The skeletal phenotypes of TRα and TRβ mutant mice

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Abstract
Analysis of mice harbouring deletions or mutations of T3 receptor α (TRα) and β (TRβ) have clarified the complex relationship between central and peripheral thyroid status and emphasised the essential but contrasting roles of T3 in skeletal development and adult bone. These studies indicate that TRα1 is the predominant TR expressed in bone and that T3 exerts anabolic actions during growth but catabolic actions in the adult skeleton. Examination of key skeletal regulatory pathways in TR mutant mice has identified GH, IGF-1 and fibroblast growth factor signalling and the Indian hedgehog/parathyroid hormone-related peptide feedback loop as major targets of T3 action in chondrocytes and osteoblasts. Nevertheless, although increased osteoclastic resorption is a major feature of thyrotoxic bone loss and altered osteoclast activity is central to the skeletal phenotype of TR mutant mice, it remains unclear whether T3 has direct actions in osteoclasts. Detailed future analysis of the molecular mechanisms of T3 action in bone will enhance our understanding of this emerging field and has the potential to identify novel strategies for the prevention and treatment of osteoporosis.

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Introduction
Thyroid hormone is essential for normal skeletal development and the maintenance of adult bone mass. Recent studies of T3 receptor (TR) mutant mice have advanced our understanding and demonstrated the relevance of this field to osteoporosis, a major public health burden that affects half of all women and one-fifth of men over the age of 50, costing the European community €31 billion per annum (Kanis & Johnell 2005).

Systemic thyroid hormone levels are maintained by the hypothalamus–pituitary–thyroid (HPT) feedback axis. The cellular actions of 3,5,3′-triiodothyronine (T3) are mediated by TRs, which act as hormone-inducible transcription factors. Unliganded TRs bind T3 response elements (TREs) in T3-target genes and mediate transcriptional repression. T3 binding results in derepression and activation of gene transcription (Yen 2001). The THRA and THRBB genes encode three functional receptors TRα1, TRβ1 and TRβ2 as well as a non-T3-binding isoform of unknown function TRα2 (Fig. 1). TRα1 and TRβ1 are expressed widely and the ratio of TRα1 to TRβ1 is spatio-temporally regulated. Thus, T3-target tissues may predominantly display either TRα1 or TRβ1 responsiveness or show no TR-isoform specificity. TRβ2 has a more restricted pattern of expression and regulates sensory organ development (Jones et al. 2007) as well as the HPT axis. In the skeleton, chondrocyte and osteoblast lineages express TRα and TRβ mRNAs, but in osteoclasts the position is less clear as studies have been restricted to precursor cells or in situ hybridisation analysis of osteoclastomas (Williams et al. 1994, Abu et al. 2000, Stevens et al. 2000, Kanatani et al. 2004).

Several studies have demonstrated apparent expression of TR proteins in all bone cell lineages, but it is well recognised within the field that available TR antibodies are of low affinity, thus compromising the detection of endogenous TRs (Abu et al. 1997, Robson et al. 2000).

For these reasons, a comprehensive understanding of TR expression in bone is lacking and a detailed analysis of cell-specific and temporal expression of TR isoforms is required.

Long bones are formed by endochondral ossification and the skull by intramembranous ossification. During endochondral ossification, mesenchyme-derived chondrocytes form a cartilage model, undergo hypertrophic differentiation and then apoptose. The surrounding collagen X-rich cartilage matrix calcifies and forms a scaffold for bone formation by osteoblasts. Organised columns of proliferating and differentiating
chondrocytes persist in the growth plate until adolescence and mediate linear growth and the acquisition of peak bone mass. By contrast, in intramembranous ossification, osteoblasts differentiate from mesenchyme to form bone directly. Adult bone structure and mechanical strength are preserved by a continuous process of skeletal remodelling during which precise coupling of osteoclastic bone resorption and subsequent osteoblastic bone formation is maintained.

The established view that skeletal responses to abnormal thyroid status result exclusively from altered $T_3$ action in bone has been challenged recently by studies proposing a direct role for TSH in bone. We recently discussed this issue elsewhere (Bassett & Williams 2008) and the present review will therefore focus on the analysis of $T_3$ and TR action in bone.

Thyroid hormone receptor mutant mice

Several TRz knockout mice have been generated and this led to the identification of additional TRz isoforms expressed from a promoter within intron 7 of the Thra gene (Chassande et al. 1997). As a result only TRz$^{0/0}$ mice lack all TRz isoforms whereas other TRz mutants retain truncated isoforms with dominant-negative activity (Fig. 1 and Table 1). This review will focus on mice lacking all TRz (TRz$^{0/0}$) or all TRβ (TRβ$^{-/-}$) isoforms and those harbouring dominant-negative mutations of either TRz (TRz1$^{PV/+}$, TRz1$^{R384C/+}$) or TRβ (TRβ$^{PV/PV}$).

Thyroid hormone actions during skeletal development are anabolic

The developing skeleton is exquisitely sensitive to thyroid status and childhood hypothyroidism is characterised by growth retardation, delayed bone age and short stature, whereas juvenile thyrotoxicosis accelerates growth and advances bone age but results in short stature due to premature fusion of the epiphyses (Rivkees et al. 1988, Boersma et al. 1996, Segni & Gorman 2001).

Although TRz$^{0/0}$ mice are systemically euthyroid, juveniles display post-natal growth retardation with delayed endochondral ossification characterised by impaired chondrocyte differentiation and decreased mineral deposition (Bassett et al. 2007b). TRz1$^{R384C/+}$ mice, which harbour a dominant-negative mutation of TRz, are also euthyroid. They display a similar period of growth retardation and delayed endochondral ossification, but additionally have reduced cortical bone thickness, abnormal cortical bone remodelling and impairment of intramembranous ossification (Fig. 2; Bassett et al. 2007a). TRz1$^{PV/+}$ mice, which express a potent dominant-negative TRz mutant, display a more severe skeletal phenotype. TRz1$^{PV/+}$ mice have persistent post-natal growth retardation, markedly
<table>
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<tr>
<th>Genotype</th>
<th>Endocrine status</th>
<th>Juvenile skeleton</th>
<th>Adult skeleton</th>
<th>Skeletal thyroid status</th>
<th>References</th>
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<tr>
<td>No TR(\alpha)1/(\alpha)2 TR(\Delta)(\alpha)1 and (\Delta)(\alpha)2 preserved</td>
<td>Hypothyroid (T(_4) 0·1 × T(_3) 0·4 ×, TSH 2 ×) GH normal</td>
<td>Severe growth delay; delayed endochondral ossification; impaired chondrocyte differentiation; reduced mineralisation</td>
<td>Die by weaning unless T(_3) treated</td>
<td>NR</td>
<td>Chassande et al. (1997), Fraichard et al. (1997) and Gauthier et al. (1999)</td>
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<td>No TR(\alpha)1/(\alpha)2 TR(\Delta)(\alpha)1 preserved; TR(\alpha)1 over-expression; TR(\Delta)(\alpha)2</td>
<td>Mild hypothyroidism (T(_4) 0·7 × T(_3) 1 ×, TSH 0·8 ×)</td>
<td>No growth retardation of skeletal phenotype</td>
<td>NR</td>
<td>NR</td>
<td>Wikstrom et al. (1998)</td>
</tr>
<tr>
<td>No TR(\alpha) transcripts TR(\beta) unaffected</td>
<td>Euthyroid; normal GH and IGF1</td>
<td>Transient growth delay; delayed endochondral ossification; impaired chondrocyte differentiation; reduced mineralisation</td>
<td>Reduced bone mineral density; reduced cortical bone Osteosclerosis; increased trabecular bone volume; reduced osteoclastic bone resorption</td>
<td>Hypothyroid</td>
<td>Gauthier et al. (2001) and Bassett et al. (2007b)</td>
</tr>
<tr>
<td>Heterozygous dominant-negative TR(\alpha) receptor</td>
<td>Mild thyroid failure (T(_4) 1·1 × T(_3) 1·2 ×, TSH 1·7 ×) GH normal</td>
<td>Severe persistent growth retardation; delayed intramembranous and endochondral ossification; impaired chondrocyte differentiation; reduced mineralisation</td>
<td>NR</td>
<td>Hypothyroid</td>
<td>Kaneshige et al. (2001) and O’Shea et al. (2005)</td>
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<td>Heterozygous dominant-negative TR(\alpha) receptor (10-fold reduced affinity for T(_3))</td>
<td>Euthyroid adults; mild hypothyroidism P10-35 (T(_4) 0·8 × T(_3) 0·7 ×, TSH 0·7 ×) (GH reduced in juveniles)</td>
<td>Transient growth delay; delayed intramembranous and endochondral ossification; impaired chondrocyte differentiation; reduced mineralisation</td>
<td>Osteosclerosis; increased trabecular bone volume; increased mineralisation; reduced osteoclastic bone resorption</td>
<td>Hypothyroid</td>
<td>Tinnikov et al. (2002) and Bassett et al. (2007a)</td>
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<td>Heterozygous dominant-negative TR(\alpha) receptor</td>
<td>Euthyroid (T(_4) 1·1 × T(_3) 1·1 ×, TSH 3·4 ×)</td>
<td>Reduced GH (0·4 ×)</td>
<td>NR</td>
<td>NR</td>
<td>Liu et al. (2003)</td>
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<td>Cre-inducible dominant-negative receptor TR(\alpha)1(L400R) (early global expression)</td>
<td>Euthyroid (T(_4) 1 × T(_3) 0·9 ×, TSH 0·3 ×) Reduced GH (0·4 ×)</td>
<td>Severe persistent growth retardation; delayed endochondral ossification</td>
<td>NR</td>
<td>NR</td>
<td>Quignodon et al. (2007)</td>
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<th>Genotype</th>
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<th>Skeletal thyroid status</th>
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<td><strong>TRβ mutants</strong></td>
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<tr>
<td>TRβ−/−</td>
<td>No TRβ transcripts</td>
<td>RTH and goitre (T₄ 4×T₃ 4×, TSH 8×)¹</td>
<td>Persistent short stature; advanced endochondral and intramembranous ossification; increased mineralisation</td>
<td>Osteoporosis; reduced mineralisation; increased osteoclastic bone resorption</td>
<td>Thyrotoxic</td>
</tr>
<tr>
<td>TRβ2−/−</td>
<td>No TRβ1 TRβ1 preserved</td>
<td>Mild RTH (T₄ 3×T₃ 1-3 ×, TSH 2-5×)¹</td>
<td>No reported growth abnormality</td>
<td>NR</td>
<td>Thyrotoxic</td>
</tr>
<tr>
<td>TRβPV/PV</td>
<td>Homozygous dominant-negative TRβ receptor</td>
<td>Severe RTH and goitre (T₄ 15×T₃ 10×, TSH &gt;400×); reduced GH</td>
<td>Accelerated prenatal growth; persistent postnatal growth retardation; advanced intramembranous and endochondral ossification; increased mineralisation</td>
<td>NR</td>
<td>Thyrotoxic</td>
</tr>
<tr>
<td>TRβΔ377T/Δ377T</td>
<td>Homozygous dominant-negative TRβ receptor</td>
<td>Severe RTH and goitre (T₄ 15×T₃ 10×, TSH &gt;50×)</td>
<td>No growth phenotype</td>
<td>NR</td>
<td>NR</td>
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<td><strong>TRα and TRβ compound mutants</strong></td>
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<tr>
<td>TRα−/−β−/−</td>
<td>No TRα1/α2 or TRβ TRα1/Δα2 preserved</td>
<td>RTH and small goitre (T₄ 10×T₃ 10×, TSH &gt;100×)</td>
<td>Growth delay similar to TRα−/−β−/−; delayed endochondral ossification; impaired chondrocyte differentiation; reduced mineralisation</td>
<td>Die at or near weaning</td>
<td>NR</td>
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<td>TRα1−/−β−/−</td>
<td>No TRα1/Δα1 or TRβ TRα2/Δα2 preserved</td>
<td>RTH and large goitre (T₄ 60×T₃ 60×, TSH &gt;160×); reduced GH/IGF1</td>
<td>Persistent growth retardation; delayed endochondral ossification; reduced mineralisation</td>
<td>Reduced trabecular and cortical bone mineral density. GH treatment corrects growth but not ossification defect</td>
<td>NR</td>
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<td>TRα2−/−β−/−</td>
<td>No TRα2/Δα2 or TRβ TRα1/Δα1 preserved, TRα1 over-expression</td>
<td>Mild hypothyroidism (T₄ 0-7×T₃ 0-8×, TSH 1×)</td>
<td>Transient growth delay</td>
<td>NR</td>
<td>NR</td>
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<td>TRα0/0β−/−</td>
<td>No TRα/ TRβ transcripts</td>
<td>RTH and goitre (T₄ 13×T₃ 14×, TSH &gt;200×); reduced GH/IGF1</td>
<td>More severe phenotype than TRα0/0; growth delay; delayed endochondral ossification; impaired chondrocyte differentiation; reduced mineralisation</td>
<td>NR</td>
<td>Hypothyroid</td>
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<tr>
<td>TRα, TRβ and Pax8 compound mutants</td>
<td>No thyroid (No T&lt;sub&gt;4&lt;/sub&gt; or T&lt;sub&gt;3&lt;/sub&gt;, TSH 1900 ×)</td>
<td>More severe growth defect than TR&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;α0&lt;/sup&gt;β&lt;sup&gt;−/−&lt;/sup&gt;; severe persistent growth retardation; severely delayed endochondral ossification; impaired chondrocyte differentiation</td>
<td>Majority die by weaning; coarse plate-like trabeculae with impaired trabecular remodelling; reduced cortical thickness; reduced mineralisation</td>
<td>Hypothyroid</td>
<td>Mansouri &lt;em&gt;et al.&lt;/em&gt; (1998), Flamant &lt;em&gt;et al.&lt;/em&gt; (2002) and Bassett &lt;em&gt;et al.&lt;/em&gt; (2008)</td>
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<td>Pax8&lt;sup&gt;−/−&lt;/sup&gt;TRα&lt;sup&gt;1−/−&lt;/sup&gt;</td>
<td>No T&lt;sub&gt;3&lt;/sub&gt; or TRα1/Δα1 TRα2/Δα2 preserved Apo TRβ receptors</td>
<td>Severe growth retardation similar to Pax8&lt;sup&gt;−/−&lt;/sup&gt;; skeletal phenotype not reported</td>
<td>Die by weaning</td>
<td>NR</td>
<td>Mittag &lt;em&gt;et al.&lt;/em&gt; (2005)</td>
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<tr>
<td>Pax8&lt;sup&gt;−/−&lt;/sup&gt;TRα&lt;sup&gt;0/0&lt;/sup&gt;</td>
<td>No T&lt;sub&gt;3&lt;/sub&gt; or TRα Apo TRβ receptors</td>
<td>Growth retardation less than Pax8&lt;sup&gt;−/−&lt;/sup&gt; and similar to TRα&lt;sup&gt;0/0&lt;/sup&gt;β&lt;sup&gt;−/−&lt;/sup&gt;; delayed endochondral ossification; mice survive to adulthood</td>
<td>NR</td>
<td>NR</td>
<td>Flamant &lt;em&gt;et al.&lt;/em&gt; (2002)</td>
</tr>
<tr>
<td>Pax8&lt;sup&gt;−/−&lt;/sup&gt;TRβ&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>No T&lt;sub&gt;3&lt;/sub&gt; or TRβ Apo TRα receptors</td>
<td>Severe growth retardation similar to Pax8&lt;sup&gt;−/−&lt;/sup&gt;; severely delayed endochondral ossification</td>
<td>Die by weaning</td>
<td>NR</td>
<td>Flamant &lt;em&gt;et al.&lt;/em&gt; (2002)</td>
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TR, T<sub>3</sub> receptor; T<sub>4</sub>, thyroxine; T<sub>3</sub>, triiodothyronine; NR, not reported; IGF1, insulin-like growth factor 1; RTH, resistance to thyroid hormone; P10-35, postnatal day 10 to 35.
delayed endochondral ossification, decreased mineralisation, reduced cortical bone thickness and impaired intramembranous ossification (O’Shea et al. 2005). Similar findings have been reported in mice expressing the potent dominant-negative receptor TRα1L400R (Quignodon et al. 2007). Consistent with the delayed bone development in juvenile TRα1/0, TRα1R384C/0 and TRα1PV/0 mice, skeletal expression of the T3-target genes, fibroblast growth factor receptors 1 and 3 (FGFR1/3; Stevens et al. 2003, Barnard et al. 2005, O’Shea et al. 2007) were reduced (Barnard et al. 2005, O’Shea et al. 2007, Bassett et al. 2007a, b). Deletion or mutation of TRα does not affect systemic thyroid status but causes local skeletal hypothyroidism while the presence of a dominant-negative TRα leads to a more severe skeletal phenotype than receptor deficiency alone.

Two TRβ−/− strains have been generated in different genetic backgrounds and both show similar skeletal phenotypes (Table 1; Forrest et al. 1996, Gauthier et al. 1999). Deletion of TRβ results in elevated circulating TSH, T4 and T3 levels consistent with resistance to thyroid hormone (RTH). In contrast to TRα mutants, juvenile TRβ−/− mice display advanced endochondral ossification, accelerated chondrocyte differentiation, increased mineral deposition and persistent short stature due to premature growth plate quiescence. Furthermore, cortical thickness is increased and intramembranous ossification advanced in TRβ−/− mice (Fig. 2; Bassett et al. 2007b). TRβPV/PV mice express a potent dominant-negative TRβ and display severe RTH with a 400-fold elevation of TSH and 15-fold elevation of T4. TRβPV/PV animals exhibit a more severe phenotype than TRβ−/− mice with advanced intrauterine growth characterised by advanced endochondral and intramembranous ossification. Premature ossification results in persistent postnatal growth retardation, premature growth plate quiescence, increased mineral deposition and craniosynostosis (O’Shea et al. 2003). Consistent with advanced skeletal development in TRβ−/− and TRβPV/PV mice, expression of the T3-target genes Fgfr1 and Fgfr3 was increased (O’Shea et al. 2003, Barnard et al. 2005, Bassett et al. 2007a, b). Thus, deletion or mutation of TRβ disrupts the HPT axis resulting in increased circulating thyroid hormone levels and skeletal thyrotoxicosis. The presence of a dominant-negative TRβ leads to a greater elevation of systemic
thyroid hormone concentration and a more severe skeletal phenotype than receptor deficiency alone.

In the developing skeleton, reduced T₃ action in TRα mutant mice results in delayed ossification and reduced mineralisation whereas increased T₃ action in TRβ mutant mice leads to advanced ossification and increased mineralisation. Thus, during growth, T₃ actions in bone are anabolic.

**Thyroid hormone actions in adult bone are catabolic**

Adult thyrotoxicosis results in both increased bone resorption and formation, but uncoupling of these processes favours osteoclastic resorption and leads to a 10% net bone loss per remodelling cycle (Mosekilde et al. 1990). Consequently, thyrotoxicosis is an important cause of secondary osteoporotic fracture (Mosekilde et al. 1990, Cummings et al. 1995, Franklyn et al. 1998, Vestergaard et al. 2000, Vestergaard & Mosekilde 2002, Murphy & Williams 2004) and even subclinical hyperthyroidism has been associated with decreased bone mineral density and increased fracture risk in postmenopausal women (Bauer et al. 2001, Quan et al. 2002, Murphy & Williams 2004, Heemstra et al. 2006, Kim et al. 2006, Morris 2007).

Remarkably, delayed ossification and reduced mineralisation in juvenile TRα²/₀ mice were accompanied by greatly increased trabecular bone mass in adults (Fig. 3; Bassett et al. 2007b). Moreover, the robust and plate-like trabeculae contained highly mineralised calcified cartilage indicating a trabecular bone remodelling defect. Consistent with such a defect, TRα²/₀ mice displayed reduced osteoclast numbers and activity (Bassett et al. 2007b). Trabecular bone mass increased progressively with age in TRα₁R384C/₀ mice with adults showing osteosclerosis (Bassett et al. 2007a). Consistent with a remodelling defect, trabeculae were of increased thickness and connectivity, showed increased mineralisation with extensive retention of calcified cartilage and reduced osteoclast numbers and activity (Fig. 4). Remarkably, brief T₃ supplementation during growth, sufficient to overcome transcriptional repression by TRα₁R384C, ameliorated the adult skeletal phenotype (Bassett et al. 2007a; Table 1). These data indicate that during development even transient relief from the transcriptional repression mediated by unliganded TRα₁ (apo-TRα₁) has long-term consequences for adult bone structure and mineralisation. Thus, in the adult skeleton, deletion or mutation of TRα results in persistently impaired bone remodelling. Similarly, the presence of a dominant-negative TRα leads to a more severe skeletal phenotype than receptor deficiency alone.

Despite a juvenile phenotype of accelerated growth and increased ossification, adult TRβ⁻/⁻ mice became progressively osteoporotic (Fig. 3; Bassett et al. 2007a).

**Mechanism of T₃ action**

The opposing skeletal phenotypes in TRα and TRβ mutant mice provide compelling evidence for the complex interaction between central and peripheral
Figure 4  Trabecular bone micro-mineralisation in adult TR mutant mice. Quantitative backscattered electron scanning electron microscopy (qBSE-SEM) images showing mineralisation densities of trabecular bone from mice lacking TRβ and mice harbouring a dominant-negative mutation of TRα (R384C) (A). Mineralisation densities were derived from halogenated standards and images pseudo-coloured according to the palette shown in which high mineralisation density is grey. (B) Relative and cumulative frequency histograms of bone micro-mineralisation densities are shown. Black arrow indicates the increased relative frequency of high mineralisation densities that correspond to retained calcified cartilage in TRα_{1R384C/+} mice. (C) Higher power views of trabecular bone are shown. Dashed white arrows indicate normal bone cement lines. Solid white arrows indicate retained calcified cartilage within which the outlines of chondrocyte lacunae remain evident. Scale bar 200 μm in all panels. ***P<0.001 micro-mineralisation density in mutant versus WT; Kolmogorov–Smirnov test.
thyroid status (Fig. 5). Thus, delayed ossification and impaired bone remodelling in TRz mutant mice are secondary to the disruption of T3 action in bone, whereas advanced skeletal development and osteoporosis in TRβ mutant mice are due to disruption of the HPT axis, elevated systemic thyroid hormone levels and local supraphysiological stimulation of TRz in bone (O’Shea et al. 2006). This model is supported by T3 target gene expression in skeletal cells of TR mutant mice and by the demonstration of higher levels of TRz mRNA expression in bone compared with TRβ (O’Shea et al. 2003, 2005, Barnard et al. 2005, Bookout et al. 2006, Bassett et al. 2007a,b). Nevertheless, it is apparent that TRz0/0 TRβ−/− mice have a more severe skeletal phenotype than TRz0/0 mice, while TRz0/0 mice also remain sensitive to T4 treatment, thus suggesting a residual role for TRβ in skeletal cells. In this context, quantitative RT-PCR analysis has shown that TRz expression is 10- to 100-fold greater than TRβ expression in adult whole bone (O’Shea et al. 2003, Bookout et al. 2006). However, since the temporospatial patterns of TRz and TRβ expression in skeletal cells are unknown, a role for TRβ is possible. Furthermore, it is unclear whether individual skeletal cell co-express both TR isoforms or whether their patterns of expression are cell type specific.

Present understanding is that TRz1 is functionally predominant in bone.

**T3 action in the developing skeleton**

In vitro T3 inhibits chondrocyte proliferation and promotes differentiation (Robson et al. 2000, Shao et al. 2006; Fig. 6). Since growth plate architecture and linear growth are frequently disrupted in TR mutant mice, key regulators of endochondral ossification have been investigated as targets of thyroid hormone action. GH and insulin-like growth factor 1 signalling pathways are thought to act as a point of convergence for many growth promoting factors since more than 80% of somatic growth can be attributable to the actions of the GH/IGF1 axis (Lupu et al. 2001). IGF1 is the major determinant of post-natal growth, it mediates both GH-dependent and -independent effects and is implicated in chondrocyte recruitment, proliferation and hypertrophic differentiation (van der Eerden et al. 2003). Examination of the GH/IGF1 pathway revealed that GH receptor (GHR) and IGF1 receptor (IGF1R) mRNA expression was reduced in growth plate chondrocytes in TRz0/0 and TRz1PV/+ mice. Furthermore, phosphorylation of their secondary messengers signal transducer and activator of transcription 5 (STAT5) and protein kinase B (AKT) was also impaired (O’Shea et al. 2005, Bassett et al. 2007b). By contrast, GHR and IGF1R expression was increased in TRβ−/− and TRβPV/PV mice (O’Shea et al. 2005, Bassett et al. 2007b). Thus, GH/IGF1 signalling is also a local downstream mediator of T3 action in the growth plate (Fig. 6).

FGFs and their receptors have key roles in skeletal development with activating mutations of FGFR3 causing achondroplasia, the commonest form of genetic dwarfism. In the developing growth plate, FGFR3 is expressed in reserve and proliferating chondrocytes and negatively regulates their proliferation and differentiation (Murakami et al. 2004). By contrast, FGFR1 is expressed in prehypertrophic and hypertrophic chondrocytes and its location suggests a role in differentiation, matrix synthesis and apoptosis (Ornitz 2005). FGFR1 is also expressed in the osteoblast lineage with activating mutations result in Pfeiffer craniosynostosis. Investigation of the FGF/FGFR signalling pathway in TR mutant mice demonstrated that Fgfr3 and Fgfr1 expression was reduced in growth plates of TRz0/0, TRz1R384C+/+ and TRz1PV/+ mice and Fgfr1 expression was reduced in osteoblasts from TRz0/0 and Pax8−/− mice (Stevens et al. 2003, Barnard et al. 2005, O’Shea et al. 2005, Bassett et al. 2007a,b, 2008). By contrast, Fgfr3 and Fgfr1 expression was increased in growth plates of TRβ−/− and TRβPV/PV mice and Fgfr1 expression was increased in osteoblasts from TRβPV/PV mice (O’Shea et al. 2005, Barnard et al. 2005, Bassett et al. 2007a,b). Thus, FGF/FGFR signalling is a downstream mediator of T3 action in chondrocytes and osteoblasts (Fig. 6).

The pace of chondrocyte differentiation is precisely regulated by the Indian hedgehog/parathyroid hormone-related peptide paracrine (Ihh/PTHrP) negative feedback loop. Prehypertrophic chondrocytes secrete Ihh which diffuses to periarticular cells to induce synthesis of PTHrP. PTHrP, acting via its receptor PTHR1, then completes the loop by stimulating chondrocyte proliferation and inhibiting further hypertrophic differentiation (Vortkamp et al. 1996, Dentice et al. 2005). Although this pathway has not been studied in TR mutant mice, previous experiments in thyroid-manipulated rats demonstrated increased PTHrP mRNA expression in hypothyroid animals and decreased PTHrP receptor mRNA expression in growth plates of thyrotoxic animals (Stevens et al. 2000). Furthermore, recent studies in chicken tibia explants have shown that Ihh stimulates degradation of the type 2 deiodinase enzyme resulting in an induction of PTHrP expression (Dentice et al. 2005). Together, these findings suggesting that thyroid hormone can inhibit chondrocyte proliferation and promote differentiation by local regulation of the Ihh/PTHrP negative feedback loop (Fig. 6).

T3 is essential for normal cartilage matrix synthesis. Heparan sulphate proteoglycans (HSPGs) are a key matrix component and are essential for functional
FGF/FGFR signalling and extracellular diffusion of Ihh. Studies in thyroid manipulated rats and TRα0/0β−/− and Pax8−/− mice revealed reduced HSPG expression in thyrotoxic animals, increased expression in TRα0/0β−/− mice and more markedly increased expression in hypothyroid rats and congenitally hypothyroid Pax8−/− mice (Bassett et al. 2008).

These studies suggest T₃ coordinately regulates FGF/FGFR and Ihh/PTHrP signalling within the growth plate.
T₃ actions in the bone remodelling cycle

In vivo studies suggest that thyroid hormone stimulates osteoblast proliferation, differentiation and apoptosis by direct and indirect actions. Thus, T₃ increases synthesis of osteocalcin, type 1 collagen, alkaline phosphatase and matrix metalloproteinases 9 and 13 (Pereira et al. 1997, 2000, Kanatani et al. 2001, Bassett & Williams 2003) and also regulates IGF1, parathyroid hormone (PTH) and FGF signalling (Huang et al. 2000, Gu et al. 2001, Pepene et al. 2003, Stevens et al. 2003, O'Shea et al. 2007; Fig. 6). In vivo, activation of FGFR1 stimulates osteoblast proliferation and differentiation (Zhou et al. 2000). Consistent with this, Fgf1 expression in osteoblasts is reduced in the hypothryoid skeleton and increased in thyrotoxic bone (O'Shea et al. 2003, Stevens et al. 2003, Bassett et al. 2007a). Although osteoclasts have been reported to express TRs (Allain et al. 1992, Abu et al. 1997, 2000, Kanatani et al. 2004), it remains uncertain whether T₃ regulates osteoclast differentiation directly or indirectly (Siddiqi et al. 1998, Miura et al. 2002, Varga et al. 2004). Previous studies are contradictory; some demonstrating that T₃ acts directly in osteoclasts while others report indirect effects mediated via osteoblasts.

Future directions

Analyses of TR mutant and congenitally hypothyroid Pax8⁻/⁻ mice have identified a key role for thyroid hormone and TR1 in both the developing skeleton and adult bone. However, it remains unclear how the skeletal effects of apo-TR1 differ from those of TR1 deficiency and to what extent the skeletal effects of thyroid hormones result from central, systemic and local actions of T₃. The more severe skeletal phenotype observed in congenitally hypothyroid Pax8⁻/⁻ mice as compared with TRZ₀/₀β⁻/⁻ mice suggests that unliganded TRs may be more detrimental to skeletal development than TR deficiency (Mansouri et al. 1998, Flamant et al. 2002, Bassett et al. 2008; Table 1). In support of this, amelioration of the Pax8⁻/⁻ skeletal phenotype in Pax8⁻/⁻ TRZ₀/₀ mice but not in Pax8⁻/⁻ TRβ⁻/⁻ mice suggests that some of the detrimental effects of hypothyroidism are mediated by unliganded TR1 in bone (Flamant et al. 2002). Despite this, it is important to note from an additional study (Mittag et al. 2005) that deletion of TR1 alone in Pax8⁻/⁻TR1⁻/⁻ mice did not prevent weight loss, early mortality and pituitary abnormalities although the skeletal consequences were not investigated. Thus, it remains possible that TR2 has an additional and essential role in the manifestation of the Pax8⁻/⁻ phenotype. This importance of apo-TR1 is further supported by the more severe skeletal phenotype present in mice harbouring dominant-negative mutations of TR1 (TR1R384C⁺/⁺ and TR1P⁻/⁻) as compared with mice lacking all TRz isofoms (TRz₀/₀; O'Shea et al. 2005, Bassett et al. 2007ab) and by the amelioration of the adult skeletal phenotype in TR1R384C apo-receptor activity during development (Bassett et al. 2007a). Nevertheless, a complete understanding of the molecular mechanism of thyroid hormone action in bone will require at least two experimental approaches. First, it is clear that phenotyping of the existing mouse models is incomplete and more detailed studies including quantitative histomorphometry, mechanical testing and analysis of primary bone cell cultures will help to clarify the picture. However, such an approach cannot identify the cellular targets of T₃ action in the skeleton in vivo and this will require the use of cell-specific gene-targeting strategies.

Analysis of TRz and TRβ mutant mice has demonstrated the complex relationship between central and peripheral thyroid status and established the predominant role of TR1 in bone. These studies also highlight contrasting responses of the skeleton to T₃ during developing and in adulthood. Although understanding of the molecular mechanism of T₃ action in bone is still limited, coordinate regulation of key signalling pathways has now been identified in chondrocytes and osteoblasts. By contrast, the molecular mechanism of T₃ action in the osteoclast lineage remains unclear. A more detailed understanding of the molecular basis of T₃ action in bone will provide the rational for the development of novel strategies for the prevention and treatment of osteoporosis.

Figure 5 Proposed molecular mechanism for TRz and TRβ mutant mice. The pituitary expresses predominantly TRβ₁ and TRβ₁ act via a negative TRE to repress TSH transcription. The skeleton expresses predominantly TRz₁ and TRz act via TRz₁ to activate target gene expression. Since the levels of TRz₁ in the pituitary are low, its absence in TRz₀/₀ mice does not disrupt pituitary negative feedback and systemic TSH, T₄ and T₃ concentrations are normal. By contrast, because TRz predominates in bone, its absence in TRz₀/₀ mice results in impaired T₃ responsiveness, skeletal hypothyroidism and reduced expression of T₃-target genes. Similarly, in TRz₁P⁺/⁺ and TRz₁R384C⁺/⁺ mice, the levels of the dominant-negative TRz receptor in the pituitary are insufficient to interfere with TRβ and disrupt TSH repression. However, in bone, the higher concentrations of mutant receptor interfere with the actions of wild-type TRz₁. Thus, TRz responses are severely impaired and the skeleton is hypothyroid. In TRβ⁻/⁻ mice, by contrast, the absence of TRβ disrupts pituitary T₃ responses and impairs TSH repression leading to high circulating TSH, T₄ and T₃ concentrations. Since TRβ₁ levels are low in bone, T₃ responses are unaffected in TRβ⁻/⁻ mice and high circulating levels of TH act via wild-type TRz₁ to induce skeletal thyrotoxicosis. Similarly in TRβP⁺/⁺ mice, the dominant-negative TRβ disrupts TSH repression in the pituitary and results in grossly elevated TSH, T₄ and T₃ concentrations. The low levels of the mutant receptor in bone are insufficient to interfere with TRz₁ and impair T₃ responses and thus high circulating levels of TH increase expression of T₃-target genes resulting in severe skeletal thyrotoxicosis.
Declaration of interest

There is no conflict of interest.

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