Paradigms of Dynamic Control of Thyroid Hormone Signaling

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ABSTRACT Thyroid hormone (TH) molecules enter cells via membrane transporters and, depending on the cell type, can be activated (*i.e.*, T4 to T3 conversion) or inactivated (*i.e.*, T3 to 3,3′-diiodo-L-thyronine or T4 to reverse T3 conversion). These reactions are catalyzed by the deiodinases. The biologically active hormone, T3, eventually binds to intracellular TH receptors (TRs), TRα and TRβ, and initiate TH signaling, that is, regulation of target genes and other metabolic pathways. At least three families of transmembrane transporters, MCT, OATP, and LAT, facilitate the entry of TH into cells, which follow the gradient of free hormone between the extracellular fluid and the cytoplasm. Inactivation or marked downregulation of TH transporters can dampen TH signaling. At the same time, dynamic modifications in the expression or activity of TRs and transcriptional coregulators can affect positively or negatively the intensity of TH signaling. However, the deiodinases are the element that provides greatest amplitude in dynamic control of TH signaling. Cells that express the activating deiodinase *DIO2* can rapidly enhance TH signaling due to intracellular buildup of T3. In contrast, TH signaling is dampened in cells that express the inactivating deiodinase *DIO3*. This explains how THs can regulate pathways in development, metabolism, and growth, despite rather stable levels in the circulation. As a consequence, TH signaling is unique for each cell (tissue or organ), depending on circulating TH levels and on the exclusive blend of transporters, deiodinases, and TRs present in each cell. In this review we explore the key mechanisms underlying customization of TH signaling during development, in health and in disease states. ***(Endocrine Reviews 40: 1 – 48, 2019)***

Multiple processes and systems in vertebrates are sensitive to the thyroid hormones (THs) T4 and T3 (1, 2). However, circulating TH levels are remarkably stable, which is difficult to reconcile with the idea that important biologic processes are initiated or terminated by T3, the most biologically active TH. Historically, this was explained by the concept of the "permissive effect" of TH; TH was thought to be necessary but not sufficient to initiate critical biologic events (3). Progress in our understanding of TH action illuminated this apparent inconsistency, with the discovery that a number of cellular and molecular processes such as gene transcription are indeed highly sensitive to T3 action *per se* (1, 2). The work of several groups resolved the logistical hurdle of steady T3 plasma levels by demonstrating the existence of "local" mechanisms that function within target cells to rapidly modulate TH signaling up or down in the short- or long-term, despite relatively stable circulating levels of T3 (4–8). The signaling TRIAD, that is, transmembrane transport, intracellular deiodination, and TH receptor (TR)–mediated gene transcription, constitutes the basis for cellular customization of TH signaling [see Ref. (9) for a comprehensive review of methods and experimental approaches to study the signaling TRIAD].

Transmembrane transport

The cellular lipid bilayer that forms the plasma membrane is not significantly permeable to T4 or T3; both molecules enter and exit cells through specific transporters that are embedded in the plasma membrane (10–12). Knowledge about these transporters originated from work with L- and T-type amino acid transporters (13), and eventually led to identification of monocarboxylate transporter (MCT) 8, a highly effective T4 and T3 transporter (14). The homologous molecule, MCT10, is also capable of T3 transport but

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ESSENTIAL POINTS

- Thyroid hormone (TH) signaling is customized to different cell types
- Customization in TH signaling is mediated by TH transporters, deiodinases, and TH receptors
- Deiodinases provide the greatest amplitude in dynamic control of TH signaling
- TH signaling is customized during development to ensure that cells are exposed to T3 at the appropriate timing, which is different among tissues
- Critical illness is associated with changes in TH signaling, which are viewed as an adaptive phenomena

less effective than MCT8 for T4 (15). In addition to the MCTs, at least two other families of TH transporters exist: the organic anion-transporting polypeptide (OATP) family, highly expressed in the brain with substrate specificity for T4, and the L-type amino acid transporters (LATs) 1 and 2, which transport both T4 and T3 but with relative low affinity (16).

Intracellular deiodination

Once inside the cells, TH molecules can be activated or inactivated by the deiodinase group of enzymes (1, 4, 5, 17) (Fig. 1). These are dimeric integral membrane selenoproteins composed of a single N-terminal transmembrane segment connected to a larger globular domain with a selenocysteine-containing active center embedded in a thioredoxin-like fold (18–21) (Fig. 2). Deiodinases modify the biologic activity of TH molecules, either by activating T4 via outer ring deiodination [type II iodothyronine deiodinase (D2)] or inactivating T4 and T3 via inner ring deiodination [type III iodothyronine deiodinase (D3)], thus modulating T3 levels inside target cells. *DIO2* is primarily expressed in the brain, pituitary gland, and brown adipose tissue (BAT), whereas *DIO3* expression predominates in most fetal tissues, subsiding after birth (22, 23). In adults, brain, placenta, skin, and pancreatic β-cells (24, 25) are the tissues with highest D3 activity. However, D3 can be expressed ectopically in almost any tissue during critical illness (26, 27). A third deiodinase gene, *DIO1*, is expressed in liver and kidney; type I iodothyronine deiodinase (D1) is capable of both outer and inner ring deiodination. However, D1 exhibits three orders of magnitude lower affinity for T4. Whereas D1 plays a role in thyroid economy (28), its low affinity for T4 and presence in the plasma membrane precludes it from significantly affecting local TH signaling; its products, T3 and reverse T3 (rT3), rapidly exit the cells and enter the systemic circulation (29, 30).

TR-mediated signaling

Two types of T3 receptors, TRα and TRβ, mediate most TH effects via interaction with transcriptional modulators to control multiple gene sets (2). Tissues vary in their expression levels of TRα and TRβ. For example, brain, heart, intestine, skeletal muscle (SKM), and skeleton are known for their predominance of TRα, whereas TRβ expression occurs primarily in liver and pituitary gland. In genes that are positively regulated by T3, unoccupied TRs are mostly bound to thyroid responsive elements (TREs) near the promoter region where they form complexes with transcriptional repressors, reducing the velocity at which target genes are transcribed. T3 binding to TRs might direct additional TRs to specific DNA sites. Furthermore, binding to T3 shifts the affinity of TRs from corepressors to coactivators, not only de-repressing but also transactivating transcription of target genes (2). In addition to transcriptional effects, TRs might also function via a noncanonical pathway that does not require binding to TREs (31). Although complete loss of canonical TH action is observed in knock-in mice with a TR mutation that abrogates binding to DNA, several important TH-dependent physiological effects are preserved, including heart rate, body temperature, blood glucose, and triglyceride concentration, indicating that they could be affected by noncanonical TR signaling (31).

The functions of all three components of the signaling TRIAD are intertwined and constitute the basis for localized control and tissue specificity displayed by TH action, with most tissues having their own unique blend of transporters, deiodinases, and TRs. The focus of this article is to review these mechanisms in the context of existing paradigms of dynamic control of TH signaling and their relevance to human disease.

Circulating T3 Underlies TH Signaling in Most Tissues

Whether a cell or tissue responds to T3 depends on the expression of TH transporters and the presence of TRs, which may vary from very few (minimally responsive cells) to as many as 8000 TR molecules per cell, as seen in liver, pituitary, and BAT (32, 33). TRs display relatively high affinity and low capacity for T3 and, as T3 levels increase, T3–TR binding increases following an asymptote curve that reflects higher TR occupancy and consequently greater intensity of

TH-dependent biologic effects. This relationship has been demonstrated in isolated cell nuclei, in intact cells, and in whole animals (34–36); it is also evident in transgenic mouse models that assess TH signaling through a reporter gene (37, 38).

Based on the known TR affinity for T_3 (K_a of ~10^{12} L/M) and euthyroid plasma levels of free T_3 (FT_3; ~10^{-12} M), it is estimated that about half of the TR pool in liver and kidney cells is occupied with T_3 derived from the circulation, a figure that has been confirmed experimentally (32, 39). In other words, the circulating level of FT_3 in euthyroid individuals provides the cell nucleus with sufficient T_3 to occupy about half of the TRs, respectively activating or suppressing genes that are positively or negatively regulated by T_3, eventually leading to downstream biologic effects. The other half of the TRs in these organs remain empty but do exert biologic effects by repressing genes that are positively regulated by T_3. Thus, the presence of TRs and the balance between occupied and unoccupied TRs are what define the type and intensity of T_3-dependent biologic effects in any given cell or tissue.

Circulating T_3 levels are important determinants of TH signaling. Indeed, in most tissues the level of TR occupancy, expression of T_3-responsive genes, and downstream biologic effects are greatly influenced by circulating T_3 levels. In other words, as long as TH transmembrane transporters are available, T_3 from plasma will enter cells at levels that occupy half of the TR pool. Conversely, a drop in plasma T_3 will reduce TR occupancy in most tissues as well. For example, studies in rats estimate that a mere 10% drop in plasma T_3 levels reduces liver and kidney TR occupancy by ~15% (40). These changes are of course magnified in patients with hypothyroidism or hyperthyroidism in whom plasma T_3 levels may fluctuate markedly. As a counterpoint, there are instances in which TR occupancy does not reflect the levels of plasma T_3. For example, in cells that express *DIO2*, intracellular T_3 levels are higher than expected from circulating T_3 (33). In contrast, in cells that are deficient in functional TH transporters, as for example in the Allan-Herndon-Dudley syndrome, extracellular T_3 only minimally enters cells, and hence there is low TR occupancy (41, 42). Additionally, in cells that express *DIO3*, T_3 can enter but could be inactivated before reaching TRs (43, 44).

It is estimated that in healthy adult individuals ~100 μg of T_4 and 30 μg of T_3 are produced daily (Fig. 3). About 5 μg/d T_3 is secreted directly from the thyroid gland into the circulation, whereas the remainder of 25 μg/d is produced outside the thyroid parenchyma via T_4 deiodination (5). Thyroidal T_3 derives from thyrocyte digestion of iodinated thyroglobulin; despite the existence of ~70 tyrosine residues distributed within thyroglobulin, formation of T_4 and T_3 happens at relatively few sites. Whereas the molar ratio of T_4 to T_3 in human thyroglobulin is 15:1, some estimates are that thyroidal secretion contains a molar

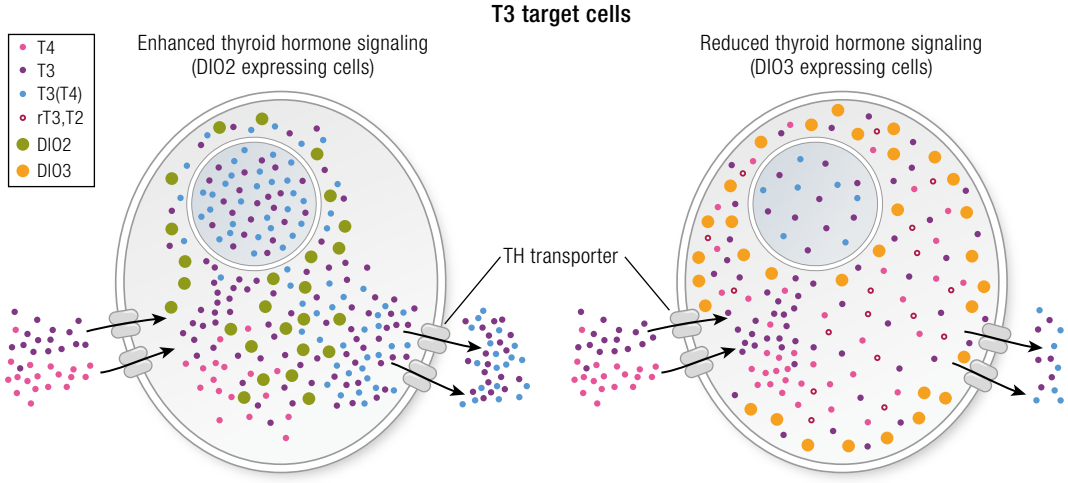
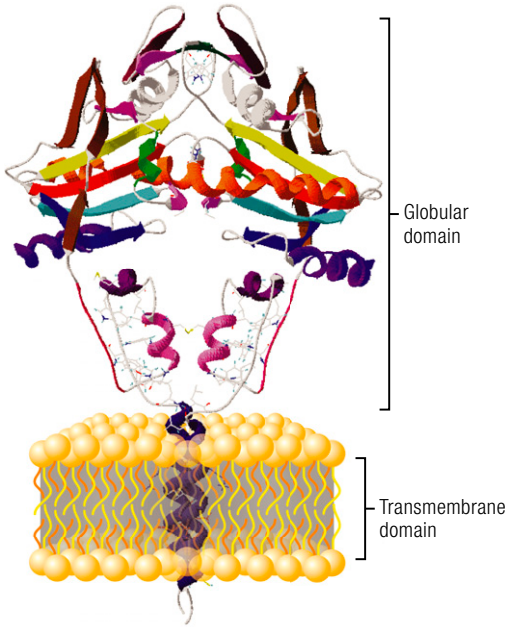


Figure 1. Deiodinases modify local TH signaling. T4 and T3 enter virtually all cells through membrane transporters. Once inside the cells, T3 diffuses to the nucleus and interacts with TRs to modulate gene expression. T3–TR complexes control specific sets of T3-responsive genes, thus promoting T3-dependent biological effects. While inside the cells, TH molecules can be modified through the deiodinase group of enzymes. Deiodinases modify the biological activity of TH molecules either activating T4 (D2) or inactivating T4 and T3 (D3). As a result, the flow of T3 molecules diffusing from the cell membrane to the nucleus can be enhanced with additional T3 supplied by the D2 pathway, which locally converts T4 to T3. In contrast, the D3 pathway decreases the flow of T3 to the nucleus because it terminally inactivates T3 to T2. D2 is an ER-resident protein, a cell compartment that is adjacent to the nucleus. This explains why D2 activity results in higher TR occupancy with locally generated T3. In contrast, D3 sorts to the plasma membrane, where it undergoes endocytosis and recycling via early endosomes. Notably, under hypoxic and/or ischemic conditions, D3 is redirected to the nuclear envelope, where it inactivates T3 and slows down cellular metabolism. See reviews for more details (1, 4, 17). [Adapted with permission from "Hypothyroidism, thyroid hormones and deiodinases." www.BiancoLab.org.]

Figure 2. Molecular structure of deiodinases. Deiodinases are homodimeric type I integral membrane selenoproteins composed of a single N-terminal transmembrane segment connected to a larger globular domain with a selenocysteine-containing active center embedded in a thioredoxin-like fold (18). The structure of the three deiodinases is similar as modeled through hydrophobic cluster analysis in combination with position-specific iterated BLAST. Their extramembrane portion belongs to the thioredoxin-fold superfamily (18). The crystal structure of an inactive catalytic domain of one of the deiodinases (mouse D3) was solved and confirmed most aspects revealed with the three-dimensional modeling (19). It also revealed a close structural similarity to 2-Cys peroxiredoxin(s) (Prx), which suggests a route for transferring protons to the substrate during deiodination and a mechanism for subsequent recycling of the transiently oxidized enzyme (19). [Adapted with permission from "Hypothyroidism, thyroid hormones and deiodinases." www.BiancoLab.org.]



ratio of 11:1, which indicates that thyroidal T_3 secretion could be enriched via intrathyroidal deiodination of T_4 to T_3 (45–47).

In healthy adult individuals, ~40% of the T_4 produced daily is converted to T_3 via D1 or D2 pathways (Fig. 3). Essentially, circulating T_4 molecules enter deiodinase-containing cells, broadly distributed throughout the body, and are deiodinated to T_3. In turn, these newly formed T_3 molecules exit cells and enter the circulation, mixing with the T_3 molecules that were secreted directly from the thyroid gland. In euthyroid individuals, D2 is thought to catalyze the bulk of daily T_3 production, ~20 μg/d, with a smaller contribution provided by D1 (5 μg/d) (5). Given the widespread *DIO2* expression throughout the body, it is likely that multiple organs/tissues collectively contribute to daily T_3 production. Relatively high D2 specific activity can be found in the brain, pituitary gland, and cold-stimulated BAT (5, 48). D2 is present in many other tissues at lower specific activity, including skin, SKM, skeleton, vascular smooth muscle, and testis (49–53). The brain, however, also expresses relatively high levels of *DIO3*, minimizing its potential as a source of plasma T_3. BAT, alternatively, does not express *DIO3*, and thus it is potentially an important source of circulating T_3. This has previously been shown in rodents (54), but the finding of D2-containing BAT in humans (55) indicates that, also in humans, BAT could be a relevant source of circulating T_3. Less is known about the contribution from tissues that express *DIO2* at low levels; given the mass of some of these organs/tissues, it is probably relevant as well.

D1 expression is limited and can be found predominantly in liver, kidney, and thyroid gland. D1 also metabolizes conjugated T_3, clearing these molecules from the circulation (28). For example, T_3 is a poor D1 substrate but, once it is sulfated in its outer ring, sulfated T_3 (T_3S) gains water solubility and is rapidly metabolized via D1, conceivably to conserve iodide before the molecule is eliminated in the urine or bile (28, 56). T_3S has no biologic activity, but sulfatases present in tissues, particularly the placenta, and the intestinal microflora can convert T_3S back to T_3. It is currently unclear the extent to which these pathways play a role in the human T_3 economy.

Adjustable T3 production and clearance preserve stability of circulating T3 levels

Two pathways cooperate to maintain stable circulating T_3 levels: (i) thyroidal T_3 secretion and (ii) the group of deiodinases. Combined, they stabilize plasma T_3 levels, preserving TH signaling and clinical euthyroidism in most tissues.

Thyroidal T3 secretion

The hypothalamic–pituitary axis adjusts thyroidal T_3 output through controlled thyroglobulin iodination (*i.e.*, the molar ratio of T_4 to T_3 in the thyroglobulin) and conversion of T_4 to T_3 within the thyrocyte. In light of studies on the *Mct8* knockout (*Mct8*-KO) mouse, it is conceivable that *Mct8* expression within the thyroid also modifies the T_4/T_3 ratio that is secreted from the thyroid gland. In such animals there is reduced efflux of T_4 from thyrocytes, thereby providing more substrate for intrathyroidal deiodination to T_3 (57). The molar ratio of T_4 to T_3 in the thyroglobulin molecule is sensitive to TSH receptor stimulation. Thyroidal stimulation by TSH increases T_3 formation within thyroglobulin (58–60), thus lowering the thyroidal T_4/T_3 molar ratio and increasing the relative secretion of T_3. Iodine deficiency and Graves disease are two extreme examples of this phenomenon, in which the molar ratio of T_4 to T_3 in the thyroglobulin can drop to 5:1 (45, 46). It is not clear

to what extent subtle changes in circulating TSH, seen for example during circadian rhythmicity, contribute to daily variations in circulating T3 as opposed to cues generated by the transition from fed to fast states (61), which affect extrathyroidal T3 production via deiodination (62, 63). A cross-sectional study in healthy individuals with 24-hour blood sampling and cosinor analysis indicated that T3 follows a circadian rhythm with periodicity that lags behind TSH, suggesting a more significant role for thyroidal secretion of T3 (64).

In a remarkable show of adaptability, the thyroidal secretion is capable of preserving serum T3 levels in mice with single or combined global inactivation of genes encoding D1 (*Dio1*) and/or D2 (*Dio2*) (65–68). In these animals, there is increased secretion of TSH that accelerates thyroidal T3 output, making up for the lack of extrathyroidal T3 production. A byproduct of the enhanced thyroidal activity is elevation of circulating T4 that is tolerated without suppression of TSH. Serum T3 is preserved even when *Dio2* is inactivated in a tissue-specific manner such as in TSH-producing cells (69), glial cells (70), SKM (71), adipose tissue, or liver (72). Notably, a similar hypothalamic–pituitary–thyroid (HPT) response is involved in the maintenance of serum T3 levels during iodine deficiency or mild hypothyroidism (73, 74). In both conditions, there is an increase in serum TSH levels due to decreased serum T4 whereas serum T3 remains within normal range or even above normal (75).

These analyses indicate that thyroidal T3 secretion is the gateway through which the HPT axis affects systemic TH signaling. Thyroidal T3 output is particularly sensitive to TSH signaling, thus explaining how the HPT axis plays such an important role (58). The HPT axis seems to be particularly driven to defend serum T3 levels (67).

The deiodinase group of enzymes

These enzymes adjust T3 production and clearance outside the thyroid parenchyma in response to fluctuations in circulating TH levels. *DIO2* and *DIO3* expression and activity exhibit inverse reciprocal relationship during hypothyroidism or hyperthyroidism (4, 76, 77). Whereas *DIO2* is negatively regulated by TH, the opposite is observed for *DIO3*. As a result, in hypothyroidism there is an increase in the fractional

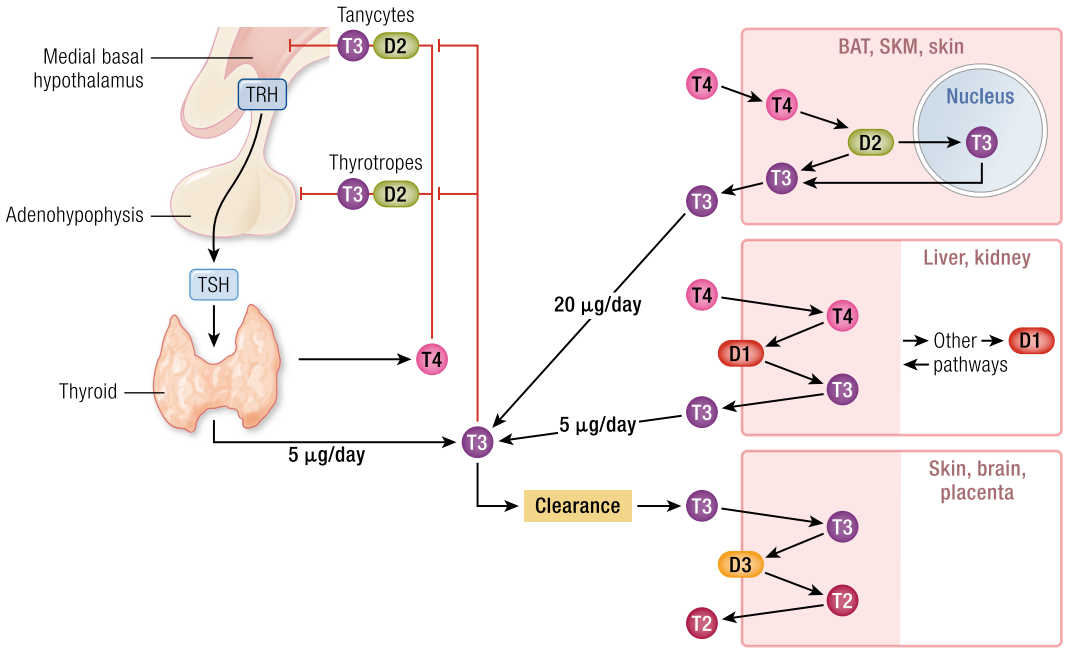


Figure 3. Sources and clearance mechanisms of circulating T3 in humans. The daily T3 production in a 70-kg adult individual is ~30 µg/d. The thyroid gland contributes with ~5 µg/d and the rest is produced outside of the thyroid parenchyma via two deiodinase-mediated pathways, D1 and D2; the latter is the most important source of circulating T3 in humans. Even though the thyroid contributes with a small fraction of the circulating T3, thyroidal T3 secretion is upregulated in response to TSH stimulation. This occurs through an increase in the T3/T4 ratio in the thyroglobulin and through increased thyroidal conversion of T4 to T3. Through this mechanism and the homeostatic changes in deiodinase activity, circulating levels of T3 are maintained fairly stable throughout the day. T3 is cleared from the circulation by deiodination via the D3 pathway that converts T3 to T2, as well as hepatic glucuronidation and sulfation, the latter followed by deiodination via the D1 pathway. In cells expressing D1, the T3 residence time inside the cells is relatively short, that is, ~30 minutes, whereas in D2-expressing cells the residence time is several hours. This is probably the result of distinct subcellular localization of D1 vs D2, plasma membrane vs ER, respectively. Additionally, T3 produced in D2-expressing cells finds its way to the cell nucleus and binds to TRs, triggering biological effects. See reviews for more details (5). [Adapted with permission from "Hypothyroidism, thyroid hormones and deiodinases." www.BiancoLab.org.]

conversion of T4 to T3, which is a reflection of the higher D2 activity; there is also decreased clearance of T3, which reflects lower D3 activity. Both adjustments contribute to maintenance of serum T3 levels within the normal range (78–80).

Although *DIO2* expression is only weakly downregulated by T3 (81), D2 activity is greatly decreased by T4 via posttranslational mechanisms. D2 protein and catalytic activity are lost upon interaction with T4 (82, 83) as a result of conjugation to ubiquitin (84, 85). This explains why D2 exhibits a variable half-life that depends on whether its natural substrate T4 is available. In the presence of T4, D2 is inactivated with an ~20-minute half-life, whereas in the absence of T4 its half-life is prolonged to hours. This provides a mechanism through which the production of T3 can be regulated according to the availability of T4.

The covalent attachment of multiple ubiquitin molecules to D2 both inactivates the enzyme and targets it to degradation in the proteasomes (85–87) (Fig. 4). Ubiquitination is thought to inactivate D2 by disrupting conformation of the D2:D2 dimer, critical for enzyme activity (21, 84). A unique 18–amino acid loop in the D2 molecule confers its intrinsic metabolic instability, facilitating binding to proteins involved in the ubiquitination process (88, 89). The ubiquitin-activating enzymes (UBCs) 6 and 7 are critical for the process of D2 ubiquitination (90, 91), as are two ubiquitin ligases, the hedgehog-inducible WD repeat and SOCS box-containing 1 (WSB1), and membrane-associated ring-CH-type finger 6 (TEB4), a ligase involved in the degradation of endoplasmic reticulum (ER) proteins (89, 92, 93). Ubiquitinated D2 (UbD2) is not immediately taken up by the proteasomes. Instead, UbD2 can be reactivated by deubiquitination, a process catalyzed by two ubiquitin-specific peptidase (USP) class D2–interacting deubiquitinases, USP20 and USP33 (94).

D2 ubiquitination occurs via K48-linked ubiquitin chains (95). Once UbD2 is formed, it can be taken up by 26S proteasomes after it is retrotranslocated to the cytoplasm via interaction with the p97–ATPase complex (Fig. 4). D2 retrotranslocation also includes deubiquitination by the p97-associated deubiquitinase Ataxin-3. Once in the cytosol, D2 is delivered to the proteasomes as evidenced by coprecipitation with 19S proteasome subunit S5a and increased colocalization with the 20S proteasome (95). Notably, the other two deiodinases, D1 and D3, are not known to be ubiquitinated or undergo posttranslational modifications.

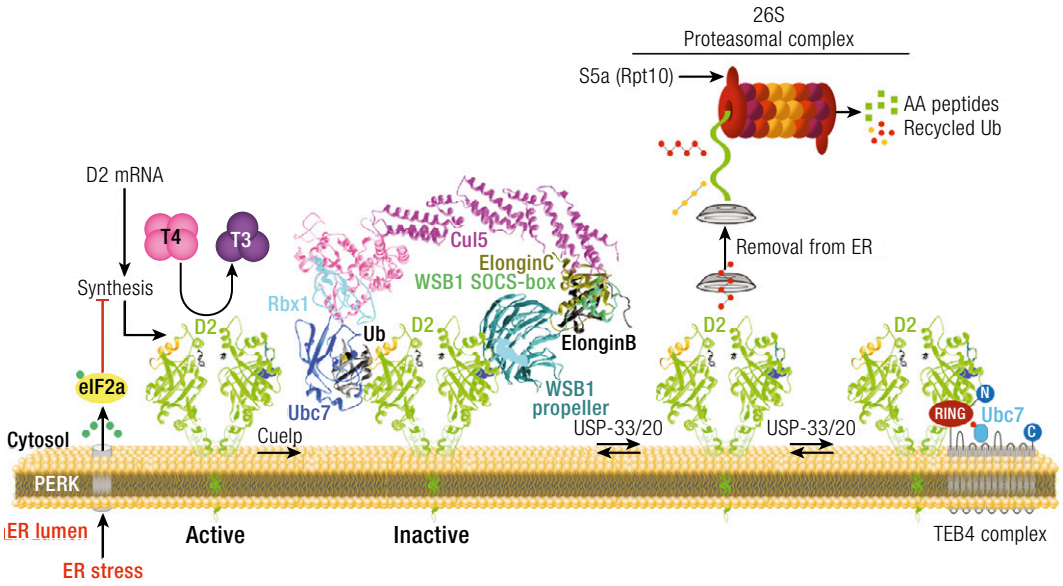
Food availability adjusts T3 production and controls TH signaling

In humans and other mammals, food availability is a key factor for stimulation of the thyroid system, ensuring coupling between caloric intake and TH signaling (96, 97). Default thyroid activity, in the absence of food, is low, along with low circulating TH levels and a slow rate of energy expenditure. Once caloric intake is initiated, thyroidal activity is accelerated and circulating TH levels increase; for example, this is seen in patients recovering from anorexia nervosa (98). In such patients, weight gain and elevation in serum T3 are closely associated with acceleration in energy expenditure (98). These mechanisms are largely driven by the hypothalamus, based on molecules that signal nutritional status, for example, leptin and insulin (99–101).

A striking feature of the thyroid system during caloric restriction or fasting in humans includes low serum T3; serum T4 may be low as well, and this coexists with normal/low serum TSH (102, 103). TSH-releasing hormone (TRH)/TSH may not be elevated because of the increase in medial basal hypothalamus (MBH) *Dio2* expression and TH signaling as seen in mice during fasting (104). As a result, serum TH levels diminish unopposed. In humans, the T3 production rate declined by ~50% after a 6-day fasting but the metabolic clearance rate of T3 remained unchanged (105). In contrast, rT3 clearance is reduced by ~40% in these individuals, without changes in rT3 production (106). The use of animal models to understand the mechanistic basis of these changes has its limitations. Fasting in rodents is associated with decreased thyroidal (107) and extrathyroidal T3 production via reduced activity of the D2 pathway (62). The activity of the D1 pathway is reduced as well (63, 108), but it has been difficult to ascertain whether this is cause or consequence given that *Dio1* is inducible by T3 as shown in rodents (107, 109). Notably, fasting for 16 to 36 hours reduced circulating levels of T4 and T3 in double D1/D2KO mice (110). In these animals, as well as in other mouse models of food deprivation (111), D3 activity was increased up to fourfold in skeletal muscle, liver, and kidney. Additionally, fasted mice also exhibit an increase in the expression of enzymes involved in glucuronidation and sulfation of iodothyronines in the liver, with the latter potentially followed by deiodination via the D1 pathway (112). These studies suggest that in rodents, as opposed to humans, an accelerated clearance of T4 and T3 plays a major role in fasting-induced changes in thyroid economy. Notwithstanding these differences, reduced levels of circulating T3 in all models of fasting diminish TH signaling in most tissues, explaining the reduction in metabolic rate.

Among different nutrients, carbohydrates are the most effective to modulate circulating T3 levels (96, 97). In fact, it is thought that our Paleolithic ancestors had low circulating T3 levels, as they subsisted on a very low–carbohydrate/high-protein diet. The agricultural revolution with the increase in dietary carbohydrate, ~10,000 years ago, might have brought circulating T3 levels to what they are today, increasing iodine requirements and hence expanding iodine deficiency (113). Mechanistically, a hint that D2-generated T3 is nutritionally regulated came from the observation that insulin stimulates D2 activity in

Figure 4. D2 is inactivated by ubiquitination. ER stress rapidly reduces D2 activity via activation of eIF2a, which inhibits translation of *Dio2* mRNA. D2 ubiquitination is the molecular mechanism underlying changes in D2 half-life, that is, the covalent attachment of multiple ubiquitin molecules to D2, which both inactivates the enzyme and targets it to degradation in the proteasomes. D2 is structured as a homodimer, D2:D2, and monomers are inactive. Ubiquitination is thought to inactivate D2 by disrupting the conformation of the D2:D2 dimer, critical for enzyme activity. A unique 18–amino acid loop confers intrinsic metabolic instability to D2, facilitating binding to proteins involved in the ubiquitination process. UBC6 and UBC7 are critical in the process of D2 ubiquitination, as well as two ubiquitin ligases, the hedgehog-inducible WSB1, and TEB4, a ligase involved in the degradation of proteins in the ER. The WD-40 propeller of WSB-1 recognizes an 18–amino acid loop in D2 that confers metabolic instability, whereas the SOCS box domain mediates its interaction with an ubiquitinating catalytic core complex, modeled as Elongin BC–Cul5–Rbx1. Ubiquitinated D2 (UbD2) can be reactivated by deubiquitination, a process catalyzed by two USP class D2–interacting deubiquitinases, USP20 and USP33. D2 ubiquitination occurs via K48-linked ubiquitin chains and exposure to its natural substrate, T4, accelerates UbD2 formation. UbD2 is retrotranslocated to the cytoplasm via interaction with the p97–ATPase complex. D2 retrotranslocation also includes deubiquitination by the p97-associated deubiquitinase Ataxin-3. Once in the cytosol, D2 is delivery to the proteasomes as evidenced by coprecipitation with 19S proteasome subunit S5a and increased colocalization with the 20S proteasome. See reviews for more details (86, 87). [Adapted with permission from "Hypothyroidism, thyroid hormones and deiodinases." www.BiancoLab.org.]



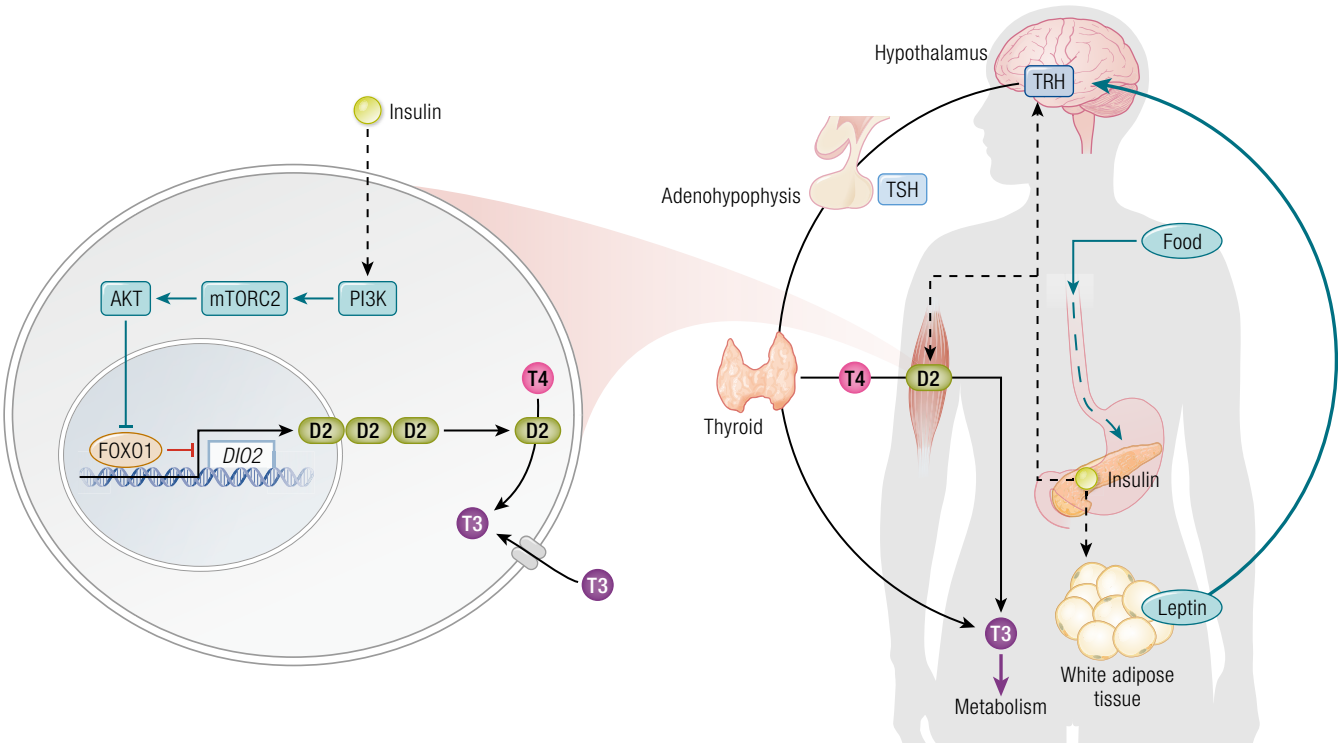
rat brown adipocytes (114) and that insulin sensitizers stimulate *Dio2* expression in cultures of skeletal myocytes (115). Additionally, D2 activity in BAT is upregulated by growth factors such as IGF-1 and insulin (114, 116), which promotes glucose uptake and growth through nutrient sensing pathways such as the phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) (117, 118) pathways (Fig. 5). Indeed, semistarvation in rats is associated with the higher gene encoding D3 (*Dio3*) and lower *Dio2* in skeletal muscle along with slower formation of T3 from T4; these changes are associated with accumulation of slow-twitch fibers at the expense of fast-twitch fibers, which is a hallmark of reduced TH signaling in the skeletal muscle. Thus, it is conceivable that diminished skeletal muscle T3 production and accelerated T3 catabolism not only explain the slower muscle energy expenditure rate following caloric restriction but also contribute to the lower circulating levels of T3 (119). Studies in cells and mice indicate that *Dio2* is normally inhibited by forkhead box, subgroup O (Foxo) 1 (*Foxo1*), a transcriptional regulator that binds the *Dio2* promoter. In turn, insulin signals through the PI3K–mammalian target of rapamycin complex (mTORC) 2–serine/threonine kinase 1 (AKT) pathway to relieve *Foxo1* repression. These studies provide a mechanistic explanation for why in humans fasting is associated with a reduction in SKM D2 activity that is partially prevented by insulin administration (120).

Dio2 expression in the cerebral cortex is not modified by fasting or refeeding, indicating that *Dio2* regulation by nutrient availability is not universal, likely occurring in tissues where the metabolic pathways are responsive to T3 and insulin such as BAT, SKM, and neonatal liver (1, 72). Thus, the balance between PI3K–mTORC2–AKT and *Foxo1* signaling in metabolically relevant tissues should provide nutritional input and fine-tuning to the regulation of circulating levels of T3 and T3-dependent processes.

Accelerated D1 activity increases T3 production in hyperthyroid patients

On the other end of the spectrum, circulating T3 might be disproportionally high in patients with hyperthyroidism, particularly with Graves disease, which

Figure 5. Nutrient availability and activation of TH signaling. Leptin is a key molecule signaling food intake and the availability of energy substrates to the hypothalamus, where it activates the HPT axis by stimulating secretion of TRH and TSH, and hence thyroidal activity. There is a drop in serum T3 levels with fasting, which reflects decreased thyroidal secretion and decreased extrathyroidal conversion of T4 to T3. The mechanism regulating *DIO2* expression in skeletal muscle in this setting was modeled by shifting cells to media containing only 0.1% fetal bovine serum, which reduces *DIO2* expression via FOXO1-mediated transcriptional repression (62). There is a FOXO1 binding site within the *DIO2* promoter, close to the transcription start site. Binding of FOXO1 to this site suppresses *DIO2* gene expression. In contrast, shifting cells back to a media containing 10% fetal bovine serum (after 24 h of fasting) increases *DIO2* expression and D2 activity through a mechanism initiated by insulin and mediated by a series of kinases (PI3K–mTORC2–AKT) that end up phosphorylating FOXO1, hence relieving *DIO2* repression. These findings are relevant for hypothyroid patients maintained on L-T4 that depend on D2 for >80% of all their T3 needs; thus, they are at greater risk to develop low serum T3 during caloric restriction (62). [Adapted with permission from "Hypothyroidism, thyroid hormones and deiodinases." www.BiancoLab.org.]



contributes with the overall enhancement of TH signaling. This has been attributed to an accelerated thyroidal T_3 secretion and T_4 to T_3 conversion via the D_1 pathway. T_3 secretion is accelerated thanks to increased T_3 synthesis (58) as well as increased intrathyroidal conversion of T_4 to T_3 (121). In a study of patients with Graves disease, higher serum T_3 levels correlated with higher thyroidal D_1 and D_2 activities (122). Similarly, patients with hyperthyroidism as part of the McCune–Albright syndrome also exhibit a lower circulating T_4/T_3 ratio thanks to accelerated D_1 and D_2 activities in the thyroid parenchyma (123). In hyperthyroid patients *DIO1* expression is also upregulated outside the thyroid gland (124), which is reminiscent of the fact that in rodents *Dio1* is highly sensitive and positively regulated by T_3 (109). Given this prominent role played by D_1 in producing T_3 in hyperthyroid patients, propylthiouracil (PTU), which specifically inhibits D_1 activity, has been advocated as a more effective antithyroid drug in severe thyrotoxicosis or thyroid storm (125).

Transmembrane transport, deiodinases, and TRs fine-tune TH signaling

The signaling TRIAD, that is, (i) transmembrane transport, (ii) intracellular deiodination, and (iii) TR-mediated gene transcription, defines TH signaling; with each component, conditions exist that may, permanently or transiently, enhance or dampen TH signaling.

Local mechanisms for customization of TH signaling

A number of physiological and pathophysiological conditions exist in which homeostatic or disease signals can transiently affect the TRIAD that controls TH action in specific organs or tissues, resulting in dynamic changes of local TH signaling. THs move across the plasma membrane (in and out of cells) via transporters following a concentration gradient of free hormone between the extracellular fluid and the cytoplasm. Thus, movement of TH molecules across the cell membrane requires the transporters, but a relative

excess of transporter molecules should not increase further intracellular levels of THs or TH signaling (126). In other words, increasing the expression of TH transporters beyond a critical minimal number will likely only speed up the time to equilibrium between the two compartments, not define the intracellular levels of THs. In fact, most of the situations in which TH transporters affect TH signaling are the result of transporter inactivation or marked downregulation. In contrast, dynamic modifications of TRs and transcriptional coregulators have been shown to affect the intensity of TH signaling both ways, but by far the most impressive dynamic control of TH action is seen as a result of the deiodinase group. It is unlikely, however, that deiodinases define TH signaling universally, at all times. It is expected that future studies will reveal new components, pathways, and nuances underlying dynamic control of TH signaling, such as, for example, posttranslational modifications of TH transporters and TRs. Furthermore, it is imperative that we better understand how cytosolic T_3 finds its way to the cell nucleus. It is generally agreed that the T_3 equilibration between cytosol and cell nucleus is defined by simple diffusion. However, estimates of the concentration of FT_3 in these compartments revealed nuclear/cytosolic T_3 ratios of ~58 in the liver, ~56 in the kidney, ~81 in the heart, and ~251 in the brain, suggesting that a specific transport mechanism exists from cytosol to the cell nucleus (127). It remains to be seen whether D2 with its perinuclear localization plays a role in the higher ratios observed in the brain.

Tissue-specific dynamic changes in TH signaling occur in a number of systems in response to multiple physiological cues, without antecedent changes in circulating levels of THs. For example, cold exposure through the sympathetic nervous system stimulates *Dio2* expression and T_3 production in BAT that adds to the intracellular T_3 entering from the circulation. As a result, there is an increase in cellular T_3 content that augments TR occupancy from its baseline level of ~75% (29, 33) to >95%, along with induction of T_3-responsive genes (128). Such a role for D2 in defining local TH signaling is not unique to BAT (4). For example, in the developing setting a timed surge in D2-generated T_3 is critical for a number of organs, including cochlea (129) and liver (72). In the adult mouse, D2-generated T_3 has also been shown to play a role in brain, lung, SKM, and skeleton (70, 130–132) (Fig. 6).

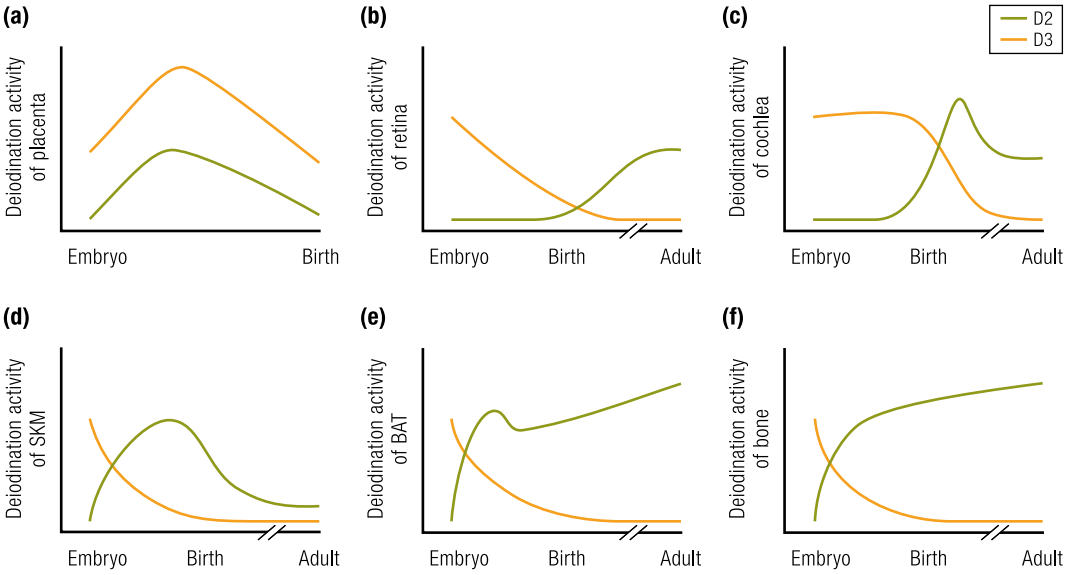
Unfortunately, measuring TR occupancy to assess changes in TH signaling is cumbersome and rarely done. Alternatively, investigators have measured tissue T_3 or relied on changes in mRNA levels of T_3-responsive genes or well-known T_3-dependent biologic effects to study TH signaling (9). For example, it is assumed that induction of *DIO3* in skin cells by members of the Hedgehog family of proteins reduces TH signaling. This is because the mRNA levels for cyclin D1, a gene that is negatively regulated by T_3, increase upon induction of *DIO3* and is followed by proliferation of keratinocytes (133). Similarly, two mouse models of *DIO2* overexpression in the myocardium further illustrate how deiodinase expression modifies TH signaling (134, 135). In both cases, modulation of T_3-responsive genes and biologic effects were documented. Notwithstanding, there is still the possibility that dynamic changes in deiodinases (or other elements of the signaling TRIAD) coincide with but do not directly affect the expression of T_3-responsive genes. Multiple approaches have been used to exclude random associations, including gene inactivation or silencing as well as phenotypic rescue with reintroduction of the targeted gene (9).

The availability of mouse models that express T_3 reporter systems has been helpful in the evaluation of the signaling TRIAD (38, 136). For example, a mouse with global *Dio3* inactivation (global-D3KO) was crossed with the transgenic mouse model $FINDT_3$ that expresses the reporter gene β-galactosidase as a readout of local TH signaling. Studies of the $FINDT_3$/D3KO litter indicate that TH signaling in the central nervous system (CNS) of these animals fluctuates throughout the animal's life. Following a period of enhanced TH signaling in early development, most regions of the D3KO brain experience reduced TH signaling. Notably, TH signaling is elevated again later in adulthood and in old age, despite reduced circulating TH levels (136). As a counterpoint, the role played by *Dio2* activation in TH signaling can be visualized through bioluminescence in the TH action indicator mouse model (38), a transgenic mouse ubiquitously expressing a luciferase reporter gene regulated by a strong TRE that operates in the context of endogenously expressed levels of TH transporters, TRs, and transcriptional coregulators. Exposing these mice to cold (4°C) caused tissue-specific bioluminescence in the interscapular region (interscapular BAT), along with an ~3.0- to 9.0-fold increase in luciferase activity and mRNA in interscapular BAT, which was eliminated after surgical denervation of the organ (38).

TH transporters

The type and expression level of TH transporters constitute an intrinsic property of each cell/tissue, which in general responds minimally to physiological or disease signals. For example, it is unclear whether the expression of TH transporters is affected by TH. Changes are small and there is conflicting evidence, with interspecies variability (137). Furthermore, TH transporters are typically multispanning plasma membrane proteins with long half-lives, unlikely to exhibit fast regulation. Notwithstanding, there are reports of increased transporter expression in liver and SKM of critically ill patients and in a rabbit model of prolonged critical illness (138), as well as in thyroid tissue of patients with Graves disease (139). In contrast, *Mct8* and *Oatp1c1* expression in the mouse

Figure 6. Developmental control of TH signaling via timed expression of deiodinases. Profiles of *DIO2* (red line) and *DIO3* (blue line) expression in (a) placenta, (b) retina, (c) cochlea, (d) SKM, (e) BAT, and (f) bone at the indicated periods of life. In most cases *DIO2* and *DIO3* exhibit a reciprocal inverse relationship. In general, D3 activity is high at early embryonic stages. Its expression drop is followed by an elevation in *DIO2*. See reviews for more details (7, 8, 48, 68, 132).



blood–brain barrier (BBB) is transiently diminished in response to an acute inflammatory challenge by lipopolysaccharide (140). *Mct8* is also downregulated in benign and malignant thyroid tumors (139) as well as in rat thyroid tissue after iodine overload (141). The impact of these changes in transporter expression on local TH signaling, if any, has yet to be determined.

Mutations may impair the function of TH transporters, which in turn may dampen cellular uptake of T_3 and TH signaling in cells that depend (mostly) on a specific transporter (142). Fortunately, this is a rare condition due to redundancy of transporter molecules, but it can be seen in carriers of *MCT8* gene mutations that result in the X-linked Allan–Herndon–Dudley syndrome (41, 42) and in carriers of *OATP1C1* gene mutation that results in dementia with spasticity and cold intolerance (143). Intellectual disability and problems with movement in patients with *MCT8* mutations stem from developmental deficit of T_3 in brain areas where neurons rely on MCT8 to take up T_3. A mouse with *Mct8* inactivation exhibits somewhat reduced TH content in the cerebrum and cerebellum despite elevated circulating levels of T_3; neurologic deficits are present, but much less intense than in humans (144–146). In this species, the combined inactivation of *Oatp1c1* is also required to lower brain T_3 levels and cause locomotor abnormalities typical for Allan–Herndon–Dudley syndrome (147). Molecules with thyromimetic activity that enter cells via different mechanisms or transporters may be useful in these syndromes, as they could restore TH signaling. For example, administration of the TH analog diiodothyropropionic acid, which is less dependent on MCT8 to enter cells, seems to rescue brain hypothyroidism (148); Triac also bypasses the plasma membrane of fibroblasts obtained from carriers of *MCT8* mutations (149). Furthermore, diiodothyropropionic acid has been used in children with Allan–Herndon–Dudley syndrome with promising results (150).

Deiodinases

The discovery that deiodinases convert T_4 to T_3 in humans heightened interest in these enzymes (151). From a physiological perspective, deiodination was found to activate T_4 to T_3 in the MBH and pituitary gland, transducing plasma T_4 levels, via the T_3 molecule, to the system that regulates TRH and TSH secretion (152, 153); this explained the effect of T_4 on TSH secretion. Subsequently, local deiodination was identified as the source of most T_3 in the brain. Additionally, because the acceleration in D_2 activity preserves T_3 content in the cerebral cortex during iodine deficiency, this pathway was identified as key to cerebral cortex adaptation to low T_4 in the circulation (29). Later, studies in cold-exposed rats led to the discovery that induction of *Dio2* expression can enhance TH signaling in a tissue-specific fashion, without antecedent changes in plasma T_4 levels (33, 128, 154, 155). This mechanism explained the molecular link between deiodination and thermogenesis, specifically that the uncoupling protein 1 (UCP1) gene is transcriptionally upregulated by D_2-generated T_3 (156). Conversely, the observations that D_3 activity correlates inversely with tissue T_3 content (157) and

that *DIO3* expression can be reactivated in almost any tissue (158, 159) led to the discovery of conditions in which *DIO3* expression dampens local TH signaling and even causes systemic hypothyroidism (160). Subsequent studies in a number of vertebrate species led to the discovery that coordinated reciprocal expression of *Dio2* and *Dio3* during development customizes TH signaling in most organs/tissues according to a predefined developmental program (22, 161, 162) (Fig. 6).

Deiodinases are anchored in cellular membranes with the catalytic active site located in the cytosol (18, 19, 30). T_3 production via D1 and D2 occurs inside cells, but T_3 molecules eventually exit such cells via TH transporters mixing with the pool of circulating T_3. In contrast, D3-expressing cells function as sinks for T_4 and T_3, dampening local TH signaling and consuming circulating TH. Important differences exist between D1 and D2; D1 has low affinity for T_4 (K_m of ~10^{-6} M), has a half-life measured in hours, is induced by TH, and is inhibited by PTU; D2 has high affinity for T_4 (K_m of ~10^{-9} M), has a half-life measured in minutes, is inhibited by TH, and is inducible by cAMP. Additionally, the differing subcellular localizations of D1 and D2 impact the fate of T_3 molecules produced by these enzymes (30, 163). D1 is located in the plasma membrane, possibly explaining why D1-generated T_3 equilibrates rapidly with plasma; the mean residence time of D1-generated T_3 inside cells is ~30 minutes (39). In contrast, D2 is an ER-resident protein, possibly explaining why D2-generated T_3 does not equilibrate rapidly with plasma; the mean residence time of D2-generated T_3 is ~8 hours (33, 39).

D3 has high affinity for T_3 (K_m of ~10^{-9} M), and it is stimulated by developmental and disease signals. D3 is anchored in the plasma membrane and is constantly internalized to early endosomes and recycled back to the plasma membrane, which accounts for its relatively long half-life (~12 hours) (163). Both T_4 and T_3 entering cells from the circulation can be deiodinated via D3 to inactive molecules rT_3 and 3,3′-diiodo-L-thyronine (T_2), respectively. Thus, D3 activity depletes cells of TH and reduces TH signaling (126). Under hypoxic/ischemic conditions D3 is redirected to the cell nucleus where it accumulates in the nuclear envelope (164); this occurs via the cochaperone heat shock protein 40 (HSP40). Preventing nuclear D3 import by HSP40 knockdown increases the metabolic effects of T_3. In contrast, HSP40 overexpression increases nuclear import of D3 and minimized TH effects in cell metabolism (164).

Structural and functional properties of both D2 and D3 place these enzymes at the crossroads of TH action. The high catalytic activity of D2 associated with the longer residence time of D2-generated T_3 link D2 to intracellular buildup of T_3 molecules and enhanced TH signaling. On the contrary, the high catalytic activity of D3 along with its potential to accumulate in the nuclear envelope link D3 to lower intracellular T_3 content and reduced TH signaling.

An alternative way through which *DIO2* and *DIO3* expression can influence local TH signaling is by reacting to changes in circulating levels of T_4 and T_3, hence minimizing the impact of these changes on TH signaling (165). For example, iodine deficiency in rats markedly lowers plasma T_4 without affecting plasma T_3. Circulating T_3 is preserved due to increased thyroidal T_3 secretion, to accelerated conversion of T_4 to T_3 in *Dio2*-expressing tissues, and to reduced T_3 clearance in *Dio3*-expressing tissues. Although the decrease in plasma T_4 does not affect TH signaling in tissues that depend on circulating T_3, it places tissues that depend on local D2-mediated T_4 to T_3 conversion, such as brain, at risk for reduced TH signaling. Therefore, adaptive changes in *Dio2* and *Dio3* expression occur under these circumstances that preserve T_3 content and TH signaling in these tissues. In the brain, iodine deficiency lowers *Dio3* mRNA expression and D3 activity several fold, whereas D2 activity is increased by ~20-fold. Thus, reciprocal changes in D2 and D3 activities are an integral component of the brain's physiological response to iodine deficiency (166). Indeed, cerebral T_3 levels are preserved in iodine-deficient mouse pups throughout most of the postnatal period of brain development (167), highlighting the homeostatic efficiency of the deiodinases.

Deiodinase genes. Inactivating mutations in the genes encoding the deiodinases have not been reported. These are probably "concealed" by the extraordinary ability of the HPT axis to preserve circulating T_3 levels as seen in mice with complete absence of deiodinases (168). However, we know of alternatively spliced *Dio2* mRNA molecules that encode an inactive D2 enzyme, including both deleted and insertion-extended *Dio2* mRNA (76). In the human *DIO2*, for example, a 108 -bp region from the middle portion of the ~8-kb-long intron was found inserted downstream of codon 74, which results in an extra in-frame UGA codon and an ~35-kDa inactive D2 enzyme (169, 170). Additionally, there is also a *Dio2* mRNA variant that lacks 77 bp in the coding region at the conserved exon/intron junction, which results in an inactive D2 protein (169). The physiological and/or clinical implications of these mRNA variants remain poorly understood.

Notwithstanding, an array of single-nucleotide polymorphisms (SNPs) of the deiodinase genes exists that could disrupt TH signaling and explain associated clinical syndromes (171). Molecular scanning of *DIO2* identified a Thr92Ala variant present in 12% to 36% of the population (172) that is associated with metabolic parameters suggestive of reduced TH signaling (173). These original findings led to population-based studies suggesting associations

between Thr92Ala-*DIO2* and hypertension (174), insulin resistance (173, 175), type 2 diabetes (176), bipolar disorder (177), mental retardation (178), low IQ (179), recovery from lung injury (180), osteoarthritis (181), and increased bone turnover (182), all of which could reflect localized disruption in TH signaling. Perhaps unsurprisingly, the associations between *DIO2* SNPs and clinical syndromes have not been universally reproduced (87, 172, 183). Racial and other background factors that are difficult to control for may play important roles in such associations. Negative studies include the evaluation of 12,625 individuals, including 364 patients on TH replacement therapy. No associations between the Thr92Ala-*DIO2* SNP and thyroid parameters, quality of life, or cognitive functioning were observed (183).

The missense amino acid exchange encoded by Thr92Ala-*DIO2* is in the D2 coding region, specifically the very first amino acid in an 18-residue instability loop that explains the relatively short D2 half-life (18). Deletion of this loop stabilizes D2 (89) whereas its transfer to stable proteins shortens half-life (88). Mapping of this mutation to such a critical D2 residue supports the possibility that it could affect D2 activity and/or its stability, decreasing D2-mediated T3 production. However, several groups have failed to detect differences in enzyme kinetics [K_m(T4) and V_{max}] of the Thr92Ala-D2 protein when assayed in sonicates of cells transiently expressing Thr92Ala-*DIO2* (184, 185) or of tissue samples obtained from a mouse carrying the Thr92Ala-*Dio2* polymorphism (186). A contrasting finding was obtained in thyroid sonicates of individuals with the Thr92Ala-*DIO2* polymorphism, which revealed decreased V_{max} (184).

Understanding the impact of the Thr92Ala-*DIO2* polymorphism of D2 activity required utilizing intact cells. T4-dependent biologic effects were less evident in proliferating murine myoblasts and thyrotropes expressing Thr92Ala-D2, suggesting reduced D2-mediated T3 production (187). Indeed, when assayed in intact cells, Thr92Ala-D2 exhibited ~20% reduced catalytic activity despite similar D2 protein levels (186). Three studies in patients indirectly support this: (i) higher doses of levothyroxine (L-T4) were needed to achieve target TSH levels in 191 thyroidectomized individuals carrying the Thr92Ala-*DIO2* polymorphism (188); (ii) the Thr92Ala-*DIO2* polymorphism is associated with delayed T3 secretion in response to TRH stimulation (189); and (iii) in a study of 140 thyroidectomized subjects on L-T4, the *DIO2* genotype revealed an association between low FT3 values and Thr92Ala, with the mean postsurgery FT3 levels significantly lower in patients carrying the Thr92Ala allele(s) (187). Notwithstanding, results of all studies showed that individual carriers of the Thr92Ala-*DIO2* polymorphism with a normal thyroid gland are systemically euthyroid (185). Phenotypes related to polymorphisms in *DIO1* and *DIO3* have also been reported, particularly in association with modifications in circulating TH levels (190–192), but their clinical significance has been less well documented [see Refs. (193, 194) for review].

Deiodinase synthesis. Genetic defects in two components of the complex machinery required for selenoprotein synthesis have been reported to cause inherited deficiencies in the deiodinases and abnormal thyroid function signaling (195, 196). One such defect is caused by mutations in the selenocysteine insertion sequence (SECIS)–binding protein 2 (*SECISBP2* or *SBP2*), MIM 607693 (195). The C-terminal region of the protein is responsible for SBP2 functions: SECIS-binding capacity, ribosome interaction, and selenocysteine insertion through two main domains in the C-terminal region [the RNA-binding domain and the selenocysteine (Sec) incorporation domain] (197). Most patients with partial SBP2 deficiency seek medical attention during childhood because of short stature and delayed bone age. These features prompt thyroid function testing, leading to the identification of thyroid abnormalities. Currently only 10 families have been reported worldwide (198, 199). Recessive mutations in SBP2 result in abnormalities in TH metabolism with characteristic thyroid test abnormalities, with high serum T4, low T3, high rT3, and normal or slightly elevated serum TSH (195), which have served as biomarkers to identify additional patients with SBP2 deficiency. Additional clinical features have been observed in some patients, such as axial muscular dystrophy, azoospermia, skin photosensitivity, abnormal immune cell function, and marked insulin sensitivity, indicating a multisystem disorder involving the defective biosynthesis of multiple selenoproteins (200). Of note, most reported patients are children, and the only known adult with this defect exhibits many more symptoms, raising the concern that additional phenotypic features can develop with age (200).

In vitro studies in cultured skin fibroblasts have demonstrated decreased D2 activity and glutathione peroxidase 1, as well as decreased selenium, selenoprotein P, and glutathione peroxidase 3 enzymatic activity in serum, which supported a generalized defect in selenoprotein biosynthesis. *In vivo* studies in patients have shown that higher amounts of L-T4 and higher circulating serum T4 levels are needed to suppress TSH in affected subjects compared with controls, although similar doses of liothyronine (L-T3) and circulating levels of T3 were able to equally suppress TSH, indicating an impairment in deiodinase-mediated T4 to T3 conversion.

Considering that complete congenital Sbp2 deficiency is not compatible with survival (201), other targeting strategies have been used (201–203). The hepatocyte and neuron-specific knockout (KO) models have not replicated the circulating thyroid function tests (201, 203). A recent mouse model of tamoxifen inducible conditional KO (iCKO) of Sbp2

has replicated most of the characteristic serum thyroid test abnormalities, with high T_4, high rT_3, and elevated TSH, with normal T_3 (202). It is notable that there seems to be an inverse relationship between T_3 and TSH between mice and humans with SBP2 deficiency. In patients, T_3 is distinctly low, although TSH is usually normal and only occasionally slightly elevated, whereas in mice, TSH is distinctly high and T_3 levels are comparable to those of wild-type controls (195, 202). Additionally, mouse models of *Dio1* KO, *Dio2* KO, double-KO, and even triple-KO *Dio1/Dio2/Dio3* maintain normal circulating T_3 levels (110, 204). Detailed studies in the *Sbp2* iCKO mice demonstrated decreased enzymatic activity of D1 in liver, of D2 in cerebrum, and decreased expression of *Dio3* in cerebrum. Additional insights from study of the *Sbp2* iCKO mice have brought up new aspects; in particular, the brain T_3 content was low, despite normal and high circulating T_3 and T_4, respectively. This relative hypothyroidism at the cerebral level is expected to have consequences at the levels of TH-regulated genes.

Another inborn error in a component of the selenoprotein synthesis machinery was recently identified in a patient presenting with abdominal pain, fatigue, muscle weakness, reduced plasma selenium, and abnormal thyroid function tests similar to those of SBP2-deficient patients (196). This patient harbored a homozygous missense mutation in the *TRU-TCA1-1* gene, which encodes for tRNA[Ser]Sec. Whereas lack of tRNA[Ser]Sec in mice is embryonically lethal (205), studies on patients' cells showed preservation of reduced levels of tRNA[Ser]Sec (196). Identification of additional patients and further *in vitro* characterization will provide more insight into this new genetic defect that seems to also alter TH metabolism.

TH receptors

At some point during early embryogenesis cells start expressing TRα and/or TRβ. The levels at which these genes are expressed might change during development but, in broad terms, stay relatively stable throughout life. Notably, hundreds of patients carrying mutations in the TR encoding genes with significant phenotypes have been reported. Mutations that inactivate TRβ cause a syndrome of TH resistance in which patients exhibit hyperthyroidism due to pituitary insensitivity to THs. There is enhanced TH signaling in tissues that predominantly express TRα, for example, brain and bone, and diminished TH signaling in tissues where TRβ predominates, for example, pituitary gland and liver (148, 206). Fewer families with TRα mutations have been reported (207, 208). These individuals have low to low-normal serum free T_4 (FT_4), high-normal to high T_3, and low rT_3, with normal TSH levels, but they exhibit localized reduction in TH in tissues where TRα predominates (brain, skeleton, and gastrointestinal tract), resulting in growth retardation, mild impairment of mental development, and constipation (209).

The expression of TRα or TRβ may fluctuate as part of normal homeostatic mechanisms or in disease states, but it is not clear that an overarching hypothesis of how TH signaling is dynamically affected by these changes can be developed at this time. The question of whether TR expression is affected by TH signaling and/or other signaling molecules remains an interesting one. TH has been shown to regulate the TR level in a number of tissues and cell lines, but the results are not always consistent. Analysis of I-125–radiolabeled T_3 binding to isolated nuclei revealed no differences (increases or decreases) in the number of TRs in response to hypothyroidism or hyperthyroidism (210, 211). In a comprehensive study, hypothyroid rats were treated with either saline or T_3 followed by analyses of mRNA encoding different TR isoforms (212). There was marked tissue-specific and differential regulation of the multiple TR transcripts by T_3. In the pituitary, the levels of *TRα-1* mRNA increased, whereas the levels of the pituitary-specific *TRβ-2* decreased with T_3 treatment. In heart, kidney, liver, and brain the levels of *TRβ-1* were unaffected by thyroid status, whereas both *TRα* mRNAs decreased with T_3 treatment in all tissues except for the brain, where there was no change. The study, however, did not assess whether/how these changes affected TH signaling. Additionally, and also very importantly, there was a discrepancy between mRNAs levels and nuclear binding sites for T_3, indicating that relying on TR mRNA levels only might not be feasible (212).

Disease signals reportedly affect TR expression and TH signaling. Patients with nonthyroidal illness syndrome (NTIS) exhibit a drop in circulating T_3 levels but no obvious signs of clinical hypothyroidism. Studies on peripheral mononuclear cells from patients admitted to an intensive care unit revealed that this could be due to changes in TR expression (213). In such patients there were increases in mRNA levels of both *TRα* and *TRβ* when compared with peripheral mononuclear cells from normal individuals. Similar findings were obtained in liver biopsy specimens of patients with liver disease (213). Although these findings suggest that increases in TR expression during NTIS may support clinical euthyroidism in the face of reduced levels of circulating TH, they are certainly not universal. For example, patients with nonseptic shock and NTIS exhibited a reduction in skeletal muscle expression of *TRβ*, *TRα1*, and retinoid X receptor (RXR)γ (214), indicating that more studies are needed before a unifying hypothesis could be formulated.

In addition to regulation at the gene expression level, TRα and TRβ properties and functions can be modified by posttranslational modifications, including phosphorylation, acetylation, and conjugation to small ubiquitin-like modifier (SUMO), a process referred to

as sumoylation (215, 216). These modifications affect TH signaling by altering TR/DNA binding, interaction with cofactors, and TR-mediated gene transcription. For example, TR phosphorylation promotes TR/DNA binding and heterodimerization with RXR. In the case of TRβ, phosphorylation is induced by TH at the cell membrane level and phosphorylation occurs via ERKs. In turn, TRα is susceptible to phosphorylation by casein 2 and protein kinase A, which reduces DNA binding (215, 216). Such TR modifications are unlikely to be permanent, but they allow for rapid crosstalk with other fast signaling networks. For example, TR phosphorylation facilitates a crosstalk with the PI3K/AKT pathway. It has been reported that an unoccupied TRβ molecule can be associated with the regulatory subunit of PI3K, an intracellular signaling kinase. Binding to T3 dissociates the TRβ–PI3K complex and increases PI3K signaling. Abrogation of TRβ–PI3K binding in mice does not affect TRβ signaling but results in deficient synaptic strength and plasticity, possibly due to a developmental defect in PI3K signaling (217). Additionally, in human umbilical vein endothelial cells both T3 and T4 rapidly stimulate AKT phosphorylation and activate Ras-related C3 botulinum toxin substrate 1 (Rac1), which results in PI3K-dependent cell migration. Human umbilical vein endothelial cells are known to express *DIO2* and have D2 activity that, when blocked, abolishes AKT phosphorylation, Rac1 activation, and cell migration induced by T4 but not by T3. These observations suggest that the D2 pathway is involved in TRα1/PI3K-mediated nongenomic actions of T4 (218). If confirmed, the crosstalk between these pathways constitutes a mechanism through which TH signaling can be modified via downstream kinase cascades.

TR coregulators. TR functions alongside transcriptional regulators to ultimately define TH signaling. In the absence of T3, empty TRs recruit nuclear corepressors, nuclear receptor corepressor (NcoR) 1 and NcoR2 (SMRT), which in turn recruit histone deacetylase 3 (HDAC3) to repress transcription via histone deacetylation (219, 220). Even in the absence of ligand, TRs bind to TRE and repress genes positively regulated by T3. T3 modifies this arrangement by disassembling the corepressor complex and recruiting members of the steroid receptor coactivators family of coactivators, p300/CREB-binding protein and other activators of histone acetylation, to accelerate transcriptional activity (221). Studies in which NcoR1 and steroid receptor coactivator-1 were selectively inactivated revealed that the target set point expression of a T3-responsive gene is affected by the balance between corepressors and coactivators (222). Therefore, TH signaling can be dynamically modified by the local levels of NcoR1/SMRT and local coactivators (223). There are numerous other potential coregulators that may play a role in TH action, including histone deacetylase Sirt1 and the mediator subunit Med1 (224, 225). In fact, the unique environment in each cell that surrounds each TRE probably allows for its own blend of TR/coregulators, which then initiate or modulate TH signaling.

NcoR1 actions are tightly regulated by metabolic signals in liver, SKM, and adipose tissue, hence allowing for metabolic regulation of TH signaling in these tissues. For example, insulin and mTORC1 increase nuclear levels of NcoR1, which leads to repression of lipid oxidation genes (226, 227). Likewise, endurance exercise, fasting, high-fat diet (HFD), aging, and accelerated fat oxidation are all conditions associated with changes in *NcoR1* mRNA levels (226); TH signaling is expected to fluctuate accordingly. Other examples include the peroxisome proliferator-activated receptor-γ (PPARγ) coactivator 1α (PGC1α), a TR coactivator that is reduced in both genetic (ob/ob) and acquired obesity (HFD), setting the stage for reduced T3 effects in individuals who are obese. Indeed, in the liver of individuals with obesity and during fatty liver disease, TH signaling is reduced, which seems to contribute to metabolic imbalance (228–230). For example, TRβ expression was inversely correlated with disease severity in 85 liver biopsies from patients with different stages of nonalcoholic steatohepatitis (231). That TH signaling is reduced during metabolic unbalance is also supported by the failure of TH to induce typical T3-responsive genes and accelerate energy expenditure in mice placed on an HFD (232). Remarkably, gene expression analyses of surgical liver biopsies from 13 subjects with obesity and five control subjects revealed that the top-ranking gene set downregulated in subjects with obesity was comprised of T3-responsive genes related to RNA metabolism, protein catabolism, and energy metabolism. Thus, despite normal serum T3 levels, there is reduction in T3 signaling in models of obesity linked to a drop in *PGC1α* levels (232).

Integrated Action of TH Transporters, Deiodinases, and TRs in Health and in Disease

Systemic and localized control of TH signaling is of paramount importance in development, growth, and normal adult life. The dynamic regulation of the elements in the signaling TRIAD allow for constant adjustment to TH signaling according to endogenous and environmental cues. At the same time, remarkable changes in TH economy and signaling occur during disease states (233). For example, most hospitalized patients exhibit a substantial drop in circulating T3, the explanation of which is multifactorial. TSH levels in these patients are inappropriately low for the reduced T4 and T3 serum levels. This is largely the result of increased *Dio2* expression in MBH tanycytes, which are specialized glial cells lining the third ventricle with projections to the median eminence. This leads to a

localized increase in TH signaling and suppression of TRH/TSH secretion at the same time that circulating TH levels are falling (234–236). In fact, the increase in MBH *Dio2* expression is the cause, rather than a consequence, of the drop in circulating TH levels (237–239). Additionally, depending on the nature of the disease, there can be also ectopic *Dio3* expression and accelerated D3 activity in one or more affected organs/tissues (27, 236, 240, 241), which dampens local TH signaling and also contributes to the reduction in circulating T3 levels. Indeed, the expression of the other elements in the TH signaling TRIAD can also be affected during NTIS in a tissue-specific fashion, depending on the nature of NTIS. This is elegantly illustrated in the study of liver and skeletal muscle of mice experiencing three models of NTIS (240). In the liver, NTIS was associated with variable degrees of reduction in Mct8, Mct10, TRβ, D1 and D3 activities, and T3 content, markedly reducing the expression of a T3-responsive gene. In contrast, the skeletal muscle of the same animals behaved quite differently, with much less impressive changes, if any, in TRIAD elements and preserved TH signaling (240).

It is notable that, in disease states, systemic signaling and local TH signaling support a proinflammatory response in innate immune cells, such as neutrophils, macrophages, and dendritic cells, via TRα (242–244). *Dio2* expression in macrophages (245) is induced during the initial phase of inflammation (234, 245, 246). There is evidence that TH signaling in macrophages can occur through genomic and nongenomic pathways via integrin $\alpha_v\beta_3$, PI3K, and ERK1/2 (247). Both pathways increase phagocytic capacity, cytokine response, inducible nitric oxide synthase, and bacterial death (248–250). Accordingly, macrophages obtained from mice with global inactivation of *Dio2* (global-D2KO) have impaired phagocytosis and decreased cytokine production, similar to macrophages obtained from TRα KO mice (245, 247). At the same time, D3 protein can be found in the cytoplasm and in granules containing either myeloperoxidase or lactoferrin of murine and human neutrophils expressing *Dio3* (251, 252). Accelerated D3 activity lowers intracellular T3 levels concomitantly with production of free iodide. The latter has been proposed as important for the generation of hypoiodite, a toxic compound that kills bacteria (253, 254). In fact, mice with global *Dio3* inactivation (global-D3KO) exhibit decreased bacterial killing ability (252), and zebrafish embryos with *Dio3* knockdown have increased mortality and reduced neutrophil infiltration during pneumococcal meningitis (255).

TH signaling triggers TRH and TSH negative feedback

The relationship between thyroid activity and the HPT axis is explained by a set point and maintained by a feedback mechanism. The set point is defined during development, including the perinatal period, and fine-tuned by the hypothalamus, where environmental and endogenous cues are integrated. The feedback mechanism is based on constant monitoring of TH levels in the systemic circulation, which then leads to adjustments in TRH and TSH secretions and hence thyroidal activity. Circulating T4 and T3 play independent roles in this process: circulating T3 is detected by TRH-expressing neurons in the paraventricular nucleus (PVN) and in the TSH-producing cells of the anterior pituitary gland; circulating T4 requires local conversion to T3 via the D2 pathway present in the MBH and in the thyrotropes of the anterior pituitary gland.

The independent role played by T4 in the feedback mechanism is illustrated by the increase in serum TSH that trails the decrease in serum T4 associated with iodine deficiency or mild hypothyroidism, whereas serum T3 remains within the normal range (73, 74). Few examples exist in which the independent role of T3 in the feedback mechanism can be documented. Most cases in which serum T3 is low in the face of normal serum T4 levels indicate altered thyroid economy due to NTIS, which does not reflect normal HPT physiology (233). A unique experimental setup that points to serum T3 *per se* as having an important role in TSH secretion is acute administration of large doses of PTU to thyroidectomized individuals kept on L-T4 replacement therapy (256). The ~20% drop in serum T3 that follows as a result of D1 inhibition is sufficient to double serum TSH levels, even as serum T4 levels remain stable (256).

The structures involved in monitoring circulating T4 and T3 levels are located inside and outside the BBB. Within the BBB, THs are transported via both MCT8 and OATP1C1, which are expressed in the barrier's endothelial cells. For example, TRH neurons in the PVN project to the outer zone of the median eminence, a region located below the floor of the third ventricle, which is outside of the BBB (238). The median eminence is a critical anatomic and functional region where the two sources of T3 are integrated: T3 from the systemic circulation and T3 produced locally via D2-mediated deiodination of T4 in the tanycytes. MCT8 is abundant in the axon varicosities of TRH neurons, suggesting that T3 is taken up by these cells via this transporter (257). Indeed, the brain of a global *Mct8*-KO mouse takes up less T3 and has decreased T3 content, with marked upregulation of *Trh* mRNA in the PVN neurons (145). The role played by Oatp1c1 is less clear (258). Whereas TH uptake is also affected in the brain of global mice with global *Oatp1c1* inactivation, *Trh* expression is not (259). In addition to expressing *Dio2*, tanycytes express both MCT8 and OATP1C1 (257, 260). It is thought that T3 produced by tanycytes exits the cells through these transporters and is taken up by axon terminals of the PVN neurons that extend to the median eminence; indeed, these

axon terminals lie in close proximity to tanycyte endfeet processes (257, 261). Thyroid hormone transporters might play similar roles in the human brain, where MCT8 is also found in PVN neurons and glial cells (262, 263).

At the same time, the presence of D2 in thyrotropes allows plasma T4 to directly inhibit production of TSH in the pituitary (264). The direct role played by T4 (and T3) in TSH secretion is critical given that not even an injection of a TRH bolus is able to elicit TSH secretion in patients with mildly elevated TH levels (265). Unfortunately, not so much is known about TH transport in the pituitary TSH-secreting thyrotropes: T3 signaling in the pituitary gland is mildly impaired in mice with *Mct8* inactivation, but the transport mechanism remains elusive (258). The role played by D2 in the tanycytes and thyrotropes for the HPT feedback mechanism was further elucidated through studies in the global-D2KO mouse (65). These animals exhibit normal serum T3, but serum TSH and T4 are elevated, hinting at a relative hypothalamic and pituitary insensitivity to T4. Indeed, pituitary D2 specifically seems to play an important role in this phenotype given that a mouse with selective inactivation of *Dio2* in the pituitary gland also exhibits normal serum T3 but elevated serum T4 and TSH; PVN *TRH* mRNA levels are reduced as well as the TSH bioactivity (69). Unfortunately, objective demonstration of the relative role of D2 in tanycytes is still missing given that selective inactivation of *Dio2* in these cells has yet to be achieved.

D3 is also expressed in the HPT structures. It is conceivable that the presence of D3 in the MBH and pituitary gland ensures that there is no local T3 buildup and accurate reading of newly arrived/formed T3 molecules. The role of D3 is illustrated in studies of the global-D3KO mouse, which exhibits central hypothyroidism, with low circulating levels of T4 and T3, and normal serum TSH. This is because *Dio3* inactivation results in neonatal thyrotoxicosis, later followed by central hypothyroidism that persists throughout life (266). A similar scenario is seen in mice exposed to high TH levels *in utero*. There is an increase in *Dio3* mRNA levels in the hypothalamus that explains the persistent central resistance to TH (267). In these mice, anterior pituitary *Dio3* mRNAs is increased, accelerating local T3 clearance. A comparable phenotype is observed in adult humans who were exposed to high TH levels *in utero* (267).

Overall, the studies in animals with disruption of the deiodinase pathways indicate that each disruption triggers adjustments in the HPT function, namely changes in T4, TSH, and TRH, that are aimed at preserving serum T3 levels (168). That serum T3 levels are the main target around which serum T4 and TSH are adjusted constitutes a shift in the paradigm traditionally accepted for the function of the HPT axis. It is unexpected that the HPT axis tolerates an elevated serum T4 to preserve serum T3 (67–69).

The idea that circulating T4 is detected by the hypothalamus and the pituitary gland via the D2 pathway has been challenged over the years because of the intrinsic homeostatic nature of D2; that is, D2 activity accelerates under low serum T4 conditions, whereas high serum T4 levels result in loss of D2 activity (29). Such D2 response at the hypothalamus and/or thyrotrope, if operational, would impair the detection of changes in serum T4, leaving TSH levels unchanged. However, studies using the TαT1 mouse tumor cell line that secretes TSH indicate that the T4-induced loss of D2 activity in these cells is offset by the combined effect of D2 reactivation via deubiquitination and a particularly rapid rate of D2 synthesis. As a result, higher T4 levels are rapidly translated into greater D2-mediated T3 production and suppression of TSHβ gene expression; this explains the operation of the T4-mediated TSH feedback mechanism (264). A similar situation is observed in the MBH. *In vitro* analysis of D2 ubiquitination driven by hypothalamic and other tissue extracts revealed less ubiquitinated D2 when hypothalamic extracts were used, including when compared with other areas of the brain (268). In other words, D2 activity does not fluctuate as much in the MBH in response to changes in T4 levels. As a result, the hypothalamus remains exquisitely sensitive to elevations or drops of circulating T4, in contrast to what is observed in other tissues (268).

Given the pivotal role played by D2 and D3 in the HPT axis, it is no surprise that drugs or pathways that influence the activity of these enzymes have the potential to interfere with the normal feedback mechanism. For example, the widely prescribed cardiac antiarrhythmic drug amiodarone (AMIO) and its main metabolite, desethylamiodarone (DEA), elevate serum TSH levels (269). This is because both AMIO and DEA behave as noncompetitive inhibitors of D2 (270), and a disruption in the D2 pathway interferes with the transduction of the T4 signal, generating less T3 and softening the TSH feedback mechanism. The underlying effect on TSH is at the pituitary gland given that in AMIO-treated mice, there is a reduction in paraventricular *TRH* mRNA levels (270). Quite the opposite is observed in mice with inactivation of the fatty acid amide hydrolase (FAAH) gene. These animals are prone to adiposity and, in humans, mutations in FAAH are associated with obesity. In these animals there is a PPARγ–mediated increase in MBH *Dio2* expression, which leads to a localized increase in TH signaling and suppressed TRH/TSH secretion (271). The reduced energy expenditure in global Faah KO mice is attributed to lower circulating THs secondary to a suppressed HPT axis (271).

Is TH signaling restored in patients with hypothyroidism on therapy with L-T4?

A significant clinical concern is whether the loss of adjustable thyroidal T3 secretion, as seen in patients who are hypothyroid, compromises the ability to preserve circulating T3, and therefore systemic TH signaling (86, 87, 272). In other words, can deiodinases alone preserve circulating T3 homeostasis in the absence of a functional thyroid gland? If not, could this compromise systemic TH signaling and be a contributing factor to residual "hypothyroid-like" symptoms among some of the L-T4-treated patients with hypothyroidism?

Historically it was assumed that circulating T3 is fully normalized in L-T4–treated patients (273–275). However, the issue has been revisited through very large studies, and serum T3 levels were found to be lower than normal in many clinically euthyroid patients maintained on L-T4. A cross-sectional study involving ~1800 patients with athyreosis with normal serum TSH levels on L-T4 monotherapy revealed that the distribution of serum FT3 levels shifted to the left (lower levels) and that of FT4 levels shifted to the right (higher levels) compared with the distribution patterns of ~3900 controls (276). In a subsequent large national study using cross-sectional data from the US National Health and Nutrition Examination Survey, L-T4–treated participants had higher serum total and FT4 and lower serum total and FT3 than did controls when matched for sex, age, ethnic background, and serum TSH. Thus, the current consensus is that although L-T4–treated patients maintain normal serum TSH levels, they also exhibit slightly lower T3 levels and slightly higher T4 levels than do control individuals (277). Although studies with a relatively small number of patients suggest that monotherapy with L-T4 is able to normalize serum T3 without suppressing serum TSH (278), the larger studies failed to replicate these findings. Normal serum T3 levels can be achieved with L-T4 alone, but at the expense of having relatively lower/suppressed serum TSH (279, 280).

What are the underlying mechanisms that explain the relatively lower levels of T3 in L-T4–treated patients? This has been addressed in rodent models, including in L-T4–treated thyroidectomized rats (67, 68). In these rats, the daily dose of L-T4 that normalizes serum TSH results in serum T4 levels above the reference range and lower than normal serum T3 levels. Indeed, only combined therapy with L-T4 and L-T3 normalize serum T4, T3, and TSH concentrations simultaneously (281). Studies of the D2 pathway in L-T4–treated mice indicate that tissue-specific differences in D2 ubiquitination account for the high T4/T3 serum ratio in L-T4–treated thyroidectomized rats (268). L-T4 administration at doses that normalize plasma TSH reduces whole-body D2-dependent T4 to T3 conversion, and a larger fraction of the circulating T3 is derived from the D1 pathway. Thus, as the dose of L-T4 given to thyroidectomized rats is increased, there is relatively less T3 being produced via the D2 pathway in peripheral tissues. Notwithstanding, D2 activity and T3 production in the hypothalamus of the same animals are only minimally affected by L-T4 treatment. This difference in the way D2 responds to therapy with L-T4 creates a situation in which TSH secretion is normalized whereas circulating T3 is not (Fig. 7).

In vitro analysis of D2 ubiquitination driven by different tissue extracts indicates that the hypothalamus is less capable of maintaining D2 in the ubiquitinated form. In contrast to other D2-expressing tissues, the hypothalamus exhibits less D2 downregulation when exposed to T4. As a consequence, fluctuations in plasma T4 are faithfully transduced as variations in local TH signaling because the rate of local T4 to T3 conversion is kept stable in the face of fluctuating plasma T4 levels. This is also supported by findings obtained in the TH action indicator mouse in which hypothalamic TH signaling in the hypothalamus is affected by hypothyroidism (38). These studies reveal that tissue-specific differences in D2 ubiquitination are an inherent property of the HPT feedback mechanism, explaining why replacement with L-T4 alone results in relatively lower plasma T3 levels (Fig. 7).

The reduction in circulating T3 seen in L-T4–treated patients is modest. There is understandable skepticism as to whether this is sufficient to cause even mild hypothyroidism. This was tested in a preclinical animal model, that is, thyroidectomized rats receiving L-T4 at doses that normalize serum TSH but not serum T3 (268). Previous studies in similarly treated animals demonstrated that T3 content in most tissues is not normalized (281). Furthermore, an in-depth analysis of multiple T3-dependent markers revealed widespread signs of hypothyroidism, including an ~20% reduction in mitochondrial content in liver and SKM, and a failure to normalize serum cholesterol, which remained ~30% elevated in L-T4–treated rats. The cerebral cortex, cerebellum, and hippocampus were also analyzed for the expression of 14 T3-responsive genes, but only 5 genes were normalized; all the other genes indicated reduction in TH signaling, despite normal serum TSH. Notably, all of these parameters were normalized in rats that received combined L-T4 and L-T3 treatment, which normalized serum T3 levels (268).

Evidence that a similar scenario happens in L-T4–treated patients already exists. In the US National Health and Nutrition Examination Survey, L-T4–treated participants with normal serum TSH differed in 12 out of 52 objective and subjective measures, including higher body mass index, despite reportedly consuming fewer calories per day per kilogram of body weight (282). This is likely explained by the fact that these patients have lower energy

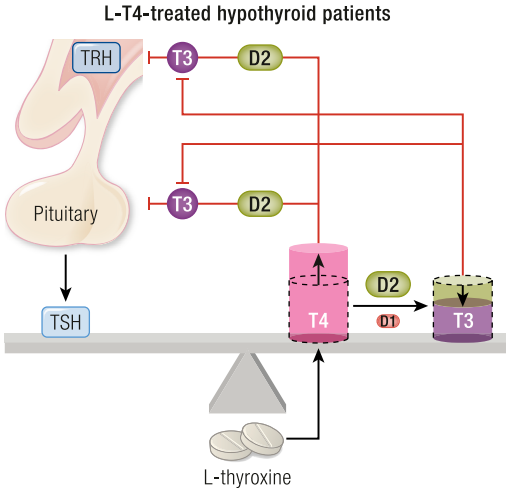


Figure 7. TSH levels are normalized to slightly higher circulating T4/lower T3 in LT4-treated patients with hypothyroidism. TSH secretion is defined by the balance between the positive input provided by TRH secretion and the negative input provided by circulating T4 and T3 levels. In LT4-treated patients with hypothyroidism the negative input is based on a slightly higher circulating T4/T3 ratio when compared with normal individuals. This is because of an imbalance between D2 ubiquitination in the hypothalamus vs the rest of the body. While outside the hypothalamus T4-induced D2 ubiquitination limits T3 production; in the hypothalamus–pituitary axis this mechanism is less efficient, preserving D2-mediated T3 production even as circulating T4 rises with LT4 administration. A growing body of work suggests that the relatively lower circulating T3 levels in LT4-treated patients with hypothyroidism are clinically relevant. LT4-treated patients weigh ~10 pounds (4.5 kg) more, exhibit higher serum cholesterol levels, are more likely to be on statin and antidepressive medications, and display a slower rate of energy expenditure. See reviews for more details (86, 87). [Adapted with permission from "Hypothyroidism, thyroid hormones and deiodinases." www.BiancoLab.org.]

expenditure (283, 284) and lower total metabolic equivalents (282). Additionally, they were more likely to be taking beta-blockers, antidepressants, and statins. Indeed, a systematic review of publications of overt hypothyroidism in which participants were treated with L-T4 and had normal serum TSH levels followed by meta-analysis showed that L-T4–treated participants had 3.3 ± 1.6 mg/dL higher serum low-density lipoprotein (LDL) levels and 9.6 ± 3.6 mg/dL higher serum total cholesterol levels compared with controls. In studies that did not concomitantly assess healthy controls, serum LDL levels were 138 ± 4.6 mg/dL (reference range, <129 mg/dL) and serum total cholesterol levels were 210 ± 3.4 mg/dL (reference range, <200 mg/dL) (285). Taken together, these studies support the idea that L-T4–treated individuals, with normal serum TSH, exhibit objective signs of mild reduction in systemic TH signaling (285).

It is intriguing that the Thr92Ala-*DIO2* polymorphism has been linked to altered responsiveness of patients with hypothyroidism to TH replacement therapy (286, 287). In a double-blind clinical trial, Thr92Ala-*DIO2* polymorphism carriers achieved better quality of life in response to combination therapy with L-T4 and L-T3 compared with L-T4 alone (288). This supports the idea that Thr92Ala-*DIO2* polymorphism carriers have systemic or localized dampening of TH signaling that can be overcome using L-T3. This outcome was reproduced in a subsequent study in which the compound Thr92Ala-*DIO2* and *MCT10* polymorphisms enhanced patients' preference for L-T4 plus L-T3 replacement therapy (289), but not in all studies (183). Subsequent studies that focused on circulating T3 levels in L-T4–treated thyroidectomized carriers of the Thr92Ala-*DIO2* polymorphism support the idea that such patients might be at a greater risk of systemic and/or localized hypothyroidism (187). However, why would such a risk be detected only after hypothyroidism is diagnosed? The answer might be in the studies of mice with combined *Dio1* and *Dio2* deficiencies. These animals maintain circulating T3 levels despite their inability to convert T4 to T3 thanks to an adjustment in thyroidal T3 secretion (67). Carriers of the Thr92Ala-*DIO2* polymorphism do well for as long as their thyroid gland is functional, probably because their HPT axis adjusts thyroidal T3 secretion up to compensate for deficiencies in the D2 pathway (67). Once they develop hypothyroidism and are treated with L-T4, they no longer have the ability to activate the thyroid and compensate, and hence become symptomatic.

The central nervous system

TH is essential for CNS development and function (290, 291), with documented effects on proliferation, differentiation, migration, synaptogenesis, and myelination (292). In fact, TH signaling not only affects neuronal development throughout embryogenesis but also in the adult brain, regulating neural stem cell function in the hippocampus and the subventricular zone, the main sites of neurogenesis in the adult mammalian brain (292, 293). Despite that D2 generates most T3 in the brain (294, 295), *Dio2* inactivation, globally or locally, results in a limited neurologic phenotype, suggesting the existence of compensatory mechanisms that minimize functional abnormalities caused by the absence of D2-generated T3 (296). Indeed, the brain has a sophisticated range of mechanisms to control TH signaling that could potentially offset a *Dio2* deficiency, including different sets of transporters, D3 and TRs (297–300) (Fig. 8). In the case of TRs, TRα is the isoform that predominates in the brain, with some areas also expressing TRβ (301).

Tissue architecture also affects how deiodinases and transporters modify TH signaling in the brain. *Dio2* is typically expressed in glial cells whereas

neurons express *Dio3* (294, 302–305). Details about this system were obtained after it was modeled *in vitro* using a coculture system of D2-expressing human glioma cells and D3-expressing human neuroblastoma cells. In this system, glial cell D2 activity produced T3 that acted in a paracrine fashion to induce T3-responsive genes in the cocultured neurons. D3 activity in the neurons responded to known stimuli and modulated T3 effects (239). Of course, these signaling pathways require transit of T4 and T3 across cell membranes. Glial cells are likely to take up T4 through OATP1C1 and release T3 that acts in neighboring neurons (239, 299). Additionally, limited amounts of circulating T3 also reach neurons through MCT8, contributing with ~20% of the intracellular T3 in the cerebral cortex. The resulting relatively high content of T3 causes higher than usual occupation of TRs in the brain, close to saturation levels. In some rare cases TH transport is limiting, such as in patients with Allan–Herndon–Dudley syndrome, in whom neurons that rely on MCT8 for T3 transport have diminished TH signaling despite normal TH levels in the circulation (41, 42).

The presence of D3 in neurons at first seems puzzling and counterintuitive. Why would neurons inhibit entry of T3 if THs are so critical for brain development and function? Indeed, this does not seem to be the case. Studies performed in rats using labeled T3 and T4 molecules indicate that TRs are almost fully occupied with T3 (295). In other words, glial cells produce so much T3 that almost all TRs in the brain are bound to T3. This suggests that D3 activity in neurons does not limit T3 entry or access to the neuronal nucleus. Although having control over both local production and catabolism of T3 is intuitively advantageous, an additional hypothesis that remains to be tested is that D3 in neurons serves to minimize cellular exit of T3, preventing neurons from becoming a secondary source of T3 in the CNS.

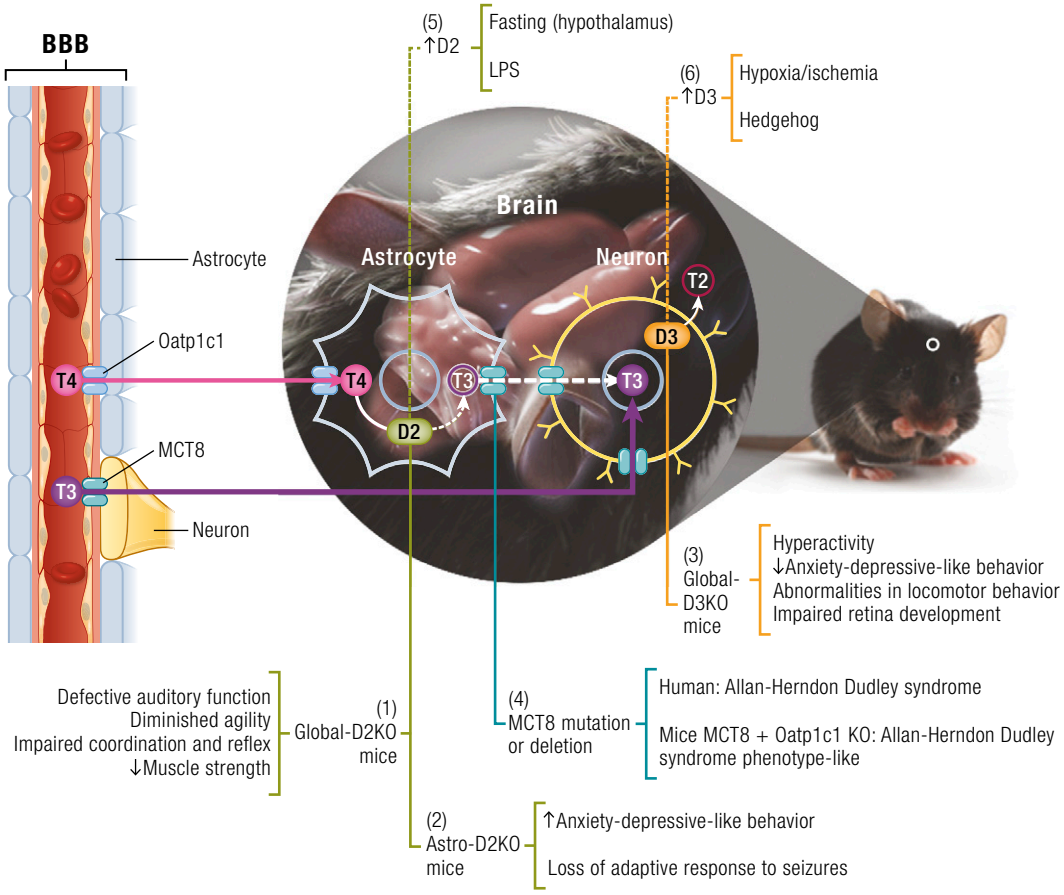


Figure 8. Transport and metabolism modulate TH signaling in the brain. T4 crosses the BBB through Oatp1c1, reaching astrocytes where it is converted to T3 via D2. T3 exits astrocytes and is likely to enter neurons via MCT8. Circulating T3 can also reach neurons crossing the BBB through MCT8. The presence of D3 in neurons inactivates T3 to T2. It is not clear whether D3 preferentially targets incoming, outgoing, or both flows of T3 molecules. Shown are conditions known to affect TH signaling along with their main characteristics: (1) global-D2KO mouse; (2) Astro-D2KO mouse; (3) global-D3KO mouse; and (4) mutations of MCT8; also shown are conditions known to stimulate (5) *Dio2* or (6) *Dio3* pathways. See reviews for more details (10, 294, 299, 300). [Adapted with permission from "Depression due to deiodinase defect, despite normal thyroid hormone levels. PMID: 27501182 ." www.BiancoLab.org.]

Development of sensory structures and function

During development, deiodinase expression is timed for each tissue to minimize or activate local TH signaling according to a predefined developmental program. The development of sensory structures in the brain constitutes elegant models in which deiodinase expression exerts a time and spatial control of TH signaling (306–308). TH acting via TRβ controls maturation of auditory function (309) by regulating the expression of fast-activating potassium conductance in the cochlea (310). During the fetal and neonatal period, there is relatively high D3 activity in the immature cochlea that dampens TH signaling (311). Later, in the postnatal phase, cochlea *Dio3* expression and D3 activity decrease at the same time that D2 activity rises and peaks around postnatal day 7, only to decline by postnatal day 10. Peak local TH signaling occurs at a critical stage of cochlear development, the absence of which causes an auditory phenotype similar to TRβ inactivation (312). In addition to deiodinases, TH transporters also play a critical role in this process. *Dio2* and TRβ are not coexpressed in the same cochlear cells. *Dio2* is expressed in periosteal connective tissue whereas TRβ expression is expressed in the sensory epithelium. Thus, D2 generates T3 in the connective tissue that acts in a paracrine-like fashion on the greater epithelial ridge and sensory epithelium residing inside the bony labyrinth to activate TRβ (129). This special compartmental anatomy of the cochlea hints to the existence of transport mechanisms that convey T3 to target tissues. Different TH transporters are involved: LAT1 is located in the cochlear blood vessels and sensory hair cells, whereas MCT8 is found in the greater epithelial ridge and other structures, partly overlapping with the TRβ expression; MCT10 and OATP1C1 can also be found in the cochlea (308). Indeed, mice with inactivating mutations in *Mct8* and *Mct10* develop hearing loss (313). These animals have retarded development of the sensory epithelium, compounded with a progressive degeneration of cochlear hair cells. This phenotype is largely rescued with the administration of T3, confirming the role played by TH transporters and TH signaling in the cochlea development and function (313).

In the development of the visual system, local TH signaling plays a role in defining the fate of distinct populations of cone photoreceptors in the retina. Rodents have dichromatic vision with two types of photoreceptors that are sensitive to middle (M, green) or short (S, blue) wavelengths, depending on the photopigment they express. Cone photoreceptors throughout the retina have the potential to follow a default S-cone pathway but, upon activation of TRβ, they commit to an M-cone identity (314). Notably, TH signaling is symmetrically distributed in the retina at birth as S-pigment expression begins, due to minimal TH signaling caused by *Dio3* expression. Over time, TH signaling is strengthened in the dorsal retina at the time of M-pigment onset (postnatal day 10), illustrating how the ratio and patterning of cone types may be determined by TH availability during retinal development (315, 316). The study of human retinal organoids confirmed that similar deiodinase-mediated control of TH signaling takes place in humans. In these organoids, S cones are specified first, followed by red (L)/M cones; TH signaling controls this temporal switch through timed expression of *DIO3* and *DIO2* within the retina. This ensures that early low TH signaling specifies S cones and high TH signaling later produces L/M cones (317). Notably, the fate of retinal cones continues to be affected by the deiodinases and TH signaling even during adulthood (318, 319). Suppression of TH signaling by overexpression of *Dio3* preserves cones in a mouse model of retinal degeneration (319). Indeed, *Dio2* inactivation improved cone survival and function in these mouse models. Additionally, cellular oxidative stress responses were increased in animal models of retina degeneration, which were improved by *Dio2* deficiency and worsened by treatment with T3. These studies suggest that dampening TH signaling in degenerating retinas might constitute a therapeutic approach aimed at cone preservation (318).

Motor activity

TH signaling controls formation of the transient external germinal layer in the cerebellum. Both *DIO2* and *DIO3* are expressed in the cerebellum, but *DIO3* expression predominates at embryonic and neonatal stages, indicating that during this period local TH signaling is limited. If this is disrupted, as in the global-D3KO mouse, there are locomotor behavioral abnormalities manifested as impaired ability in descending a vertical pole (320). Following the perinatal period of reduced TH signaling, *Dio2* expression increases, and the enhanced local TH signaling plays a role in the adult cerebellum. Adult global-D2KO mice exhibit diminished agility and an altered global gait pattern (they walk slower, with shorter strides and with a hindlimb wider base of support than do wild-type mice). There is also impaired coordination and prehensile reflex and decreased muscle strength (321). This phenotype in the global-D2KO mouse is associated with structural cerebellar alteration, with reduced foliation, accelerated disappearance of the cerebellar external germinal layer, and premature expansion of the molecular layer at juvenile ages.

Cognition and mood

DIO2 and *DIO3* are expressed in multiple brain areas involved in behavioral and mood processes, with important roles played by local TH signaling. Indeed, global-D2KO mice, which have reduced brain T3 content and TH signaling (304), have increased anxiety and fear memory (322). Furthermore, a mouse in

which *Dio2* has been selectively inactivated in astrocytes (Astro-D2KO) has anxiety/depression-like behavior (70). Notably, the opposite phenotype, that is, hyperactivity and significantly decreased anxiety-like behavior, was observed in the global-D3KO mouse, a model of enhanced TH signaling in the brain (323).

The effects of a more subtle impairment of T4 to T3 conversion in the brain were studied in a mouse carrier of the Thr92Ala-*Dio2* polymorphism, in which there was an ~20% reduction in D2-mediated T3 production (186). Despite normal serum T3 levels, microarray analyses of the Ala92-*Dio2* brain revealed reduced TH signaling in the striatum, amygdala, prefrontal cortex, hippocampus, and cerebellum (186). These mice underwent testing for mood and behavior and were found to have higher exploratory and more risk assessment behavior than did control mice. The pattern of higher mobility in Ala92-*Dio2* mice was maintained during the highly anxiogenic tail suspension studies (186). Notably, once settled in the environment, Ala92-*Dio2* mice traveled ~30% shorter distances and slept ~4.2-fold longer than did control mice. Cognition in these animals was tested through standard memory tests, with the Ala92-*Dio2* mice failing the 3-hour recall test. Increasing TH signaling with L-T3 administration partially rescued the Ala92-*Dio2* mouse phenotype, confirming that localized reduction in TH signaling plays a role in the phenotype (186).

In contrast, the localized increase in brain TH signaling seen in the global-D3KO mouse was associated with a significant increase in aggression-related behaviors and mild deficits in olfactory function (324). Additionally, 85% of global-D3KO dams manifested no pup-retrieval behavior and increased aggression toward newborns. The abnormal social behaviors of global-D3KO mice are associated with sexually dimorphic alterations in oxytocin and arginine vasopressin, two neuropeptides that affect social interactions. Global-D3KO mice exhibited lower serum oxytocin and arginine vasopressin levels, as well as abnormal expression of both peptides and their receptors in the neonatal and adult hypothalamus (324). Developmental overexposure to T3 as a result of *Dio3* inactivation changed hypothalamic gene expression of more than a thousand genes in postnatal day 15 mice. The alterations in gene expression extended to other brain regions and, in adulthood, were associated with decreased anxiety-like behavior, increased marble burying, and reduced physical activity (325). Overall, these studies indicate that *Dio2* and *Dio3* are important in establishing mood, with *Dio3* also involved in aggression and maternal behaviors.

Traumatic and hypoxic-ischemic brain injury

TH signaling in the brain can be disrupted by a severe insult such as traumatic brain injury (TBI) (326). In rats, TBI is associated with reduction in *MCT8* and *Dio2* mRNA levels in the brain, as well as an elevation of *Dio3* mRNA levels, which is compatible with a reduction in TH signaling. The cortex, compared with the hippocampus and cerebellum, sustained the greatest injury and displayed the most significant change in gene expression as a result of injury (326). Insults such as ischemia or hypoxia, in which there is induction of *DIO3* as a result of hypoxia-inducible factor (HIF)1α activation in neurons, also leads to reduced TH signaling (239). Hypoxia (HIF1α) induction of *DIO3* has also been observed in the hypertrophic ventricular myocardium (43, 44, 327) and in the postinfarction myocardium (327, 328). *DIO3* induction in the brain during hypoxic or ischemic disease is associated with incorporation of D3 into the nuclear membrane, which in cell models reduces the paracrine effects of T3 (164). After unilateral ischemia in the rat brain, D3 protein is increased predominantly in the neuronal nuclei in the pyramidal and granular ipsilateral layers, as well as in the hilus of the dentate gyrus of the hippocampal formation (239). Similar observations were made in hippocampal neurons in culture as well as in a human neuroblastoma cell line (164). Incorporation of D3 into the nuclear membrane dampens TH signaling and may reduce brain damage caused by hypoxic or ischemic disease. Notably, concentration of D3 in the nuclear membrane of neurons was also seen in hippocampal sections of mice after brain hypoxia was induced by status epilepticus, an abnormally prolonged seizure that lasted 3 hours (329).

Other studies in mice, however, indicate that ischemia increases D2 activity in cerebral cortex and striatum, whereas D3 activity remains stable (330). In another study, D2 activity was induced in a cell model by hypoxia without changes in *Dio2* mRNA levels (331). In this case, hypoxia stabilized and prolonged the half-life of D2 by decreasing its susceptibility to the ubiquitin–proteasome pathway, whereas D3 was not affected. TBI also increases *Dio2* mRNA expression, although it is not clear how much of this effect is due to changes in plasma and local levels of TH (332, 333). In the mouse model of status epilepticus, there was also a rapid increase in *Dio2* expression and reduction in *Dio3* expression in hippocampus, amygdala, and prefrontal cortex (329). An analysis of the hippocampal transcriptome of mice undergoing status epilepticus revealed changes in a number of genes, including those involved with response to oxidative stress, cellular homeostasis, cell signaling, and mitochondrial structure. In contrast, when Astro-D2KO mice underwent status epilepticus, the highly induced genes in the hippocampus were related to inflammation, apoptosis, and cell death (329).

Collectively, these studies suggest that a severe brain insult affects *Dio2* and *Dio3* expression, most of the time in a reciprocal fashion, modifying TH signaling in localized brain areas, which could affect the balance between adaptive and maladaptive mechanisms. The reduction in TH signaling seen during

hypoxic-ischemic events reduces energy expenditure and oxygen consumption, and it could be interpreted as an adaptive mechanism (334). Indeed, administration of the D2 inhibitor rT3 in rats undergoing middle cerebral artery occlusion reduced neuronal injury markers, infarct size, and neurologic deficit. Similarly, rT3 increased cellular survival in primary cerebral neurons under oxygen glucose deprivation/reoxygenation stress (335). However, it is clear that not all types of brain injuries/insults result in the same changes in deiodinase and TH signaling. Notably, in the setting of TBI, treatment with T4 significantly increased the expression of mRNA from B-cell lymphoma 2 (*Bcl2*), vascular endothelial growth factor (*VEGF*)*A*, SRY box 2 (*Sox2*), and neurotrophin, genes important for neuronal survival and recovery (326). Also, under hypoxic conditions, treatment with T3 accelerated by 8 hours the expression of hypoxia-mediated genes (*VEGF*, enolase, *HIF2α*, *c-Jun*). Thus, although it is clear that injury to the brain reduces TH signaling, more studies are needed to establish whether these changes are adaptive or maladaptive, and a unifying hypothesis can be formulated (336).

Demyelination syndromes

Demyelinating disease is any disease of the nervous system in which the neuronal myelin sheath is damaged. This damage impairs the transmission of signals that, in turn, impairs sensation, movement, cognition, or other functions. Oligodendrocytes produce myelin, and investigators have looked for molecules that promote proliferation, differentiation, and maturation of oligodendrocyte precursor cells (OPCs). TH signaling has a well-established role in promoting oligodendrocyte differentiation and maturation, and it has been used in some settings to accelerate remyelination (337–339).

At the same time, TH signaling also plays a role upstream of the OPC maturation, namely in the differentiation of neuronal stem cells located in the subventricular zone into OPCs. Investigators noted that in the adult subventricular zone, the fate of differentiating neuronal stem cells that can give rise to neurons or OPCs depends on *Dio3* expression (340). Those cells that express *Dio3* experience a transient period of reduced TH signaling that promotes differentiation of stem cells into OPCs. As a result, there is functional remyelination and restored neural conduction, with important clinical implications (340). Thus, D3-mediated dampening of TH signaling accelerates generation of OPCs whereas exposure of OPCs to T3 accelerates maturation into oligodendrocytes.

Intraventricular hemorrhage (IVH) that compromises blood flow to different brain areas remains a major cause of white matter injury in preterm infants. TH signaling seems to play a role in how the brain responds to such injury. In both autopsy materials from human preterm infants and a rabbit model of IVH there was a reduction in D2 levels, whereas D3 levels were increased compared with controls without IVH; TRα expression was also increased in infants with IVH (341). Notably, treatment with TH accelerated recovery, which included proliferation and maturation of OPCs, augmented myelination, and restored neurologic function in pups with IVH. Furthermore, in TH-treated human preterm infants the density of myelinating oligodendrocytes was almost doubled as compared with controls. Thus, the combined elevation in D3 and reduction in D2 activity levels decreases TH signaling, which could be worsened by the increase in unliganded TRα. Given that TH promotes neurologic recovery in IVH, TH treatment should be further explored to improve the neurodevelopmental outcome of preterm infants with IVH (341).

Brain degenerative disease

Carriers of the Thr92Ala-*DIO2* polymorphism exhibit alterations in the transcriptome of the temporal lobe, which are typically associated with neurodegenerative diseases, such as amyloid-β peptide processing (342). This observation led to a study designed to test the hypothesis that carriers of the Thr92Ala-*DIO2* polymorphism have increased risk for incident Alzheimer's disease (AD). Although this locus has not been identified in previous genome-wide association studies (343–345), the candidate gene approach could still lead to identification of a moderate association that provides insight into AD pathogenesis (346, 347). Knowing that the epidemiology and tissue pathology of AD vary by ethnicity (348), large cohorts comprised of thousands of blacks were compared with European Americans. The assessment indicated that black carriers of Thr92Ala-*DIO2* have 1.3-fold higher odds of developing AD. In a second cohort, Thr92Ala-*DIO2* blacks exhibited 1.35-fold higher odds of developing cognitive impairment. In contrast, in European Americans there was no association between Thr92Ala-*DIO2* and AD or dementia (349).

These findings prompted more detailed studies of cells expressing Ala92-D2, which led to the discovery that D2 is normally a cargo protein in ER-Golgi intermediary compartment (ERGIC) vesicles, recycling between ER and Golgi. The Thr92 to Ala substitution (Ala92-D2) causes ER stress and activates the unfolded protein response. This pushes Ala92-D2 to the Golgi apparatus via the adaptor protein ERGIC53, and accumulation in the *trans*-Golgi. Remarkably, all of these changes are restored by eliminating ER stress with the chemical chaperone 4-phenyl butyric acid (4-PBA) (186, 342). A detailed study of mice carrying the Thr92Ala-*DIO2* polymorphism revealed that different areas of their brain also exhibit ER stress and activation of the unfolded protein response, which could contribute to the phenotype of impaired cognition and

motivation for physical activity (186). Furthermore, treatment with 4-PBA for 10 days reversed most of this phenotype, indicating a potential mechanism through which cognition is affected in these animals (186).

Metabolic control

TH synergizes with the sympathetic nervous system to markedly accelerate the rate of energy expenditure, which in patients with severe hypothyroidism can fall as much as ~50% and in thyrotoxic patients can be increased by ~50%, an approximately threefold excursion over the hypothyroid baseline (350, 351). Most effects of T3 are direct and take place in metabolically relevant tissues, such as BAT, liver, SKM, heart, and pancreatic islets, where dynamic regulation occurs through the action of the deiodinases. Important metabolic effects of T3 have also been reported in different areas of the brain. For example, central administration of T3 promotes *de novo* lipogenesis in liver and lipid oxidation in BAT through the autonomic nervous system (352, 353). Evidence exists that the TH derivative 3,5-diiodo-L-thyronine also exerts thermogenic effects by directly influencing mitochondrial activity (354, 355). However, the pathways leading to the endogenous synthesis of this molecule are presently unknown, downplaying its physiological role and potential as a signaling molecule.

Mice with targeted disruption of deiodinases exhibit a variety of metabolic phenotypes. The expression of *Dio2* and *Dio3* in the MBH strategically places both enzymes at the crossroads of neural regulation of metabolism. For example, studies in mice show that food deprivation increases hypothalamic *Dio2* mRNA levels and D2 activity. *Dio2* mRNA levels are also increased in the MBH of mice fasted for 48 hours (356). Hence, this localized increase in TH signaling could explain the reduction in *Trh* mRNA observed in fasted rats (104, 357). Deiodinase-mediated control of TH signaling in the hypothalamus might also play a role in regulating the torpor state, in which there is a dramatic reduction in metabolism and in body temperature, diminishing the energy requirements of the animal (358). In hamsters, hypothalamic *Dio2* expression is decreased during spontaneous daily torpor as well as fasting-induced torpor, indicating reduced hypothalamic TH signaling in these animals (358). Additionally, reciprocal expression of *Dio2* and *Dio3* in the MBH was shown to be critical for photoperiodically induced gonadal growth in birds (359). Long photoperiods induce hypothalamic *Dio2* expression and simultaneously reduce *Dio3* expression, indicating that long days enhance TH signaling in the MBH (359, 360). Notably, the global-D3KO mouse exhibits increased TH signaling in the hypothalamus, with abnormal expression and T3 sensitivity of genes in the melanocortin system, suggesting leptin resistance. They also have decreased adiposity, reduced BAT size, and accelerated fat loss in response to treatment with L-T3. Notably, global-D3KO mice display increased locomotor activity and an increased rate of energy expenditure along with expanded nighttime activity periods, suggesting a disrupted circadian rhythm (361).

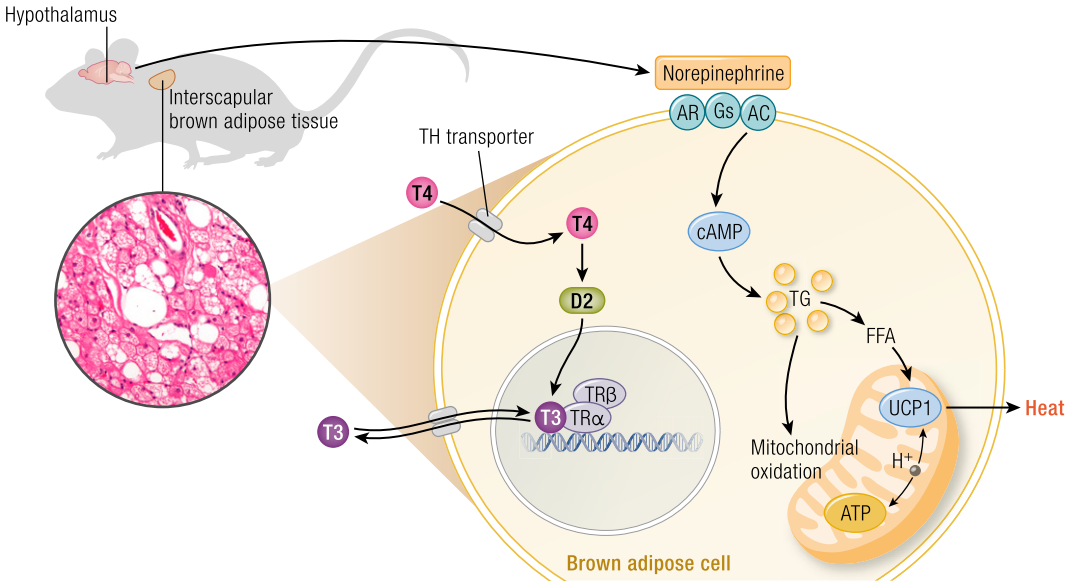
Dynamic changes in TH signaling affect adaptive thermogenesis and metabolism

The ability to thermoregulate is an evolutionary advantage of mammals. In the warm climate, most animals can dissipate heat efficiently. However, when exposed to cold, mammals not only minimize heat losses but at the same time increase heat production by accelerating their metabolic rate, a process known as adaptive thermogenesis (362). This is accomplished in large part due to release of norepinephrine (NE) in a number of tissues, including BAT, which upregulates cAMP-responsive genes, such as *Dio2*, *PGC1α*, and *UCP1* (363, 364) [Fig. 9 (365)]. All three β-adrenergic receptor subtypes respond to NE and increase cAMP production, but each play slightly different roles in thermogenesis and metabolic control (366–368).

Dio2 is a cAMP-responsive gene, hence cold exposure enhances local TH signaling in BAT, essential for adaptive thermogenesis (351). When exposed to cold, thyroidectomized rats become hypothermic and fail to induce *UCP1* expression; this response is limited to ~20% of what is observed in controls. L-T4 is particularly effective in restoring thermogenesis given its activation to T3 in BAT via D2 (154). Circulating T3 is important, but NE-induced several-fold activation of D2 activity and local T3 production is critical for thermogenic function. Local TH signaling amplifies cAMP production and directly induces *UCP1* expression (155, 369, 370) and the activity of malic enzyme, glucose-6-phosphate dehydrogenase, and acetyl–coenzyme A carboxylase, key enzymes involved in BAT lipogenesis (371–373). The latter is likely to involve T3 induction of the carbohydrate response element–binding protein, a T3-target gene in BAT that mediates glucose regulation of key lipogenic genes (374). The overwhelming data available on the role of *Dio2* in BAT catapulted this gene into the thermogenic program that includes *PGC1α* and *UCP1* (375).

BAT expresses both TRα and TRβ (365). Indeed, when a selective TRβ agonist is given to hypothyroid mice there is stimulation of the BAT thermogenic program, for example, induction of *Dio2*, *UCP1*, and *PGC1α*, but these animals fail to generate normal amounts of cAMP and to produce heat in response to NE (376). These observations suggest that TH signaling augments BAT thermogenesis via a coordinated effort between TRα and TRβ. Induction of the thermogenic program depends on TRβ, whereas potentiation of cAMP generation and heat production in response to NE infusion cannot be elicited by

Figure 9. Local TH signaling accelerates BAT thermogenesis. BAT is a specialized organ that produces heat in response to cold exposure or excessive caloric intake (362). BAT expresses both TRα and TRβ (365). Heat is generated due to mitochondrial uncoupling triggered by the sympathetic nervous system [*i.e.*, NE-induced adenylyl cyclase (AC) activation and cAMP production] that also stimulates *Dio2*, increases T3, and leads to the induction of T3-responsive thermogenic genes, including *Ucp1*. Moreover, D2-generated T3 also stimulates BAT lipogenesis, which generates fatty acids used to sustain accelerated mitochondrial activity. Hypothyroid animals have impaired the ability to thermoregulate in the cold due to decreased BAT function. Global-D2KO animals exhibit a reduction in the expression of genes that define the tissue thermogenic identity (*i.e.*, *Ucp1*, *Pgc1α*, and *Dio2*) and exhibit impaired oxidative capacity. See reviews for more details (350, 362–364). [Adapted with permission from "Hypothyroidism, thyroid hormones and deiodinases." www.BiancoLab.org.]



treatment with TRβ agonist alone, depending on activation of TRα as well (376). It is notable that expression of the thermogenic program in white adipose cells, also known as browning of adipose tissue, can also be activated via stimulation of TRβ (377).

***Dio2* inactivation and BAT thermogenesis.** Inhibition of BAT D2 with iopanoic acid *in vivo* (378) and in freshly isolated brown adipocytes decreases adrenergic induction of *UCP1* expression (370) and local lipogenesis (371). Studies in the global-D2KO mouse revealed an impaired thermogenic response to cold and to NE infusion (379). These animals survive by compensating for reduced TH signaling with increased sympathetic activity (380) and shivering, a behavior not typically observed in cold-exposed rodents (379). Room temperature of 21°C is sufficiently low to activate cold-induced thermogenesis in small mammals. Therefore, the overall compensatory increase in sympathetic activity renders global-D2KO mice resistant to diet-induced obesity, even when kept at room temperature. Remarkably, this phenotype is reversed when NE turnover is minimized by acclimatization at thermoneutrality (30°C), which "turns off" sympathetic activity to the BAT. As a result of the unopposed reduction in TH signaling, global-D2KO mice become markedly sensitized to diet-induced obesity, not only gaining excessive weight but also developing severe hepatic steatosis (381).

***Dio2* plays a role in defining BAT identity during development.** BAT development is a coordinated process during which local TH signaling reflects synchronized changes in deiodinase expression and activity. In the mouse, BAT develops between embryonic day (E)16.5 and E18.5, during which time *Dio2* expression is increased and *Dio3* expression is decreased, thus increasing local TH signaling (382) (Fig. 6). Targeted disruption of *Dio2* results in defective brown adipocytes, including impaired expression of genes in the adipogenic program (fatty acid–binding protein 4, cell death–inducing DNA fragmentation factor, α subunit–like effector A, and acyl–coenzyme A synthetase long-chain family member 5) and thermogenic identity of these cells (*Ucp1*, *Pgc1α*, and *Dio2*). Global-D2KO preadipocytes exhibit delayed maturation, with fewer cells terminally differentiating into brown adipocytes (382).

Other metabolic signals affect *Dio2* expression and local TH signaling. A number of endogenous and exogenous molecules modulate BAT function via the *Dio2* pathway, including bile acids (383), flavonols (384), chemical chaperones (385), and the adipokine adipocyte-specific fatty acid–binding protein (AFABP) (386). Bile acids activate BAT D2 and UCP1-mediated thermogenesis via the G-protein–coupled bile acid receptor 1 (TGR5) pathway; TGR5 is a G-protein–coupled receptor that

accelerates cAMP production and BAT thermogenesis, protecting against diet-induced obesity and insulin resistance. Treatment of human skeletal myocytes with bile acid increases D2 activity and O_2 consumption through a cAMP-dependent process (383). In fact, the TGR5 selective agonist INT-777 is efficacious *in vivo*, increasing energy expenditure and reducing adiposity in a mouse model of diet-induced obesity (387). Oral supplementation of the bile acid chenodeoxycholic acid in 12 healthy female subjects for 2 days increased BAT activity and whole-body energy expenditure (388). Additionally, the study of 10 healthy subjects and 8 patients with liver cirrhosis revealed a positive correlation between circulating bile acids and whole-body energy expenditure (389). *In vitro* treatment of primary human brown adipocytes with chenodeoxycholic acid or specific TGR5 agonists increased mitochondrial uncoupling and *DIO2* expression, an effect that was absent in human primary white adipocytes (388). Along the same lines but through a different pathway, kaempferol and other flavonols stimulate *DIO2* expression via a cAMP-mediated mechanism in primary cultures of human skeletal myocytes, leading to D2-mediated T3 production, expression of thermogenically relevant genes, and acceleration of O_2 consumption (384).

ER stress constitutes a link between metabolic homeostasis and D2-mediated TH signaling (186, 390). ER stress is present when its functions are affected by factors such as buildup of misfolded proteins, disruption of redox state, or calcium homeostasis (391). In general, cells respond to ER stress in a number of ways, including blockade of mRNA translation and protein synthesis, which then minimizes ER stress (392). From a metabolic perspective, ER stress is recognized as an important pathway that can be triggered by an HFD and obesity; in adipose tissue, ER stress downregulates insulin sensitivity (393). In this regard, it is notable that ER stress leads to rapid loss of D2 activity, to as low as 30% of control levels, without affecting *Dio2* mRNA levels; this drop in D2 activity is accompanied by a slowdown in intracellular D2-mediated T3 production, hence TH signaling (390). This drop in D2 levels involves eukaryotic initiation factor 2, which blocks *Dio2* mRNA translation, hence D2 synthesis. These data seem to be clinically relevant. For example, primary human airway cells normally exhibit D2 activity. However, in cells obtained from patients with cystic fibrosis, the ensuing ER stress results in complete loss of D2 activity (390). Notably, the chemical chaperones tauroursodeoxycholic acid (TUDCA) and 4-PBA, both molecules that minimize ER stress, increase *Dio2* expression, D2 activity, and local T3 production (385). In brown adipocytes, treatment with TUDCA or 4-PBA enhances TH signaling, expression of T3-dependent genes, and acceleration of O_2 consumption. In control mice, but not in global-D2KO mice, treatment with TUDCA accelerates BAT D2 activity, lowers the respiratory quotient, and normalizes the glucose intolerance associated with feeding an HFD (385).

Human BAT is sensitive to TH signaling. *Dio2* is present in interscapular BAT depots in premature and full-term neonatal humans in amounts comparable to rodents in terms of onset of development and peak distribution (394). In adult humans with positive fluorodeoxyglucose uptake in positron emission tomography scans, biopsies have proven the presence of D2 at levels higher than in corresponding white adipose depots (395). BAT in humans seems to be responsive to TH signaling as studied in a patient with thyroid cancer who underwent positron emission tomography and CT scanning while systemically hypothyroid and during suppressive treatment with L-T4. The transient systemic hypothyroid state suppressed fluorodeoxyglucose uptake in BAT that had been previously active during the systemic hyperthyroid state created by suppressive L-T4 therapy (55).

Metabolic roles of *Dio2* in tissues other than BAT. Studies in mice with fat-specific, Astro-specific, or skeletal muscle–specific D2KO have shed light on the role of *Dio2* in metabolically relevant tissues. The Astro-D2KO mice exhibit lower diurnal respiratory quotient and greater contribution of fatty acid oxidation to energy expenditure, but no differences in food intake. In contrast, the mice with fat-specific *Dio2* inactivation (Fat-D2KO) exhibit greater contribution of carbohydrate oxidation to energy expenditure, as illustrated by a sustained (24-hour) increase in respiratory quotient, food intake, glucose tolerance, and insulin sensitivity. Furthermore, Fat-D2KO animals that were kept on an HFD gained more body weight and fat, indicating impaired BAT thermogenesis and/or inability to oxidize the fat excess. Acclimatization of Fat-D2KO mice at thermoneutrality dissipated both features of this phenotype. Notably, muscle D2 does not seem to play a significant metabolic role given that mice with skeletal muscle-specific *Dio2* inactivation (Skm-D2KO) exhibited no metabolic phenotype (71). The interpretation of these findings must also take into consideration the fact that there is BAT mixed with muscle fibers (396), suggesting that some of the local D2-mediated TH signaling and consequent metabolic effects are mediated at the BAT level and not SKM fiber.

***Dio2* polymorphism and metabolism.** Reduced catalytic activity of Thr92Ala-D2 could potentially dampen TH signaling in metabolically relevant tissues that express *DIO2* (186, 187), similar to what was observed in the brain of mice carrying the Thr92Ala-*Dio2* polymorphism (186). Indeed, the first description of the Thr92Ala-*DIO2* polymorphism was described in patients at higher risk of exhibiting insulin resistance (397) and type 2 diabetes mellitus (184). Furthermore, individuals with compounded

Thr92Ala-*DIO2* and Trp64Arg β_3-adrenergic receptor polymorphism, a receptor variant that generates less cAMP (398), exhibit higher body mass index (173); this suggests an interaction between these variants. Of note, the current literature about the Thr92Ala-*DIO2* polymorphism is controversial, with poor reproducibility among different studies (176, 399–403). It is likely that additional unidentified linkage factors such as ethnic background play a significant role in the physiological and clinical relevance of the Thr92Ala-*DIO2* polymorphism (171, 176, 404).

Susceptibility to hepatic steatosis is defined by a perinatal surge in hepatic Dio2

TRβ predominates in the adult liver, where TH signaling is mostly a reflection of circulating levels of T3. Liver has high D1 activity, but D1-generated T3 equilibrates rapidly with plasma, not contributing significantly to local TH signaling (39). TH signaling is a potent stimulus for lipogenesis, which is in agreement with the coupling between food intake and thyroid activity. Many of the key lipogenic enzymes and transcriptional factors are induced by T3 in the liver and adipose tissue. Whereas D2-generated T3 is key to ensuring high lipogenic rates in cold-stimulated BAT (371), circulating T3 is thought to be the key factor defining TH signaling in liver and white adipose tissue, as in the adult mouse *Dio2* is not expressed in these tissues (405).

Notwithstanding, the global-D2KO mouse exhibits intense steatosis when placed on an HFD (381, 406, 407). Additionally, *Dio2* is ectopically expressed in the liver of mice with targeted deletion of both liver X receptor (LXR) α and β (407), suggesting that LXR and RXR signaling inhibit *Dio2* expression. Indeed, 22(*R*)-OH-cholesterol negatively regulates human *DIO2* in a dose-dependent manner through a specific region, −901 to −584 bp, within its promoter (406). Remarkably, the adipokine AFABP induces expression of *Dio2* in BAT via inhibition of the nuclear receptor LXRα, thereby increasing local TH signaling. AFABP accelerates thermogenesis by activating D2-mediated T3 production in brown adipocytes. The thermogenic responses to T4 are abrogated in *Afabp*-KO mice but enhanced by AFABP (386).

These observations prompted follow-up studies that revealed, at around the first day of life, a transient surge in hepatocyte *Dio2* expression activates T4 to T3 and local TH signaling. This T3 surge doubles local T3 concentration and modifies the expression of ~165 genes involved in broad aspects of hepatocyte function, including lipid metabolism (72, 408) (Fig. 10). The role of *Dio2* expression was further investigated through the creation of a mouse with liver-specific *Dio2* inactivation (Alb-D2KO). These animals exhibit delay in neonatal liver expression of key lipid-related genes and a persistent reduction in *PPAR*γ expression. Notably, the absence of a neonatal *Dio2* peak significantly modifies the baseline and long-term hepatic transcriptional response to an HFD. In control animals, feeding an HFD changes the expression of ~400 genes involved in synthesis of fatty acids and triglycerides, whereas in Alb-D2KO animals, the response to an HFD is restricted to a different set of only ~200 genes associated with reverse cholesterol transport and lipase activity (72). A whole-genome methylation profile coupled to multiple analytical platforms indicates that 10% to 20% of these differences can be related to the presence of differentially methylated local regions mapped to sites of active/suppressed chromatin, thus qualifying as epigenetic modifications occurring as a result of neonatal *Dio2* inactivation. The resulting phenotype of the adult Alb-D2KO mouse is dramatic, with greatly reduced susceptibility to diet-induced steatosis, hypertriglyceridemia, and obesity (72).

One of the genes that underlies the Alb-D2KO phenotype is zinc finger protein-125 (*Zfp125*) (72, 408). *Zfp125* is a Foxo1-inducible transcriptional repressor that causes lipid accumulation in the alpha mouse liver 12 cell line (AML12) and liver steatosis in mice by reducing liver secretion of triglycerides and hepatocyte efflux of cholesterol (408). *Zfp125* acts by repressing 18 genes involved in lipoprotein structure and lipid binding, as well as transport. The apolipoprotein E (*APOE*) promoter contains a functional *Zfp125*-binding element that is also present in 17 other lipid-related genes repressed by *Zfp125*. Whereas liver-specific knockdown of *Zfp125* causes an "Alb-D2KO–like" metabolic phenotype, liver-specific normalization of *Zfp125* expression in Alb-D2KO mice rescues the phenotype, restoring normal susceptibility to diet-induced obesity, liver steatosis, and hypercholesterolemia (408). Overall, these studies indicate that the neonatal liver is particularly sensitive to TH signaling. The transient peak of D2-generated T3 on the first day of life that doubles the local T3 concentration mediates a series of epigenetic events (72), including expression of the transcriptional repressor *Zfp125*, that defines the future ability of the liver to secrete very LDL (VLDL) (408). These mechanisms are aligned with the overall positive effect of TH on liver lipogenesis and carry significant implications for future development of obesity and liver steatosis.

Dio3 *expression in pancreatic* β*-cells dampens TH signaling*

TH is transported into pancreatic islets via MCT8 (409) and OATP1C1, with T3 playing a metabolic role in these cells (410). Islet cells express both *TR*α*1* and *TR*β*1*, with a relative higher level of *TR*α*1* found in murine pancreas α-cells (411); however, glucagon secretion does not seem to be regulated by TH signaling. Exposure of zebrafish during the larval to juvenile transition to exogenous TH precociously activates the β-cell differentiation genes paired box 6b (*Pax6b*) and motor neuron and pancreas homeobox 1

(*Mnx1*) while downregulating aristaless related homeobox a (*Arxa*), a master regulator of α-cell development and function (412). *In vivo* studies in neonatal rats indicate that TH accelerates metabolic development of β-cells by inducing expression of a transcription factor, v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog a (*Mafa*), via TRβ. Additionally, THs also accelerate β-cell senescence through a TRα-dependent mechanism (413). Interspecies variability in the effects of TH signaling on β-cell maturation might exist vis-à-vis the observation that hypothyroidism *in utero* stimulates pancreatic β-cell proliferation and hyperinsulinemia in the ovine fetus during late gestation (414).

Studies involving isolated mature murine pancreatic islets indicate that TH inhibits insulin secretion (24, 25). Furthermore, T_3 suppresses glucagon-like peptide 1 (*GLP1*)–stimulated insulin secretion in MIN6 cells (415), which are derived from a mouse insulinoma cell line. However, the presence of *Dio3* mRNA and D3 protein in embryonic and adult human and murine pancreatic β-cells minimizes the inhibitory effects of T_3 (24, 25). In MIN6 cells, *Dio3* expression is stimulated by *GLP1*, an effect mediated via the cAMP–protein kinase A pathway. Exendin-4, a GLP1 receptor agonist, also stimulates *Dio3* expression in MIN6 cells. Accordingly, mice with *Dio3* inactivation in pancreatic islets are glucose intolerant due to *in vitro* and *in vivo* impaired glucose-stimulated insulin secretion, without changes in peripheral sensitivity to insulin (24, 25). In these animals, neonatal (postnatal day 0) and adult pancreas exhibited reduced total islet area due to reduced β-cell mass, insulin content, and impaired expression of key β-cells genes. It is conceivable that *Dio3* expression in perinatal pancreatic β-cells prevents untimely exposure to TH, the absence of which leads to impaired β-cell function, insulin secretion, and disruption of glucose homeostasis (25). Studies in adult heterozygous mice with *Dio3* inactivation indicate that *Dio3* is preferentially expressed from the maternal allele in pancreatic islets and that inactivation of this allele is sufficient to disrupt glucose homeostasis by reducing pancreatic islet area, insulin gene expression, and glucose-stimulated insulin secretion (24).

Heart

TRα1 is present in the myocardium and in the peripheral ventricular conduction system, whereas the TRβ1 isoform can be found in cells that form the peripheral ventricular conduction system. In the atria and in the proximal conduction system (sinoatrial node, atrioventricular node), TRα1 and TRβ1 isoforms are coexpressed (416). TH acts in the myocardium, triggering chronotropic and inotropic effects. TH signaling not only affects electrical transmission and rhythmicity but also myocardial energy metabolism, changing the types of energy substrates and the rate at which they are used. An important component of TH effects in the heart is mediated indirectly via acceleration of the rate of oxygen consumption throughout the body. By increasing global demand for oxygen, TH causes vasodilation that requires an increase in cardiac output to sustain mean arterial blood pressure.

The finding of *DIO2* mRNA in the healthy human (but not rodent) myocardium along with the identification of *DIO3* mRNA in human cardiomyocytes that were differentiated from human induced pluripotent stem cells (417) and in the myocardium of patients with various cardiac diseases suggest the existence of local mechanisms that control TH signaling (405, 418). These observations sparked general interest, given that the commonly prescribed antiarrhythmic AMIO and its active metabolite, *N*-DEA, inhibit D2 activity (270). In fact, it has been proposed that inhibition of myocardial D2 and consequent reduction in local TH signaling contributes to the antiarrhythmic efficacy of AMIO (419). Given the

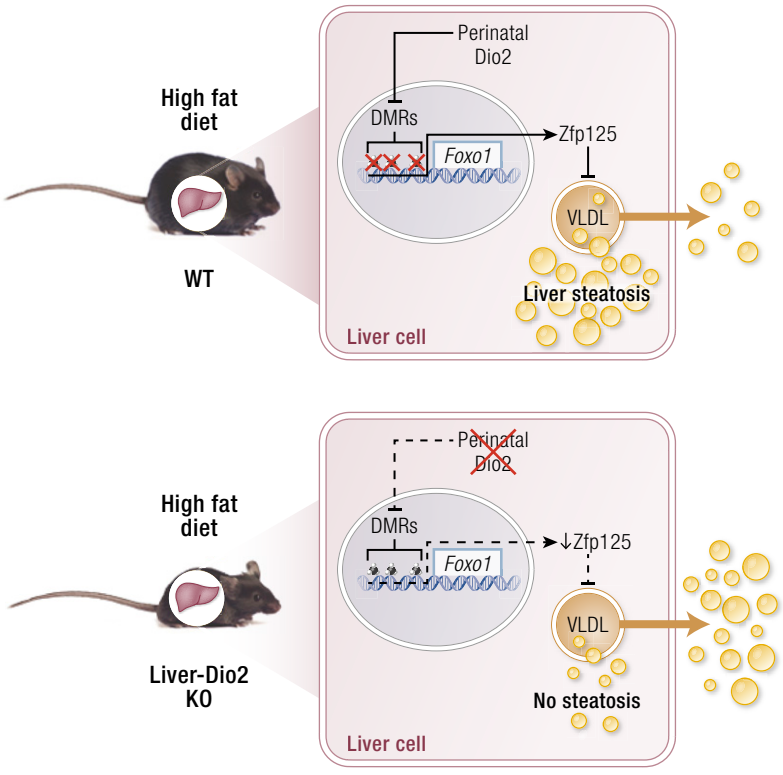


Figure 10. Perinatal *Dio2* liver expression defines future susceptibility to obesity and liver steatosis. A brief surge in *Dio2* expression in the liver around the first day of life affects the methylation status [differentially methylated region (DMR)] of hundreds of genes, including *Foxo1*. The neonatal surge in TH signaling prevents methylation of three sites within the Foxo1 promoter, allowing the gene to be expressed and stimulate *Zfp125*, a liver transcription factor that suppresses the expression of 18 genes involved in the assembly and secretion of VLDL particles. As a result, normal mice develop steatosis when placed on an HFD. In contrast, mice in which liver *Dio2* was inactivated exhibit three DMRs in the *Foxo1* gene, reducing its expression to about half of that in control mice. Consequently, the expression of the *Foxo1* downstream target *Zfp125* is also greatly reduced in the absence of the perinatal surge in *Dio2*. The reduction in *Zfp125* expression accelerates VLDL secretion, minimizing lipid deposition and steatosis when animals are fed with an HFD (72, 408).

similarities between these molecules with T3, AMIO is also thought to reduce cardiac TH signaling by inhibiting TH transport across the plasma membrane (420), and/or direct binding to TRα and TRβ (421, 422), and even TR-dependent gene transcription (423).

The role played by D2-generated T3 in the myocardium was investigated in transgenic mice that express *DIO2* in the myocardium under the α-myosin heavy chain (*MHC*) promoter (134). This mouse has normal thyroid function but exhibits a discrete increase in myocardial T3 content and a gene expression profile compatible with increased TH signaling, that is, increased mRNA levels of hyperpolarization-activated cyclic nucleotide–gated potassium and sodium channel 2 (*HCN2*; an ion channel that is key to the cardiac pacemaker) and decreased *βMHC* mRNA levels (134). In perfused *ex vivo* studies, the αMHC-D2 heart had an ~20% higher heart rate and decreased levels of phosphocreatine and adenosine diphosphate, indicating accelerated metabolic rates. This is supported by results of *in vivo* studies in which glucose uptake is increased by ~2.5-fold in the αMHC-D2 heart (424). The increase in TH signaling is associated with an enhanced capacity of the αMHC-D2 heart to generate cAMP in response to catecholamine stimulation (425). The effects of increased cardiac-specific TH signaling were studied in a second αMHC-D2 mouse model conditionally expressing *DIO2* in the myocardium (135). Myocardium *DIO2* was found to be protective against adverse myocardial remodeling caused by pressure overload (135) or doxorubicin-induced chemical injury (424). D2-generated T3 provided a host of mechanical improvements to the heart such as increased fractional shortening, velocity of circumferential fiber shortening, peak aortic outflow velocity, and aortic velocity acceleration (135). It is thus conceivable that the increase in *Dio2* mRNA observed in some murine models of cardiac remodeling is an attempt to increase cardiac performance. For example, a knock-in mouse model of inherited dilated cardiomyopathy with a deletion mutation (ΔK210) in the cardiac troponin T gene exhibits an increase in myocardial *Dio2* mRNA and D2 activity, likely as a result of generalized activation in cAMP-dependent pathways (426). Similarly, postmyocardial infarction mice develop markedly enlarged hearts with left ventricle systolic dysfunction and upregulation of *Dio2* mRNA expression in the heart (426).

Taken together, these studies support the assumption that myocardial TH signaling might enhance cardiac performance in some settings. For example, in pediatric patients undergoing cardiac surgery with cardiopulmonary bypass, the use of L-T3 infusion in the postoperative period decreased the requirement for inotropic support, increased spontaneous conversion to normal sinus rhythm, and improved clinical outcomes (427). In adult high-risk patients undergoing coronary artery bypass grafting, randomized postoperative administration of L-T3 was associated with a higher mean cardiac index and lower systemic vascular resistance, but it did not change outcomes or alter the need for standard postoperative therapy (428). Additionally, in patients with depressed left ventricle function undergoing coronary artery bypass grafting, perioperative administration of L-T3 resulted in a lower incidence of atrial fibrillation, fewer required instances of cardioversion or anticoagulation during hospitalization, and decreased required antiarrhythmic therapy at discharge (429). Beneficial effects of short-term L-T3 replacement therapy, such as improved ventricular performance, were also observed in stable patients with ischemic or nonischemic dilated cardiomyopathy (430).

Myocardial injury dampens local TH signaling

In most models of ischemic heart disease with myocardial injury, there is ectopic cardiac expression of *Dio3*, which inactivates TH and dampens local TH signaling (27, 431, 432). It remains controversial as to whether this is an adaptive response in view of the positive effects of T3 on the myocardium (see above). Dampening of TH signaling reverses the myocardial gene expression profile to that observed during embryonic development and might enhance the regeneration potential as seen in a mouse expressing a dominant-negative *TRα* (433). Dampening of TR signaling occurs through reactivation of *Dio3* (432) and increased expression of *TRα* (434), thereby shifting the balance toward unoccupied TRs. In fact, *Dio3* is expressed in human cardiomyocytes differentiated from human induced pluripotent stem cells. Exposure of these cells to iopanoic acid, a competitive inhibitor of deiodinases, changes the expression of downstream targets of T3, that is, *αMHC* and *βMHC*, ATPase sarcoplasmic/ER Ca^{2+} (*SERCA*), and phospholamban (*PLB*) mRNA levels, confirming dampening of TH signaling (417).

Dio3 expression has also been observed in animal models of adverse remodeling such as myocardial infarction (328) and chronic pulmonary hypertension with right ventricular hypertrophy and ventricular failure (treatment with monocrotaline) (43, 44). Subsequent studies identified induction of cardiac *Dio3* and dampening of TH signaling in cardiomyocytes obtained from a transgenic model of progressive dilated cardiomyopathy (435). Studies in the postmyocardial infarction heart suggested that miRNAs (miRs) might play a role as well. These are noncoding RNA molecules that bind to complementary sequences of target mRNAs and function as RNA silencers and posttranscriptional regulators of gene expression, interfering with translation or causing target degradation. In the postmyocardial infarction, miR-214 is the miRNA with the highest potential to target *Dio3* mRNA (436). In this setting, miR-214 and

D3 protein are coexpressed in cardiomyocytes, but *Dio3* mRNA expression precedes *miR-214* expression. This suggests that a D3-mediated decrease in TH signaling induces cardiac *miR-214* expression, which in turn suppresses both mRNA and protein D3 expression. These results support the existence of a negative feedback mechanism regulating *Dio3* expression in the heart during myocardial injury (436).

Taken together, these studies served as the basis for a clinical trial that enrolled patients undergoing elective open heart surgery to assess TH deiodination in the human heart (437). Myocardial TH metabolism was assessed by analyzing the difference in serum TH levels between the aortic root (incoming blood) and the coronary sinus (outgoing blood) of patients undergoing cardiac surgery. Immediately before cardiopulmonary bypass, blood flowing through the myocardium of patients with aortic stenosis (with left ventricular hypertrophy) exhibited ~5% reduction in T3 and ~7% increase in rT3 levels, a decrease in the T3/rT3 ratio of ~10%. In contrast, no myocardial TH metabolism was observed in patients with coronary artery disease (no ventricular hypertrophy). The accelerated TH inactivation in the myocardium of patients with aortic stenosis is likely the result of *DIO3* expression. Notably, there was no evidence to suggest TH activation in the myocardium in this study (437).

Whereas the injured myocardium could benefit from dampening of TH signaling, activation of TH signaling in other tissues of the heart seems to improve outcomes for myocardial injury. For example, activation of TH signaling in endothelial cells by selective expression of TRα1 in a transgenic mouse model increased coronary blood flow by 77%, coronary conductance by 60%, and coronary reserve by 47% (438). Notably, systemic blood pressure decreased by 20% in these transgenic mice after *TRα1* expression, with no effects on heart rate. Furthermore, these animals exhibited much improved performance in response to myocardial ischemia followed by reperfusion, and reduced infarct size by 45%. It is thus conceivable that selective activation of TRα1 in endothelial cells protects the heart against injury after an ischemic insult and does not result in adverse cardiac or systemic effects (438).

Lung

Normal human lung exhibits both D1 and D2 activity (439), hinting that deiodinases control how local TH signaling affects fetal lung development and function. In fact, it is well known that TH signaling and steroids affect the maturation of pneumocytes. Prenatal administration of glucocorticoids is commonly used to accelerate lung maturation and attenuate the severity of respiratory distress syndrome (RDS). Knock-in mutations of the nuclear corepressor *SMRT* in mice (SMRT mRID), which specifically disrupt the interaction between SMRT and TRs, produces RDS caused by prematurity of the alveolar type I epithelial cells (440). Remarkably, administration of anti-TH drugs rescues SMRT-induced RDS, indicating that an untimely increase in TH signaling is detrimental for lung development. Subsequent studies indicate that TR affects alveolar type I epithelial cell differentiation through Krüppel-like factor 2, a transcription factor that activates specific gene programs in these cells (440).

Dio2 *activation plays a role in pulmonary response to injury*

In addition to normal lung development and physiology, local TH signaling also plays a role in how the lung responds to injury. In mouse models of acute lung injury (lipopolysaccharide- and ventilator-induced lung injury), there is induction of both *Dio2* mRNA and D2 protein in lung, with expression directly increasing with the extent of lung injury. Mice with *Dio2* knockdown exhibit increased lung injury, suggesting a protective role for *Dio2* in acute lung injury (180). In subsequent studies of ventilator-induced lung injury (VILI), global-D2KO mice exhibit greater susceptibility to VILI when compared with control mice, with poorer alveoli integrity and greater induction of lung chemokine and cytokine gene expression (441). Systemically hypothyroid mice exhibited a similar response to VILI, suggesting that the global-D2KO lungs were functionally hypothyroid. Treatment of global-D2KO mice with T3 rescued many of the lung chemokine and cytokine profiles in response to VILI, suggesting that administration of T3 could be beneficial for the treatment of lung injury (441).

Indeed, this was tested in a mouse model of pulmonary fibrosis, a condition in which the normal lung tissue is replaced as a result of active remodeling; there is deposition of extracellular matrix and dramatic changes in the phenotype of both fibroblasts and alveolar type II epithelial cells (442, 443). Idiopathic pulmonary fibrosis (IPF) affects ~120,000 patients in the United States (444, 445). IPF is the result of multiple cycles of epithelial cell injury and activation that provoke the migration, proliferation, and activation of mesenchymal cells with the formation of fibroblastic/myofibroblastic foci, accumulation of extracellular matrix, and abnormal wound repair (442, 446). A search in a database of IPF lungs for abnormal expression of genes involved in lung bioenergetics revealed *DIO2* among the top 20 significantly increased genes (447). *DIO2* expression was eightfold higher in lungs of patients with IPF compared with controls. Subsequent studies indicated that disruption of TH signaling via *Dio2* inactivation or systemic hypothyroidism enhanced bleomycin-induced fibrosis whereas local or systemic supplementation with TH after bleomycin administration blunted fibrosis. Aerosolized TH delivery increased survival and resolved fibrosis in two models of pulmonary fibrosis in mice (447). Sobetirome, a TRβ-selective agonist,

also blunted bleomycin-induced lung fibrosis. After bleomycin-induced injury, TH promoted mitochondrial biogenesis, improved mitochondrial bioenergetics, and attenuated mitochondria-regulated apoptosis in alveolar epithelial cells both *in vivo* and *in vitro*. TH did not blunt fibrosis in PGC1α- or PTEN-induced putative kinase 1 KO mice, suggesting dependence on these pathways (447). It is conceivable that the antifibrotic properties of TH are associated with protection of alveolar epithelial cells and restoration of mitochondrial function and that TH may thus represent a potential therapy for pulmonary fibrosis (447).

Musculoskeletal system

Skeletal muscle

SKM is a target of TH signaling, with T3 affecting differentiation, development, regeneration, and metabolism (448–450). TH gains access to skeletal myocytes predominantly via MCT8/OATP1C1 and signal through TRα (451). *DIO2* is expressed in human and murine SKM (4, 115, 450–452), with higher levels in slow-twitch compared with fast-twitch muscle (453). However, an issue remains, which is low D2 activity level observed in the tissue, at least two orders of magnitude lower than brain (453–455). Is this low baseline D2 activity sufficient to affect local TH signaling?

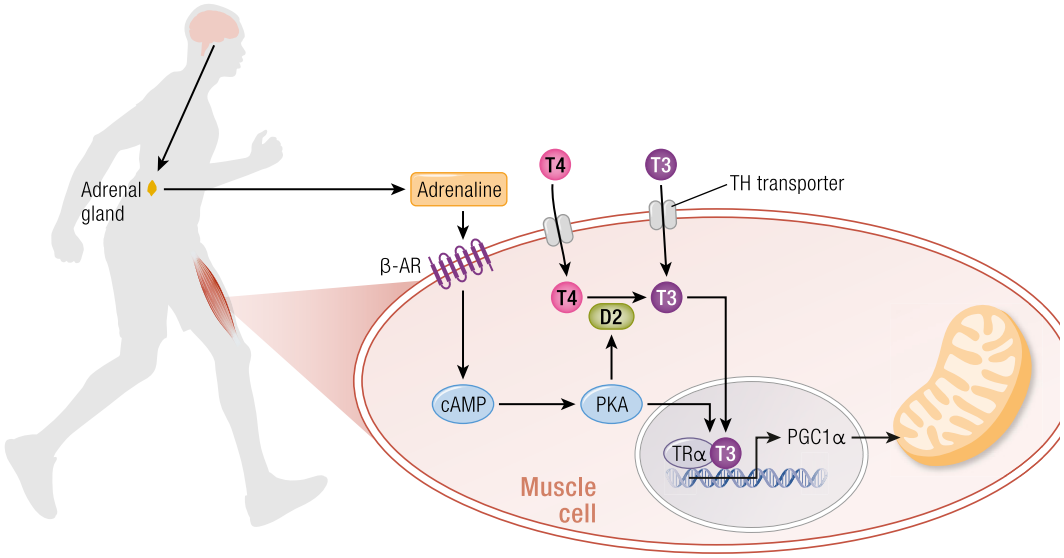
Although the role of SKM *Dio2* in TH signaling remains controversial, in some settings the global-D2KO mouse does exhibit signs of reduced TH signaling in SKM (456). In this regard, deiodinases have been studied in the context of SKM development (457, 458). Cell culture studies indicate that D2 activity is increased during maturation of mouse myoblasts (115). Indeed, there is a temporal association between induction of *Dio2* and expression of developmental genes in primary muscle precursor pp6 cells (456). If these cells are obtained from global-D2KO animals, they remain in the proliferating phase and do not differentiate into myotubes, a phenotype that is rescued by addition of T3 (456). Notably, during this process, the *Dio3* expression pattern is reciprocal to that of *Dio2*, a mechanism that is controlled by the histone H3 demethylating enzyme (LSD-1) that induces *Dio2* and represses *Dio3* (450) (Fig. 6).

Other studies on the role of *Dio2* in defining TH signaling in SKM used animals in which SKM *Dio2* inactivation was limited to SKM (452). Floxed-*Dio2* mice were crossed with mice expressing Cre-recombinase under the myosin light chain 1f to disrupt *Dio2* expression in the late developmental stages of skeletal myocytes (Skm-D2KO). This led to an ~50% loss in D2 activity in neonatal and adult SKM and ~75% loss in isolated Skm-D2KO myocytes. However, soleus (SOL) T3 content was not affected. The expression of several T3-responsive genes in SKM was also preserved in neonatal Skm-D2KO hindlimb muscles, at a time that coincides with a peak of D2 activity in control animals. In adult SOL the baseline level of D2 activity was about sixfold lower, and in the Skm-D2KO SOL, the expression of only one of five T3-responsive genes was reduced (452). Despite these results, adult Skm-D2KO animals performed indistinguishably from controls on a treadmill test of endurance and on muscle strength (452). These studies indicate the existence of multiple sources of *Dio2* expression in mouse SKM, with limited roles in postnatal SKM fibers.

The *Dio2* role on SKM development was further tested in mice with disruption of *Dio2* driven by two early developmental SKM promoters: myogenic regulator factor-5 (*Myf5*) and muscle determination gene (*MyoD*) (459). *Myf5* myoblasts in culture differentiate normally into myotubes, despite loss of almost all D2 activity. *Dio2* mRNA levels in developing SKM obtained from *Myf5*-D2KO embryos (E18.5) were ~54% of control littermates, but the expression of the T3-responsive genes myosin, heavy polypeptide (*Myh*) 1 and 7, and ATPase sarcoplasmic/ER Ca^{2+} transporting (*Atp2a*) 1 and 2 was not affected. In *Myf5*-D2KO and *Myod*-D2KO neonatal hindlimb muscle, the expression of *Myh1* and *Myh7* and *Atp2a2* remained unaffected, despite a 60% to 70% loss in D2 activity and/or mRNA. Only in *Myo*D-D2KO neonatal muscle was there a 40% reduction in *Atp2a1* mRNA (459). Postnatal growth of both mouse models and SKM function as assessed by exercise capacity and measurement of muscle strength were normal. Furthermore, an analysis of the adult SOL revealed no changes in the expression of T3-responsive genes, except for an ~18% increase in *MyoD*-D2KO SOL *Myh7* mRNA.

The report of two mouse models of early developmental disruption of *Dio2* in myocyte precursors with no significant SKM phenotype adds to the controversy regarding the role of D2 during SKM development and as a determinant of TH signaling in adult SKM. However, there are studies indicating that TH signaling can be enhanced in SKM via induction of *Dio2* expression by physical activity (130) (Fig. 11). An acute treadmill exercise session (20 minutes at 70% to 75% of maximal aerobic capacity) increased *Dio2* expression/activity (1.5- to 2.7-fold) as well as *PGC1α* mRNA levels (1.5- to 5-fold) in rat SOL muscle and white gastrocnemius muscle and in mouse SOL muscle. However, induction of *PGC1α* was only partial (~40% less) in the Skm-D2KO mice by acute treadmill exercise as well as in primary Skm-D2KO myocytes stimulated with cAMP. Chronic exercise training (6 weeks) increased SOL muscle *PGC1α* mRNA levels (~25%) and the mitochondrial enzyme citrate synthase (~20%). In contrast, *PGC1α* expression did not change and citrate synthase decreased by ~30% in Skm-D2KO mice. SOL muscle *PGC1α* response to chronic exercise was also blunted in *Myf5*-D2KO

Figure 11. Physical exercise enhances TH signaling in skeletal muscle via induction of *Dio2*. One of the downstream targets of T3 is the thermogenic coactivator PGC1α that is key to mitochondrial function. Physical exercise accelerates cAMP production within skeletal myocytes, which induce the expression of both *Dio2* and PGC1α. *Dio2* expression accelerates local activation of T4 to T3, which enhances TH signaling and further stimulates PGC1α expression. This TH-mediated mechanism for induction of *PGC1α* is a component of the mitochondrial adaptation induced by exercise, which is lost in animals with skeletal muscle–specific *Dio2* inactivation (130). [Adapted with permission from "Physical exercise activates thyroid hormone in skeletal muscle." www.BiancoLab.org.]



mice (130). These studies indicate that *Dio2* expression mediates part of *PGC1α* induction by treadmill exercise and its downstream effects on mitochondrial function.

Skeleton

TH plays a key role on postnatal bone development and metabolism, as undiagnosed congenital TH deficiency can lead to delayed growth, and hyperthyroidism in adults can lead to osteoporosis (460). TH signaling in bone cells occurs predominantly via MCT8 and TRα; other TH transporters such as LATs and MCT10 are also expressed. TH induces endochondral ossification and linear bone growth by acting directly in reserve and proliferating TR-expressing chondrocytes to induce differentiation. In turn, hypertrophic chondrocytes do not express TRs (460, 461). T_3 acts in osteoblastic cells via TRα to induce differentiation and increase bone formation. Bone resorption is also accelerated by T_3 via induction of osteoclastic activity; however, this is an indirect effect via osteoblasts that is likely to involve expression of osteoblastic–osteoclastic coupling factors (460, 461). Studies in *Mct8*-KO animals indicate that postnatal endochondral ossification and linear growth are delayed. Furthermore, bone mass and mineralization are decreased in adult *Mct8*-KO mice (462), a phenotype that is consistent with decreased TH signaling in growth plate chondrocytes and increased TH signaling in adult bone. It is conceivable that in addition to the essential physiological requirement for MCT8 in chondrocytes, other TH transporters in other skeletal cells play a role in adult bone maintenance (462).

Fetuses harvested from pregnant hypothyroid mice exhibited marked reduction in tissue concentration of both T_4 and T_3, but bone development, as assessed at the distal epiphyseal growth plate of the femur and vertebra, is largely preserved up to E16.5 (463). Only at E18.5 do hypothyroid fetuses exhibit a reduction in femoral type I and type X collagen and osteocalcin mRNA levels, in the length and area of the proliferative and hypertrophic zones, in the number of chondrocytes per proliferative column, and in the number of hypertrophic chondrocytes, in addition to a slight delay in endochondral and intramembranous ossification. This suggests that up to E16.5, TH signaling in bone is kept to a minimum. In fact, *Dio3* mRNA, which in mice is present in growth plate chondrocytes, osteoblasts, and osteoclasts (460), is readily detected as early as E14.5 and its expression decreases markedly (~10-fold) at E18.5, and even more at 14 days after birth (463). In contrast, *Dio2* mRNA expression increases significantly by E18.5 and markedly (~2.5-fold) by postnatal day 14. *Dio2* mRNA was detected in growth plate chondrocytes, in osteoblasts and osteoclasts, but D2 activity was only detected in osteoblasts (460, 461). Reciprocal expression patterns of *Dio2* and *Dio3* during early bone development along with the absence of a "hypothyroid-like" bone phenotype at this time suggest that coordinated reciprocal deiodinase expression keeps TH signaling in bone to very low levels up until E18.5 (463). Indeed, activation of TH signaling accelerates differentiation of

chondrogenic cells and cultured mouse tibias, but T4 is as potent as T3, which indicates that T4 is converted locally to T3 (464). *Dio2* mRNA is present in neonatal mouse tibias (464), and D2 activity can be detected in bone extracts at multiple sites of the mouse skeleton, bone marrow, and in osteoblastic cell line (53). Treatment with vitamin D [1,25(OH)2VD] induces D2 activity by twofold to threefold, but estradiol, parathyroid hormone, forskolin, leptin, TNFα, TGFβ, and dexamethasone do not affect D2 (53).

Dio2 continues to play an important physiological role in TH signaling in adult animals. Studies in global-D2KO mice revealed bones that have reduced toughness and are brittle, displaying increased susceptibility to fracture (131). This phenotype is characterized by a 50% reduction in bone formation and a generalized increase in skeletal mineralization resulting from local deficiency of T3 in osteoblasts. Articular cartilage is preserved in adult global-D2KO mice, but they exhibit increased subchondral bone mineral content (465). Therefore, osteoblast *Dio2* expression plays an essential role in the optimization of bone strength and mineralization (131).

Reproductive organs

Gonads

Ovaries express multiple elements of the signaling TRIAD but little is known about dynamic control of TH signaling in this organ (466). TRα predominates and Lats and Mct8 are the most abundant transporters in the mouse ovary (467, 468). Expression of Dio1 and Dio2 have also been detected at different neonatal ages, with Dio2 mRNA levels predominating over Dio1 (161). Indeed, evidence exists that ovarian steroidogenesis is affected by TH signaling. Ovarian granulosa cells obtained from infertile women exhibit a reduction in biological markers of fertility, which is associated with reduced expression of TRs (469). However, it is unclear whether this relationship involves modifications in TH signaling. A clearer but incomplete picture exists for the testis. The rat and human testes express *TRα* during development and adulthood (mostly in the Sertoli cells) and thus are a potential target of TH (470). Both MCT and OATP types of transporters are present in the testis (471, 472). *OATP1C1* has been identified in the Leydig cells (473) whereas *MCT8* is also expressed in the Sertoli cells. *DIO2* and *DIO3* are also expressed in the testis, suggesting that local control of TH signaling plays a role. In rodents, D2 activity is located in germ cells at late stages of differentiation (51). The highest D2 activity level coincides with the peak of TH in the circulation at ~3 weeks of age (161), a time that marks the end of the high proliferation rate of Sertoli cells and spermatogonia (474). This suggests that *DIO2* may assist the rising circulating TH levels in enhancing local TH signaling to trigger differentiation processes in both cell types (470). However, adult global-D2KO mice have no specific testicular phenotype, suggesting the existence of redundant mechanisms (471). At the same time, *Dio3* expression is high in the mouse developing testis, peaking around the first 2 weeks of life. Although global-D3KO mice exhibit a dramatic testicular phenotype, it is not clear how much of the phenotype is due to deficient local D3-mediated regulation of TH signaling as opposed to systemic neonatal thyrotoxicosis that is typical in these animals, or to both (471).

Uterus

In uterus, TRα is expressed in the uterine luminal epithelium, endometrial gland epithelium, and endometrial stromal cells and, moderately, with myometrial smooth muscle. In oviduct, they were observed moderately in the epithelium of the tube and the smooth muscle cells of the muscular layer (475). *Dio2* and *Dio3* are expressed in the mouse and human uterus, suggesting that local TH signaling and/or the flow of TH to the fetus is regulated by these enzymes (476–478). *Dio2* expression is in the murine endometrial stromal cells, particularly in the region adjacent to the epithelial cells of the uterine lumen (478). D3 can also be found in the endometrial glands of nonpregnant human uteri, and endometrial activity approximated that of term placenta (477). Once the embryo implants into the receptive mouse uterus, *Dio3* expression and D3 activity are induced in the stromal cells via progesterone and cAMP, leading to a drop in uterine T3 levels and TH signaling (476). Notably, addition of T3 or *Dio3* knockdown compromises decidualization (479).

Placenta

Rat placenta expresses significant levels of TRα and TRβ transcripts and proteins (480). In human term placenta the use of laser capture microdissection revealed that trophoblasts express substantially less mRNA encoding TRα and TRβ than do stromal cells (481). Human term placenta expresses different TH transporters, including *MCT8*, *MCT10*, *LAT1*, *LAT2*, *OATP1A2*, and *OATP4A1*. These transporters are often present in the apical (maternal-facing) microvillous membrane of syncytiotrophoblasts (STBs). Studies using Mct8, Mct10, and Lat2 KO mice indicate that none of these transporters, independently, is essential for fetal development, albeit the study of Lat1 is inconclusive, as its inactivation is embryonic lethal (482).

Human placenta expresses *DIO2* and *DIO3* during the entire gestational period, which may affect TH signaling in the fetus (483) (Fig. 6). Before 16 weeks' gestation, the fetus relies on transplacental delivery of maternal TH. Maternofetal TH transfer is regulated by trophoblast cell membrane transporters, which mediate influx and efflux of THs, as

well as placental D3 and D2 that control intraplacental TH levels. *DIO2* is expressed mainly in mixed fetal membranes, but also in trophoblasts (484). D2 activity declines during pregnancy, to hardly detectable levels at term. Notably, D2 activity is ~200-fold lower than D3 activity at all gestational ages, suggesting that placental D2 plays no significant role in fetal TH levels, but it may play a role in local TH signaling, inducing differentiation of trophoblasts (485–490).

In humans, high *DIO3* expression is present in the placental STBs and cytotrophoblasts, endothelium of fetal vessels, and maternal decidua. D3 is also present at other sites of maternal–fetal interface, including the umbilical arteries and vein and the fetal respiratory, digestive, and urinary tract epithelium (477). This is likely to account for the low fetal serum T3 and high serum rT3 levels. Placental D3-specific activity also decreases during gestation and is likely to function as a barrier for maternal TH to reach the fetus, possibly contributing to low T3 and high rT3 serum concentrations observed in the fetus (491). The disappearance of D3 at birth is likely to explain many of the changes in neonatal TH economy occurring early in the postnatal life (492). It is uncertain exactly which cells in the placenta constitute a barrier for maternal–fetal TH transfer in the different stages of gestation, but it is clear that STBs play a role in this process. Given all the D3 activity, it is unexpected that some T4 molecules undergo transcellular transport across STBs without being inactivated. This suggests an alternative mechanism for T4 transport, namely complexed with transthyretin (TTR) that is produced by STBs (482).

Tumors and kinase inhibitors

TH signaling affects the progression of certain tumors to the point that it can halt growth of basal cell carcinomas (BCCs) (133). *DIO3* is the element in the signaling TRIAD that plays the most relevant role, but other deiodinases, TRs, and TH transporters have been studied in this context as well.

Deiodinases

DIO3 is predominantly expressed during embryonic development, coordinated with *DIO2* expression to fine-tune TH signaling in different tissues (5, 451). As an oncofetal protein, D3 is only minimally expressed in most adult tissues (except for brain and placenta), with much higher expression levels seen in many malignant tumors (493, 494). In this regard, TGFβ and the Hedgehog family of proteins are known to stimulate *DIO3* expression and dampen TH signaling (133, 495, 496). TGFβ transcriptionally induces *DIO3* expression in human cells, including SKM myoblasts, fibroblasts, fetal epithelium, endometrium, and tumors such as hemangioma and glioma (77). Hedgehog proteins also transcriptionally induce *DIO3*, but they also dampen TH signaling through coordinated inhibition of D2 activity (133, 495, 496) and by inducing expression of *SMRT* through Gli, the effector of the Hedgehog pathway (497). The effects of the Hedgehog family of proteins are illustrated in the chicken developing growth plate, where Indian Hedgehog signaling inhibits D2-mediated T3 production by inducing the ubiquitin ligase WSB1 (84, 89) and at the same time stimulates *Dio3* expression, further dampening TH signaling.

In the skin setting, the Shh pathway is constitutively active in BCCs, creating an example of how TH signaling is fine-tuned by the coordinated expression of deiodinases. Shh, signaling through Gli2, induces *DIO3* in proliferating keratinocytes, in mouse and human BCCs. Gli-induced *DIO3* dampens TH signaling, thus increasing cyclin D1 and keratinocyte proliferation (498, 499). *DIO3* knockdown reduces growth of BCC xenografts in nude mice by about fivefold. This crosstalk between Shh/Gli2 and TH explains how Shh induces keratinocyte proliferation (133). Notably, BCC cells express not only *DIO3* but also substantial levels of *DIO2*. In these cells, *DIO2* inactivation accelerates cell cycle progression, thereby enhancing the proportion of S-phase cells and cyclin D1 expression. Furthermore, the basal apoptotic rate is oppositely regulated in D2- and D3-depleted cells. The dual regulation of D2 and D3 expression plays a critical role in cell cycle progression and cell death by influencing cyclin D1–mediated entry into the G_1–S phase, and may constitute a potential therapeutic approach to BCC (498, 499).

High levels of *DIO3* expression have been reported in a number of tumors, including gliomas, gliosarcomas, glioblastomas, TSH/adrenocorticotropic hormone–producing tumors, papillary thyroid carcinoma, as well as tumor-derived cell lines of breast cancer (MCF7 cells), colon adenocarcinoma (Caco2, SW280, and HCT116 cells), endometrial cancer (ECC-1 cells), and neuroblastoma (SH-SY5Y cells), as a result of activation of ERK and p38 pathways (160, 493, 500–509). *DIO3* lies downstream of the Wnt/β-catenin pathway and contributes to colon carcinoma cell growth (493) and tumorigenic capacity in stem cells via T3-induced bone morphogenetic protein 4 gene, which exhibits high antitumor activity in colorectal cancer (510). *DIO3* expression in vascular tumors such as hepatic hemangiomas is most striking. Hemangiomas are common tumors of infancy that express variable levels of *DIO3*. Depending on the size of the tumor, D3 levels can be so high that the tumor inactivates circulating TH faster than the thyroid gland can secrete, resulting in what it is known as consumptive hypothyroidism (505).

In recent studies, a similar condition has been observed in patients with metastatic renal cell carcinoma or gastrointestinal stromal tumors receiving

treatment with the tyrosine kinase inhibitor sunitinib (511, 512). Hepatic D3 activity increases markedly in rats undergoing similar treatment with this kinase inhibitor, indicating that *DIO3* induction plays a role in sunitinib-induced hypothyroidism (511). Similar to hemangiomas, gastrointestinal stromal tumors themselves can produce consumptive hypothyroidism caused by marked overexpression of *DIO3* within the tumor (513, 514). Some kinase inhibitors might have a broader effect on TH signaling by affecting other components of the TRIAD. For example, in a study of 57 consecutive patients with hepatocellular carcinoma who were treated with sorafenib, 4 patients developed thyroiditis and 16 had elevation of TSH or FT4 above the normal range; simultaneously, the serum T3/rT3 ratio decreased (515). In cellular studies, sorafenib decreased T3 uptake via MCT8 and to a lesser extent via MCT10 (515).

Conversely, some tumors overexpress *DIO1* and *DIO2*, with systemic consequences for thyroid economy. There are reports of patients with large or widely metastatic follicular thyroid carcinoma who had a persistently increased ratio of serum T3/T4 and exhibited increased D2 activity in their tumors (516, 517). In turn, *DIO1* expression can be reduced in several types of human malignancies such as papillary thyroid carcinoma, clear cell renal cell carcinoma, lung cancer, gastric cancer, hepatic adenoma, and some pituitary tumors, whereas in breast cancer, follicular thyroid carcinoma, and anaplastic thyroid cancer there is an increase in D1 levels (439, 498, 518–524). Loss of *DIO1* expression is associated with proliferation and migration of renal cancer cells, downregulating oncoproteins and affecting key metabolic pathways (499, 525). In this context, miRNAs seem to play an alternative role as regulators of *DIO1* expression (521). The use of bioinformatics analyses revealed that the *DIO1* 3′ untranslated region is targeted by two miRNAs, miR-224 and miR-383, with the former mediating loss of D1 in renal cancer and reducing the intratumoral levels of T3. This suggests that in renal cancer cells, miR-224 dampens D1-mediated TH signaling (521). Stable reexpression of *DIO1* in these cells downregulated 26 proteins consisting mainly of oncoproteins (*e.g.*, STAT3, ANPEP, TGFBI, TGM2) that promote proliferation, migration, and invasion. Furthermore, *DIO1* reexpression enhanced expression of LAT1 components and elevated intracellular concentration of T4. Expression of *DIO1*-affected genes strongly correlated with *DIO1* mRNA levels in biopsies of renal cancer patients as well as with their poor survival (499). Overall, this is a unique situation given that D1 is not known for affecting local TH signaling in other systems.

TRs and transcriptional coregulators

Studies in a renal cell cancer (RCC)–derived cell line indicate a possible disruption of TH signaling due to decreased TR-interacting protein 11 (TRIP11) levels, a TR coactivator that is regulated by T3 (526). Additionally, the expression of TRα and TRβ are also reduced in RCC tumor samples and correlated with poor prognosis in pairs of RCC tumor-control samples (526). Whether this disruption in TH signaling plays a role in tumor progression or it is just an associated event remains to be determined. However, in other tumor types a causal relationship has been established. Studies in cultured cells *in vitro* and in xenograft models *in vivo* indicate that TRβ1 could function as a tumor suppressor (527, 528). At the same time, NCoR depletion enhances cancer cell invasion and increases tumor growth and metastatic potential in nude mice (529). Expression of TRβ increases NCoR levels, an essential step to inhibit tumor growth and metastasis. Indeed, NCoR is downregulated in human hepatocarcinomas and in the more aggressive breast cancer tumors, and its expression correlates positively with that of TRβ (529). The TRα pathway has also been associated with tumorigenesis: increased expression of *TRα1* has been reported in cohorts of patients with colorectal tumors. In these cases, *TRα1* gene expression correlates directly with Wnt activity (530). In fact, ectopic expression of *TRα1* in the intestine epithelium of mice accelerates tumorigenesis and the development of more aggressive tumor phenotypes (531). In colon cancer cells, *TRα1* levels regulate Wnt activity to affect cell proliferation and migration: increased expression of *TRα1* was accompanied by decreased levels of several cellular inhibitors of Wnt signaling (530). Such inverse correlation found in mouse models was also demonstrated in cohorts of patients with colorectal tumors. This accounts for how the elevated *TRα1* led to the activation of Wnt signaling (530), thereby establishing the potential oncogenic role of TRα1 in the intestine epithelium (532). The contrasting functions of TR isoforms in tumorigenesis are puzzling. A better understanding of how both TRα and TRβ crosstalk with other cellular networks of tumor promoters and suppressors is necessary to characterize their role in tumorigenesis (532).

Tissue regeneration

The observations that TH signaling as modulated by the deiodinases plays a role in tumor cell proliferation sparked interest regarding a possible similar role in tissue regeneration. In the SKM, tissue damage caused by turpentine injection results in *Dio3* expression, suggesting that reduced TH signaling is important for the initial steps of muscle regeneration (451). In fact, satellite cell–specific inactivation of *Dio3* severely impairs SKM regeneration due to massive satellite cell apoptosis (533). The proapoptotic program requires an intact *FoxO3*/*MyoD* axis, with both genes positively regulated by TH signaling. Induction of *Dio3* is followed by a several-fold induction of *Dio2* in the later stages of muscle

regeneration, enhancing TH signaling that possibly plays a role in muscle differentiation (130, 456, 495).

Partial hepatectomy in rodents is another well-known model of tissue regeneration. *Dio3* mRNA and D3 activity are several-fold increased hours after partial hepatectomy (534). This increase in D3 reduces serum and liver T3 and T4 levels by twofold to threefold, which coincides with a peak in hepatocyte proliferation. This temporal profile also suggests that in this model dampening TH signaling via induction of *Dio3* expression favors cellular proliferation. Similar observations of reduced local TH signaling were made in rats with cholestatic liver injury and fibrosis caused by bile duct ligation (535). In this model there is strong induction of hepatic *Dio3* expression in stromal cells, whereas *Dio1* expression, which is typical in hepatocytes, decreases to low levels. *Dio3* expression occurs in the injury-activated hepatic stellate cells, which play important roles in hepatic wound healing and regeneration. Notably, hepatic stellate cell activation and *Dio3* expression are controlled by Hedgehog signaling (535). The Hedgehog family of proteins also plays a role in advanced liver fibrosis that might be present in patients with nonalcoholic fatty liver disease (535). In these patients, the reduced FT3/rT3 ratio confirms the switch from *DIO1* to *DIO3* expression, reducing local TH signaling as evidenced by lower mRNA levels of T3-responsive genes.

These studies indicate that the Hedgehog-dependent changes in liver stromal cells drive repair-related changes in hepatic deiodinase expression that dampens local TH signaling and is likely to affect cellular differentiation (535). However, this could not be verified when a novel mutant mouse with hepatocyte-specific *Dio3* deficiency was studied (536). These animals exhibited normal local responses to toxin-induced hepatonecrosis, including normal levels of tissue necrosis and regeneration. Notably, these mice exhibited accelerated systemic recovery from NTIS-induced hypothyroxinemia and low serum T3 levels, confirming that peripheral reactivation of *Dio3* expression is an important factor in the pathogenesis of NTIS (536).

Conclusions

The tranquility of the plasma T3 levels contrasts with a stormy intracellular environment of a large number of tissues, in which T3 levels and TH signaling rapidly increase or decreases whereas serum T3 concentration remains unchanged. This is possible due to the signaling TRIAD, namely the TH transporters, deiodinases, and TRs, which modulate entry and metabolism of TH molecules as well as transduction of TH signaling. These mechanisms control how the biologic activity of the thyroid secretion impacts tissues at various life moments, during health and disease states. Understanding these mechanisms should allow for the development of customized approaches to manipulate TH signaling, with enormous therapeutic implications.

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Abbreviations

4PBA, 4-phenyl butyric acid; AD, Alzheimer's disease; AFABP, adipocyte-specific fatty acid–binding protein; AKT, serine/threonine kinase 1; Alb-D2KO, (mouse with) liver-specific *Dio2* inactivation; AMIO, amiodarone; Astro-D2KO, (mouse with) astrocyte-specific *Dio2* inactivation; ATP2a, ATPase sarcoplasmic/ER Ca^{2+} transporting; BAT, brown adipose tissue; BBB, blood–brain barrier; BCC, basal cell carcinoma; CNS, central nervous system; D1, type I iodothyronine deiodinase; D2, type II iodothyronine deiodinase; D3, type III iodothyronine deiodinase; DEA, desethylamiodarone; *Dio1*, gene encoding D1; *Dio2*, gene encoding D2; *Dio3*, gene encoding D3; E, embryonic day; ER, endoplasmic reticulum; FAAH, fatty acid amide hydrolase; Fat-D2KO, (mouse with) fat-specific *Dio2* inactivation; FT3, free T3; FT4, free T4; Foxo, forkhead box, subgroup O; global-D2KO, (mouse with) global *Dio2* inactivation; global-D3KO, (mouse with) global *Dio3* inactivation; GLP1, glucagon-like peptide 1; HFD, high-fat diet; HIF, hypoxia-inducible factor; HPT, hypothalamic–pituitary–thyroid; HSP40, heat shock protein 40; iCKO, inducible conditional KO; IVH, intraventricular hemorrhage; IPF, idiopathic pulmonary fibrosis; KO, knockout; LAT, L-type amino acid transporter; LDL, low-density lipoprotein; L-T3, liothyronine; L-T4, levothyroxine; LXR, liver X receptor; MBH, medial basal hypothalamus; MCT, monocarboxylate transporter; *Mct8*-KO, (mouse with) global *Mct8* inactivation; MHC, myosin heavy chain; miR, miRNA; mTOR, mammalian target of rapamycin; mTORC, mammalian target of rapamycin complex; MYF5, myogenic regulator factor-5; MYH, myosin, heavy polypeptide; MYOD, muscle determination gene; NCOR, nuclear receptor corepressor; NE, norepinephrine; NTIS, nonthyroidal illness syndrome; OATP, organic anion-transporting polypeptide; OPC, oligodendrocyte precursor cell; PGC1α, PPARγ coactivator 1α; PI3K, phosphatidylinositol 3-kinase; PPARγ, peroxisome proliferator-activated receptor-γ; PTU, propylthiouracil; PVN, paraventricular nucleus; RCC, renal cell cancer; RDS, respiratory distress syndrome; rT3, reverse T3; RXR, retinoid X receptor; SBP2, selenocysteine insertion sequence binding protein 2; SECIS, selenocysteine insertion sequence; SKM, skeletal muscle; Skm-D2KO, (mouse with) skeletal muscle-specific *Dio2* inactivation; SMRT, NcoR2; SNP, single-nucleotide polymorphism; SOL, soleus; STB, syncytiotrophoblast; T2, 3,3′-diiodo-L-thyronine; T3S, sulfated T3; TBI, traumatic brain injury; TGR5, G-protein–coupled bile acid receptor 1 (GPBAR1); TH, thyroid hormone; TR, thyroid hormone receptor; TRE, thyroid responsive element; TRH, TSH-releasing hormone; TRIAD, transmembrane transport, intracellular deiodination, and TR-mediated gene transcription; TUDCA, tauroursodeoxycholic acid; UBC, ubiquitin-activating enzyme; UbD2, biquitinated D2; UCP1, uncoupling protein 1; USP, ubiquitin-specific peptidase; VILI, ventilator-induced lung injury; VLDL, very LDL; WSB1, WD repeat and SOCS box-containing 1; Zfp125, zinc finger protein-125.