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Review

Activation and inactivation of thyroid hormone by deiodinases: Local action with general consequences

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Abstract. The thyroid hormone plays a fundamental role in the development, growth, and metabolic homeostasis in all vertebrates by affecting the expression of different sets of genes. A group of thioredoxin fold-containing selenoproteins known as deiodinases control thyroid hormone action by activating or inactivating the precursor molecule thyroxine that is secreted by the thyroid gland. These pathways ensure regulation of the availability of the biologically active molecule T3, which occurs in a time- and tissue-specific fashion. In addition, because cells and plasma are in equilibrium and deiodination affects central thyroid hormone regulation, these local deiodinase-mediated events can also affect systemic thyroid hormone economy, such as in the case of non-thyroidal illness. Heightened interest in the field has been generated following the discovery that the deiodinases can be a component in both the Sonic hedgehog signaling pathway and the TGR-5 signaling cascade, a G-protein-coupled receptor for bile acids. These new mechanisms involved in deiodinase regulation indicate that local thyroid hormone activation and inactivation play a much broader role than previously thought.

Keywords. Thyroid, hormone metabolism, deiodinases, non-thyroidal illness syndrome, obesity, Sonic hedgehog pathway, bile acid.

Introduction

The major secretory product of the thyroid gland is thyroxine, a precursor molecule that has to be converted to 3,5,3′-triiodothyironine (T3), the compound that can effectively bind to the nuclear thyroid hormone receptor (TR) and mediate thyroid hormone-dependent transcriptional activation or repression [1, 2]. Since T4 half-life is long (1 week in humans) [3], the role played by the hypothalamo-pituitary-thyroid axis in regulating T3 availability is complemented by the function of the deiodinases, enzymes that can activate or inactivate in a time- and tissue-specific fashion [4–7].

Deiodinase proteins could not be purified by classic biochemical techniques due to their low expression and membrane-bound nature. Cloning of the type 1, 2,

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and 3 (D1, D2, and D3, respectively) deiodinases [5, 8–14] tremendously facilitated the understanding of their cellular and molecular biology. D2 is the major T4 activating deiodinase, producing T3 by the removal of an iodine residue from the outer (phenolic) ring of thyroxine. Conversely, D3 inactivates T3 by deiodination of the inner (tyrosyl) ring of T4. The physiological role of the rather inefficient D1 enzyme, which has three orders of magnitude less affinity for thyroid hormones, remains to be determined.

Despite their distinct roles in thyroid hormone metabolism, members of the deiodinase family share important common features [15]. All are selenoproteins and contain an in-frame UGA codon for selenocysteine (Sec) incorporation in their active center along with a selenocysteine insertion sequence (SECIS) element in the 3′ untranslated region (UTR) of their mRNA [5]. The SECIS element is required for SECIS-binding protein 2 (SBP2) and elongation factor Sec (EFsec) mediated co-translational insertion of Sec at the UGA codon in the active center of the enzyme [16]. The presence of the rare amino acid Sec in deiodinases makes these proteins highly active and yet tightly expressed oxido-reductases, a feature that allows precise regulation of T3 generation and inactivation. Furthermore, D1, D2, and D3 share important structural similarities. All are homodimer forming integral membrane proteins [17–19], belong to the thioredoxin-fold protein family, and contain a glycoside hydrolase clan GH-A-like structure [20]. Knockout mice were generated for each deiodinase and the phenotypes of these animal models have been reviewed recently [21].

In the first part of this review, we summarize the current knowledge on the general and specific properties of deiodinases, including structure, gene organization, characteristics of the D1, D2, and D3 mRNA and protein, subcellular localization/topology, tissue distribution, and regulation. In the second part, we focus on specific issues, including the role of deiodinases in non-thyroidal illness, the role of D2 in metabolic control, and recent progress in D3 pathophysiology.

The 3-D structure of deiodinases

The three deiodinase proteins (D1, D2, and D3) are highly similar in their active center. All are integral membrane proteins of 29–33 kDa, and have regions of high homology in the area surrounding the active center [22–24]. Insights into the structures of these proteins were obtained through protein modeling using hydrophobic cluster analysis (HCA) [20]. Based on the HCA analysis, it is clear that the three deiodinases share a common general structure composed of a single trans-membrane segment, which is present in the N termini of D1, D2, and D3, and several clusters, typical of α-helices or β-strands, corresponding to core secondary structures of the deiodinase globular domains (Fig. 1). A striking common feature is the presence of the thioredoxin (TRX) fold, defined by the βαβ and ββα motifs. It is interesting that, within the canonical TRX fold, the relationship between the βαβ and ββα motifs is locally interrupted by interfering elements. These sequences correspond to distinct secondary structure elements added to the canonical TRX fold core, a feature also observed in other proteins of the TRX fold family [25]. A unique aspect of the deiodinases, however, is that one of these highly conserved intervening elements shares similarities with α-L-iduronidase (IDUA; 47% identity with D1 and D3, 60% with D2), a lysosomal enzyme that cleaves α-linked iduronic acid residues from the nonreducing end of glycosaminoglycans [26].

The 3-D general model of the deiodinases predicts that the active center is a pocket defined by the β1-α1-β2 motifs of the TRX fold and the IDUA-like insertion. A striking feature of this pocket is the

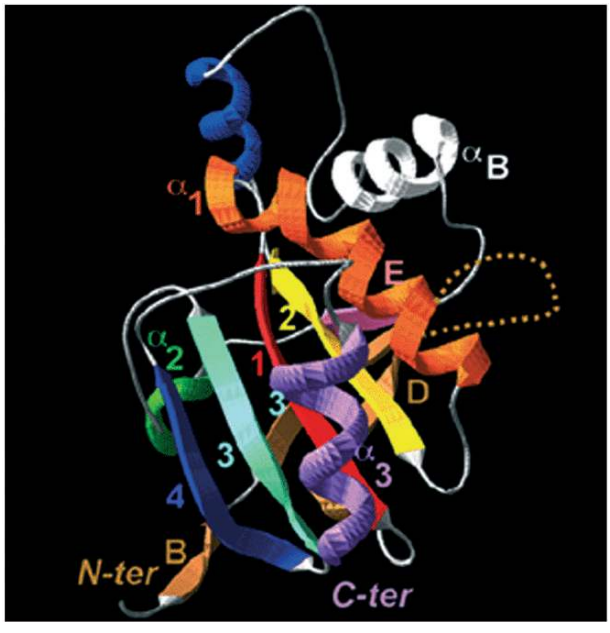

Figure 1. 3-D structure of deiodinases. In the absence of experimental data on deiodinase crystals, protein modeling was used to assess the 3-D structure of these enzymes. Modeling indicates that deiodinases share a common general structure composed of a single N-terminal-anchoring segment, a short hinge region, and a thioredoxin fold-containing globular domain [20] demonstrated here on the D2 globular domain. Letters and numbers indicate different β-sheets and α -helices as previously reported [20]. The orange dotted line indicates the D2-specific loop that mediates interaction with the E3-ubiquitin ligase WSB-1 [145]. Reprinted with permission from [145].

Table 1. Basic properties of deiodinases.

Parameter	D1 (ORD, IRD)	D2 (ORD)	D3 (IRD)
Molecular mass of monomer (kDa)	27	31	32
Preferred substrate	rT3 (5′), T3S (5)	T4, rT3	T3, T4
Apparent K_m (M)	10^{-7}, 10^{-6}	10^{-9}	10^{-9}
Susceptibility to PTU	High	Very low	Very low
Susceptibility to IOP	Yes	Yes	Yes
Protein half-life	Hours	~20 minutes	Hours
Subcellular localization	Plasma membrane	Endoplasmic reticulum	Plasma membrane
Homodimerization	Yes	Yes	Yes
Predominant tissue source	Liver, kidney	Pituitary, brown adipose tissue, placenta, brain (tanycytes, astrocytes)	Placenta, brain, hemangioma
Response to thyroid hormone			
Transcriptional	↑↑	↓	↑↑
Post-translational	↓↓ (oxidation of active center)	↓↓↓ (ubiquitination)	?
Physiological role	rT3 and T3S clearance	Intra- and extracellular T3, development, thermogeneis	T3, T4 clearance, development
Pathophysiological role	Plasma T3 generation in hyperthyroid patients	Nonthyroidal illness, obesity?	Consumptive hypothyroidism, nonthyroidal illness
Knockout models			
Serum T4	↑	↑	↑
Serum T3	Normal	Normal	↓
TSH	Normal	↑	Normal
systemic phenotype	Euthyroid	Euthyroid	Hypothyroid

Up and down arrows indicate increases and decreases, respectively; the number of arrows corresponds with the intensity of the change. ORD, outer ring deiodination; IRD, inner ring deiodination; PTU, 6-*n*-propyl-2-thiouracil; IOP, iopanoic acid; rT3, reverse T3; T3S, triiodothyronine sulfate.
Detailed properties of the deiodinase enzymes can be obtained in previously published reviews [5–7, 21, 239].

amino acid Sec, critical for the deiodination reaction catalyzed by all three deiodinases. This was first identified when the rat D1 cDNA became available, the analysis of which revealed the presence of the Sec encoded by UGA, which is recognized in the vast majority of mRNAs as a STOP codon [8]. However, a specific RNA stem-loop immediately downstream of the UGA codon allows Sec incorporation in the STOP codon. This structure is termed the Sec insertion sequence, or SECIS element, which is present in the deiodinases and all other selenoproteins [27].

Specific properties of deiodinases

Type 1 iodothyronine deiodinase

Type 1 deiodinase (D1) is capable of both inner (IRD) and outer ring deiodination (ORD), thus it can convert T4 to T3 or rT3 and can produce 3,3′-T2 from T3 or rT3. D1 was the first deiodinase to be studied. Originally, D1 was viewed as the major activating deiodinase responsible for the bulk of the extrathyroidal T3 production. However, this view has been challenged over the years, with several studies in humans demonstrating that D2 and not D1 is the major source of circulating T3 [28–32]. Only in the hyperthyroid patients is D1 an important factor in extrathyroidal T3 generation, given that the *dio*1 gene is exquisitely up-regulated by T3 with the opposite happening with *dio*2. In rodents, D1 is likely to play a more important role and, in fact, studies in D1 knockout mice suggest that D1 may act by balancing the consequences of altered thyroid function, including thyrotoxicosis, iodine deficiency, and in rT3 clearance [31].

Gene structure, chromosomal localization, and 5′ flanking region. The human *dio*1 gene is located on chromosome 1 p32-p33 [33]. Both the human and mouse *dio*1 genes consist of four exons with the UGA Sec codon located in exon 2 and the UAG STOP codon and the SECIS element in exon 4 [33, 34]. The

human *dio*1 gene is under the control of GC-rich SP1 promoters and contains two TREs in the 5′ FR, both contributing to the T3 responsiveness of the human *dio1* promoter [35–37]. The TRE-2 is a typical DR+4 direct repeat with 4 bp separating the RXR-T3-TR binding half-sites, which makes D1 responsive both to T3 and retinoic acid [37]. In contrast, TRE-1 is an unusual *cis*-acting element in which two TR-binding octameric half-sites are separated by 10 bp, binding TR but not RXR [35]. A 21-bp insert containing five CTG repeats in the 5′ FR of the *dio*1 gene of the C3H mice is considered responsible for the impaired D1 expression in this strain [34].

The D1 mRNA. The D1 mRNA (~2 kb) was the first to be cloned among the deiodinases *via* the use of expression cloning [8]. Identification of the SECIS element in the D1 3′ UTR recognized D1 as only the second eukaryotic mRNA encoding a selenoprotein [27]. Rat D1 is encoded by at least two different transcripts that vary in length at the 3′ UTR, but their significance, if any, has not been clarified [38, 39]. Two single nucleotide polymorphisms (SNPs) were identified in the 3′ UTR of the human D1 mRNA, D1a-C/T at position 785 and D1b-A/G at position 1814, the latter being 33 nucleotides 3′ to the SECIS element [40]. The frequency of the T variant of the D1a-C/T polymorphism was positively correlated with rT3/T4 and negatively with T3/rT3 ratios, suggesting a negative effect of this variant on total D1 expression and/or activity. The G variant of the D1b-A/G polymorphism was negatively correlated with rT3/T4 and positively with T3/rT3 ratio, suggesting a higher D1 expression and/or activity of the D1b-G variant.

The D1 protein. Outer ring deiodination by D1 activity was first reported in the 1970 s as a 6-*n*-propyl-2-thiouracil (PTU)-sensitive T4 to T3 conversion [41–43] that is dependent on thiol group-containing cofactors [44]. D1 activity follows ping-pong kinetics [8] and the best substrates are rT3 and sulfated T3, while its K_m(T4) is in the micromolar range (1000-fold higher than that of D2). Sulfation of the phenolic hydroxyl group blocks outer ring deiodination of T4, while it strongly stimulates T4 inactivation by favoring inner ring deiodination [45], resulting in enhanced degradation of thyroid hormones by D1. Although D1 is PTU sensitive, in some vertebrates the enzyme has reduced (killfish, *Tilapia*) or no (*Xenopus*) sensitivity toward inhibition by PTU [46–48]. The D1 protein is ~27 kDa and contains Sec in its active center [8, 27]. The core catalytic center consists of approximately 15 amino acid residues surrounding the Sec residue and is highly conserved among species. Within this core, the Ser128Pro modification in D1 makes the enzyme resistant to PTU and changes the kinetics for sequential compared to the ping-pong kinetics of the wild type [20]. Interestingly, the substitution of Ser for Pro in the equivalent position of D2 (135) makes D2 kinetics similar to D1 [20]. The Pro135Ser D2 mutant has a two order of magnitude increase in K_m(T4) to 250 nM that is just tenfold lower than that of D1 for T4. Furthermore, compared to the sequential kinetics of the wild type, this D2 mutant has ping-pong kinetics and is sensitive to inhibition by PTU, just like D1. In D3, substitution of Ser for Pro in the equivalent position (146) also makes the enzyme kinetics similar to D1, *i.e.*, the K_m(T3) becomes fivefold higher and the enzyme becomes very sensitive to inhibition by PTU [20].

The D1 protein has a long half-life (>12 h) and its activity is subjected to substrate-induced inactivation that can be blocked by PTU [49–51]. However, in contrast to D2, D1 is not ubiquitinated and its activity decrease is not associated with decrease of the D1 protein [50]. Early attempts to purify D1 identified activity in higher molecular weight forms [52, 53]. Subsequent studies using different approaches confirmed that D1 forms dimers [17, 18, 54, 55]. The existence of a well-conserved deiodinase dimerization domain (DDD) has been also suggested [54].

Subcellular localization and topology. Studies in cell-free systems indicated that D1 is an integral membrane protein with its active site in the cytosol [56]. In fact, D1 was found to be localized to the basolateral plasma membrane in renal cortical epithelial cells and thyroid cells [57, 58], as well as HEK-293 cells transiently expressing tagged D1 (Fig. 2) [59].

Tissue distribution. D1 is localized predominantly in the liver and kidney of vertebrates. In mammals it is also expressed in the thyroid gland, pituitary, intestine and placenta [4, 5]. Notably, D1 is expressed in the rat but not human central nervous system (CNS) [60, 61]. In lower vertebrates, D1 activity can be found abundantly in the gills and brain of fish, in the pancreas of reptiles, and in the gut of birds and mammals [62]. Interestingly, the recently identified D1 in *Xenopus* is expressed in marked, spatially defined patterns during embryogenesis [63].

Transcriptional regulation of the dio1 gene. The *dio*1 gene is markedly stimulated by T3 due to the presence of well-characterized TREs in the human *dio*1 promoter [35, 36]. Thus, one would expect that the significance of the T3 responsiveness of the human *dio1* gene would be most obvious in patients with thyrotoxicosis, such as in Graves disease. Semi-quantitative PCR of D1 mRNA in human peripheral

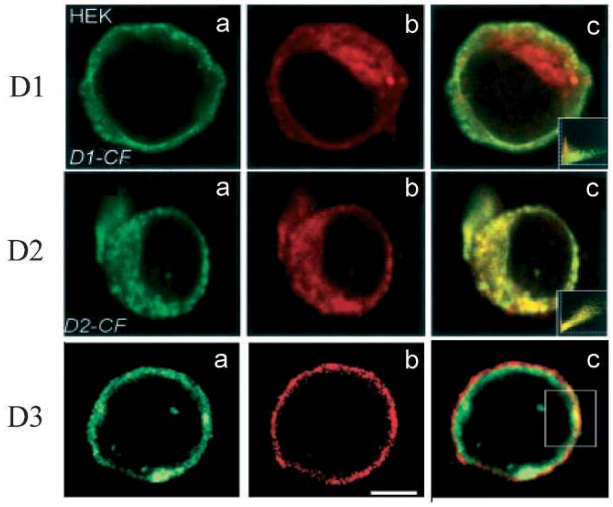

Figure 2. Subcellular localization of deiodinases. D1 is excluded from the endoplasmic reticulum (ER), while D2 is co-localized with the ER marker Grp78/BiP in HEK-293 cells transiently expressing D1C-FLAG or D2C-FLAG. Specimens were processed for indirect immunofluorescence with anti-FLAG (*a*, green) and anti-Grp78/BiP (*b*, red) antibodies as assessed by confocal microscopy; co-localization is shown in (*c*). Scale bar: 10 μm. Reprinted with permission from [59] (© The Endocrine Society). D3 is excluded from ER and co-localizes with Na,K-ATPase in the plasma membrane in HEK-293 cells transiently expressing D3C-FLAG processed for immunofluorescence with anti-FLAG (*a*, green) and anti-Na,K-ATPase α (*b*, red) antibodies; the co-localization is shown in (*c*). Reprinted with permission from [166] (© ASBMB, Inc).

blood mononuclear cells demonstrates that it is increased in proportion to the degree of hyperthyroidism [64]. The T3 responsiveness of the *dio*1 gene can explain the marked increase in the ratio of PTU-sensitive plasma T3 production in patients with hyperthyroidism [65]. The more distal TRE-1 binding site responds also to retinoic acid, which makes D1 expression retinoic acid sensitive [36, 37, 66].

Other signaling pathways also participate in *dio*1 regulation. For example, cAMP stimulates rat *dio*1 transcription in a T3-sensitive manner and this process requires protein synthesis [67]. The mechanism of cAMP-mediated D1 expression has not yet been resolved. Data on the regulation of D1 by glucocorticoids are conflicting. *In vitro* studies showed that glucocorticoids induced D1 activity and mRNA expression in rat hepatocytes [68, 69] and hepatic D1 activity of fetuses of dexamethasone-treated ewes was higher [70]. Dexamethasone treatment had no effect on hepatic D1 activity or mRNA level in chicken embryos [71]. Recent data suggest that glucocorticoids regulate thyroid hormone metabolism in a tissue- and age-dependent manner, suggesting a mechanism mediated rather by D3 [72, 73].

The cytokines TNF-α, IL-1β, and IFN-γ were shown to decrease D1 activity and mRNA in FRTL5 cells, a rat thyroid cell line [67]. In HepG2 cells, a human hepatic cell line, TNF-α was shown to inhibit T3 induction by D1, mediated by induction of NF-κB [74]. In addition, IL-1 and IL-6 were shown to block T3 induction of D1 in hepatic cells (rat primary hepatocytes) [75]. However, in pituitary cells, cytokines appear to have an opposite effect; in rat pituitary cells, IL-1 stimulated D1 [76]. The effect of cytokines on hepatic D1 mRNA is in line with animal models of illness associated with decreases in hepatic D1 [77, 78] (see also *Deiodinases in nonthyroidal illness* below).

Type 2 iodothyronine deiodinase

Type 2 deiodinase (D2) catalyzes the first step in thyroid hormone action by converting T4 to T3. As an outer ring deiodinase, it also deiodinates reverse T3 to 3,3′-T2. The D2 homodimer is a highly active oxidoreductase, which is under complex transcriptional and post-transcriptional control. D2 plays a crucial role in regulating intracellular plasma T3 levels. This is especially significant in the brain, where D2-mediated T3 production is responsible for more than 50 % of the nuclear T3 in the rat cortex [79]. In addition, D2-mediated T3 generation is a major source of plasma T3 in euthyroid humans [80]. The generation of *dio*2 gene knockout mice paved the way to studies on the physiological role of D2 and demonstrated its critical relevance in the feedback regulation of TSH secretion [81], brain T3 content [82], and adaptive thermogenesis [83, 84].

Gene structure, chromosomal localization, and 5′ flanking region. D2 is encoded by the single-copy *dio*2 gene located on the long arm of the 14th human chromosome in position 14q24.3 [85, 86]. The coding region is divided into two exons by an ~ 7.4-kb intron [85]. The human gene has three transcriptional start sites (TSS), 707, 31, and 24 bp 5′ to the initiator ATG [87]. The human, mouse, and rat *dio*2 5′-flanking regions (FRs) contain a functional cAMP responsive element (CRE) [87–89]. In the human, *dio*2 5′FR functional, thyroid transcription factor-1 (TTF-1 or Nkx-2.1), Nkx-2.5, AP-1, and NF-κB sites have also been described [89–91].

The D2 mRNA. D2 is encoded by long mRNAs in higher vertebrates. The rat and human D2 mRNA are ~ 7.5 kb and the chicken ~ 6 kb. The coding region is just ~ 800 bp; whereas the 5′ UTR is ~ 600–700 bp, the 3′UTR is ~ 5 kb and contains a SECIS element in its 3′ region [11, 12, 92, 93]. This element is necessary for Sec incorporation into the active center of the protein at the UGA codon [16]. The long and structured D2 5′ UTR, which contains several short open reading frames (sORF), decreases the efficiency of D2 trans-

lation [94]. Furthermore, the D2 mRNA has a short half-life (~2 h in GC cells) [95] and the 3′ UTR contributes to the short D2 half-life probably *via* the numerous AUUUA mRNA instability motifs within the 3′UTR [94].

The D2 mRNA is subjected to alternative splicing. This mechanism can affect the D2 5′ UTR or coding region in the human thyroid [87, 94]. Splicing limited to the coding region is found in the human umbilical vein endothelial cell line ECV304, in the human brain, lung, kidney, heart, and trachea by inserting 108 or 242 bp genomic fragments originating from the *dio*2 intron [87, 94, 96]. In contrast, the chicken D2 mRNA has an alternatively spliced D2 variant in the brain and liver that lacks 77 bp of the coding region [94]. In the mouse cochlea, a D2 mRNA variant has been found that has a truncated coding region, followed by the 3′ UTR [97]. The spliced coding region variants do not encode an active enzyme [94], and the role of these variants in D2 regulation is presently unknown.

Numerous SNPs are known for *dio*2. Here we discuss only the well-studied human D2 SNPs. The Thr92Ala missense mutation was identified in the human D2 coding region [98] and found not to affect the D2 kinetics [40]. Notably, the Thr92Ala SNP was found to be associated with insulin resistance in obese Caucasians [98] but not with type 2 diabetes or insulin resistance in the Old Order Amish, in Danes, or in the Framingham Heart Study [99–101]. The Thr92Ala increased the risk for the development of hypertension [102] but did not explain differences in well-being, neurocognitive functioning, or the response to T4/T3 combination therapy in patients treated for hypothyroidism in comparison to controls [103]. The D2-ORFa-Gly3Asp SNP was found in the human D2 5′ UTR and was associated with serum thyroid hormone levels in blood donors but not in elderly men [104]. Three human *dio*2 SNPs were studied in subjects living in iodine-deficient areas of China. Two of them were associated with mental retardation, whereas the Thr92Ala was not [105]. It has also been proposed that the Thr92Ala D2 SNP is associated, or may be in linkage disequilibrium, with a functional D2 SNP that could be involved in the development of Graves disease in a Russian population [106].

The D2 protein. D2 follows sequential kinetics [92] and its existence was first suggested by the presence of PTU-insensitive T4 5′-monodeiodinase activity in the rat pituitary [107, 108]. Its K_m(T4) was in the nM range, ~1000-fold lower than that of D1 [60] and rT3 is almost as good a substrate for D2 as T4.

The D2 protein is ~31 kDa with a highly conserved Sec residue in the active center [10–12, 92, 109]. A second in-frame UGA codon serves also as Sec incorporation site close to the C terminus, but it does not affect the biochemical properties of the D2 enzyme [110]. Substitution of cysteine for Sec in the active center increases K_m(T4) by approximately three orders of magnitude, while it increases translation of the enzyme by ~100-fold. These data indicate that Sec greatly increases the affinity for substrate but decreases translational efficiency [24].

It has been suggested that D2 is a homodimer [17, 54]. Recent studies using fluorescence resonance energy transfer (FRET) on living cells and bioluminescence fluorescence energy transfer (BRET) confirmed that the D2 homodimer consisted of two functional monomers and showed that interaction at the globular domains is sufficient and necessary to keep the monomers in the conformation required for catalytic activity [19].

Subcellular localization and topology. D2 activity was found in membrane fractions but differences in the subcellular localization of PTU-sensitive and -insensitive 5′ deiodinase activities could not be resolved by classical techniques [111]. D2 cloning facilitated studies on D2 topology and proved that D2 is an endoplasmic reticulum (ER)-resident protein (Fig. 2) with its N terminus located in the ER lumen and its catalytic globular domain in the cytosol [59, 112].

Tissue distribution. D2 expression is widespread. D2 activity has been found in the rat pituitary, brain, and brown adipose tissue (BAT), in rat gonads, pineal gland, and thymus, and in mouse mammary gland (extensively reviewed in [5]). In the brain, D2 is predominantly glial and is highly expressed in tanycytes, the specialized ependymal cells lining the third ventricle in the mediobasal hypothalamus and in astrocytes [113, 114]. D2 activity in these cells is thought to provide T3 for neighboring neurons, cells that have TRs, but lack T3-generating capacity [5, 115, 116]. D2 is also expressed in the mouse cochlea in a temporally highly regulated manner and plays an important role in cochlear maturation [97]. D2 expression also has species-specific characteristics. Unlike the rat, D2 is the only 5′ deiodinase in the adult human CNS [61] and its mRNA is also expressed in human heart and skeletal muscle [11]. Similarly, D2 expression in the human thyroid is much more abundant than in the rat thyroid gland [89]. D2 is not expressed in the human or rat liver [11, 92] but it is present in the liver of adult chicken and teleost fish [12, 117, 118].

Transcriptional regulation of the dio2 gene. Here we review the factors that are transcriptional regulators of the *dio*2 gene. Other factors that affect D2 mRNA

levels *via* a yet unidentified mechanism are extensively reviewed elsewhere [119].

Thyroid status controls D2 activity both at the pre- and post-translational levels (see *Post-translational regulation of D2 activity* below) [120–123]. T4 robustly decreases D2 activity, but not D2 mRNA level, while T3 treatment decreased D2 mRNA in the rat cortex [124]. Hypothyroidism elevates D2 mRNA in the pituitary gland and, albeit to a much lesser extent, in various brain regions [11, 113, 124]. The effect of T3 on the *dio*2 promoter is transcriptional. T3 does not affect the D2 mRNA half-life and does not require protein synthesis [95]. However, a negative TRE in the *dio*2 promoter has not yet been identified.

Early studies demonstrated that norepinephrine-induced increase in cAMP in brown fat rapidly activates lipolysis, heat production, and increases T4-to-T3 conversion *via* type 2 iodothyronine deiodinase [125]. This suggested that the *dio*2 gene is regulated *via* a cAMP-mediated pathway. Thus, it was hardly surprising to find a canonical CRE in the *dio*2 5′ FR approximately 90 bp 5′ to the first TSS [87–89, 126]. CREB binding to the human *dio*2 promoter has been also demonstrated by chromatin immunoprecipitation in JEG3 cells [127]. In contrast to mammals, the *dio*2 gene of *Fundulus heteroclitus* seems to lack a functional CRE [128].

Glucocorticoids affect D2 expression transcriptionally. The transcriptional nature of the effect of glucocorticoids on D2 expression was established in GC pituitary tumor cells and in the chicken brain [71, 95]. In GC cells, the increase in D2 mRNA induced by dexamethasone is actinomycin dependent and does not affect the half-life of D2 mRNA. Cortisol increased D2 activity in rat astrocytes [129], whereas dexamethasone decreased D2 activity in cultured mouse neuroblastoma cells [130], suggesting that the effect exerted by glucocorticoids on D2 expression is cell specific.

There are clear species-specific differences in expression of D2 in the thyroid and heart. For example, D2 mRNA expression in the thyroid is much higher in humans than in rats [89, 131]. In parallel, the human *dio*2 gene is transcriptionally induced by TTF-1 *via* two DNA binding sites that are not present in the rat *dio*2 5′FR [89]. The heart is highly sensitive to the thyroid hormone [3]. The human heart expresses high levels of D2 mRNA in contrast to the low D2 levels in the rodent heart [11, 92]. Transgenic mice overexpressing D2 in the myocardium have mild chronic cardiac thyrotoxicosis [132] associated with increased β-adrenergic responsiveness of cardiomyocytes [133, 134]. It has been demonstrated that Nkx-2.5 and GATA-4 interact with the TTF-1 binding sites of the human *dio*2 5′FR and induce D2 expression while the absence of these binding sites results in a lower D2 mRNA level in the rat heart [90].

The basal promoter activity of the human *dio*2 promoter is twofold down-regulated by an activator protein-1 (AP-1) binding site in COS-7 cells, as was demonstrated by a transient transfection approach [89]. This was confirmed *in vivo* in the rat pineal gland where the Fos-related antigen 2 (Fra-2), a heterodimerization partner of Jun proteins, suppress D2 expression [135, 136]. The protein kinase C-mediated regulation of D2 expression seems cell-type specific since phorbol ester decreased D2 mRNA in cultured human thyroid cells, whereas it caused a tenfold induction in glial cells [129, 137, 138].

It has been demonstrated in a rat infection model that systemic administration of bacterial lipopolysaccharide (LPS) induced D2 mRNA and expression in the mediobasal hypothalamus [139]. This has been confirmed in mice [140]. It has been shown that NF-κB increased the expression of the human and rat *dio*2 gene, and detailed analysis of the human *dio*2 promoter identified an NF-κB binding site that is responsible for this effect [91, 139]. The potential consequences of these findings in relation to the pathogenesis of the non-thyroidal illness syndrome are discussed below in *Deiodinases in non-thyroidal illness*.

Post-translational regulation of D2 activity. The D2 protein has a short (~40 min) half-life and is further destabilized by its substrate [120, 123]. MG132, a proteasome uptake blocker, could stabilize D2 activity, suggesting that proteasomal degradation could be involved in D2 instability [141]. Identification of high molecular weight ubiquitin D2 conjugates (but not D1) demonstrated that D2 is ubiquitinated and identified D2 as the first ER resident enzyme that undergoes substrate-induced ubiquitination [50]. It has been demonstrated in yeast and mammals that UBC6 and UBC7 ubiquitin conjugases (E2 proteins) are involved in the ER-associated degradation (ERAD) of D2 [142, 143]. The deubiquitinases, USP-33 and USP20 (VDU-1 and VDU-2), bind to and deubiquitinate D2, increasing its half-life [144]. Moreover, it has been demonstrated that the Sonic hedgehog (Shh)-inducible protein, WSB-1, serves as the ubiquitin ligase (E3) adaptor for D2 (Fig. 3) [145]. The structural features of the D2 protein, required for WSB-1-mediated ubiquitination, have been characterized (Fig. 1) [146]. Recently, FRET studies on live HEK-293 cells transiently expressing D2 showed that the D2 dimer is associated with WSB-1, UBC-7, and USP-33, and that substrate-mediated ubiquitination destabilizes the dimer, thereby functioning as a switch that regulates enzymatic activity [19].

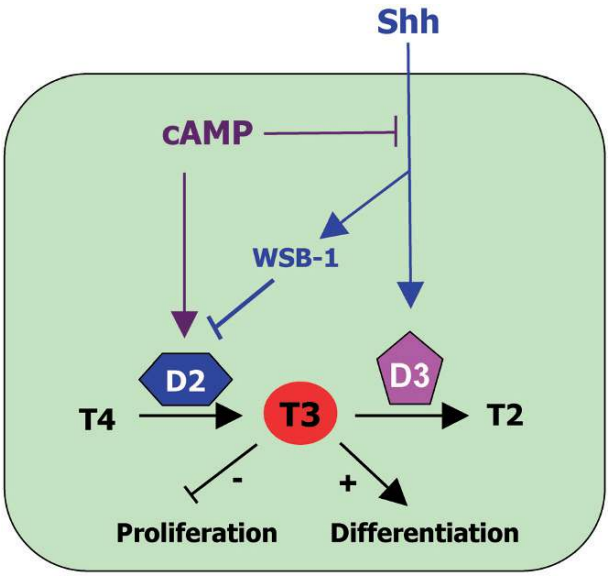

Figure 3. Schematic diagram of deiodination-mediated proliferation/differentiation along the Sonic hedgehog (Shh) pathway. Shh-induced proliferation includes tissue-specific reduction of T3, the potent differentiation factor. This is achieved by the acceleration of WSB-1-mediated ubiquitination of the T3 generating D2 enzyme and transcriptional activation of the T3 degrading D3. cAMP exerts an opposite effect on this system [145, 158].

Type 3 iodothyronine deiodinase

Type 3 deiodinase (D3), the main physiological inactivator of thyroid hormones, metabolizes T4 and T3 into inactive compounds. In rodents, D3 is expressed in the pregnant uterus and placenta and in most fetal tissues [147, 148], including the CNS [149]. After birth, the expression of D3 is more restricted and in rats occurs primarily in the skin [149] and CNS. The pattern of D3 expression suggests that in the developing fetus it plays a role in limiting tissue exposure to thyroid hormone. This is critical since serum thyroid hormone levels in the fetal rat are much lower than those present in the mother. While the D3 knockout mice identified a critical role of D3 in the maturation and function of the thyroid axis [150], D3 overexpression in vascular tumors results in consumptive hypothyroidism [151, 152].

Gene structure, chromosomal localization, and 5′ flanking region. The D3 protein is coded by the *dio*3 gene, localized on chromosome 14q32 in the human and on the chromosome 12F1 in the mouse [153]. Characterization of the mouse *dio*3 gene has shown that the coding regions and the 3′ UTR are contained in a single exon, ~1.9 kb long. Analysis of the *dio*3 genomic region led to the identification of an additional gene designated *dio*3 opposite strand (*dio*3os) that is transcribed in the antisense orientation [154]. The *dio*3os gene is expressed in most tissues and multiple transcripts are detected by Northern analysis. The sequences of the *dio*3os transcripts, which consist of at least six exons and two alternate polyadenylation sites, showed a complex and tissue-specific pattern of expression [155]. The D3 promoter contains a TATA box, two CAAT boxes, and several GC boxes in the proximal 180-bp region of the 5′ FR [156]. A conserved 180-bp-long enhancer was identified ~6 kb 3′ to the *dio*3 transcriptional start site, and this region contains a consensus AP-1 site and serum response element [157]. A conserved Gli-2 binding site, D3-A, is located in the mouse and human *dio*3 5′ FR [158]. Human and mouse *dio*3 genes map to chromosomal regions known to include imprinted genes. D3 knockout mice display high growth retardation and neonatal mortality [150], which may result from exposure to excessive levels of maternal thyroid hormone. We noted that D3 expression was almost normal or greatly decreased in heterozygous D3 knockout mice depending on whether the defective allele was inherited from the mother or the father, respectively. These data led to the conclusion that the *dio*3 gene is imprinted, with preferential expression from the paternal chromosome [159]. At the mRNA level, the expression from the paternal allele is 4–12 times higher than that from the maternal allele. *dio*3 is the last imprinted gene at the telomeric end of a mouse-imprinted domain of ~1 Mb defined by the imprinted genes Dlk1 and Gtl2; the Dlk1 gene is located at the centromeric end of the domain [160]. Tsai et al. [161] studied D3 expression and T3 levels in UDP12 mice that received both homologues of chromosome 12 from only one parent. Disruption of the imprinting status of *dio*3 in UDP12 mice resulted in abnormal serum T3 levels: mUDP12 mice had significantly higher T3 levels than normal, which concurs with the lower D3 expression. Moreover, patients with uniparental disomy of human chromosome 14 (UDP14) show phenotypic characteristics such as growth retardation [162], which is consistent with the phenotype of D3-deficient mice as well as UDP12 mice. Imprinting of *dio*3 does not appear to be regulated by differential methylation at CpG islands in the CpG-rich *dio*3 promoter [161].

The D3 mRNA. The expression of the *dio*3 gene results in a transcript of 2.1 kb that is most abundant in those rat and mouse tissues that have the highest D3 activity, such as decidual tissue and placenta, and in growth factor-stimulated cell culture systems. However, larger transcripts have been detected in the brain of hyperthyroid adult rats when a rat D3 cDNA is used as a probe. A consensus polyadenylation signal (AATAAA) is present at position 2034-9 and is followed by a short polyA tail. The human D3

cDNA contains a 5′ UTR of 220 bp, an 834-bp ORF that contains an in-frame TGA codon at position 650–652, and a 3′ UTR of 1012 bp [14].

The D3 protein. D3 was first identified in the NCLP-6E hepatocarcinoma cell line [163]. D3 catalyzes the IRD of T4 and T3 with a K_m in the nanomolar range. D3 follows sequential kinetics, is inhibited by iopanoic acid, and is insensitive to inhibition by PTU [14].
D3 is a ~32-kDa selenoprotein with a Sec residue in its catalytic center [14, 23]. Kuiper et al. [164] investigated the role of the Sec residue in the catalytic center of the D3 protein. Replacing Sec with alanine inactivated the D3 enzyme, as described for the D1 [165] and D2 enzymes [24]. The Sec residue in the catalytic center is essential for maximal catalytic efficiency. In fact, substitution of cysteine for Sec did not eliminate D3 activity [166] but reduced the turnover number 2-fold in the case of T3 and 6-fold in the case of T4 deiodination. In addition, the K_m(T3) increased 5-fold, whereas the K_m(T4) increased 100-fold, compared with the wild-type D3 enzyme [164].
D3 forms dimers [17] and has a long half-life (~12 h). To date, there is no evidence of regulation of *dio3* expression at the post-transcriptional level.

Subcellular localization and topology. Type 3 deiodinase is an integral membrane protein resistant to extraction from microsomal membranes by high pH [167, 168]. D3 contains a single predicted transmembrane domain between residues 16 and 41 and is located in the plasma membrane (Fig. 2) [166]. This location is consistent with the role of D3 in the placenta, uterus, and fetal liver to block entry of maternal thyroid hormone to the fetus. The rapid accumulation of biotinylated D3 in the plasma membrane in the presence of endocytosis inhibitors indicates that D3-containing membrane regions are normally internalized, and thereby become part of endosomal vesicles [166]. These vesicles seem to be predominantly clathrin coated, suggesting that, during endocytosis, internalized D3 is selected for recycling back to the cell surface. Thus, newly synthesized D3 migrates to the plasma membrane and rapidly undergoes endocytosis to the early endosomal pool. The signal controlling D3 partition between these two pools is not known. While studies with anti-D3 antibodies place the D3 catalytic center in the extracellular space [166], more recent studies indicate that co-expression of D3 with the T3 transporter MCT8 increases D3-mediated deiodination [169]. Whether this apparent inconsistency indicates that MCT8 is also necessary for T3 entry into endosomal vesicles or that D3 is incorporated in two opposite orientations in the plasma membrane remains to be investigated.

Tissue distribution. D3 expression is high in the embryonic liver, brain, gonads, lung, heart, intestine, and skin [9, 71, 170, 171]. In humans, D3 mRNA or protein has been detected in the fetal liver, cerebral cortex, and the epithelial structures of the embryonic lung, intestine, skin, and urinary tract [147]. In *Xenopus laevis* tadpoles, D3 activity is present in limb buds, retina, and tail in various stages of development [172, 173]. D3 activity in the liver decreases rapidly after birth and, within a few weeks of life, circulating iodothyronine concentrations in the newborn reach those measured in the adult [174]. D3 mRNA has been identified throughout the brain in the adult rat. It was widely distributed throughout the forebrain but was particularly striking in layers II–IV of the cerebral cortex, hippocampal pyramidal cells, granule cells of the dentate, and layer II of the pyriform cortex [175]. It is interesting that the same regions contain the highest concentrations of TRs in the CNS [176]. *In situ* hybridization experiments showed that D3 expression changes during early differentiation of neonatal rat brain [177]. At postnatal day 0, D3 is selectively expressed in the bed nucleus of the stria terminalis, the preoptic area, and in other such areas as the central amygdala [178]. In contrast to D2, which is found in glial cells, D3 expression occurs predominantly in neuronal cells.
High levels of D3 are expressed in the human placenta, where it blocks the maternal-to-fetal transfer of T4. Consistent with this function, high levels of D3 have been found in the syncytiotrophoblast and cytotrophoblast layers as well as in the fetal endothelium of the chorionic villi. D3 staining was also strong in the maternal decidua of the human placenta and in the amnion sheath of the umbilical cord [147]. In the same study, Huang and colleagues [147] provided the first evidence of D3 expression in normal human endothelium. Previously, endothelial D3 activity had been identified only in infantile hemangiomas and hemangioendothelioma [151, 152].
The skin is the largest organ in humans, and functions as a metabolically active biological barrier separating internal homeostasis from the external environment. Both the epidermal and dermal tissues are target organs for the thyroid hormone. D3 expression in the mouse embryonic and adult skin has been analyzed by immunohistochemistry [158]. D3 first appears in the mouse embryonic epidermis at E13.5 and is highly expressed in the epidermal layers and in hair follicle keratinocytes by E17.5. In the growing phase of the hair follicle cycle (anagen), D3 staining was intense in the hair matrix and in the surrounding outer root

sheath. It progressively decreased during catagen and was almost absent in telogen.

D3 activity is closely correlated with the rate of proliferation in differentiating brown preadipocytes. In this system, differentiation of precursor cells to adipocytes is associated with decreased levels of D3 expression, while the *dio3*os gene is markedly up-regulated, suggesting that D3 is a marker of preadipocytes rather than of mature adipocytes, whereas *dio3*os expression is associated with mature adipocytes [179].

Transcriptional regulation. The expression of the *dio3* gene is regulated *in vivo* and *in vitro* by a number of agents. Changes in the level of D3 mRNA and D3 activity are well correlated, suggesting that the changes in *dio3* expression after treatment with various agents are primarily the result of changes in gene transcription. Although several factors are known to affect *dio3* expression, the underlying mechanism and binding sites in the *dio3* 5′ FR are poorly defined.

Thyroid status has been widely demonstrated to modulate D3 activity in different tissues, especially in the CNS [175, 180]. Thyroid hormone up-regulates D3 activity in the rat brain [175] and in cultured glial cells. Brain D3 activity is up-regulated in hyperthyroidism and down-regulated in hypothyroidism, and D3 mRNA levels increase 4–50-fold from the euthyroid to the hyperthyroid state. D3 is predominantly present in neuronal cells, which are the main cells that express TRs. T3 has been shown to stimulate D3 expression rapidly in the *X. laevis* tadpole before the metamorphic climax [173].

In different cell culture models, D3 expression is regulated by the action of serum, phorbol esters, and the epidermal and fibroblast growth factors (EGF, FGF) [181–183]. The important role of basic FGF (bFGF) in angiogenesis and the high D3 activity reported in human infant hemangiomas, a tumor enriched in blood vessels [151], indicate a relationship between D3 expression and angiogenic processes. In glial cells, induction of the *dio3* gene by growth factors appears to be mediated by the extracellular signal regulated kinases (ERKs) [183]. In particular, D3 expression in astroglial cells is regulated by mitogens, growth factors, and hormones, and exposure to certain combinations of these agents results in synergistic induction of D3 mRNA levels and activity. The compounds that generate signals from the cell surface [tetradecanoyl phorbol acetate (TPA) and bFGF] induce rapid increases in D3 mRNA and activity, whereas treatment with ligands that interact with nuclear receptors (T3 and retinoic acid) result in slower effects. TPA-mediated induction of D3 mRNA is transient: it peaks at 7 h of treatment and returns to baseline levels by 12 h. Furthermore, downstream activation of the MEK/Erk cascade appears to be essential for D3 induction by both bFGF and TPA [183]. No specific response element in the *dio3* promoter has been found to be responsible for these stimulatory effects. However, CAAT boxes can mediate a response to serum. Therefore, it is reasonable to speculate that the response to these stimuli is mediated by one or both the conserved CAAT boxes present in the basal promoter.

The highly conserved enhancer region (see *Gene structure, chromosomal localization, and 5′ flanking region* above) further enhances the response to serum when placed within the context of the *dio3* proximal promoter [157]. The enhancer region contains an AP-1 and a serum response element, both known to mediate responses to serum and growth factor.

Glucocorticoids and growth hormone affect D3 expression. In the chicken embryo liver, dexamatasone and growth hormone decrease D3 expression followed by increased serum T4 and T3, which suggests that hepatic D3 plays a role in the regulation of serum thyroid hormone levels in the chicken [184]. In the rat brown fat vascular-stromal (BVS-1) cell line, dexametasone both suppresses basal D3 expression and prevents D3 induction by serum and growth factors [182].

Using non-transformed human cells, Huang et al. [185] showed that TGF-β stimulates transcription of the human *dio3* gene *via* a Smad-dependent pathway. Combinations of Smad2 or 3 with Smad4 stimulate the human *dio3* gene transcription only in cells that express endogenous D3 activity, indicating that Smads are necessary but not sufficient for D3 induction. TGF-β induces endogenous D3 in diverse human cell types, including fetal and adult fibroblasts from several tissues, hemangioma cells, and fetal epithelia. Maximum stimulation of D3 by TGF-β also requires MAPK and is synergistic with phorbol ester and several mitogens but not with estradiol. Interestingly, the D3-stimulating effect of TGF-β occurs rapidly, within 5 h, and terminates within approximately 10 h of exposure. These data revealed a previously unrecognized interaction between two pluripotent systems, TGF-β and thyroid hormone, both of which have major roles in the regulation of cell growth and differentiation. The four- to fivefold increase in D3 promoter activity induced by estradiol could contribute to the increased T4 requirements during human pregnancy. During embryonic development, secondary epithelia trans-differentiates into mature epithelia or, under the influence of TGF-β and other paracrine factors, undergoes epithelial-mesenchymal transition to produce the various cell types of con-

nective tissue. Thus, D3 expression in fetal epithelia can be retained through the process of epithelial-mesenchymal transition or reactivated after terminal differentiation by the action of TGF-β.
D3 expression in the skin is positively regulated by Gli-2, a member of the Gli transcription factor family that mediates Shh signaling. A conserved Gli-2 binding site, D3-A, located within the mouse and human *dio*3 promoter, is critical for the human *dio*3 response to Gli2 in transfected keratinocytes [158].

Deiodinases in illness

Although patients with genetic defects in deiodinase genes have not yet been identified (for SNPs, see mRNA sections above), data are accumulating on altered deiodinase activities in different clinical settings. Increased D2 activity was observed in patients with follicular thyroid carcinoma, follicular adenoma, or Graves disease [131, 186]. Patients with mutation of SBP2 had decreased D2 activity and abnormal thyroid hormone metabolism [187], while amiodarone, an anti-arrhytmic drug, is known to alter thyroid hormone metabolism *via* inhibition of T4 activation [188]. Increased T3 inactivation and consumptive hypothyroidism was observed in children and adults with D3-overexpressing vascular tumors [151, 152].
We focus below on the complex changes in thyroid hormone metabolism in non-thyroidal illness, the role of D2 in metabolic control, and recent developments in D3 pathophysiology.

Deiodinases in non-thyroidal illness. It has been known for 30 years that sepsis, starvation, malignancy, life-threatening trauma, major surgery, and other critical illness are accompanied with low T3 and sometimes even with low T4 serum levels, and associated with non-elevated or inappropriately elevated TSH levels, referred to as non-thyroidal illness syndrome, euthyroid sick syndrome, or low T3 syndrome [189–192].
No consensus has yet been reached on whether the changes of thyroid hormone profile provide physiological compensation for illness or if it represents pathological conditions [193–195]. Interestingly, pregnant rats near term show an attenuated negative feedback to decreased thyroid hormone levels, indicating that at least under specific conditions low thyroid hormone levels could provide an adaptive mechanism for energy preservation [196]. However, these findings cannot be generalized because of the complex etiology of this syndrome.
Early data suggested different mechanisms in rats and human for reduced circulating T3. Although T4 5′-deiodination was diminished in the livers of starved rats, the reduced thyroidal T4 secretion seemed to be the primary cause for reduced serum T3 levels [197], while impaired T4 to T3 conversion and undecreased T4 production was demonstrated in starving euthyroid obese patients [198]. The first candidate for decreased T4 to T3 conversion was D1 and its decreased activity was demonstrated in several models [199]. For example, different mechanisms for cytokine-mediated inhibition of T3-induced D1 transcription have been proposed including the IL-1/IL-6/SRC-1- and the TNF-α/NF-κB-mediated pathways [74, 75, 200]. However, it should be also noted that the *dio*1 gene is one of the most T3-sensitive promoters known (see *Transcriptional regulation of the dio1 gene* above) and this feature makes its reduced expression explainable by the decreasing T3 levels. In addition, D2 and not D1 is responsible for the vast majority of circulating T3 in non-hyperthyroid humans and mice [30–32], indicating that D1 is not likely to be a major factor in the pathogenesis of the syndrome. This view is further supported by the observation in a mouse infection model demonstrating decreased T4 output from the thyroid but no sign for reduced D1 expression assessed by decreased rT3 levels [201].
Data are accumulating concerning the potential role of D2 and D3 deiodinases in the pathogenesis of the syndrome. Studies on samples of critically ill patients obtained just after death demonstrated that D2 activity in skeletal muscle was diminished, while D3 activity was ectopically induced in the liver and skeletal muscle [202]. These findings indicate that increase of the D3-mediated T3 degradation could also contribute to the decreased T3 levels observed in the syndrome. The role of increased T3 degradation by D3 in this mechanism has been further documented by data demonstrating that local inflammation strongly induces D3 activity in inflammatory cells, especially in invading polymorphonuclear granulocytes [203].
The observed changes in peripheral deiodination could contribute to the pathogenesis of the syndrome, but the role of a central component based on altered function of the hypothalamic-pituitary-thyroid axis has been also proposed [204]. In critically ill patients, TSH secretion is decreased and TRH infusion significantly improved serum T4 and T3 levels [205]. The pathways underlying this modulation of the axis are not understood in detail but several factors have been suggested. While dopamine and glucocorticoids can suppress TSH [206], leptin administration increased TSH, T4, and T3 levels in fasting rats and humans [207, 208].
T3-mediated down-regulation of TRH expression could be another pathway that has the potential to suppress the hypothalamic-pituitary-thyroid axis in

non-thyroidal illness. Systemic administration of bacterial lipopolysaccharide (LPS) to rats resulted in reduced plasma T4, T3, and TSH, but this was not associated with an increased TRH expression, a situation highly similar to parameters in non-thyroidal illness [209]. Using this system, it has been demonstrated that D2 expression and activity is induced by LPS in tanycytes of the mediobasal hypothalamus [139]. It has been also shown that NF-κB, a potential effector of this pathway, can induce transcription of the human and rat *dio*2 gene and a potent NF-κB binding site has been characterized in the human *dio*2 5′ flanking region [91, 210]. The LPS-induced increase of D2 mRNA in the hypothalamus was also observed in mice, immediately followed by decreased expression of thyroid receptor β2, TSHβ in the pituitary, and decreased D1 mRNA in the pituitary and liver [140]. LPS-induced decreased RXR/TR DNA binding, and reduced TR and RXR expression has been also reported in the rat liver [211].

Since tanycytes probably serve as a cytoplasmic passage between the CSF and the blood circulating in the area of the arcuate nucleus and median eminence of the mediobasal hypothalamus [212], changes in NF-κB-mediated D2 induction and the consequent T3 generation in tanycytes could have important consequences. The upstream signaling components of the NF-κB pathway have not yet been resolved for D2-expressing cells in the brain. This is a complex task since LPS induces a variety of cytokines in a mouse model for non-thyroidal illness [213].

Although there is presently no direct evidence that D2-mediated T3 production in tanycytes is responsible for the suppression of TRH secretion, circumstantial evidence suggests that this pathway could be functional. For example, both key components of LPS/TLR4 signaling (*i.e.*, CD14 and TLR4 mRNA) have been detected in the median eminence of the mediobasal hypothalamus, and systemic injection of LPS up-regulates CD14 in this region [214, 215]. It has been recently demonstrated that TLR and FC receptor pathways of mast cells are involved in the generation of infection-induced non-thyroidal illness in mice [201]. This study indicated that the release of proinflammatory cytokines by mast cells is crucial in this process and cytokines could act centrally by the inhibition of TRH release [201]. Since the p55 TNF-1 receptor is expressed in the median eminence and it is increased by TNF-α [216, 217], and TNF-α release can be both the consequence and the inducing factor of NF-κB activation [218, 219], we can speculate that TNF-α is one of the mast cell-released cytokines that could play a role in hypothalamic NF-κB-mediated up-regulation of D2 and consequent TRH suppression. Recent observations on the kinetics of NF-κB activation in the mediobasal hypothalamus of rats suggest that LPS-induced NF-κB activation is more involved in the maintenance of the up-regulated D2 expression than in the initiation of the process [220]. Similar to the LPS model, D2 expression is also induced in the hypothalamus of mice with chronic local inflammation, and IL-1β is increased in this region of the brain [221]. Importantly, the suppressed TRH in the paraventricular nucleus of these mice occurs simultaneously with decreased D3 expression. These findings indicate that hypothalamic changes of T3 levels during non-thyroidal illness are affected both by increased D2-mediated T3 generation and decreased D3-mediated T3 degradation.

D2-mediated tissue-specific control of thyroid hormone action. Given the generalized metabolic sensitivity to the thyroid hormone, one would anticipate the existence of mechanisms that allow for some degree of tissue specificity. This is possible due to the deiodinases. Thyroid hormone action is initiated through its binding to nuclear receptors, which are high-affinity nuclear T3 binding proteins that regulate transcription of T3-dependent genes. TR occupancy is determined by the T3 concentration in the nucleus, which in most tissues depends on serum T3 concentration. However, tissues expressing D2 have an additional source of T3, the intracellular conversion of T4 to T3 [107, 222–224]. Generation of T3 by D2 occurs in the perinuclear region and contributes to TR occupation, which in contrast to D1, is localized to the plasma membrane, from which the T3 produced more readily enters the plasma [59]. In contrast, D3 function results in cell hypothyroidism [225]. Its location in the plasma membrane creates a barrier that prevents the thyroid hormone from entering the cell.

Nowhere has the role of deiodinases in determining tissue-specific thyroid hormone signaling been more studied than in the brown adipose tissue (BAT), the main site of adaptive thermogenesis in small mammals. Sympathetic activation of this tissue such as during exposure to cold increases cAMP generation and induces *dio*2 expression, stimulating intracellular conversion of T4 to T3 by up to ~50-fold [125]. As a result, T3 concentration in cold-stimulated BAT is about 4- to 5-fold higher within a few hours after cold exposure being initiated [226], creating an isolated thyrotoxicosis in the tissue [227]. Notably, targeted inactivation of *dio*2 impairs BAT thermogenesis by precluding the adaptive increase in T4 to T3 conversion [83].

That the action of D2 has relevance for metabolism beyond its role in cold-induced thermogenesis was

recently established with the discovery that bile acids can confer resistance to diet-induced obesity in mice *via* up-regulation of D2 expression in BAT [228]. In this tissue, binding of bile acids to the plasma membrane G protein-coupled receptor TGR-5 triggers an increase in cAMP formation and subsequently D2 expression. In normal mice fed a high-fat diet supplemented with bile acids, oxygen consumption increases and the mice did not gain weight or become as insulin resistant as mice fed the high-fat diet alone. However, this effect is lost in D2 knockout mice. It is noteworthy that D2 is overexpressed in two other rodent models of resistance to diet-induced obesity. UCP-1 knockout mice are paradoxically lean [229], and have ectopic expression of D2 in their white fat, while the double liver X receptor (LXR) knockout mice express D2 ectopically in the liver [230]. If the ectopic expression of D2 in these animals results in tissue-specific thyrotoxicosis, as is suggested by gene expression profiling in the case of the LXR double knockout mice, this would certainly support the concept that the D2 signaling pathway increases energy expenditure.

D3 in illness. Several animal studies have shown elevated D3 expression in embryonic organs, whereas D3 has been identified in only a limited number of postnatal tissues, which led to the categorization of D3 as an oncofetal protein. However, recent data indicate that postnatal expression can be reactivated in tissues during critical illness and other pathological conditions. In this section, we focus on recent data on D3 expression under pathological conditions.

The Shh pathway determines patterns of cell growth and differentiation in a wide variety of developmental and disease processes, including cancer, and in continuously remodeling organs such as skin [231–233]. In the developing chicken growth plate, Indian hedgehog regulates WSB-1, the E3 ligase adaptor that inactivates D2 [145]. It has been suggested that Shh might control thyroid hormone action in the epidermis by affecting the balance between inactivating (D3) and activating deiodinases (D2), which could be critical in modulating the balance between proliferation and differentiation of keratinocytes (Fig. 3). Shh is known to be overactive in basal cell carcinomas (BCCs) and related skin tumors [234]. Dentice et al. [158] have shown that D3 is significantly higher in two BCC cell lines (G2N2C and Tb3A) derived from Gli2N-expressing transgenic mice and in human BCC samples *versus* surrounding normal skin. This regulation represents a novel route by which Shh exerts its proliferative effects, *i.e.*, by attenuating thyroid hormone signaling.

D3 mRNA and/or activity has been identified in some malignant cell lines and in a number of human tumors, including astrocytomas, oligodendromas, gliosarcomas, glioblastoma multiforme, and basal cell carcinomas [158, 163, 235, 236]. Moreover, the highest D3 activity reported in any tissue thus far has been in vascular tumors, including hemangiomas and hemangioendotheliomas with consequent consumptive hypothyroidism [151, 152]. The tumoral D3 expression in these vascular lesions combined with the disappearance of hypothyroidism after hemangioma involution supports the concept that this endocrinopathy is due to an excessive degradation of thyroid hormone relative to the functional reserve of the infant thyroid. Kester et al. [237] have evaluated the activity of deiodinases, in particular, D3 activity and mRNA expression, in human cell lines. They identified D3 activity and mRNA in ECC-1 endometrium carcinoma cells, MCF-7 mammacarcinoma cells, WRL-68 embryonic liver cells, and SH-SY5Y neuroblastoma cells, but not in the HepG2 hepatocarcinoma cell line or in any choriocarcinoma or astrocytoma cell line. They demonstrated that the phorbol ester TPA increased D3 activity two- to ninefold in ECC-1, MCF-7, WRL-68, and SH-SY5Y cells.

Estradiol increased D3 activity threefold in ECC-1, but not in any other cells. Dexamethasone decreased D3 activity in WRL-68 cells only in the absence of fetal calf serum. Incubation with retinoids increased D3 activity two- to threefold in ECC-1, WRL-68, and MCF-7 cells but decreased D3 activity in SH-SY5Y cells. D3 expression in the different cells was not affected by cAMP or thyroid hormone. Interestingly, D3 mRNA expression in the different cell lines strongly correlated with *dio*3os mRNA expression, and also with Dlk1 expression in a large set of neuroblastoma cell lines.

Changes in D3 expression was also demonstrated in cardiac disorders. The similarities between the changes in cardiac gene expression in pathological ventricular hypertrophy and hypothyroidism suggest that impaired cardiac thyroid hormone action is involved in the development of contractile dysfunction during chronic cardiac pressure overload. Using a rat model of right-ventricular (RV) hypertrophy induced by pressure-overload, Wassen et al. [225] showed that D3 activity in ventricular tissues was stimulated up to fivefold in hypertrophic RV, but remained unaltered in the non-hypertrophic left ventricle. Stimulation of RV D3 activity was higher in those animals that developed compensatory hypertrophy. Induction of a cardiac thyroid hormone-degrading deiodinase may be expected to result in reduced cellular levels of T3 and thereby contribute to

a local hypertrophy state in the hypertrophic and in the failing ventricle.

Olivares et al. [238] recently reported a time-course study of pituitary-thyroid function and thyroid hormone metabolism in rats subjected to myocardial infarction by left coronary ligation. The severe cardiac dysfunction in rats induced an early decrease of both T4 and T3 combined with high-serum TSH levels, indicating that the transient hypothyroidism is established secondary to modifications in the peripheral thyroid hormone metabolism. The authors found high D3 activity in infracted cardiac tissue, which supports the possibility that D3-mediated inactivation is the major cause of the drastic decrease in serum thyroid hormone levels after myocardial infarction. Changes in systemic thyroid status during several illnesses, *e.g.*, a rat model of cardiac hypertrophy, have been correlated with reactivation of D3, which would have a crucial role as a modulator of thyroid status in euthyroid sick syndrome.

Conclusions

Sixteen years have passed since the first cloning of a deiodinase enzyme, followed by the identification of the genetic code for the whole deiodinase family. This accelerated the understanding of their regulation, structure, and role in thyroid hormone economy. Rapid progress is now being made in the characterization of their cellular biology, and the discovery of new pathways that regulate these enzymes such as the Shh and the TGR5-bile acid. The realization that deiodinases allow for thyroid hormone action to be regulated in a time- and tissue-specific fashion has been critical for implicating these enzymes in novel physiological and pathophysiological functions such as the non-thyroidal illness syndrome, metabolic control, and balance between cell proliferation/differentiation.

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