormone.

(Fraichard *et al.* 1997), although both male and female TRα$^{-/-}$ mice are infertile. These data indicate a stimulatory role for TRα1 or α2 in the initiation of thyroid hormone production at weaning or a suppressive role for the truncated Δα1 and Δα2 products that continue to be expressed in TRα$^{-/-}$ mice.

TRα1$^{-/-}$ knockout

Both male and female TRα1$^{-/-}$ mice are fertile and display mild abnormalities of the pituitary–thyroid axis. Male TRα1$^{-/-}$ mice have reduced free T4 concentrations but normal free T3 concentrations compared with wild-type mice whereas females are euthyroid. Thyroid gland histology in TRα$^{-/-}$ mice was normal. Reduced T4 levels in males occurred in association with a small reduction in TSH concentrations and reduced pituitary TSHα, but not TSHβ, mRNA expression. These data suggest that the mild hypothyroidism in male TRα1$^{-/-}$ mice results from impaired TSHα gene regulation (Wikstrom *et al.* 1998). In combination with findings in TRβ$^{-/-}$ mice (Forrest *et al.* 1996*a*,*b*), these studies suggest a model in which TSH gene expression is dynamically regulated by interactions between individual isoforms expressed by both TRα and β genes (Wikstrom *et al.* 1998).

TRα2$^{-/-}$ knockout

TRα2$^{-/-}$ mice overexpress TRα1 as an inevitable consequence of gene targeting and are hypothyroid (Salto *et al.* 2001). Free T3 and T4 concentrations are reduced, but TSH concentrations are inappropriately normal and there is impaired hormone production from the thyroid gland. Nevertheless, there are also features of hyperthyroidism such as increased heart rate, weight loss and elevated body temperature, suggesting that tissue-specific differences in thyroid hormone responsiveness may depend on the amount of TRα1 expressed. Thus, the TRα1:α2 ratio may regulate hormone responsiveness in specific tissues. Female TRα2$^{-/-}$ mice have a prolonged oestrous cycle due to abnormal patterns of ovulation that are reflected by impaired fertility. In contrast, the TRα2$^{-/-}$ mice have normal GH levels but there is a decrease in circulating insulin-like growth factor-I (IGF-I) concentrations, suggesting that GH signalling requires tissue euthyroidism.

TRβ$^{-/-}$ knockout

TRβ$^{-/-}$ mice, which lack expression of all TRβ isoforms (Table 1), display resistance to thyroid hormone (RTH) and demonstrate the key role for TRβ in set-point control of the pituitary–thyroid feedback axis (Forrest *et al.* 1996*b*, Gauthier *et al.* 1999). There is enlargement of the thyroid gland with an increased size and number of follicles. The inappropriate elevation of TSH is accompanied by selective 2·5- and 3·3-fold rises in pituitary TSHα and TSHβ mRNA expression, suggesting that RTH results from defective thyrotroph function rather than hyperplasia or pituitary gland malformation (Forrest *et al.* 1996*b*). Following the suggestion that TRβ may regulate TSH gene transcription directly (Forrest *et al.* 1996*b*), further studies (Weiss *et al.* 1997) revealed that TRβ is not required for increased TSH expression in hypothyroidism and is not essential for T3-mediated repression of TSH. Thus, TRβ enhances the sensitivity of the system and is necessary for complete inhibition of TSH, thereby resulting in the RTH phenotype in TRβ$^{-/-}$ mice (Weiss *et al.* 1997). These data are in accord with previous studies *in vitro*, which indicate that TRH is negatively regulated by T3 acting via TRβ (Lezoualc'h *et al.* 1992) and suggest that TRβ-mediated control of the pituitary–thyroid axis operates at the level of the hypothalamus as well as the pituitary. Although TRβ$^{-/-}$ mice display features of RTH similar to those observed in humans, they provide a recessive model for this condition whereas RTH in man is autosomal dominant (Forrest *et al.* 1996*a*,*b*). TRβ$^{-/-}$ mice also exhibit impaired T3-dependent regulation of cholesterol metabolism (Gullberg *et al.* 2000, Macchia *et al.* 2001), supporting previous observations showing that basal cholesterol levels were elevated in TRβ$^{-/-}$ mice, despite elevated basal T4 concentrations (Weiss *et al.* 1998), thus demonstrating peripheral as well as pituitary RTH.

TRβ2$^{-/-}$ knockout

To elucidate further the role of TRβ in pituitary–thyroid feedback control, TRβ2$^{-/-}$ mice were generated (Abel *et al.* 1999*a*) (Table 1). These mice also have RTH with a 3-fold increase in basal T4 concentrations, a 40% increase in T3, a 2·5-fold elevation in TSH and defective TSH suppression following T3 administration. Pituitary TSHβ mRNA was increased in TRβ2$^{-/-}$ mice and there was impaired stimulation of pituitary GH mRNA following T3 administration, adding to previous evidence indicating that TRβ2 plays a major role in T3-stimulated GH mRNA expression (Ball *et al.* 1997). Thus, deletion of TRβ2 is sufficient to cause RTH, suggesting that TRβ1 cannot adequately substitute for β2 and that β2 primarily mediates feedback control. This hypothesis is supported by studies which indicate that TRβ2 is a more potent regulator of TSH mRNA than β1 (Ng *et al.* 1995, Safer *et al.* 1997). More recently, Ng *et al.* (2001) developed Thrb$^{\mathrm{tm2Df/tm2Df}}$ mutant mice, which also lack expression of TRβ2. These mice display milder RTH than observed by the same group previously in TRβ$^{-/-}$ mice (Forrest *et al.* 1996*b*) and milder than observed in TRβ2$^{-/-}$ mice reported by Abel *et al.* (1999*a*). Thus, Ng *et al.* (2001) concluded that β2 acts co-operatively with β1 to regulate the pituitary–thyroid axis, in contrast to conclusions from the studies discussed above that suggest that β2 is the primarily important isoform (Abel *et al.* 1999*a*). Although the two laboratories each report mice that lack TRβ2, their phenotypic differences may result from the differing gene targeting strategies employed by the two groups or from differences in genetic background of the mice. Thus, it is difficult to conclude at this stage precisely how much contribution to control of the pituitary–thyroid axis is made by TRβ2 relative to β1 *in vivo* or whether other modifier genes are influential.

TR$\alpha^{0/0}\beta^{-/-}$ double knockout

Deletion of all TR isoforms in TR$\alpha^{0/0}\beta^{-/-}$ mice (Gauthier *et al.* 2001) results in profound abnormalities of the pituitary–thyroid axis with concentrations of T4, T3 and TSH elevated 14-fold, 13-fold and more than 200-fold respectively, which are similar to findings in TR$\alpha1^{-/-}\beta^{-/-}$ (Gothe *et al.* 1999) and TR$\alpha^{-/-}\beta^{-/-}$ mice (Gauthier *et al.* 1999). Pituitary GH mRNA expression is markedly reduced in TR$\alpha^{0/0}\beta^{-/-}$ double mutant mice in contrast to TR$\alpha^{0/0}$ animals, in which expression is preserved (Gauthier *et al.* 2001), providing further evidence that T3 regulation of GH occurs via TRβ.

The high levels of TSH in TR$\alpha^{0/0}\beta^{-/-}$ mice are around half those reported in Pax8$^{-/-}$ mice (Flamant *et al.* 2002) in which the primary defect is an absence of thyroid follicular cells (Mansouri *et al.* 1998) (Table 1). The number of pituitary thyrotrophs is markedly elevated in both Pax8$^{-/-}$ and TR$\alpha^{0/0}\beta^{-/-}$ mice and TSHα gene expression is similar in both genotypes (Flamant *et al.* 2002). These similar features occur despite the fact that Pax8$^{-/-}$ mice have no circulating T3 and T4 whilst TR$\alpha^{0/0}\beta^{-/-}$ mice have markedly elevated peripheral hormone concentrations and suggest that programming of the numbers of developing pituitary thyrotrophs occurs independent of thyroid hormones or TRs.

We have selected Pax8$^{-/-}$ mice (Mansouri *et al.* 1998) as the best available genetic model of isolated thyroid hormone deficiency for comparison with mice harbouring TR deletions. Whilst it remains possible that Pax8 has additional and uncharacterised functions in tissues other than the thyroid gland, the phenotype of Pax8$^{-/-}$ mice appears to result solely from hypothyroidism. Furthermore, Pax8$^{-/-}$ mice have been crossed with various TR-deficient mice (Flamant *et al.* 2002) enabling objective phenotypic comparisons to be made with TR-null mice. Nevertheless, alternative genetic models of hypothyroidism including the Ames (*df/df*) and Snell (*dw/dw*) dwarf and *hyt/hyt* (Beamer *et al.* 1981) hypothyroid mutants have been reported. Whilst these mice provide further useful tools for the further understanding of T3 action, the *df/df* and *dw/dw* mutants develop hypothyroidism secondary to hypopituitarism resulting from mutations of the prophet of Pit-1 (Sornson *et al.* 1996) and Pit-1 (Li *et al.* 1990) transcription factors respectively, whilst congenital hypothyroidism in *hyt/hyt* mice results from a mutation of the TSH receptor (Stein *et al.* 1994), which is expressed widely. These complexities suggest that comparison of the phenotypes of TR-null mice with the *df/df*, *dw/dw* and *hyt/hyt* mutants should be viewed cautiously. For the purposes of this review, therefore, we have focused on Pax8$^{-/-}$ mice as a standard genetic model of hypothyroidism.

Pax8$^{-/-}$ mice have a complete absence of T4 in postnatal life, when autonomous production of thyroid hormone replaces the maternal supply, and survival is not seen beyond postnatal day 30 (Flamant *et al.* 2002). However, lethality following weaning is not necessarily a direct consequence of hypothyroidism. Studies of Pax8$^{-/-}$TR$\alpha^{0/0}$ and Pax8$^{-/-}$TR$\beta^{-/-}$ mutants revealed that Pax8$^{-/-}$TR$\alpha^{0/0}$ were viable and similar in phenotype to TR$\alpha^{0/0}\beta^{-/-}$ double receptor mutants, whilst the Pax8$^{-/-}$TR$\beta^{-/-}$ mutation was lethal and similar to Pax8$^{-/-}$ single mutants. These data indicate detrimental developmental effects of unliganded TRα receptor isoforms in Pax8$^{-/-}$ mice (Flamant *et al.* 2002) and support similar conclusions derived from TR$\alpha^{-/-}$ mice (Fraichard *et al.* 1997).

TR$\alpha^{-/-}\beta^{-/-}$ double knockout

In TR$\alpha^{-/-}\beta^{-/-}$ mice, T4 and T3 levels were elevated 10-fold and TSH increased more than 100-fold (Gauthier *et al.* 1999). There was concomitant thyroid gland enlargement with follicular cell hyperplasia, an increase in the number of follicles, which were smaller than seen in TR$\beta^{-/-}$ mice, and evidence of abnormal colloid production and increased vascularity of the gland (Gauthier *et al.* 1999). As in the case of TR$\alpha^{-/-}$ animals (Fraichard *et al.* 1997), TR$\alpha^{-/-}\beta^{-/-}$ mice die following weaning (Gauthier *et al.* 1999), further implicating the TR$\Delta\alpha$1 and $\Delta\alpha$2 isoforms in the generation of this phenotype.

TR$\alpha1^{-/-}\beta^{-/-}$ double knockout

Gothe *et al.* (1999) reported the phenotype of TR$\alpha1^{-/-}\beta^{-/-}$ mice. Free T3 and T4 concentrations were elevated 60-fold with a 160-fold increase in TSH in 4- to 8-week-old mice that reduced to a 60-fold elevation at 5 months of age. There was a 15-fold increase in thyroid gland weight with hyperplasia and proliferation of follicular cells leading to progressive goitre. TSHα and TSHβ mRNA levels were increased 3·3- and 26-fold respectively in double mutant pituitary glands, although the pituitary gland was not malformed or enlarged. Similar to Pax8$^{-/-}$ and TR$\alpha^{0/0}\beta^{-/-}$ mice (Flamant *et al.* 2002), there were increased numbers of pituitary thyrotrophs reflecting the enhanced TSH secretion in TR$\alpha1^{-/-}\beta^{-/-}$ mice (Gothe *et al.* 1999).

The growth-promoting actions of GH have been shown to occur, in part, via IGF-I (Ohlsson *et al.* 1998). IGF-I and GH levels were reduced in TR$\alpha1^{-/-}\beta^{-/-}$ mice and may be a cause of retarded growth (Gothe *et al.* 1999). The decrease observed in GH mRNA in TR$\alpha1^{-/-}\beta^{-/-}$ mice, but not in TR$\alpha1^{-/-}$ or TR$\beta^{-/-}$ single receptor knockout models suggests that both TRα1 and

Table 2 Transgenic over-expression of mutated TRβ

Model	Reference	Mutation	Promoter	Expression pattern	Thyroid status
G345R	Hayashi *et al.* (1998)	G345 (human)	TSHβ	Pituitary	RTH
Δ337T	Abel *et al.* (1999*b*)	Δ337T (human)	Glycoprotein α-subunit	Pituitary	RTH
Cardiac Δ337T	Pazos-Moura *et al.* (2000)	Δ337T (human)	α-MHC	Cardiac	Euthyroid
PV mutant	Wong *et al.* (1997)	C insertion at nucleotide 1627	β-actin	Ubiquitous	RTH
Pituitary PV mutant	Zhu *et al.* (1999)	C insertion at nucleotide 1627	Glycoprotein α-subunit	Pituitary	Euthyroid

MHC, myosin heavy chain.

TRβ have regulatory roles in GH gene transcription, although studies described above suggest that the effects of TRβ may predominate.

Transgenic models of human RTH

RTH has been classified as generalised (GRTH) or pituitary (PRTH) according to the absence or presence of thyrotoxicosis. Hayashi *et al.* (1998) selected the natural TRβ mutant G345R which causes severe RTH in man (Sakurai *et al.* 1989), and produced a transgenic mouse with the mutant TR targeted to the pituitary, under the control of the TSHβ promoter (Table 2). Pituitary-specific expression of TRβ^{G345R} resulted in PRTH with increased serum T4 and reduced cholesterol levels but normal TSH concentrations. These findings reflected thyrotroph resistance to T3 and normal hepatic responsiveness, although the phenotype was milder than predicted from the human disease. TRβ^{G345R} transgenic mice were normal in terms of fertility, survival and growth (Hayashi *et al.* 1998).

The naturally occurring mutation Δ337T (Usala *et al.* 1991, Baniahmad *et al.* 1992) was introduced into human TRβ1 (Abel *et al.* 1999*b*). The TRβ$^{\Delta337T}$ mutant does not bind T3 and is associated with severe GRTH (Usala *et al.* 1991). Abel *et al.* (1999*b*) generated TRβ$^{\Delta337T}$ transgenic mice, selectively targeted to the pituitary via the common α-subunit promoter (Table 2). There was a 3-fold increase in TSH but normal circulating T4 suggesting the possibility of reduced TSH bioactivity. Previous studies have indicated that TRH enhances the biological activity of TSH via alteration of TSH glycosylation (Beck-Peccoz *et al.* 1985, Taylor *et al.* 1988, Taylor & Weintraub 1989). This concept was supported recently by Yamada *et al.* (1997) who demonstrated that mice with a targeted ablation of TRH have T4 levels 50% lower than wild-type mice despite 2-fold elevated TSH concentrations. RTH patients have been observed to have increased bioactive TSH (Persani *et al.* 1994) and, thus, differences in TRH production may explain differences between human RTH patients and pituitary-specific TRβ$^{\Delta337T}$ transgenic mice (Abel *et al.* 1999*b*). TRβ$^{\Delta337T}$ animals also show impaired ligand-independent activation of TSHβ gene expression *in vivo* and deficient TSH responsiveness to hypothyroidism. The results indicate that the pituitary-specific expression of TRβ$^{\Delta337T}$ leads to impaired T3-mediated repression, with defective T3-independent activation of TSH production, and that resistance at the level of both the pituitary and the hypothalamus is required for thyroid hormone elevation in RTH (Abel *et al.* 1999*b*).

A third RTH mutant, TRβ^{PV}, was introduced into transgenic mice under the control of the human β-actin promoter to ensure ubiquitous expression (Table 2). Mice displayed features of RTH with elevated thyroid hormone levels and inappropriately normal TSH (Wong *et al.* 1997). Subsequently, Zhu *et al.* (1999) generated a transgenic mouse with the TRβ^{PV} mutant targeted to the pituitary using the common α-subunit promoter. Male TRβ^{PV} mutant mice exhibited impaired weight gain but there were no alterations in T3, T4 or TSH compared with wild-type mice. Levels of GH and IGF-I were also unaffected, suggesting that impaired growth might be mediated by alternative factors (Zhu *et al.* 1999).

A major problem with the transgenic approach is that the mutant TRβ is introduced and expressed under the control of a heterologous promoter that might be ubiquitous or tissue specific but does not possess the regulatory features of the endogenous TRβ promoter. Furthermore, random integration of the transgene into host DNA may result in altered expression of other unknown potential modifier genes; the mutant receptor is introduced in the presence of both normal alleles of the native receptor; and transgene copy number may be important. These features suggest that a transgenic approach is unlikely to produce a faithful mouse model of RTH in which to understand physiological activities of TR isoforms in specific tissues.

Models of RTH using targeted mutagenesis

Kaneshige *et al.* (2000) addressed these issues by targeting the TRβ^{PV} mutation to the endogenous mouse TRβ gene

Table 3 Knock-in models using homologous recombination

Model	Reference	Mutation	Other information	Thyroid status
TRβ^{PV}	Kaneshige *et al.* (2000)	C insertion at 1627 bp of mouse TRβ1; frameshift and generation of 463 aa mutant protein	*Cre/lox* P system	Profound RTH
TR$\alpha 1^{PV}$	Kaneshige *et al.* (2001)	C insertion at 1180 bp of mouse TRα1; frameshift and generation of 409 aa mutant protein		RTH

PV, mutation derived from a patient with severe resistance to thyroid hormone (RTH).

by homologous recombination to generate a TRβ^{PV} 'knock-in' mutant model of RTH (Table 3). The PV mutation was derived from a patient with severe RTH, who had elevated T4 and T3 levels, normal TSH, short stature, goitre and tachycardia (Parrilla *et al.* 1991). The mutation in exon 10 is a C insertion at codon 448 which produces a frameshift of the carboxy-terminal 14 amino acids (aa) of TRβ, resulting in severe impairment of T3 binding and transactivation activity by the mutant TRβ^{PV} protein, which acts as a dominant negative TR *in vitro* (Kaneshige *et al.* 2000).

Heterozygous TR$\beta^{PV/+}$ mice showed biochemical features of RTH that were typical of those in human RTH, whilst homozygous TR$\beta^{PV/PV}$ mutants had severe RTH with disruption of the pituitary–thyroid feedback axis and impaired growth (Kaneshige *et al.* 2000). T3 concentrations were elevated 2- and 9-fold in TR$\beta^{PV/+}$ and TR$\beta^{PV/PV}$ mice respectively, and T4 levels were increased 2·5- and 15-fold in heterozygous and homozygous mutant mice. RTH was profound in TR$\beta^{PV/PV}$ mice in which 412-fold increased TSH concentrations were seen. TRβ^{PV} mice possessed thyroid glands of increased size with extensive hyperplasia seen in homozygous mutants. The PV mutation interfered with the activities of wild-type receptors on the expression of both α-glycoprotein subunit and TSHβ genes and GH expression was repressed to 20% of wild-type values in TR$\beta^{PV/PV}$ pituitaries. In heterozygous TR$\beta^{PV/+}$ mice, there was a reduced GH mRNA response to elevated T3 levels (Kaneshige *et al.* 2000). Previous biochemical data have shown that TRβ^{PV} mutant receptors form inactive heterodimers with wild-type TRβ or TRα1, with TRβ having a higher interaction affinity than TRα1 for TRβ^{PV} (Zhu *et al.* 1996*a*). However, several mechanisms can be proposed to account for the dominant negative effect of mutant TRβ^{PV}. Thus, the mild pituitary–thyroid abnormalities in TR$\beta^{PV/+}$ mice might result from the formation of inactive or repressive TRβ^{PV}-containing heterodimers, from indirect interference with the actions of one or both TRα1 and TRβ by TRβ^{PV}, from haplo-insufficiency of wild-type TRβ or a combination of such factors (Kaneshige *et al.* 2000).

Kaneshige *et al.* (2001) investigated this by generating mice with the PV mutation in TRα1 (Table 3). TR$\alpha 1^{PV/+}$ mice are dwarfs and have impaired fertility with reduced litter size and frequency of pregnancy. Although viable, mortality was high, a clear difference from the TRβ^{PV} mice. TR$\alpha 1^{PV/PV}$ homozygotes were especially rare after mating TR$\alpha 1^{PV/+}$ animals and when born they died in the early neonatal period. TR$\alpha 1^{PV/+}$ mice had normal T4, but elevated T3 and TSH levels with no histological abnormality of the thyroid gland. The elevated TSH, together with reduced T4:T3 ratio, suggests that mild thyroid failure occurs in TR$\alpha 1^{PV/+}$ mice. There was no effect observed on pituitary GH expression but a 2- to 3-fold increased common α-subunit mRNA expression was observed. TSHβ mRNA was unaffected, suggesting that increased circulating TSH concentrations may result from increased TSH protein translation, secretion or stability. The distinct phenotypes seen in the TR$\alpha 1^{PV}$ (Kaneshige *et al.* 2001) and TRβ^{PV} (Kaneshige *et al.* 2000) mice demonstrate that functions of the TR mutants are isoform specific *in vivo* and show that, although no TRα mutations have been documented in human RTH, dominant mutations affecting TRα are neither silent nor lethal. Thus, it seems probable that mutations of TRα are likely to exist in man, but that their resulting biochemical and general phenotypes would be predicted to be quite distinct from the RTH associated with TRβ mutations.

Skeleton and growth

TR$\alpha^{0/0}$ homozygous mice are proportionately growth retarded with reduced weight gain and preservation of a normal weight-to-length ratio, whereas heterozygotes grow normally (Gauthier *et al.* 2001). Growth retardation results from delayed endochondral bone formation. There is a failure of progression of hypertrophic chondrocyte differentiation in the epiphyseal growth plate, which is disorganised and immature. Bone mineralisation is impaired and trabecular bone density in the tibial metaphysis is reduced compared with wild-type mice

(Gauthier *et al.* 2001). These features are similar to those seen in hypothyroidism (Stevens *et al.* 2000), although in the basal state TR$\alpha^{0/0}$ mice are biochemically euthyroid and have normal levels of pituitary GH (Gauthier *et al.* 2001), suggesting a defect in skeletal T3 responsiveness due to deletion of TRα.

TR$\alpha^{-/-}$-null mice are growth retarded with markedly delayed endochondral ossification (Fraichard *et al.* 1997), although these features are not manifest until the end of the second postnatal week. By the fifth postnatal week, TR$\alpha^{-/-}$ mice lose up to 50% body weight and die, failing to survive beyond weaning. Skeletal analyses revealed normal morphology with significantly delayed ossification and mineralisation and a hypoplastic bone marrow. Histomorphometry showed reduced bone mass and increased areas of disorganised epiphyseal cartilage containing reduced numbers of hypertrophic chondrocytes (Fraichard *et al.* 1997, Gauthier *et al.* 1999). In contrast, deletion of TR$\alpha1^{-/-}$ (Wikstrom *et al.* 1998) does not result in growth abnormality and no skeletal abnormalities have been reported in TR$\alpha1^{-/-}$ mutants.

TR$\alpha2^{-/-}$ mice have a slightly reduced growth rate after 5 weeks of age. Nevertheless, pituitary GH mRNA and protein levels are not different from wild-type animals, although serum IGF-I concentrations are diminished. Analysis of the skeleton revealed no evidence of altered longitudinal bone growth or change in growth plate width in TR$\alpha2^{-/-}$ mice. However, TR$\alpha2^{-/-}$ and TR$\alpha2^{+/-}$ heterozygotes showed evidence of impaired mineralisation in endochondral, but not intra-membranous bones, manifest by reduced bone mineral density and content (Salto *et al.* 2001). There was evidence of reduced tibial cortical bone in TR$\alpha2^{-/-}$ mice, suggesting late-onset growth retardation as these parameters may result from reduced periosteal bone growth in adults. A possible explanation for the adult onset growth retardation with reduced cortical bone in TR$\alpha2^{-/-}$ mice is that lack of TRα2 or over-expression of TRα1 results in late-onset IGF-I deficiency (Salto *et al.* 2001).

Complete deletion of TRβ or TRβ2 results in PRTH as indicated above, but does not cause growth retardation and no skeletal abnormalities have been reported in TR$\beta^{-/-}$- or TR$\beta2^{-/-}$-null mice (Abel *et al.* 1999*a*, Gauthier *et al.* 1999).

Growth retardation in TR$\alpha^{0/0}\beta^{-/-}$ double mutant mice is more severe than that seen in TR$\alpha^{0/0}$ single mutant animals despite the observation that TR$\beta^{-/-}$ mice grow normally (Gauthier *et al.* 2001). These data suggest that some important growth-promoting actions may be performed by TRβ in TR$\alpha^{0/0}$ mutant mice. Nevertheless, GH production is also diminished in TR$\alpha^{0/0}$ $\beta^{-/-}$ double mutants relative to wild-type and TR$\alpha^{0/0}$ mice, suggesting an additional cause of the profound growth retardation in TR$\alpha^{0/0}\beta^{-/-}$ animals (Gauthier *et al.* 2001). The phenotype of delayed bone maturation and impaired ossification was seen in TR$\alpha^{0/0}\beta^{-/-}$ mice and is similar to that in TR$\alpha^{0/0}$ mice (Gauthier *et al.* 2001). Comparison of TR$\alpha^{0/0}\beta^{-/-}$ double mutants with hypothyroid Pax8$^{-/-}$ animals indicated that more severe growth retardation and further delay in endochondral ossification occurs in Pax8$^{-/-}$ mice, suggesting that unliganded apo-TRs also exert deleterious effects during bone formation in the complete absence of thyroid hormones (Flamant *et al.* 2002).

TR$\alpha^{-/-}\beta^{-/-}$ mice exhibit a similar skeletal phenotype to TR$\alpha^{-/-}$ animals (Gauthier *et al.* 1999). There are no morphological alterations at birth whilst, similar to TR$\alpha^{-/-}$ mice (Fraichard *et al.* 1997), double mutants undergo growth arrest and die at 5 weeks of age. Examination of the skeleton demonstrated delayed endochondral ossification with reduced mineralisation at 2 weeks of age, findings similar to those in TR$\alpha^{-/-}$ mice (Gauthier *et al.* 1999). At this age, thyroid hormone concentrations in TR$\alpha^{-/-}\beta^{-/-}$ mice did not differ from wild-type mice, indicating that skeletal abnormalities did not result from circulating hypothyroidism but were due to the TR$\alpha^{-/-}\beta^{-/-}$ deletion (Gauthier *et al.* 1999).

TR$\alpha1^{-/-}\beta^{-/-}$ mice (Gothe *et al.* 1999) are growth retarded from 3 weeks of age and by adulthood weigh 30% less than wild-type mice, with a disproportionate reduction in long bone length, delayed ossification and disorganisation of the growth plate. The bone defects resemble hypothyroidism, although in hypothyroidism the width of the tibial growth plate is reduced (Lewinson *et al.* 1989), whereas in TR$\alpha1^{-/-}\beta^{-/-}$ mice it is the reverse (Gothe *et al.* 1999). The skeletal abnormalities in TR$\alpha1^{-/-}$ $\beta^{-/-}$ mice are associated with inhibition of the GH–IGF-I axis. Interestingly, growth retardation in TR$\alpha1^{-/-}$ $\beta^{-/-}$ mice is reversed following GH replacement, but the growth plate ossification abnormalities are not rescued (Kindblom *et al.* 2001), indicating that TRs provide important actions to regulate the activity of the GH–IGF-I axis and also exert direct effects on the skeleton.

Transgenic TRβ^{PV} mutant mice exhibited impaired weight gain compared with wild-type mice that was sexually dimorphic. Impairment of weight gain was evident in males expressing the transgene in all tissues (Wong *et al.* 1997) or in the pituitary gland only (Zhu *et al.* 1999). In females, growth retardation was less prominent and did not occur in animals with restricted expression of TRβ^{PV} in the pituitary.

Reduced bone length and impaired weight gain was also seen in TRβ^{PV} knock-in mice (Kaneshige *et al.* 2000), in which significantly shortened tibiae and femora were documented in both TR$\beta^{PV/+}$ and TR$\beta^{PV/PV}$ mice, with homozygotes more severely affected but no sexual dimorphism identified. Homozygous mutants also displayed a reduced growth spurt by up to 40% between 3 and 7 weeks of age. No differences were seen in TR$\beta^{PV/+}$ heterozygotes. The same mutation of TRα1 in TR$\alpha1^{PV/+}$ mice (Kaneshige *et al.* 2001) resulted in severe growth impairment shortly after birth with resultant dwarfism.

Four-week-old male TRα1$^{PV/+}$ mice weighed 40% less and were 17% shorter than wild-type siblings, whilst females weighed 30% less than wild-type mice and were 15% shorter (Kaneshige *et al.* 2001).

Skeletal muscle

Hypo- and hyperthyroidism in humans cause skeletal muscle weakness with fatigue and T3 plays an important role during skeletal muscle development (Gambke *et al.* 1983) by repressing or activating expression of MHC genes (Yu *et al.* 2000). Johansson *et al.* (2000) found that alterations in thyroid hormone levels induce changes in maximum shortening velocity (V0) and in isometric twitch contraction or relaxation times. V0 depends mainly upon myosin composition of the contractile proteins, the slow type MHC I and the faster MHC II isoforms (Schiaffino *et al.* 1989, Ennion *et al.* 1995). Thyrotoxicosis induces a shift towards faster MHC isoforms (Caiozzo *et al.* 1993) and hypothyroidism reverses this equilibrium (Johansson *et al.* 2000). Contraction and relaxation times also depend on the Ca^{2+} transport capacity of the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCa), of which there are fast (SERCa1) and slow (SERCa2) types in skeletal muscle (Johansson *et al.* 2000). Hypothyroidism causes a decrease in both SERCa1 and SERCa2 activity (Sayen *et al.* 1992); this trend is reversed in thyrotoxicosis (Muller *et al.* 1994).

The actions of T3 in skeletal muscle have been studied in only a few of the TR mutant mice. TRα1$^{-/-}$ mice exhibit a hypothyroid phenotype with longer muscle contraction and relaxation times and soleus muscle twitches extended by 40%. In TRβ$^{-/-}$ mice, the soleus muscle was less fatigue resistant than in wild-type mice although contraction and relaxation times were unchanged (Johansson *et al.* 2000). SERCa1 expression was reduced in TRα$^{-/-}$ mice but unaffected in TRβ$^{-/-}$ animals. In the soleus, total fibre number was reduced in TRα1$^{-/-}$β$^{-/-}$ mice: a 51% increase in MHC I fibres was seen in TRα1$^{-/-}$β$^{-/-}$ double mutant mice, a 21% difference was recorded in TRα1$^{-/-}$ animals but no change was evident in TRβ$^{-/-}$ mice. These results corresponded with lower proportions of fast MHC II fibres in TRα1$^{-/-}$β$^{-/-}$ and TRα1$^{-/-}$ mice (Yu *et al.* 2000) and suggest that both TRα1 and TRβ are required for normal skeletal muscle responses to T3 (Johansson *et al.* 2000).

Visual and auditory systems

Retinal cone photoreceptors facilitate colour vision (Ng *et al.* 2001). Cones expressing different opsin photopigments are sensitive to short (S, 'blue') and middle (M, 'green') wavelengths in rodents, and these cones are differentially localised in the retina (Wang *et al.* 1992, Szel *et al.* 2000). Thyroid hormone has been implicated in the differentiation of cones (Kelley *et al.* 1995*a*,*b*) and mutations of TRβ may be associated with cone disorders in man (Newell & Diddie 1977). TRβ1 is widely expressed, whereas the expression of TRβ2 is largely restricted to the hypothalamus, pituitary, inner ear (Hodin *et al.* 1989, Bradley *et al.* 1994) and the outer nuclear layer of the embryonic retina that contains developing photoreceptors (Sjoberg *et al.* 1992). The mouse retina contains S- and M-opsin specific cones as well as cones expressing both (Rohlich *et al.* 1994, Szel *et al.* 1994, Lyubarsky *et al.* 1999). Deletion of TRβ2 causes a selective loss of M-cones together with an increase in S-opsin immunoreactive cones. The cone distribution gradient is also disturbed with the usually focussed S-cones becoming widespread across the TRβ2$^{-/-}$ retina. The presence of S-opsin in all TRβ2$^{-/-}$ photoreceptors indicates that, in the absence of TRβ2, all cones follow a default S-cone pathway and suggests a critical role for TRβ2 in the commitment of photoreceptors to an M-cone or 'dual-cone' phenotype. The effect of TRβ2 in cone photodevelopment is not compensated by TRβ1 (Ng *et al.* 2001).

Thyroid hormones are also critical for development of the auditory system, an observation supported by clinical findings that demonstrate frequent associations between congenital thyroid disorders and hearing impairment or deafness (Refetoff *et al.* 1967, Stanbury 1984, DeLong 1993). Hypothyroidism has been reported to cause cochlear malformation (Deol 1973, Uziel *et al.* 1981). Recent studies reveal a period extending from late embryonic development to the second postnatal week where T3 is required for normal cochlea development (Deol 1973, Hebert *et al.* 1985, Uziel 1986). Exposure to thyroid hormone excess during the same period in rats causes an accelerated onset of auditory function development (Freeman *et al.* 1993). The cochlea appears to be the major T3-sensitive auditory organ and TRα mRNA is widely distributed in both cochlear and vestibular structures in the developing inner ear (Bradley *et al.* 1994). TRβ expression is more restricted and predominantly seen in the organ of Corti, which contains primordial hair cells, supporting cells and cells secreting tectorial membrane components. Both TRα and TRβ have been implicated in development of auditory function (Forrest *et al.* 1996*a*), although TRβ plays the major role.

TRβ$^{-/-}$ mice exhibit impaired auditory function. Auditory-evoked brainstem response (ABR) studies (Forrest *et al.* 1996*a*) have demonstrated that the threshold sound pressure levels required for detection of an ABR were significantly elevated for click stimuli and for all tones tested. Ninety-five percent of TRβ$^{-/-}$ mice tested were severely impaired with 10% of these being completely deaf and lacking a response to any stimulus. In mice with some preservation of hearing, the amplitude of ABR waveforms was diminished although their pattern was normal, suggesting that the primary defect in $TR\beta^{-/-}$ mice lies in the cochlea. The findings indicated that TRβ controls the maturation of auditory function but is not required for cochlear morphogenesis (Forrest *et al.* 1996*a*). Recent data suggest that the basis for hearing impairment in TRβ-null mice is a delay in the development of the expression of the fast-activating potassium conductance (IK,f) inner hair cells of the cochlea (Rusch *et al.* 1998). In wild-type mice, half-maximal IK,f expression is reached at postnatal day 17·5 whereas, in $TR\beta^{-/-}$ animals, IK,f expression is absent up to day 18. This difference, at a critical developmental period, may explain the auditory phenotype of TRβ-null mice (Rusch *et al.* 1998). TRβ1 and TRβ2 isoforms are coexpressed in the developing cochlea, but studies in $TR\beta2^{-/-}$ mice (Abel *et al.* 1999*a*) revealed no hearing deficit, indicating that TRβ2 is not necessary for cochlear development and function.

Rusch *et al.* (1998) determined ABR thresholds in $TR\alpha1^{-/-}$ knockout mice (Wikstrom *et al.* 1998). Over a wide range of frequency stimuli, these mice show no impairment, indicating that TRα1 is not essential for auditory function (Rusch *et al.* 1998). $TR\alpha^{0/0}$ mice exhibit a normal hearing threshold at low or middle frequencies, but a marked sensitivity loss at high tone frequencies (Gauthier *et al.* 2001). In $TR\alpha^{0/0}\beta^{-/-}$ mice, the effects on hearing thresholds were significantly more severe than in $TR\beta^{-/-}$ mice (Gauthier *et al.* 2001). In summary, TRβ1 appears to be the major mediator of T3 action in the ear, although TRα is also required for full auditory function.

Small intestine

Thyroid hormones influence postnatal intestinal maturation by stimulating intestinal crypt cell proliferation and the onset of brush border enzyme expression (Castillo *et al.* 1991, Hodin *et al.* 1996). Thus, T3 enhances the dramatic developmental changes in the gastrointestinal tract that lead to its complete remodelling by adulthood (Tata 1993, Shi *et al.* 1996).

The TRΔα1 and Δα2 mRNA isoforms are transcribed from an internal promoter, located in intron 7 of the *TRα* gene (Chassande *et al.* 1997). Their expression is highest in a limited number of organs including the lung and small intestine (Fraichard *et al.* 1997). TRΔα1 mRNA is expressed mainly in the epithelium and lamina propria of the distal ileum, with barely detectable levels being seen in the muscular layers of the distal ileum and proximal colon. TRΔα2 mRNA is similarly distributed, except that maximal expression occurs in the lamina propria of the proximal jejunum (Plateroti *et al.* 2001).

Fraichard *et al.* (1997) described delayed small intestine development in $TR\alpha^{-/-}$ mice with no overall alterations in stomach, duodenum or colon. The small intestine was smaller, softer and more fragile than in wild-type mice. The diameters of the jejunum and ileum were reduced, together with the number and size of villi, and the total number of epithelial cells per crypt villus unit was reduced by 65% in the ileum. The frequency of goblet cells was reduced 2·5-fold in the ileum of $TR\alpha^{-/-}$ animals, but Paneth cell frequency was unaffected. Circular and smooth muscle layers were reduced, correlating with altered motility in the $TR\alpha^{-/-}$ ileum, which exhibited no spontaneous contraction or response to pharmacological stimuli. Small intestine dipeptidase and disaccharidase activities were reduced 2-fold in $TR\alpha^{-/-}$ mice relative to wild-type mice, although peptide and disaccharide absorption was normal. These are features of delayed intestinal maturation as a similar phenotype is characteristic in young mice.

The small intestine was further characterised by Plateroti *et al.* (1999). In $TR\alpha^{-/-}$ mice, the crypt–villus length was shortened in proximal jejunum and distal ileum compared with wild-type mice. These findings correlate with a decreased proliferation rate of $TR\alpha^{-/-}$ intestinal crypt cells and a decreased number of goblet cells in the $TR\alpha^{-/-}$ ileum. Furthermore, $TR\alpha^{-/-}$ villous epithelial cells had reduced cellular and brush border height compared with wild-type mice. mRNA expression of the intestine-specific Cdx-1 and Cdx-2 homeobox genes was reduced in the $TR\alpha^{-/-}$ mice. These transcription factors are expressed normally in a specific pattern with an increasing gradient along the longitudinal axis of the gut (James & Kazenwadel 1991, Freund *et al.* 1992). Cdx-1 and Cdx-2 play a key regulatory role in normal intestinal cell proliferation and differentiation (Traber 1994, Suh & Traber 1996, Duluc *et al.* 1997, Lorentz *et al.* 1997) and reduced expression in $TR\alpha^{-/-}$ mice may account, in part, for the severe small intestine phenotype.

Plateroti *et al.* (1999) observed that a single T3 injection stimulated recovery of intestinal morphology in $TR\alpha^{-/-}$ mice. Furthermore, digestive enzyme activities were partially rescued and Cdx-1 mRNA was restored to normal wild-type levels. Cdx-2 mRNA expression, however, was reduced. In follow-up studies, Plateroti *et al.* (2001) investigated mechanisms responsible for the intestinal abnormalities in $TR\alpha^{-/-}$ mice. They retain expression of TRΔα1 and Δα2, suggesting that expression of these isoforms in the absence of TRα1 may be deleterious to intestinal development. Gauthier *et al.* (2001) found that, in $TR\alpha^{0/0}$ animals, the size of the small intestine mucosa was reduced compared with wild-type animals, due to decreased villus length and reduced number of epithelial cells per crypt–villus axis. The features were not as severe as in $TR\alpha^{-/-}$ mice (Plateroti *et al.* 2001). Furthermore, in $TR\alpha^{0/0}$ mice, expression of Cdx-1 and Cdx-2 mRNAs remained unchanged relative to wild-type mice, indicating that the TRΔα1 and Δα2 mRNAs may exert an inhibitory role to regulate Cdx gene transcription in the absence of TRα1. Thus, the relative level of expression of

TRα and Δα isoforms in the intestine may be a critical determinant in normal small intestinal development (Plateroti *et al.* 2001). In order to investigate this, TR$\alpha^{7/7}$ mutant mice were generated, in which expression of TRΔα1 and Δα2 transcripts was almost completely abolished whilst expression of TRα1 and α2 was retained at normal levels. These mice display no morphological alterations in the ileum compared with wild-type mice and, in contrast to both TR$\alpha^{-/-}$ and TR$\alpha^{0/0}$ mice, TR$\alpha^{7/7}$ mutants had increased numbers of goblet cells compared with wild-type mice, whilst levels of Cdx-1 and Cdx-2 mRNAs were unchanged (Plateroti *et al.* 2001). Thus, the severity of the intestinal phenotype correlates with the level of expression of TRΔα transcripts and not with the absence of TRα1 or TRα2 isoforms (Gauthier *et al.* 2001). Balanced expression of all TRα isoforms is essential for normal small intestine development at weaning (Plateroti *et al.* 2001).

TR$\beta^{-/-}$ mice display no intestinal phenotype, indicating that only TRα is essential for postnatal small intestine development (Plateroti *et al.* 1999). Indeed, ablation of TRβ in TR$\alpha^{0/0}\beta^{-/-}$ mice does not worsen the intestinal phenotype seen in TR$\alpha^{0/0}$ animals (Gauthier *et al.* 2001). In contrast, however, TR$\alpha^{-/-}\beta^{-/-}$ mice have more severe intestinal abnormalities than their TR$\alpha^{-/-}$ counterparts (Gauthier *et al.* 1999, Plateroti *et al.* 1999). Despite high circulating thyroid hormone levels, there was a marked decrease in the number of epithelial and goblet cells per crypt–villus unit in the distal ileum of TR$\alpha^{-/-}\beta^{-/-}$ mice, together with severely reduced expression of lactase mRNA and protein and Cdx-1 and Cdx-2 mRNAs (Plateroti *et al.* 1999). These data demonstrate that ablation of the TRβ gene significantly worsens the phenotype observed in TR$\alpha^{-/-}$ mice. Thus, expression of the non-T3-binding TRΔα variants in the absence of all T3-binding TRα and β isoforms is deleterious to intestinal development, confirming further that a balance between hormone-bound TRs and apo-TRΔα isoforms is essential for intestinal development. Interestingly, distal small intestine epithelial cell proliferation and differentiation are more compromised in Pax8$^{-/-}$ mice relative to TR$\alpha^{0/0}\beta^{-/-}$ double mutants, although the phenotype can be completely rescued in Pax8$^{-/-}$ mice following T3 administration. Furthermore, in compound Pax8$^{-/-}$TR$\beta^{-/-}$ mutants, a severe phenotype similar to that in Pax8$^{-/-}$ mice is seen, whereas in Pax8$^{-/-}$TR$\alpha^{0/0}$ compound mutants there is partial rescue (Flamant *et al.* 2002). These findings provide evidence to support the hypothesis that the TRΔα isoforms play the key role in postnatal small intestinal development (Flamant *et al.* 2002).

Liver

TRβ is the major functional TR in liver, accounting for 80% of hepatic T3-binding activity, with the remainder resulting from TRα1 (Weiss *et al.* 1998). Accordingly, TR$\beta^{-/-}$ mice display altered hepatic T3 responsiveness (Weiss *et al.* 1998). Thyroid hormone deprivation causes reduced expression of spot 14 (S14) in wild-type mice, but no change is seen in TR$\beta^{-/-}$ mice. Similarly, T3 stimulates S14 and malic enzyme (ME) expression in wild-type animals but not in TR$\beta^{-/-}$ mice. One of the best characterised hepatic T3 target genes is the type I 5′-deiodinase enzyme (D1) which catalyses the conversion of T4 to T3 in liver and kidney (Sato *et al.* 1984, Maia *et al.* 1995). D1 is induced by T3 and the human promoter contains several well-studied T3 response elements (Toyoda *et al.* 1995). Amma *et al.* (2001) studied D1 expression in TR-deficient mice and showed, in TR$\beta^{-/-}$ mice, that liver and kidney D1 mRNA levels were reduced to less than 30% and 50% of wild-type levels respectively. Thus, TRβ is required for the maintenance of basal D1 expression. The finding that liver D1 mRNA is undetectable in TR$\alpha1^{-/-}\beta^{-/-}$ mice, with enzyme activity less than 0·03% of wild-type mice (Amma *et al.* 2001), suggests, however, that TRα and TRβ exert some common functions in liver, although TRβ appears predominant. In accord with this, TR$\alpha1^{-/-}$ mice were found to have a 2-fold elevation in liver D1 expression, presumably resulting from increased TRβ activity due to elevated T3 concentrations in these animals. Interestingly, renal D1 mRNA expression was not elevated in TR$\alpha1^{-/-}$ mice (Wikstrom *et al.* 1998), suggesting that the kidney is predominantly a TRα-sensitive target organ.

Flamant *et al.* (2002) reported that hepatic D1 expression is significantly reduced in TR$\alpha^{0/0}\beta^{-/-}$ mice and undetectable in Pax8$^{-/-}$ mutants. The presence of D1 expression in the TR$\alpha^{0/0}\beta^{-/-}$ liver but absent expression in Pax8$^{-/-}$ mutants indicates that unliganded TRs may actively repress basal transcription of D1. Both Pax8$^{-/-}$TR$\alpha^{0/0}$ and Pax8$^{-/-}$TR$\beta^{-/-}$ compound mutants do not express detectable D1, implicating both TRα and TRβ unliganded receptors in active repression of the D1 promoter.

Expression of the mutant TRβ^{PV} which results in RTH was also observed to suppress the magnitude of hepatic T3-target gene responses. Expression of S14, ME and D1 was significantly reduced in TR$\beta^{PV/+}$ heterozygotes compared with wild-type levels and a further reduction in ME and S14, together with undetectable D1 expression, was evident in TR$\beta^{PV/PV}$ mutants (Kaneshige *et al.* 2000). These findings contrast with results obtained in TRβ-null mice, where no change in ME expression was detected (Weiss *et al.* 1998). In contrast, a different profile of abnormal T3 target gene regulation was reported in TR$\alpha1^{PV/+}$ mice. Activity of ME and D1 was increased 2·2-fold and 9·2-fold respectively, indicating hypersensitivity to T3 (Kaneshige *et al.* 2001). These findings may result from the markedly increased T3 concentrations in TR$\alpha1^{PV/+}$ mice, which may overcome some of the dominant negative activity of the $\alpha1^{PV}$ mutant receptor and increase T3 responses mediated via TRβ (Kaneshige *et al.* 2001).

Hepatic cholesterol metabolism is also influenced by T3, which regulates cholesterol 7α-hydroxylase (CYP7A), the rate-limiting enzyme in bile acid synthesis (Gullberg *et al.* 2000). Hypothyroidism is associated with increased serum cholesterol, especially affecting low density lipoprotein (LDL) cholesterol, which can be normalised following T4 replacement (O'Brien *et al.* 1993). T3 also influences hepatic synthesis and uptake of cholesterol, controlled largely by hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase and LDL receptors (Ness *et al.* 1973, 1990, Mathe & Chevallier 1976, Staels *et al.* 1990, Ness & Zhao 1994), and the degradation of cholesterol into bile acids, where the rate-limiting CYP7A is transcriptionally induced by T3 (Mathe & Chevallier 1976, Crestani *et al.* 1994, Pandak *et al.* 1997). The normal stimulation of CYP7A mRNA and enzyme activity is unaffected in TRα1$^{-/-}$ mice, whereas T3 responsiveness is absent in TRβ$^{-/-}$ animals, indicating that TRβ plays a key role in the regulation of hepatic cholesterol metabolism. Further work, however, has demonstrated an enhanced CYP7A response in T3-deficient TRβ$^{-/-}$ mice challenged with dietary cholesterol such that mutant mice did not develop hypercholesterolaemia to the same extent as wild-type controls. The results suggest that TRs influence a range of regulatory effects that may be independent of circulating T3 (Gullberg *et al.* 2000).

Heart and thermogenesis

Thyroid hormones influence cardiac conductance, heart rate, cardiac output and cardiac muscle growth. Hypothyroidism leads to bradycardia, whereas hyperthyroidism induces tachycardia. Thyroid hormone plays a crucial role in the control of thermogenesis with hypothyroidism leading to reduced oxygen consumption and heat production. The action of T3 in the heart is mediated predominantly by TRα1 (Gloss *et al.* 2001) and TRβ1 is expressed in the heart at low levels (Falcone *et al.* 1992). Wikstrom *et al.* (1998) reported that TRα1-deficient mice have a lower heart rate than control animals of the same genetic background. However, TRα1$^{-/-}$ mice are hypothyroid and when hyperthyroidism is induced the heart rate increases in both TRα1$^{-/-}$ and wild-type animals. Nevertheless, the response in TRα1$^{-/-}$ mice is suboptimal indicating that ablation of TRα1 reduces intrinsic heart rate and cardiac responsiveness to T3. Thus, TRβ may influence heart rate via mechanisms that are distinct from the actions of TRα1 or that compensate for it. Electrocardiograph (ECG) recordings revealed a prolonged QRS complex and QT end duration in TRα1$^{-/-}$ mice, findings that are similar to those in hypothyroid rat cardiomyocytes (Shimoni & Severson 1995) and hypothyroid patients.

Thyroid hormones control body temperature by stimulating gluconeogenesis and increasing thermogenesis in brown adipose tissue. The 24-hour mean core body temperature was 0·5 °C lower in TRα1$^{-/-}$ mice, with no significant difference in locomotor activity compared with heterozygotes and wild-type animals. TRα1$^{-/-}$ mice also have normal brown adipose tissue mass, suggesting that reduced body temperature results from altered metabolic activity (Wikstrom *et al.* 1998). TRβ$^{-/-}$ mice, however, have normal body temperature suggesting that the involvement of TRβ in basal thermogenesis is minor, or may be substituted by TRα1 (Gauthier *et al.* 2001). There is also a slight increase in heart rate in TRβ$^{-/-}$ mice compared with wild-type mice that is not relieved by T3 administration (Weiss *et al.* 1998, Johansson *et al.* 1999). TRβ$^{-/-}$ mice have a reduced QT end time, but exhibit no other ECG abnormalities (Johansson *et al.* 1999). HCN2 and HCN4 are cardiac genes that contribute to pacemaker activity by coding for the hyperpolarisation-activated current (Ludwig *et al.* 1998). In TRβ$^{-/-}$ mice, levels of HCN2 and HCN4 are significantly increased, leading to an increase in contractile function. However, when TRβ$^{-/-}$ mice were rendered euthyroid, heart rate and HCN gene expression was normalised (Gloss *et al.* 2001), suggesting that T3 responsiveness is mediated via TRα. Furthermore, in Pax8$^{-/-}$ mice, HCN2 mRNA expression is extremely low, although it is inducible by T3. In compound Pax8$^{-/-}$ TRα$^{0/0}$ mutants, HCN2 expression is restored whereas in Pax8$^{-/-}$TRβ$^{-/-}$ animals expression remains low. These results indicate that repression of basal HCN2 expression is mediated via unliganded TRα1 (Flamant *et al.* 2002).

Deletion of both TRα1 and TRα2 in TRα$^{-/-}$ mice results in bradycardia and decreased HCN2 and HCN4 gene expression, together with reduced myocardial contractility (Gloss *et al.* 2001). TRα$^{0/0}$ mice, similarly, exhibit a reduced basal heart rate (Macchia *et al.* 2001) and also have a 0·4 °C reduction in body temperature (Gauthier *et al.* 2001). Reduced cardiac sensitivity to dynamic testing in TRα$^{0/0}$ mice indicates that TRα is the major functional cardiac TR isoform (Macchia *et al.* 2001). Furthermore, body temperature data from TRα$^{0/0}$ mice imply that TRα2 has no effect on thermogenesis under basal conditions (Gauthier *et al.* 2001). TRα1$^{-/-}$β$^{-/-}$ animals have a similar phenotype to TRα$^{-/-}$ animals, exhibiting basal bradycardia, prolonged PQ and QT end durations, and a 0·4 °C reduction in body temperature relative to wild-type mice (Johansson *et al.* 1998, 1999). TRα$^{0/0}$β$^{-/-}$ mice have a marked mean body temperature reduction of 4 °C compared with wild-type mice (Gauthier *et al.* 2001) and reduced HCN2 gene expression (Flamant *et al.* 2002). Thus, comparison of temperature reductions in TRα$^{-/-}$β$^{-/-}$ (0·5 °C) and TRα$^{0/0}$ β$^{-/-}$ (4 °C) mice reveals that TRα2 may exert important actions to regulate thermogenesis (Gauthier *et al.*

2001). Nevertheless, in TRα2$^{-/-}$ mice (Salto *et al.* 2001) a 10% decrease in basal heart rate together with a 0·4 °C increase in body temperature was reported. These data are difficult to interpret, however, because of the overexpression of TRα1 in TRα2$^{-/-}$ mice that results from the targeting strategy.

In TRβ$^{\Delta 337T}$ transgenic mice, two groups assessed the cardiac phenotype by expressing the transgene either generally (Gloss *et al.* 1999) or specifically in the myocardium (Pazos-Moura *et al.* 2000). The expression of cardiac myosin genes is influenced by T3 (Hoh *et al.* 1978, Everett *et al.* 1984, Klein & Hong 1986). In euthyroidism, αMHC is the predominant MHC isoform, its expression is increased in thyrotoxicosis, whereas βMHC is predominant in hypothyroidism (Izumo *et al.* 1986). In both Δ337T transgenic models, basal heart rate was reduced. Expression of βMHC was increased together with a parallel reduction in αMHC expression and ECG studies demonstrated broadening of the QRS complex, indicating prolongation of ventricular depolarisation time. In myocardium-specific Δ337T mice, the PQ, QR and ST intervals were also prolonged (Pazos-Moura *et al.* 2000), which was not the case in mice harbouring the ubiquitously expressed Δ337T transgene (Gloss *et al.* 1999); although both models displayed a hypothyroid pattern of gene expression, ECG changes and contractile function despite normal T3, T4 and TSH levels.

Central nervous system (CNS) and behaviour

The importance of thyroid hormones in the development of the CNS is well known. TRs are distributed widely throughout the brain, in neurons and glial cells, and the profile of TR expression in different brain regions has been suggested to implicate specific functions for different TR isoforms in CNS development (Forrest *et al.* 1990, Bradley *et al.* 1992).

In TRα$^{-/-}$ mice, brain size was marginally reduced, but no morphological or cellular differences were observed (Fraichard *et al.* 1997). No differences were noted in laminar organisation or brain cytoarchitecture by Nissl staining. Histochemical analysis of acetylcholine esterase and cytochrome oxidase showed no differences in cortical structure or subcortical nuclear organisation between TRα$^{-/-}$ and wild-type mice (Fraichard *et al.* 1997). Studies were also performed in TRβ$^{-/-}$ mice (Forrest *et al.* 1996*b*). TRβ$^{-/-}$ animals completed the Morris water maze test with equal proficiency to wild-type controls. Open field and Y-Maze testing produced similar results. No abnormalities in brain anatomy, including T3-sensitive structures such as the cerebellum and hippocampus, were observed following histological analysis. No overt neurological defects were seen, indicating that the TRβ gene may have a subtle rather than a major role in CNS development (Forrest *et al.* 1996*b*). TRβ is induced in neonatal CNS at a critical T3-sensitive period (Forrest *et al.* 1991, Mellstrom *et al.* 1991) and the rise in TRβ1 occurs with the onset of the T3-dependent regulation of genes including myelin basic protein (MBP) and Purkinje cell protein-2 (Pcp-2) (Strait *et al.* 1992). However, whilst the onset of MBP expression is delayed in the hypothyroid mouse (Farsetti *et al.* 1992), no differences in MBP or Pcp-2 mRNA levels were observed in TRβ$^{-/-}$ mice compared with wild-type controls, suggesting that TRβ is not critical for the normal postnatal expression of these T3-dependent genes (Sandhofer *et al.* 1998). Taken together, these data suggest that TRα and TRβ mediate redundant actions in the CNS.

To investigate further, TRα1$^{-/-}$β$^{-/-}$ mice, lacking both TRα1 and TRβ, were examined (Gothe *et al.* 1999). Although these mice show hypothyroid features, they have normal activity and do not fatigue (Johansson *et al.* 1999). Macroscopic examination of TRα1$^{-/-}$β$^{-/-}$ brains showed no gross cellular or morphological abnormalities compared with wild-type controls. However, TRH mRNA levels were markedly elevated in the paraventricular nucleus (PVN) and medullary raphe nuclei of TRα1$^{-/-}$β$^{-/-}$ animals, whereas substance P mRNA, a peptide coexpressed with TRH in the medullary raphe nuclei, was unchanged. Differential regulation of TRH receptor mRNA was observed in specific regions of the TRα1$^{-/-}$β$^{-/-}$ brain, with levels increased in the motor neurons of the trigeminal nucleus, unchanged in subiculum and decreased in the amygdala. Galanin mRNA was also reduced in the PVN of TRα1$^{-/-}$β$^{-/-}$ mice, although levels were unchanged in the dorsomedial and arcuate nuclei. In the arcuate nucleus, glutamic acid decarboxylase (GAD) and prepro-neuropeptide Y (NPY) mRNA levels were not altered. However, tyrosine hydroxylase (th) mRNA levels were decreased. In the olfactory bulb, reduced calbindin immunostaining in the glomerular layer with a decrease of calbindin-immunoreactive neurons was seen in TRα1$^{-/-}$β$^{-/-}$ mice, and reduced mRNA levels were recorded in the glomerular layer. Also, GAD mRNA levels were selectively reduced in the glomerular layer of the olfactory bulb of double mutant mice, but galanin and NPY mRNA expression was unchanged. The regulation of TRH signalling pathways in TRα1$^{-/-}$β$^{-/-}$ mice is, therefore, complex and regionally selective. In TRα1$^{-/-}$β$^{-/-}$ animals, the reduction in calbindin- and th-positive dendrites may also indicate impaired neuronal maturation in the olfactory bulb (Calza *et al.* 2000). Galanin expression is also selectively altered in the PVN of double mutant mice. It has a role in the regulation of pulsatile GH secretion (Maiter *et al.* 1990) and reduced galanin mRNA levels in the PVN may be involved in growth impairment seen in TRα1$^{-/-}$β$^{-/-}$ mice (Calza *et al.* 2000). Since thyroid hormones are such key regulators of CNS development, it is surprising that more extensive abnormalities were not observed in TRα1$^{-/-}$β$^{-/-}$ mice. It is likely that redundancy between complex compensatory pathways may account for the minimal phenotype.

In behavioural tests, TRβ$^{-/-}$ animals show no abnormalities and no gross CNS phenotype is observed in TRα- or TRβ-deficient animals. However, recent data suggest that the TRα1 and TRβ genes may have opposite effects on the sexual behaviour of female mice (Dellovade *et al.* 2000). Both molecular (Zhu *et al.* 1996*b*, Dellovade *et al.* 1999*a*,*b*) and behavioural (Dellovade *et al.* 1996) studies have shown that thyroid hormones affect the neural systems mediating oestrogen-dependent sexual behaviour in rodents. Dellovade *et al.* (2000) reported that TRβ$^{-/-}$ female mice show increased sexual behaviour, whereas, female TRα1$^{-/-}$ animals display a reduced lordosis pattern. In the brain, the number of oestrogen-receptor immunoreactive neurons were similar across wild-type, TRα1$^{-/-}$ and TRβ$^{-/-}$ genotypes. However, the number of oxytocin immunoreactive neurons in the PVN was increased in TRβ$^{-/-}$ animals, and a concomitant reduction was observed in TRα1$^{-/-}$ mice (Dellovade *et al.* 2000). Oxytocin has been reported to facilitate a variety of reproductive and affiliative behaviours (Pedersen *et al.* 1992). Thus, oestrogen-influenced oxytocin immunoreactivity in the PVN may explain, in part, the behavioural results of TRα1$^{-/-}$ and TRβ$^{-/-}$ mice. Although, in many physiological systems, there is co-operation between TRs, this specific behaviour pattern reveals functional antagonism between TRα1 and TRβ (Dellovade *et al.* 2000).

In mice with the RTH TRβ$^{\Delta337T}$ mutation, marked impairment in balance and co-ordination was observed (Hashimoto *et al.* 2001) following beam and rotorod testing (Scherbel *et al.* 1999). In the Morris water maze test (Forrest *et al.* 1996*b*), both heterozygous and homozygous mutants exhibited a learning deficit (Hashimoto *et al.* 2001). The defect was similar to that seen in congenital hypothyroidism (Anthony *et al.* 1993). TRβ$^{\Delta337T}$ mice have a smaller cerebellum, although they exhibit no reduction in the size of the forebrain. Haematoxylin and eosin staining of the hippocampus and dentate gyrus revealed no gross morphological differences. However, hippocampal brain-derived neurotrophic factor immunoreactivity was reduced in heterozygous and homozygous mutants. The number of stained granule cells and fibres was especially reduced in the CA3 hippocampal region. TRβ$^{\Delta337T}$ mice exhibit hypothyroid CNS changes, despite elevated thyroid hormone levels (Hashimoto *et al.* 2001), indicating a dominant negative effect of the mutant receptor *in vivo*. A transgenic mouse with TRβ$^{\Delta337T}$ selectively targeted to the pituitary has also been developed (Abel *et al.* 1999*b*). These mice show significant down-regulation of prepro-TRH mRNA in the caudal paraventricular hypothalamus. Regional differences in TRH gene expression within the PVN and reduced PVN prepro-TRH mRNA were also observed, suggesting that hypothalamic TRH neurons are extremely sensitive to changes in thyroid status (Abel *et al.* 1999*b*).

Transgenic mice expressing the PV mutant TRβ exhibit a hyperactivity behavioural phenotype (Wong *et al.* 1997). A clinical feature of human RTH is attention deficit hyperactivity disorder, which affects 73% of children and 42% of adults (Brucker-Davis *et al.* 1995). The development of structure and function in the mammalian brain is accompanied by marked increases in local rates of glucose utilisation (Duffy *et al.* 1982, Kennedy *et al.* 1982), which may be sensitive to circulating thyroid hormone levels (Dow-Edwards *et al.* 1986). In targeted knock-in mutant mice, TRβ^{PV} mRNA has been detected in cerebrum, cerebellum, pituitary gland and systemic organs (Kaneshige *et al.* 2000) and expression of TRα1^{PV} mRNA has been identified in both cerebrum and cerebellum (Itoh *et al.* 2001). Following determination by the quantitative autoradiographic 2-[^{14}C]deoxyglucose method (Sokoloff *et al.* 1977), cerebral glucose utilisation was significantly lower in 4-week-old TRα1$^{PV/+}$ mice compared with wild-type mice. TRβ$^{PV/PV}$ animals, of the same age, showed no change (Itoh *et al.* 2001). Expression of Srg1, a member of a protein family involved in the regulation of neurotransmitter release (Fernandez-Chacon *et al.* 2001) and a T3-positively regulated gene expressed in several brain regions (Thompson 1996), was reduced to 40% in the cerebellum of TRα1$^{PV/+}$ animals, with no change seen in TRβ$^{PV/+}$ or TRβ$^{PV/PV}$ mice (Itoh *et al.* 2001). Abnormal expression of T3 target genes was also observed in the cerebellum of TRα1$^{PV/+}$ mice. No change of MBP and Pcp-2 expression was seen in TRβ$^{PV/+}$ animals, although activations of 1·4- and 1·2-fold were recorded for MBP and Pcp-2 genes respectively in TRα1$^{PV/+}$ mice (Kaneshige *et al.* 2001).

Immune system

A role for thyroid hormones in B and T lymphocyte development has been revealed in Snell dwarf mice (Fabris *et al.* 1971, Murphy *et al.* 1992, 1993, Montecino-Rodriguez *et al.* 1996, 1997). Arpin *et al.* (2000) studied TRα$^{-/-}$ mice to determine T3 targets in the immune system and the TRs involved in lymphoid development. They found that the size of the mature B cell pool is altered due to a reduction in progenitor cell proliferation. In TRα$^{-/-}$ mice, no change in primary (thymus and bone marrow) or secondary (spleen) lymphoid organ cell numbers were seen at 10 days of age; however, by postnatal day 24, cell numbers in all lymphoid organs were reduced. The cellularity of T and B cells was reduced by 30% and 60% respectively, and the number of spleen granulocytes and macrophages was also reduced. There was also evidence of haemopoiesis in the TRα$^{-/-}$ spleen (Arpin *et al.* 2000). TRα$^{-/-}$ mice suffer from stress-induced defects, which may influence immune function.

Glucocorticoids down-regulate numerous immune processes and induce lymphocyte apoptosis (Wilder 1995). Cortisol levels were, therefore, measured in TR$\alpha^{-/-}$ mice, but no differences between TR$\alpha^{-/-}$ and wild-type mice were observed. Double positive thymocytes are sensitive to glucocorticoid-induced depletion of lymphocytes (Gruber *et al.* 1994) providing an objective index of stress. Up to 24 days of age, CD4^{+}CD8^{+} cells remained unaffected in TR$\alpha^{-/-}$ mice suggesting an absence of stress-induced immune modifications. Thus, T3 acts directly on B cells and this may be via control of progenitor cell proliferation or differentiation by TRα1 or α2 (Arpin *et al.* 2000). Nevertheless, it has been proposed that T3 may act via stimulation of GH–IGF-I secretion from B cells (Weigent *et al.* 1992, Geffner 1997), but this appears unlikely since GH cannot restore the pre-B cell compartment in Snell mice (Montecino-Rodriguez *et al.* 1996). Thus, development of the immune system may be influenced by TRα, acting directly on B cells and via the cellular environment on T cells. Data from TR$\beta^{-/-}$ and TR$\alpha^{-/-}\beta^{-/-}$ mice reveal decreased macrophage, granulocyte and T and B lymphocyte cellularity in TR$\beta^{-/-}$ mice, with a further reduction in TR$\alpha^{-/-}$ $\beta^{-/-}$ animals, indicating that lymphocyte development requires both TRα and β (Arpin *et al.* 2000).

Finally, Flamant *et al.* (2002) reported that TR$\alpha^{0/0}$ $\beta^{-/-}$ mice show a marked reduction in the size of the spleen, a finding that is more pronounced in Pax8$^{-/-}$ mice. Pax8$^{-/-}$ animals were observed to show a marked reduction in splenic follicle number and size. The spleen was also severely affected in Pax8$^{-/-}$TR$\beta^{-/-}$ double mutants (Flamant *et al.* 2002), whilst Pax8$^{-/-}$TR$\alpha^{0/0}$ double mutants showed a recovery of splenic phenotype (Flamant *et al.* 2002), suggesting that unliganded TRα isoforms, which may include the TRΔα variants, play an important role in spleen development.

Summary

Analysis of the literature indicates that understanding the mechanism of T3 action *in vivo* has been advanced significantly by the generation of transgenic and knockout mice involving the TRα and β genes. These mice have provided detailed insight into the roles of the various TR isoforms. However, it is prudent to interpret data obtained from genetically modified mice with caution because of the unavoidable compensatory biological responses that occur following gene deletion or over-expression. Such responses may reveal phenotypes that are indirectly related to TR function or may mask important direct actions of TRs that can be performed by other, normally redundant, pathways. Furthermore, whilst comparisons between genetically modified mice can be very informative, an additional drawback is that different laboratories engineer mutations in mice with different genetic backgrounds. Such differences may result in significant phenotypic modification. In future studies, therefore, it will be helpful to compare mutations of the TRα and β genes on uniform genetic backgrounds. In spite of these caveats, genetically modified mice have enabled T3-signalling pathways to be dissected in the intact animal. They have revealed the enormous complexity of TR function and provided a new and deeper understanding of the physiological actions of thyroid hormones.

References

Abel ED, Boers ME, Pazos-Moura C, Moura E, Kaulbach H, Zakaria M, Lowell B, Radovick S, Liberman MC & Wondisford F 1999*a* Divergent roles for thyroid hormone receptor beta isoforms in the endocrine axis and auditory system. *Journal of Clinical Investigation* **104** 291–300.

Abel ED, Kaulbach HC, Campos-Barros A, Ahima RS, Boers ME, Hashimoto K, Forrest D & Wondisford FE 1999*b* Novel insight from transgenic mice into thyroid hormone resistance and the regulation of thyrotropin. *Journal of Clinical Investigation* **103** 271–279.

Amma LL, Campos-Barros A, Wang Z, Vennstrom B & Forrest D 2001 Distinct tissue-specific roles for thyroid hormone receptors beta and alpha1 in regulation of type 1 deiodinase expression. *Molecular Endocrinology* **15** 467–475.

Anthony A, Adams PM & Stein SA 1993 The effects of congenital hypothyroidism using the hyt/hyt mouse on locomotor activity and learned behavior. *Hormones and Behaviour* **27** 418–433.

Arpin C, Pihlgren M, Fraichard A, Aubert D, Samarut J, Chassande O & Marvel J 2000 Effects of T3R alpha 1 and T3R alpha 2 gene deletion on T and B lymphocyte development. *Journal of Immunology* **164** 152–160.

Ball SG, Ikeda M & Chin WW 1997 Deletion of the thyroid hormone beta1 receptor increases basal and triiodothyronine-induced growth hormone messenger ribonucleic acid in GH3 cells. *Endocrinology* **138** 3125–3132.

Baniahmad A, Tsai SY, O'Malley BW & Tsai MJ 1992 Kindred S thyroid hormone receptor is an active and constitutive silencer and a repressor for thyroid hormone and retinoic acid responses. *PNAS* **89** 10633–10637.

Beamer WG, Eicher EM, Maltais LJ & Southard JC 1981 Inherited primary hypothyroidism in mice. *Science* **212** 61–63.

Beck-Peccoz P, Amr S, Menezes-Ferreira MM, Faglia G & Weintraub BD 1985 Decreased receptor binding of biologically inactive thyrotropin in central hypothyroidism. Effect of treatment with thyrotropin-releasing hormone. *New England Journal of Medicine* **312** 1085–1090.

Bianco AC, Salvatore D, Gereben B, Berry MJ & Larsen PR 2002 Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocrine Reviews* **23** 38–89.

Bradley DJ, Towle HC & Young WS 1992 Spatial and temporal expression of alpha- and beta-thyroid hormone receptor mRNAs, including the beta 2-subtype, in the developing mammalian nervous system. *Journal of Neuroscience* **12** 2288–2302.

Bradley DJ, Towle HC & Young WS 1994 Alpha and beta thyroid hormone receptor (TR) gene expression during auditory neurogenesis: evidence for TR isoform-specific transcriptional regulation *in vivo*. *PNAS* **91** 439–443.

Brucker-Davis F, Skarulis MC, Grace MB, Benichou J, Hauser P, Wiggs E & Weintraub BD 1995 Genetic and clinical features of 42 kindreds with resistance to thyroid hormone. The National Institutes of Health Prospective Study. *Annals of Internal Medicine* **123** 572–583.

Caiozzo VJ, Swoap S, Tao M, Menzel D & Baldwin KM 1993 Single fiber analyses of type IIA myosin heavy chain distribution in hyper- and hypothyroid soleus. *American Journal of Physiology* **265** C842–C850.

Calza L, Forrest D, Vennstrom B & Hokfelt T 2000 Expression of peptides and other neurochemical markers in hypothalamus and olfactory bulb of mice devoid of all known thyroid hormone receptors. *Neuroscience* **101** 1001–1012.

Castillo RO, Glasscock GF, Noren KM & Reisenauer AM 1991 Pituitary regulation of postnatal small intestinal ontogeny in the rat: differential regulation of digestive hydrolase maturation by thyroxine and growth hormone. *Endocrinology* **129** 1417–1423.

Chassande O, Fraichard A, Gauthier K, Flamant F, Legrand C, Savatier P, Laudet V & Samarut J 1997 Identification of transcripts initiated from an internal promoter in the c-erbA alpha locus that encode inhibitors of retinoic acid receptor-alpha and triiodothyronine receptor activities. *Molecular Endocrinology* **11** 1278–1290.

Crestani M, Karam WG & Chiang JY 1994 Effects of bile acids and steroid/thyroid hormones on the expression of cholesterol 7 alpha-hydroxylase mRNA and the CYP7 gene in HepG2 cells. *Biochemical and Biophysical Research Communications* **198** 546–553.

Dellovade TL, Zhu YS, Krey & Pfaff DW 1996 Thyroid hormone and estrogen interact to regulate behavior. *PNAS* **93** 12581–12586.

Dellovade TL, Kia HK, Zhu YS & Pfaff DW 1999*a* Thyroid hormone coadministration inhibits the estrogen-stimulated elevation of preproenkephalin mRNA in female rat hypothalamic neurons. *Neuroendocrinology* **70** 168–174.

Dellovade TL, Zhu YS & Pfaff DW 1999*b* Thyroid hormones and estrogen affect oxytocin gene expression in hypothalamic neurons. *Journal of Neuroendocrinology* **11** 1–10.

Dellovade TL, Chan J, Vennstrom B, Forrest D & Pfaff DW 2000 The two thyroid hormone receptor genes have opposite effects on estrogen-stimulated sex behaviors. *Nature Neuroscience* **3** 472–475.

DeLong GR 1993 Effects of nutrition on brain development in humans. *American Journal of Clinical Nutrition* **57** 286S–290S.

Deol MS 1973 An experimental approach to the understanding and treatment of hereditary syndromes with congenital deafness and hypothyroidism. *Journal of Medical Genetics* **10** 235–242.

Dow-Edwards D, Crane AM, Rosloff B, Kennedy C & Sokoloff L 1986 Local cerebral glucose utilization in the adult cretinous rat. *Brain Research* **373** 139–145.

Duffy TE, Cavazzuti M, Cruz NF & Sokoloff L 1982 Local cerebral glucose metabolism in newborn dogs: effects of hypoxia and halothane anesthesia. *Annals of Neurology* **11** 233–246.

Duluc I, Lorentz O, Fritsch C, Leberquier C, Kedinger M & Freund JN 1997 Changing intestinal connective tissue interactions alters homeobox gene expression in epithelial cells. *Journal of Cell Science* **110** 1317–1324.

Ennion S, Sant'ana Pereira J, Sargeant AJ, Young A & Goldspink G 1995 Characterization of human skeletal muscle fibres according to the myosin heavy chains they express. *Journal of Muscle Research and Cell Motility* **16** 35–43.

Everett AW, Sinha AM, Umeda PK, Jakovcic S, Rabinowitz M & Zak R 1984 Regulation of myosin synthesis by thyroid hormone: relative change in the alpha- and beta-myosin heavy chain mRNA levels in rabbit heart. *Biochemistry* **23** 1596–1599.

Fabris N, Pierpaoli W & Sorkin E 1971 Hormones and the immunological capacity. 3. The immunodeficiency disease of the hypopituitary Snell–Bagg dwarf mouse. *Clinical and Experimental Immunology* **9** 209–225.

Falcone M, Miyamoto T, Fierro-Renoy F, Macchia E & DeGroot LJ 1992 Antipeptide polyclonal antibodies specifically recognize each human thyroid hormone receptor isoform. *Endocrinology* **131** 2419–2429.

Farsetti A, Desvergne B, Hallenbeck P, Robbins J & Nikodem VM 1992 Characterization of myelin basic protein thyroid hormone response element and its function in the context of native and heterologous promoter. *Journal of Biological Chemistry* **267** 15784–15788.

Fernandez-Chacon R, Konigstorfer A, Gerber SH, Garcia J, Matos MF, Stevens CF, Brose N, Rizo J, Rosenmund C & Sudhof TC 2001 Synaptotagmin I functions as a calcium regulator of release probability. *Nature* **410** 41–49.

Flamant F, Poguet AL, Plateroti M, Chassande O, Gauthier K, Streichenberger N, Mansouri A & Samarut J 2002 Congenital hypothyroid Pax8(−/−) mutant mice can be rescued by inactivating the TRalpha gene. *Molecular Endocrinology* **16** 24–32.

Forrest D, Sjoberg M & Vennstrom B 1990 Contrasting developmental and tissue-specific expression of alpha and beta thyroid hormone receptor genes. *EMBO Journal* **9** 1519–1528.

Forrest D, Hallbook F, Persson H & Vennstrom B 1991 Distinct functions for thyroid hormone receptors alpha and beta in brain development indicated by differential expression of receptor genes. *EMBO Journal* **10** 269–275.

Forrest D, Erway LC, Ng L, Altschuler R & Curran T 1996*a* Thyroid hormone receptor beta is essential for development of auditory function. *Nature Genetics* **13** 354–357.

Forrest D, Hanebuth E, Smeyne RJ, Everds N, Stewart CL, Wehner JM & Curran T 1996*b* Recessive resistance to thyroid hormone in mice lacking thyroid hormone receptor beta: evidence for tissue-specific modulation of receptor function. *EMBO Journal* **15** 3006–3015.

Fraichard A, Chassande O, Plateroti M, Roux JP, Trouillas J, Dehay C, Legrand C, Gauthier K, Kedinger M, Malaval L, Rousset B & Samarut J 1997 The T3R alpha gene encoding a thyroid hormone receptor is essential for post-natal development and thyroid hormone production. *EMBO Journal* **16** 4412–4420.

Freeman S, Geal-Dor M, Shimoni Y & Sohmer H 1993 Thyroid hormone induces earlier onset of auditory function in neonatal rats. *Hearing Research* **69** 229–235.

Freund JN, Boukamel R & Benazzouz A 1992 Gradient expression of Cdx along the rat intestine throughout postnatal development. *FEBS Letters* **314** 163–166.

Gambke B, Lyons GE, Haselgrove J, Kelly AM & Rubinstein NA 1983 Thyroidal and neural control of myosin transitions during development of rat fast and slow muscles. *FEBS Letters* **156** 335–339.

Gauthier K, Chassande O, Plateroti M, Roux JP, Legrand C, Pain B, Rousset B, Weiss R, Trouillas J & Samarut J 1999 Different functions for the thyroid hormone receptors TRalpha and TRbeta in the control of thyroid hormone production and post-natal development. *EMBO Journal* **18** 623–631.

Gauthier K, Plateroti M, Harvey CB, Williams GR, Weiss RE, Refetoff S, Willott JF, Sundin V, Roux JP, Malaval L, Hara M, Samarut J & Chassande O 2001 Genetic analysis reveals different functions for the products of the thyroid hormone receptor alpha locus. *Molecular and Cellular Biology* **21** 4748–4760.

Geffner M 1997 Effects of growth hormone and insulin-like growth factor I on T- and B-lymphocytes and immune function. *Acta Paediatrica* **423** (Suppl) 76–79.

Gloss B, Sayen MR, Trost SU, Bluhm WF, Meyer M, Swanson EA, Usala SJ & Dillmann WH 1999 Altered cardiac phenotype in transgenic mice carrying the delta337 threonine thyroid hormone receptor beta mutant derived from the S family. *Endocrinology* **140** 897–902.

Gloss B, Trost S, Bluhm W, Swanson E, Clark R, Winkfein R, Janzen K, Giles W, Chassande O, Samarut J & Dillmann W 2001 Cardiac ion channel expression and contractile function in mice with deletion of thyroid hormone receptor alpha or beta. *Endocrinology* **142** 544–550.

Gothe S, Wang Z, Ng L, Kindblom JM, Barros AC, Ohlsson C, Vennstrom B & Forrest D 1999 Mice devoid of all known thyroid hormone receptors are viable but exhibit disorders of the pituitary–thyroid axis, growth, and bone maturation. *Genes and Development* **13** 1329–1341.

Gruber J, Sgonc R, Hu YH, Beug H & Wick G 1994 Thymocyte apoptosis induced by elevated endogenous corticosterone levels. *European Journal of Immunology* **24** 1115–1121.

Gullberg H, Rudling M, Forrest D, Angelin B & Vennstrom B 2000 Thyroid hormone receptor beta-deficient mice show complete loss of the normal cholesterol 7 alpha-hydroxylase (CYP7A) response to thyroid hormone but display enhanced resistance to dietary cholesterol. *Molecular Endocrinology* **14** 1739–1749.

Hashimoto K, Curty FH, Borges PP, Lee CE, Abel ED, Elmquist JK, Cohen RN & Wondisford FE 2001 An unliganded thyroid hormone receptor causes severe neurological dysfunction. *PNAS* **98** 3998–4003.

Hayashi Y, Xie J, Weiss RE, Pohlenz J & Refetoff S 1998 Selective pituitary resistance to thyroid hormone produced by expression of a mutant thyroid hormone receptor beta gene in the pituitary gland of transgenic mice. *Biochemical and Biophysical Research Communications* **245** 204–210.

Hebert R, Langlois JM & Dussault JH 1985 Permanent defects in rat peripheral auditory function following perinatal hypothyroidism: determination of a critical period. *Brain Research* **355** 161–170.

Hodin RA, Lazar MA, Wintman BI, Darling DS, Koenig RJ, Larsen PR, Moore DD & Chin WW 1989 Identification of a thyroid hormone receptor that is pituitary-specific. *Science* **244** 76–79.

Hodin RA, Shei A, Morin M & Meng S 1996 Thyroid hormone and the gut: selective transcriptional activation of a villus-enterocyte marker. *Surgery* **120** 138–143.

Hoh JF, McGrath PA & Hale PT 1978 Electrophoretic analysis of multiple forms of rat cardiac myosin: effects of hypophysectomy and thyroxine replacement. *Journal of Molecular and Cellular Cardiology* **10** 1053–1076.

Itoh Y, Esaki T, Kaneshige M, Suzuki H, Cook M, Sokoloff L, Cheng SY & Nunez J 2001 Brain glucose utilization in mice with a targeted mutation in the thyroid hormone alpha or beta receptor gene. *PNAS* **98** 9913–9918.

Izumo S, Nadal-Ginard B & Mahdavi V 1986 All members of the MHC multigene family respond to thyroid hormone in a highly tissue-specific manner. *Science* **231** 597–600.

James R & Kazenwadel J 1991 Homeobox gene expression in the intestinal epithelium of adult mice. *Journal of Biological Chemistry* **266** 3246–3251.

Johansson C, Vennstrom B & Thoren P 1998 Evidence that decreased heart rate in thyroid hormone receptor-alpha1-deficient mice is an intrinsic defect. *American Journal of Physiology* **275** R640–R646.

Johansson C, Gothe S, Forrest D, Vennstrom B & Thoren P 1999 Cardiovascular phenotype and temperature control in mice lacking thyroid hormone receptor-beta or both alpha1 and beta. *American Journal of Physiology* **276** H2006–H2012.

Johansson C, Lannergren J, Lunde PK, Vennstrom B, Thoren P & Westerblad H 2000 Isometric force and endurance in soleus muscle of thyroid hormone receptor-alpha(1)- or -beta-deficient mice. *American Journal of Physiology. Regulatory, Integrated and Comparative Physiology* **278** R598–R603.

Kaneshige M, Kaneshige K, Zhu X, Dace A, Garrett L, Carter TA, Kazlauskaite R, Pankratz DG, Wynshaw-Boris A, Refetoff S, Weintraub B, Willingham MC, Barlow C & Cheng S 2000 Mice with a targeted mutation in the thyroid hormone beta receptor gene exhibit impaired growth and resistance to thyroid hormone. *PNAS* **97** 13209–13214.

Kaneshige M, Suzuki H, Kaneshige K, Cheng J, Wimbrow H, Barlow C, Willingham MC & Cheng S 2001 A targeted dominant negative mutation of the thyroid hormone alpha 1 receptor causes increased mortality, infertility, and dwarfism in mice. *PNAS* **98** 15095–15100.

Kelley MW, Turner JK & Reh TA 1995*a* Ligands of steroid/thyroid receptors induce cone photoreceptors in vertebrate retina. *Development* **121** 3777–3785.

Kelley MW, Turner JK & Reh TA 1995*b* Regulation of proliferation and photoreceptor differentiation in fetal human retinal cell cultures. *Investigative Ophthalmology and Visual Science* **36** 1280–1289.

Kennedy C, Sakurada O, Shinohara M & Miyaoka M 1982 Local cerebral glucose utilization in the newborn macaque monkey. *Annals of Neurology* **12** 333–340.

Kindblom JM, Gothe S, Forrest D, Tornell J, Vennstrom B & Ohlsson C 2001 GH substitution reverses the growth phenotype but not the defective ossification in thyroid hormone receptor alpha 1-/-beta-/- mice. *Journal of Endocrinology* **171** 15–22.

Klein I & Hong C 1986 Effects of thyroid hormone on cardiac size and myosin content of the heterotopically transplanted rat heart. *Journal of Clinical Investigation* **77** 1694–1698.

Lazar MA 1993 Thyroid hormone receptors: multiple forms, multiple possibilities. *Endocrine Reviews* **14** 184–193.

Lewinson D, Harel Z, Shenzer P, Silbermann M & Hochberg Z 1989 Effect of thyroid hormone and growth hormone on recovery from hypothyroidism of epiphyseal growth plate cartilage and its adjacent bone. *Endocrinology* **124** 937–945.

Lezoualc'h F, Hassan AH, Giraud P, Loeffler JP, Lee SL & Demeneix BA 1992 Assignment of the beta-thyroid hormone receptor to 3,5,3′-triiodothyronine-dependent inhibition of transcription from the thyrotropin-releasing hormone promoter in chick hypothalamic neurons. *Molecular Endocrinology* **6** 1797–1804.

Li S, Crenshaw EB, Rawson EJ, Simmons DM, Swanson LW & Rosenfeld MG 1990 Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene Pit-1 *Nature* **347** 528–533.

Lorentz O, Duluc I, Arcangelis AD, Simon-Assmann P, Kedinger M & Freund JN 1997 Key role of the Cdx2 homeobox gene in extracellular matrix-mediated intestinal cell differentiation. *Journal of Cellular Biology* **139** 1553–1565.

Ludwig A, Zong X, Jeglitsch M, Hofmann F & Biel M 1998 A family of hyperpolarization-activated mammalian cation channels. *Nature* **393** 587–591.

Lyubarsky AL, Falsini B, Pennesi ME, Valentini P & Pugh EN 1999 UV- and midwave-sensitive cone-driven retinal responses of the mouse: a possible phenotype for coexpression of cone photopigments. *Journal of Neuroscience* **19** 442–455.

Macchia PE, Takeuchi Y, Kawai T, Cua K, Gauthier K, Chassande O, Seo H, Hayashi Y, Samarut J, Murata Y, Weiss RE & Refetoff S 2001 Increased sensitivity to thyroid hormone in mice with complete deficiency of thyroid hormone receptor alpha. *PNAS* **98** 349–354.

Maia AL, Kieffer JD, Harney JW & Larsen PR 1995 Effect of 3,5,3′-triiodothyronine (T3) administration on dio1 gene expression and T3 metabolism in normal and type 1 deiodinase-deficient mice. *Endocrinology* **136** 4842–4849.

Maiter DM, Hooi SC, Koenig JI & Martin JB 1990 Galanin is a physiological regulator of spontaneous pulsatile secretion of growth hormone in the male rat. *Endocrinology* **126** 1216–1222.

Mansouri A, Chowdhury K & Gruss P 1998 Follicular cells of the thyroid gland require Pax8 gene function. *Nature Genetics* **19** 87–90.

Mathe D & Chevallier F 1976 Effects of the thyroid state on cholesterol metabolism in the rat. *Biochimica et Biophysica Acta* **441** 155–164.

Mellstrom B, Naranjo JR, Santos A, Gonzalez AM & Bernal J 1991 Independent expression of the alpha and beta c-erbA genes in developing rat brain. *Molecular Endocrinology* **5** 1339–1350.

Montecino-Rodriguez E, Clark R, Johnson A, Collins L & Dorshkind K 1996 Defective B cell development in Snell dwarf (dw/dw) mice can be corrected by thyroxine treatment. *Journal of Immunology* **157** 3334–3340.

Montecino-Rodriguez E, Clark RG, Powell-Braxton L & Dorshkind K 1997 Primary B cell development is impaired in mice with defects of the pituitary/thyroid axis. *Journal of Immunology* **159** 2712–2719.

Muller A, van der Linden GC, Zuidwijk MJ, Simonides WS, van der Laarse WJ & van Hardeveld C 1994 Differential effects of thyroid hormone on the expression of sarcoplasmic reticulum Ca(2+)-ATPase isoforms in rat skeletal muscle fibers. *Biochemical and Biophysical Research Communications* **203** 1035–1042.

Murphy WJ, Durum SK, Anver MR & Longo DL 1992 Immunologic and hematologic effects of neuroendocrine hormones. Studies on DW/J dwarf mice. *Journal of Immunology* **148** 3799–3805.

Murphy WJ, Durum SK & Longo DL 1993 Differential effects of growth hormone and prolactin on murine T cell development and function. *Journal of Experimental Medicine* **178** 231–236.

Ness GC & Zhao Z 1994 Thyroid hormone rapidly induces hepatic LDL receptor mRNA levels in hypophysectomized rats. *Archives of Biochemistry and Biophysics* **315** 199–202.

Ness GC, Dugan RE, Lakshmanan MR, Nepokroeff CM & Porter JW 1973 Stimulation of hepatic beta-hydroxy-beta-methylglutaryl coenzyme A reductase activity in hypophysectomized rats by L-triiodothyronine. *PNAS* **70** 3839–3842.

Ness GC, Pendleton LC, Li YC & Chiang JY 1990 Effect of thyroid hormone on hepatic cholesterol 7 alpha hydroxylase, LDL receptor, HMG-CoA reductase, farnesyl pyrophosphate synthetase and apolipoprotein A-I mRNA levels in hypophysectomized rats. *Biochemical and Biophysical Research Communications* **172** 1150–1156.

Newell FW & Diddie KR 1977 Typical monochromacy, congenital deafness, and resistance to intracellular action of thyroid hormone. *Klinische Monatsblatter fur Augenheilkunde* **171** 731–734.

Ng L, Forrest D, Haugen BR, Wood WM & Curran T 1995 N-terminal variants of thyroid hormone receptor beta: differential function and potential contribution to syndrome of resistance to thyroid hormone. *Molecular Endocrinology* **9** 1202–1213.

Ng L, Hurley JB, Dierks B, Srinivas M, Salto C, Vennstrom B, Reh TA & Forrest D 2001 A thyroid hormone receptor that is required for the development of green cone photoreceptors. *Nature Genetics* **27** 94–98.

O'Brien T, Dinneen SF, O'Brien P & Palumbo PJ 1993 Hyperlipidemia in patients with primary and secondary hypothyroidism. *Mayo Clinic Proceedings* **68** 860–866.

Ohlsson C, Bengtsson BA, Isaksson OG, Andreassen TT & Slootweg MC 1998 Growth hormone and bone. *Endocrine Reviews* **19** 55–79.

Pandak WM, Heuman DM, Redford K, Stravitz RT, Chiang JY, Hylemon PB & Vlahcevic ZR 1997 Hormonal regulation of cholesterol 7 alpha-hydroxylase specific activity, mRNA levels, and transcriptional activity *in vivo* in the rat. *Journal of Lipid Research* **38** 2483–2491.

Parrilla R, Mixson AJ, McPherson JA, McClaskey JH & Weintraub BD 1991 Characterization of seven novel mutations of the c-erbA beta gene in unrelated kindreds with generalized thyroid hormone resistance. Evidence for two 'hot spot' regions of the ligand binding domain. *Journal of Clinical Investigation* **88** 2123–2130.

Pazos-Moura C, Abel ED, Boers ME, Moura E, Hampton TG, Wang J, Morgan JP & Wondisford FE 2000 Cardiac dysfunction caused by myocardium-specific expression of a mutant thyroid hormone receptor. *Circulation Research* **86** 700–706.

Pedersen CA, Caldwell JD, Peterson G, Walker CH & Mason GA 1992 Oxytocin activation of maternal behavior in the rat. *Annals of the New York Academy of Sciences* **652** 58–69.

Persani L, Asteria C, Tonacchera M, Vitti P, Krishna V, Chatterjee K & Beck-Peccoz P 1994 Evidence for the secretion of thyrotropin with enhanced bioactivity in syndromes of thyroid hormone resistance. *Journal of Clinical Endocrinology and Metabolism* **78** 1034–1039.

Plateroti M, Chassande O, Fraichard A, Gauthier K, Freund JN, Samarut J & Kedinger M 1999 Involvement of T3 Ralpha- and beta-receptor subtypes in mediation of T3 functions during postnatal murine intestinal development. *Gastroenterology* **116** 1367–1378.

Plateroti M, Gauthier K, Domon-Dell C, Freund JN, Samarut J & Chassande O 2001 Functional interference between thyroid hormone receptor alpha (TRalpha) and natural truncated TRDeltaalpha isoforms in the control of intestine development. *Molecular and Cellular Biology* **21** 4761–4772.

Refetoff S, DeWind LT & DeGroot LJ 1967 Familial syndrome combining deaf-mutism, stuppled epiphyses, goiter and abnormally high PBI: possible target organ refractoriness to thyroid hormone. *Journal of Clinical Endocrinology and Metabolism* **27** 279–294.

Rohlich P, van Veen T & Szel A 1994 Two different visual pigments in one retinal cone cell. *Neuron* **13** 1159–1166.

Rusch A, Erway LC, Oliver D, Vennstrom B & Forrest D 1998 Thyroid hormone receptor beta-dependent expression of a potassium conductance in inner hair cells at the onset of hearing. *PNAS* **95** 15758–15762.

Safer JD, Langlois MF, Cohen R, Monden T, John-Hope D, Madura J, Hollenberg AN & Wondisford FE 1997 Isoform variable action among thyroid hormone receptor mutants provides insight into pituitary resistance to thyroid hormone. *Molecular Endocrinology* **11** 16–26.

Sakurai A, Takeda K, Ain K, Ceccarelli P, Nakai A, Seino S, Bell GI, Refetoff S & DeGroot LJ 1989 Generalized resistance to thyroid hormone associated with a mutation in the ligand-binding domain of the human thyroid hormone receptor beta. *PNAS* **86** 8977–8981.

Salto C, Kindblom JM, Johansson C, Wang Z, Gullberg H, Nordstrom K, Mansen A, Ohlsson C, Thoren P, Forrest D & Vennstrom B 2001 Ablation of TRalpha2 and a concomitant overexpression of alpha1 yields a mixed hypo- and hyperthyroid phenotype in mice. *Molecular Endocrinology* **15** 2115–2128.

Sandhofer C, Schwartz HL, Mariash CN, Forrest D & Oppenheimer JH 1998 Beta receptor isoforms are not essential for thyroid hormone-dependent acceleration of PCP-2 and myelin basic protein gene expression in the developing brains of neonatal mice. *Molecular and Cellular Endocrinology* **137** 109–115.

Sato K, Mimura H, Han DC, Tsushima T & Shizume K 1984 Ontogenesis of iodothyronine-5′-deiodinase. Induction of 5′-deiodinating activity by insulin, glucocorticoid, and thyroxine in cultured fetal mouse liver. *Journal of Clinical Investigation* **74** 2254–2262.

Sayen MR, Rohrer DK & Dillmann WH 1992 Thyroid hormone response of slow and fast sarcoplasmic reticulum Ca2+ ATPase mRNA in striated muscle. *Molecular and Cellular Endocrinology* **87** 87–93.

Scherbel U, Raghupathi R, Nakamura M, Saatman KE, Trojanowski JQ, Neugebauer E, Marino MW & McIntosh TK 1999 Differential acute and chronic responses of tumor necrosis factor-deficient mice to experimental brain injury. *PNAS* **96** 8721–8726.

Schiaffino S, Gorza L, Sartore S, Saggin L, Ausoni S, Vianello M, Gundersen K & Lomo T 1989 Three myosin heavy chain isoforms in type 2 skeletal muscle fibres. *Journal of Muscle Research and Cell Motility* **10** 197–205.

Schwartz HL, Lazar MA & Oppenheimer JH 1994 Widespread distribution of immunoreactive thyroid hormone beta 2 receptor (TR beta 2) in the nuclei of extrapituitary rat tissues. *Journal of Biological Chemistry* **269** 24777–24782.

Shi YB, Wong J, Puzianowska-Kuznicka M & Stolow MA 1996 Tadpole competence and tissue-specific temporal regulation of amphibian metamorphosis: roles of thyroid hormone and its receptors. *BioEssays* **18** 391–399.

Shimoni Y & Severson DL 1995 Thyroid status and potassium currents in rat ventricular myocytes. *American Journal of Physiology* **268** H576–H583.

Sjoberg M, Vennstrom B & Forrest D 1992 Thyroid hormone receptors in chick retinal development: differential expression of mRNAs for alpha and N-terminal variant beta receptors. *Development* **114** 39–47.

Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD, Sakurada O & Shinohara M 1977 The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. *Journal of Neurochemistry* **28** 897–916.

Sornson MW, Wu W, Dasen JS, Flynn S, Norman DJ, O'Connell SM, Gukovsky I, Carriere C, Ryan AK, Miller AP, Zuo L, Gleiberman AS, Andersen B, Beamer WG & Rosenfeld MG 1996 Pituitary lineage determination by the Prophet of Pit-1 homeodomain factor defective in Ames dwarfism. *Nature* **384** 327–333.

Staels B, Van Tol A, Chan L, Will H, Verhoeven G & Auwerx J 1990 Alterations in thyroid status modulate apolipoprotein, hepatic triglyceride lipase, and low density lipoprotein receptor in rats. *Endocrinology* **127** 1144–1152.

Stanbury JB 1984 The pathogenesis of endemic cretinism. *Journal of Endocrinological Investigation* **7** 409–419.

Stein SA, Oates EL, Hall CR, Grumbles RM, Fernandez LM, Taylor NA, Puett D & Jin S 1994 Identification of a point mutation in the thyrotropin receptor of the hyt/hyt hypothyroid mouse. *Molecular Endocrinology* **8** 129–138.

Stevens DA, Hasserjian RP, Robson H, Siebler T, Shalet SM & Williams GR 2000 Thyroid hormones regulate hypertrophic chondrocyte differentiation and expression of parathyroid hormone-related peptide and its receptor during endochondral bone formation. *Journal of Bone and Mineral Research* **15** 2431–2442.

Strait KA, Zou L & Oppenheimer JH 1992 Beta 1 isoform-specific regulation of a triiodothyronine-induced gene during cerebellar development. *Molecular Endocrinology* **6** 1874–1880.

Suh E & Traber PG 1996 An intestine-specific homeobox gene regulates proliferation and differentiation. *Molecular and Cellular Biology* **16** 619–625.

Szel A, van Veen T & Rohlich P 1994 Retinal cone differentiation. *Nature* **370** 336.

Szel A, Lukats A, Fekete T, Szepessy Z & Rohlich P 2000 Photoreceptor distribution in the retinas of subprimate mammals. *Journal of the Optical Society of America A (Optics, Image Science and Vision)* **17** 568–579.

Tata JR 1993 Gene expression during metamorphosis: an ideal model for post-embryonic development. *Bioessays* **15** 239–248.

Taylor T & Weintraub BD 1989 Altered thyrotropin (TSH) carbohydrate structures in hypothalamic hypothyroidism created by paraventricular nuclear lesions are corrected by *in vivo* TSH-releasing hormone administration. *Endocrinology* **125** 2198–2203.

Taylor T, Gesundheit N, Gyves PW, Jacobowitz DM & Weintraub BD 1988 Hypothalamic hypothyroidism caused by lesions in rat paraventricular nuclei alters the carbohydrate structure of secreted thyrotropin. *Endocrinology* **122** 283–290.

Thompson CC 1996 Thyroid hormone-responsive genes in developing cerebellum include a novel synaptotagmin and a hairless homolog. *Journal of Neuroscience* **16** 7832–7840.

Toyoda N, Zavacki AM, Maia AL, Harney JW & Larsen PR 1995 A novel retinoid X receptor-independent thyroid hormone response element is present in the human type 1 deiodinase gene. *Molecular and Cellular Biology* **15** 5100–5112.

Traber PG 1994 Differentiation of intestinal epithelial cells: lessons from the study of intestine-specific gene expression. *Journal of Laboratory and Clinical Medicine* **123** 467–477.

Usala SJ, Menke JB, Watson TL, Wondisford FE, Weintraub BD, Berard J, Bradley WE, Ono S, Mueller OT & Bercu BB 1991 A homozygous deletion in the c-erbA beta thyroid hormone receptor gene in a patient with generalized thyroid hormone resistance: isolation and characterization of the mutant receptor. *Molecular Endocrinology* **5** 327–335.

Uziel A 1986 Periods of sensitivity to thyroid hormone during the development of the organ of Corti. *Acta Oto-Laryngologica Supplement* **429** 23–27.

Uziel A, Gabrion J, Ohresser M & Legrand C 1981 Effects of hypothyroidism on the structural development of the organ of Corti in the rat. *Acta Oto-Laryngologica* **92** 469–480.

Wang Y, Macke JP, Merbs SL, Zack DJ, Klaunberg B, Bennett J, Gearhart J & Nathans J 1992 A locus control region adjacent to the human red and green visual pigment genes. *Neuron* **9** 429–440.

Weigent DA, Baxter JB & Blalock JE 1992 The production of growth hormone and insulin-like growth factor-I by the same subpopulation of rat mononuclear leukocytes. *Brain, Behavior and Immunity* **6** 365–376.

Weiss RE, Forrest D, Pohlenz J, Cua K, Curran T & Refetoff S 1997 Thyrotropin regulation by thyroid hormone in thyroid hormone receptor beta-deficient mice. *Endocrinology* **138** 3624–3629.

Weiss RE, Murata Y, Cua K, Hayashi Y, Seo H & Refetoff S 1998 Thyroid hormone action on liver, heart, and energy expenditure in thyroid hormone receptor beta-deficient mice. *Endocrinology* **139** 4945–4952.

Wikstrom L, Johansson C, Salto C, Barlow C, Campos Barros A, Baas F, Forrest D, Thoren P & Vennstrom B 1998 Abnormal heart rate and body temperature in mice lacking thyroid hormone receptor alpha 1. *EMBO Journal* **17** 455–461.

Wilder RL 1995 Neuroendocrine–immune system interactions and autoimmunity. *Annual Review of Immunology* **13** 307–338.

Williams GR 2000 Cloning and characterization of two novel thyroid hormone receptor beta isoforms. *Molecular and Cellular Biology* **20** 8329–8342.

Wong R, Vasilyev VV, Ting YT, Kutler DI, Willingham MC, Weintraub BD & Cheng S 1997 Transgenic mice bearing a human mutant thyroid hormone beta 1 receptor manifest thyroid function anomalies, weight reduction, and hyperactivity. *Molecular Medicine* **3** 303–314.

Yamada M, Saga Y, Shibusawa N, Hirato J, Murakami M, Iwasaki T, Hashimoto K, Satoh T, Wakabayashi K, Taketo MM & Mori M 1997 Tertiary hypothyroidism and hyperglycemia in mice with targeted disruption of the thyrotropin-releasing hormone gene. *PNAS* **94** 10862–10867.

Yu F, Gothe S, Wikstrom L, Forrest D, Vennstrom B & Larsson L 2000 Effects of thyroid hormone receptor gene disruption on myosin isoform expression in mouse skeletal muscles. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **278** R1545–R1554.

Zhu XG, Yu CL, McPhie P, Wong R & Cheng SY 1996*a* Understanding the molecular mechanism of dominant negative action of mutant thyroid hormone beta 1-receptors: the important role of the wild-type/mutant receptor heterodimer. *Endocrinology* **137** 712–721.

Zhu YS, Yen PM, Chin WW & Pfaff DW 1996*b* Estrogen and thyroid hormone interaction on regulation of gene expression. *PNAS* **93** 12587–12592.

Zhu XG, Kaneshige M, Parlow AF, Chen E, Hunziker RD, McDonald MP & Cheng SY 1999 Expression of the mutant thyroid hormone receptor PV in the pituitary of transgenic mice leads to weight reduction. *Thyroid* **9** 1137–1145.

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