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Role of the type 2 iodothyronine deiodinase (D2) in the control of thyroid hormone signaling☆

Rafael Arrojo Drigo[a], **Tatiana L. Fonseca**[a], **Joao Pedro Saar Werneck-de-Castro**[a,b,c], and **Antonio C. Bianco**[a,*]

[a]Division of Endocrinology, Diabetes and Metabolism, University of Miami, Miller School of Medicine, Miami, FL, USA

[b]Instituto de Biofisica Carlos Chagas, Brazil

[c]Escola de Educacao Física e Desportos, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

Abstract

Scope of the review—This review covers the recent advances in D2 biology, a member of the iodothyronine deiodinase family, thioredoxin fold-containing selenoenzymes that modify thyroid hormone signaling in a time- and cell-specific manner. The type II (D2) deiodinase catalyzes T4-to-T3 conversion as opposed to the type III (D3) deiodinase that terminates thyroid hormone action.

Major conclusions—D2-catalyzed T3 production increases thyroid hormone signaling whereas blocking D2 activity or disruption of the Dio2 gene leads to a state of localized hypothyroidism. D2 expression is regulated by different developmental, metabolic or environmental cues such as the hedgehog pathway, the adrenergic-and the TGR5-activated cAMP pathway, by xenobiotic molecules such as flavonols and by stress in the endoplasmic reticulum, which specifically reduces de novo synthesis of D2 via an eIF2a-mediated mechanism. Thus, D2 plays a central role in important physiological processes such as determining T3 content in developing tissues and in the adult brain, and promoting adaptive thermogenesis in brown adipose tissue. Notably, D2 is critical in the T4-mediated negative feed-back at the pituitary and hypothalamic levels, whereby T4 inhibits TSH and TRH expression, respectively. Notably, ubiquitination is a major step in the control of D2 activity, whereby T4 binding to and/or T4 catalysis triggers D2 inactivation by ubiquitination that is mediated by the E3 ubiquitin ligases WSB-1 and/or TEB4. Ubiquitinated D2 can be either targeted to proteasomal degradation or reactivated by deubiquitination, a process that is mediated by the deubiquitinases USP20/33 and is important in adaptive thermogenesis.

General significance—Here we review the recent advances in the understanding of D2 biology focusing on the mechanisms that regulate its expression and their biological significance in metabolically relevant tissues. This article is part of a Special Issue entitled Thyroid hormone signalling.

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*Corresponding author at: 1400 NW 10th Avenue, Suite 816, Miami, FL, 33136, P.O. Box 016960 (D-56), USA. abianco@deiodinase.org (A.C. Bianco).

Keywords

Thyroid hormone; Deiodinase; Metabolism; Selenoprotein

1. Introduction

It has been 60 years since the identification of the 3,5,3′-triiodothyronine (T3) molecule in human plasma [1]. Today, it is well accepted that T3 is the biologically active thyroid hormone that initiates its signaling by interacting with thyroid hormone receptors (TR), ligand-dependent transcription factors that control the expression of T3-dependent genes. The historical view was that circulating T3 diffuses into cells and gains access to the nucleus, hence interacting with TRs. Today it is recognized that circulating thyroid hormone levels hardly ever fluctuate, remaining fairly constant during the entire adult life of healthy individuals [2]. Therefore, it is fair to ask "how can thyroid hormone signaling initiate or terminate important biological processes if not through changes in plasma levels?" The answer is deiodination.

The modern paradigm of thyroid hormone action recognizes that T3 and thyroxine (T4) enter target cells through specific thyroid hormone transporters [3], are metabolized through thioredoxin fold-containing selenoenzymes, the deiodinases, and finally diffuse into the cell nucleus. Deiodination either activates T4 to T3 (type I (D1) and type II (D2) deiodinases) or irreversibly inactivates T3 to T2 and T4 to rT3 (D1 and the type III deiodinase, D3). The net amount of T3 eventually occupying the TR defines the thyroid hormone transcriptional footprint in each cell type and is strongly influenced by the activity of the deiodinases [4]. Thus, by differentially expressing D2 or D3, T3-target cells do play an active role in customizing thyroid hormone signaling, a mechanism that is tissue-specific and not at all apparent by simply studying circulating levels of thyroid hormone.

A better understanding of deiodinase structure was achieved through hydrophobic cluster analysis (HCA), a computer-based molecular modeling that revealed a high degree of homology (~50%) among the three deiodinases. All are dimeric type I integral membrane proteins anchored through a single transmembrane domain located in the amino terminus [5]. Whereas D1 is a plasma membrane-resident protein, D2 resides in the endoplasmic reticulum (ER) [6] (Fig. 1). In contrast, D3 distributes to the plasma membrane or the nuclear membrane depending on the oxygen availability [7–9]. Whereas under normoxic conditions D3 is sorted to the plasma membrane, ischemia or hypoxia rapidly redirects D3 from the ER to the nuclear envelope via a HSP40-mediated shuttle mechanism, where it inactivates T3 [8].

2. Mechanisms controlling D2-mediated T3 production

D2 is under transcriptional and post-transcriptional control (Table 1) that is triggered by different developmental, metabolic or environmental cues such as the adrenergic-, the TGR5- and the xenobiotic-activated cAMP pathway [10]. In fact, Dio2 is a cAMP-responsive gene [11,12] and therefore the adrenergic/cAMP signaling pathway mediates the predominant transcriptional control of D2 [13,14]. D2 expression is also importantly

regulated by post-translational mechanisms with ubiquitination [15] and ER stress [16] reducing D2 activity by direct inactivation and inhibiting D2 de novo synthesis, respectively.

2.1. Ubiquitination, a molecular switch that inactivates D2

Evidence of the post-translational mechanism arose from the observations that cerebral cortical D2 activity was rapidly lost after acute administration of thyroid hormone [17,18]. This thyroid hormone-induced loss of D2 activity was found to be independent of transcriptional mechanisms [19] and was prevented by inhibitors of the 26S proteasomal pathway [20]. Subsequent studies have shown that D2 is inactivated by ubiquitination, an event that is triggered by interaction with its substrate, i.e. T4 [6,21]. Ubiquitination is a multi-enzymatic multi-step event that results in the conjugation of the small molecule ubiquitin (Ub) to a target protein. Additional Ub molecules may be added and a poly-Ub chain is formed, eventually targeting the protein for proteolytic breakdown in the inner chamber of the 26S proteasome system [22].

Several proteins involved in the process of D2 ubiquitination have been identified (Fig. 1), including the evolutionarily conserved E2 ubiquitin conjugases 6 and 7 (UBC6 and 7) [23] that interact with the E3 ligase WD-repeat and suppressor of cytokine signaling (SOCS)-box containing protein 1 (WSB-1) and with other accessory proteins form a complex termed Elongin BC–Cullin5–Rbx1 (ECS^{WSB-1}) to ubiquitinate D2 [24]. Studies using fluorescence resonance energy transfer (FRET) favor the model under which binding (and/or catalysis) of the T4 molecule to D2 provokes a conformational change in D2:D2 dimer that increases binding of WSB-1 to D2 and exposes two critical lysine residues at positions 237 and 244 that become available for conjugation with Ub [25]. This process has been shown to have physiological relevance in explants of chicken tibia growth plate, where the sonic hedgehog (Shh)-inducible WSB-1-mediated inhibition of D2 activity promotes chondrocyte proliferation and parathyroid hormone-related protein (PTHrP) secretion [24]. Besides WSB-1, the human ortholog of the yeast E3 ligase degradation of alpha-10 (Doa10) termed TEB4 has also been shown to interact with UBC6/7 [26] and participate in D2 ubiquitination [27]. It is possible that both WSB-1 and TEB4 work sequentially in regulating D2 activity given that WSB-1 and TEB4 are co-expressed in several D2-expressing tissues. Alternatively, it is also conceivable that these two ligases control different D2 ubiquitinating pathways of different physiological relevance. Interestingly, in the thyroid gland (a D2-expressing tissue) both WSB-1 and TEB4 are only minimal, indicating that other D2 regulatory pathways are likely to exist [27].

Ubiquitination is a reversible reaction, where conjugated Ub molecules can be removed from ubiquitinated proteins by the action of deubiquitinating (DUB) enzymes. These enzymes are divided in five different classes, including the ubiquitin-specific proteases (USPs) [28]. A yeast two-hybrid screen identified USP33 and USP20 (also known as VDU-1 and VDU-2, respectively) as D2-interacting partners [29]. The interplay between D2 and USP20/33 was characterized in vitro and in vivo, where both USPs deubiquitinate Ub-D2 and thus neutralize the effects of substrate-induced D2 ubiquitination, rescuing D2 from irreversible degradation by the proteasome system. The relevance of this process is highlighted by the significant contribution the cAMP-inducible USP33 plays in regulating D2 levels in cold-

activated BAT, maximizing local D2-mediated T3 production that is critical for the thermogenic program [29].

2.2. ER stress inhibits D2 synthesis. Reversal by chemical chaperones

ER stress is a cellular condition caused by a disruption in ER homeostasis that is associated with obesity, insulin resistance and type 2 diabetes [30]. This can result from ionic imbalance or the accumulation of misfolded proteins in the ER lumen [31]. As a result, there is a coordinated cellular effort to (i) decrease the input of newly synthesized protein in the ER lumen by temporarily turning off protein translation via the PERK–eIF2a pathway, (ii) increase ER protein folding capacity by upregulating the expression of chaperones (e.g. glucose-regulated precursor 78 [GRP78] and the transcription factor x-box protein-1 [XBP-1]), and (iii) eliminate misfolded proteins targeting them to the ER associated degradation (ERAD) pathway that is mediated by the ubiquitin–proteasome system [31].

Given the interaction between D2 and known ERAD components, i.e. UBC6 and TEB4, one would logically expect that D2 is the subject of ubiquitination and proteasomal degradation during ER stress, possibly by the increased activity of the ERAD pathway. In fact, when D2-expressing cells undergo ER stress D2 activity is rapidly lost, without changes in Dio2 transcriptional activity [16]. However, the loss of D2 activity is not mediated by increased ubiquitination but rather by translational arrest mediated by the PERK–eIF2a pathway (Fig. 2). This ER stress-mediated arrest in D2 synthesis is highly efficient, even blocking the increase in D2 activity that normally follows the induction of its mRNA by cAMP [16]. The loss of D2 activity during ER stress is marked, so that there is substantial and rapid reduction in T3 production and hence a state of relative cellular hypothyroidism [16]. This pathway is likely to have physiological relevance as it was found to take place in primary human epithelial cells expressing the mutant variant ΔF508 of the cystic fibrosis transmembrane conductance regulator (CFTR), which is known to cause the ER stress response due to a failure in conformational maturation [16]. In this setting, D2 activity is decreased despite elevated D2 mRNA levels, and it is correlated with an increased activation of the ER stress pathway [16].

The finding that ER stress regulates D2 expression and D2-mediated T3 production in cells from the airway tissues gains significant relevance given that thyroid hormone plays a critical role in this tissue's development and function. In the developing fetus thyroid hormone economy depends on maternal iodothyronines, with T3 participating in the branching process [32], alveolar formation and function [33,34], mucus secretion [35] and type 1 pneumocyte development [36]. In addition, a protective role for D2-mediated T3 production has emerged in a murine model of acute lung injury (ALI), with $Dio2^{-/-}$ mice being more susceptible to LPS- and ventilator-induced lung injury (VILI)-driven ALI phenotype [37,38].

ER stress can be attenuated or completely resolved by chemical chaperones that act as molecular stabilizing agents [39]. Three chemical chaperones, 4-phenyl butyric acid (4-PBA), taurourso-deoxycholic acid (TUDCA) and trimethylamide-N-oxide (TMAO) have been shown to attenuate ER stress and modulate D2 activity levels [16,40]. While exposure of cells to 4-PBA or TMAO reverses the effects of ER stress on D2 activity, 4-PBA or

TUDCA successfully attenuates ER stress in a mouse model of obesity and insulin resistance. In this model, treatment with either compound was able to restore glucose homeostasis, leptin and insulin sensitivity [41]. Interestingly, 4-PBA or TUDCA also stimulates Dio2 expression and D2 activity in vitro and in vivo, with the majority of the metabolic effects (i.e. the increase in oxygen consumption and normalization of glucose homeostasis) attributed to the chemical chaperones being mediated through the D2 pathway, as these effects are lost in Dio2$^{-/-}$ mice [40].

2.3. Nutritional signals upregulate D2 expression

The fact that adult humans exhibit metabolically active BAT depots [42] highlights the potential for development of therapeutical agents to accelerate energy expenditure by targeting D2-mediated T3 production. With this goal in mind, screening studies for compounds activating the D2-pathway were set up and have identified the compound kaempferol (KPF) as the most potent xenobiotic activator of D2 activity in vitro using a human skeletal cell line [43]. KPF increases D2 activity by upregulating cAMP-dependent Dio2 transcription. As a consequence, there is a 2.5-fold increase in intra-cellular T3 production that coincides with a significant increase in metabolically relevant genes, such as PGC1α and carnitine palmitoyl transferase-1 (CPT-1), and also with a 30% increase in cellular oxygen consumption [43]. Similarly, D2 has been found to be a key player in the metabolic effects of bile acids (BAs), small molecules that not only assist in the digestion and absorption of fatty nutrients but also integrate complex signaling pathways [44]. Mice fed a high-fat diet containing BA gain less weight than their control counterparts because the elevation in serum BA activates BAT, inducing key genes involved in the thermogenic process, i.e. Dio2 and PGC1α [14]. BAs activate the D2 pathway by interacting with the BA G-protein-coupled receptor TGR5 and activating the cAMP–PKA pathway, leading to a dose dependent increase in Dio2 expression and D2 activity. Most importantly, Dio2$^{-/-}$ mice are resistant to the protective effects of BAs against diet-induced obesity, indicating that some of the BA-mediated effects on metabolism depend on the TGR5–D2 pathway [14].

3. Physiological roles played by D2-mediated thyroid hormone activation

D2-mediated T3 production serves as an additional source of T3 to the TRs, increasing thyroid hormone signaling in discrete cells or tissues [45]. This pathway has been shown to play a significant role in a number of biological systems such as the pituitary and hypothalamic T4-mediated feedback mechanism [46–48], cochlear and retina development [49,50], brown adipose tissue (BAT) development [51], metabolic control [52], bone maturation [24,53] and myogenesis and muscle regeneration [54]. Furthermore, in the central nervous system (CNS), D2 is expressed in glial cells whereas most TRs are expressed in the neurons. Thus, glial cell-derived T3 enters the nearby neurons via thyroid hormone transporters and establishes a transcriptional footprint, constituting a paracrine signaling that activates neuronal gene expression in the rodent brain and human cells [55].

While these experimental studies indicate that D2 plays a number of significant roles in different biological systems, an increasingly large number of clinical studies also seem to indicate that D2 is critical in controlling thyroid hormone signaling. These studies stem from

the observation that about 15% of the normal population exhibits a Dio2 gene polymorphism (Thr92AlaD2) [56], which could potentially affect D2 activity and thus localized T3 production [57]. In addition to being associated with insulin resistance and increased BMI [56], and subsequently with diabetes mellitus type II [58], a much broader spectrum of diseases and conditions has also been associated with the Thr92AlaD2 polymorphism, including mental retardation [59], hypertension [60], osteoarthritis [61], bipolar disorder [62], clinical course and myocardial remodeling [63], accelerated bone turnover [64], and response to lung injury [37,38], indicating that indeed this locus via its control of T4 to T3 conversion, is physiologically and clinically relevant in a broad number of systems. Below we focus on the role played by D2 in the T4-mediated TRH–TSH feedback mechanism, adaptive thermogenesis and myogenesis and muscle regeneration. The reader is referred to other publications for a more comprehensive analysis of the role played by D2 in different biological systems [10,45,65–71].

3.1. D2 is critical in the T4-mediated feedback mechanism at the hypothalamus–pituitary axis

A remarkable feature of the feedback mechanism regulating the hypothalamic–pituitary–thyroid axis is that both T4 and T3 can independently repress TRH and TSH expression and secretion. During iodine deficiency there is a drop in serum T4 in the face of normal serum T3, which results in elevation in serum TSH levels [46]. Likewise, the reduction in serum T3 in the face of stable serum T4 levels in patients acutely given large amounts of propylthyouracyl (PTU) results in elevation of serum TSH levels [72]. The drop in serum T3 is sensed by the TRH-expressing hypophysiotropic neurons in the paraventricular nucleus (PVN) and the pituitary TSH-secreting thyrotrophs, de-repressing the expression of TRH and TSH-β genes, respectively. In contrast, the drop in serum T4 can only de-repress TRH and TSH expression thanks to D2, which transduces the T4 signal by locally converting it to T3 in the PVN and in the thyrotrophs, therefore establishing the critical role D2 plays in thyroid hormone economy (Fig. 2) [45,73].

In the medial basal hypothalamus (MBH), astrocyte D2 activity is dwarfed by the level of D2 expression found in tanycytes, specialized ependymal cells located on the lining floor and infralateral wall of the third ventricle, near the TRH-expressing neurons located in the PVN [74,75]. Tanycyte D2 has access to plasma-born or cerebral-spinal fluid-born T4 to produce T3 that can then reach the PVN or the pituitary gland via portal blood [74]. Thus, it is accepted that tanycyte D2 plays an important role in the T4-mediated feedback regulation of TRH and TSH [76] as well as in the central hypothyroidism that takes place during non-thyroidal illness [77].

In fact, systemic administration of bacterial lipopolysaccharide (LPS) to normal mice increases D2 mRNA and activity in the MBH and reduces TRH expression. In addition, the T3 generated via the D2 pathway can directly affect the thyrotrophs and inhibit the secretion of TSH [78]. Notably, the transcription factor NF-kappaB has been shown to be a key mediator of the effects of LPS on the *Dio2* gene in tanycytes [78]. Furthermore, the Dio2$^{-/-}$ mice treated with LPS indicated that TRH suppression is through a D2-dependent

mechanism [55,78], placing D2 as a key player in a mechanism that allows energy conservation during severe illness.

D2 is co-expressed with TSH in pituitary thyrotrophs [47] and the TR-β2 isoform is the dominant isoform that mediates the negative regulation of the TSH-β gene expression in the pituitary, while it is also the dominant isoform regulating the TRH gene expression in the hypothalamus [79,80]. The targeted inactivation of the Dio2 gene in mice leads to elevated serum TSH and T4 but normal serum T3 levels, a complex phenotype that is compatible with impaired transduction mechanism in the T4-mediated TSH suppression [73]. A similar phenotype was also observed in amiodarone-treated mice, a non-competitive inhibitor of D2 [81]. However, neither the Dio2$^{-/-}$ mouse nor the amiodarone-treated mouse models allow a detailed mechanistic understanding of the relative roles played by the pituitary D2 versus the hypothalamic D2 in thyroid hormone-mediated feedback mechanism.

Here also the D2 Thr92Ala polymorphism [82] has been implicated as playing a role in modulating the hypothalamus–pituitary axis in humans, but the results are not conclusive. Whereas this polymorphism is associated with lower serum TSH levels in heterozygous individuals, no association with circulating iodothyronine levels was found [83]. At the same time, others have shown that in homozygous individuals this polymorphism predicts the need for higher levothyroxine intake to normalize serum TSH in thyroidectomized patients [84], a finding that was not confirmed in a subsequent study [85].

3.2. Sympathetic-mediated acceleration in the metabolic rate and adaptative thermogenesis depend on D2-mediated T3 generation

The BAT is an important organ in energy homeostasis in small mammals, including humans [86]. BAT functionality relies mainly in the activity of a protein located in the mitochondrial inner membrane called uncoupling protein 1 (UCP-1), which acts as a gated channel that controls the proton (H^+) flux between the inter-membrane space and the mitochondrial matrix [87]. When activated, UCP-1 allows the flow of protons from the inner membrane to the mitochondrion matrix, bypassing the ATP generating enzyme, thus dissipating chemical energy as heat [87,88]. In response to environmental and/or endogenous cues, the sympathetic nervous system (SNS) accelerates the release of norepinephrine in the BAT and activates the β-adrenergic family of G-coupled receptors (β1, β2 and β3) in brown adipocytes. As a result, there is a spike in the intracellular production of cAMP and activation of protein kinase A (PKA) that up-regulates Dio2 mRNA and D2 activity levels [4,89], rapidly saturating nuclear TRs [90] and inducing the expression of several key metabolic genes, like the peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC1α) and UCP-1 [45,52].

The critical role of D2 in BAT physiology is underscored by studies of mice with a null mutation of the Dio2 gene (Dio2$^{-/-}$), which are sensitive to thermal stress and develop hypothermia when exposed to cold temperatures [52]. Underlying this phenotype is the fact that Dio2$^{-/-}$brown adipocytes show an increased proliferation rate combined with an impaired maturation rate, thus leading to a reduced number of mature brown adipocytes [51]. Interestingly, room temperature (23 °C) is a significant thermal stress for Dio2$^{-/-}$ mice, leading to increased sympathetic activity and a paradoxical protection against diet-induced

obesity [91,92]. This is similar to what has been observed in the UCP-1 null mice (UCP-1$^{-/-}$) [93] and in systemically hypothyroid rats and mice [94,95], which is reversed by acclimation at thermoneutrality (30 °C) [96]. Similarly, housing Dio2$^{-/-}$ mice at thermoneutrality switches fuel utilization from preferentially oxidizing fatty acids to carbohydrate oxidation [92]. In addition, only at thermoneutrality that the obesity-prone metabolic phenotype of Dio2$^{-/-}$ mice becomes evident when placed on a high-fat diet, developing diet-induced obesity, glucose intolerance and hepatic steatosis [92].

3.3. D2 expression, activity and regulation in the skeletal muscle

Thyroid hormone plays a pivotal role in skeletal muscle physiology, determining the expression of key genes involved in muscle fiber phenotype, metabolism and thermogenesis, force production and relaxation, development and regeneration [54,97]. While there is evidence that D2 plays a local role in skeletal muscle biology, its contribution to extra-thyroidal T3 production is debatable (Fig. 3).

T4-to-T3 conversion in skeletal muscle was first observed in perfused rat hindlimb, where in red slow contracting muscles the uptake of T4 and T3 was 50% higher than that in white fast-twitch contracting muscles, with T3 formation in red muscles increasing with hypothyroid status [98]. Later, D2 expression and activity were found in human skeletal muscle [99], after the mammalian D2 gene was cloned [100]. In subsequent studies, D2 mRNA and activity were reported in primary cultures of human skeletal muscle [101] and human rhabdomyosarcoma cells [102].

However, it has been challenging establishing a method to measure D2 activity levels in biopsy samples of skeletal muscle. D2 activity was found to be 0.35±0.12 fmol/min/mg of protein in human sternocleidomastoid and rectus abdominis [58]. Despite the presence of D2 mRNA, much lower D2 activity levels (two orders of magnitude) were later reported in the human Vastus lateralis muscle, i.e. 0.005–0.008 fmol/min/mg of protein, even after four weeks of hypo-thyroidism or 5 h of hyperinsulinemia [85]. Interestingly, D2 mRNA and activity were found to be 2–3-fold elevated in rectus abdominis muscle of patients with critical illness associated with the low T3 syndrome, but again, muscle D2 activity was found to be very low, between 0.003 and 0.008 fmol/min/mg of protein [103].

Subsequent studies in skeletal muscle homogenates of experimental animals also reported low or undetectable D2 activity [104–107] that decreased further with postnatal age [54,105]. In Hanaford minipigs, hindlimb muscles exhibited D2 activities ranging from 0.01 to 0.08 fmol/min/mg of protein, similar to the activity levels seen in Sinclair and Yucatan minipigs [104]. However, in murine muscle homogenates, the net amount of free iodine was quite low and not linear with the amount of protein used or time of incubation. Altering buffer composition and sample processing failed to bring out any additional measurable D2 activity in skeletal muscle and similar results were obtained using rat muscle, human strap muscle, and human rectus abdominis muscle [104]. Nevertheless, primary skeletal myocytes isolated from mouse hindlimb exhibited D2 activity only when differentiated to myotubes and stimulated with forskolin [104]. Using a similar cell culture setup with human skeletal myocytes, both D2 activity and mRNA increased only after the administration of the PPARγ

agonist pioglitazone, insulin and the BA lithocholic acid (LA), with the latter acting via the TGR5 pathway [14,104] (Fig. 2).

An improved method for measuring D2 activity in muscle biopsy samples has been reported, providing new insights into D2's physiological role and regulation in skeletal muscle [105,108,109]. This approach requires measuring D2 activity in the microsomal fraction of muscle samples and the use of Dio2$^{-/-}$ skeletal muscle homogenates as a tissue-specific negative control. As a result, microsomal D2 activity was ~6-fold higher when compared to those of tissue homogenate samples (0.070±0.007 vs. 0.012±0.001 fmol/min/mg of protein, respectively, in Anterior tibialis muscles). Under these conditions, D2 activity was concentrated in slow twitch muscles, i.e. Soleus and Vastus intermedius (0.40±0.06 and 0.16±0.02 fmol/min/mg of protein, respectively), whereas low activity was observed in fast twitch muscles i.e. Vastus lateralis, Gastrocnemius and Anterior tibialis (0.057±0.01; 0.07±0.01 and 0.09±0.01 fmol/min/mg of protein, respectively).

Moreover, short and long term exposure to hypothyroid conditions increased D2 activity in slow and fast muscles, where D2 activity levels of 1.56±0.07 fmol/min/mg of microsomal protein were reported [105]. Importantly, D2 activity increased after 4 h of cold exposure, an effect that was reduced by pharmacological manipulation using an adrenergic receptor antagonist and a blocker of catecholamine synthesis [109]. Notably, while isolating microsomes reportedly provides a technically feasible approach to monitor D2 in skeletal muscle samples, even with this methodology D2 activity in this tissue remains remarkably low when expressed in terms of total tissue protein, downplaying the previous estimate that skeletal muscle D2 is the main source of extra-thyroidal T3 production [110], which was calculated based on figures of muscle D2 activity and that did not take into account the most recent approach to measure D2 activity in this tissue [58].

3.4. Muscle development and regeneration

Skeletal muscle is a tissue with high plasticity and is capable to adapt to a new metabolic demand by regulating cell growth and metabolism [111]. This tissue has a great capacity of regeneration following damage and this regenerative capacity is due to the presence of muscle satellite cells, myogenic precursors localized in the periphery of the skeletal muscle myofiber underneath the basal lamina [112,113] (Fig. 3).

That D2 activity is markedly increased during the maturation of mouse myoblasts into myocytes, suggests a role for the D2-generated T3 in muscle development [104]. In fact, skeletal muscle of Dio2$^{-/-}$mice exhibits a mild hypothyroid phenotype characterized by low expression of MyoD and Myogenin (members of the myogenic regulatory factor family MRF; for review see [111]) and SERCA2 [54]. In addition, during the differentiation of the primary muscle precursor pp6 cells, there is a temporal association between the induction of D2 and the MyoD mRNA levels, where both genes peak 48 h after induction of the differentiation program [54]. Remarkably, pp6 cells from Dio2$^{-/-}$mice remain in the proliferating phase, incapable of differentiating into myotubes, with similar results being found in myoblasts derived from the C2C12 cell line transduced with a D2-targeting RNAi molecule [54]. Moreover, in contrast to wild-type muscle cells, Dio2$^{-/-}$pp6 cells do not express MyoD, Myogenin or MHC2 when the differentiation program is triggered, whereas

the addition of T3 to the media rescues this phenotype by resuming the normal differentiation expression profile, highlighting the importance of local D2-mediated T3 production for adequate muscle myogenesis [54]. Accordingly, in a cardiotoxin-induced injury murine model, D2 expression and D2 activity are increased after cardiotoxin injection to the Anterior tibialis muscle, with $Dio2^{-/-}$ mice failing to achieve proper muscle regeneration due to lack of muscle fiber maturation [54]. Lastly, in a similar experimental setup, D2 activation after muscle injury leads to increase in localized T4-to-T3 deiodination [114].

4. Conclusion

D2 is a major checkpoint in thyroid hormone signaling, controlling cell-specific thyroid hormone activation. This pathway plays an important role in thyroid economy and homeostasis, in development and metabolic control. D2 activity level is controlled by an array of transcriptional and post-transcriptional mechanisms that integrate environmental, neural and metabolic signals. The observation that affects the metabolic rate and fuel economy in mice is striking, and makes D2 a suitable target for pharmacological intervention.

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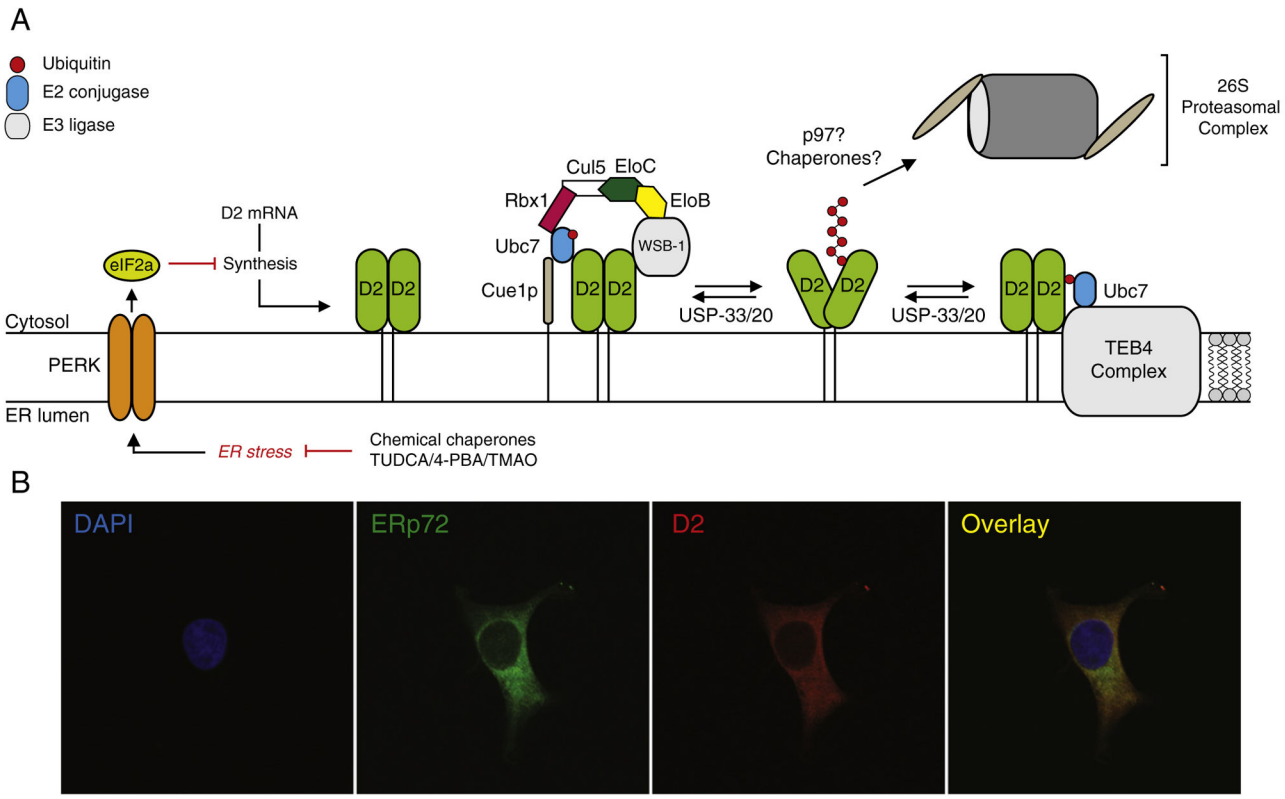

Fig. 1.

D2 is regulated at the post-transcriptional level. The short-lived dimeric enzyme type 2 iodothyronine deiodinase (D2) is an endoplasmic reticulum (ER) resident protein regulated by the ubiquitin–proteasome pathway. (A) D2 ubiquitination is triggered by substrate (T4 or rT3) binding to D2's catalytic core, when two different E3 ubiquitin ligases, WSB-1 and TEB4, play key roles in regulating cellular D2 levels. Ubiquitinated D2 complexes (Ub-D2) are catalytically inactive and can either be de-ubiquitinated by the action of the deubiquitinates (DUBs) USP20 and USP30, rescuing D2 activity; or are directed to the 26S proteasomal complex for terminal degradation. Besides ubiquitination, cellular D2 levels are regulated at the translational level by the ER stress pathway, which blocks D2 protein synthesis upon disruption of ER homeostasis and activation of the PERK–eIF2a pathway. Conversely, ER stress can be attenuated or even reversed by treatment of cells with chemical chaperones, thus lifting the negative effect of ER stress on D2 synthesis and finally increasing D2 activity. In (B), immunocytochemistry staining and confocal imaging of HEK-293 cells stably expressing a YFP-D2 construct. From left to right: nuclei (DAPI, blue); ERp72, an ER marker (green); and D2 (red). The overlay of all signals (yellow) is shown on the last picture on the right.

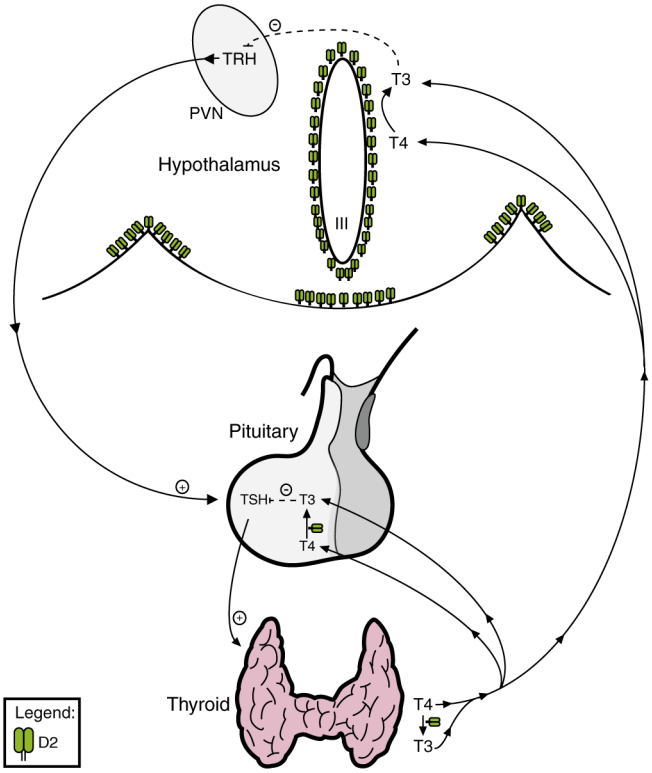

Fig. 2.

D2 is expressed in skeletal muscle and is involved in muscle differentiation and regeneration. T4 and T3 reach the myoplasm by MCT8/10-mediated transport [115]. T4-to-T3 activation is mediated by the type 2 deiodinase (D2), whereby T3 acts by binding to nuclear thyroid hormone receptors (TRs) and activates the expression of key muscle proteins, such as myosin heavy chain (MHC) and sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA). Muscle D2 is subject to regulation by (i) bile acids via the TGR5–cAMP pathway, and (ii) via adrenergic stimulation [109]. In addition, D2-generated T3 could reach the blood stream via the iodothyronine transporters and contribute to serum T3 supplies. After muscle injury, muscle precursor cells (here represented by satellite cells – SC) are activated and D2 expression and D2-dependent T3 production are upregulated locally, driving differentiation and complete skeletal muscle regeneration via T3-dependent expression of MyoD, a master myogenic regulatory factor. In the figure: SR, sarcoplasmic reticulum and TGR5, G protein-coupled bile acid receptor 1 (GPBAR1).

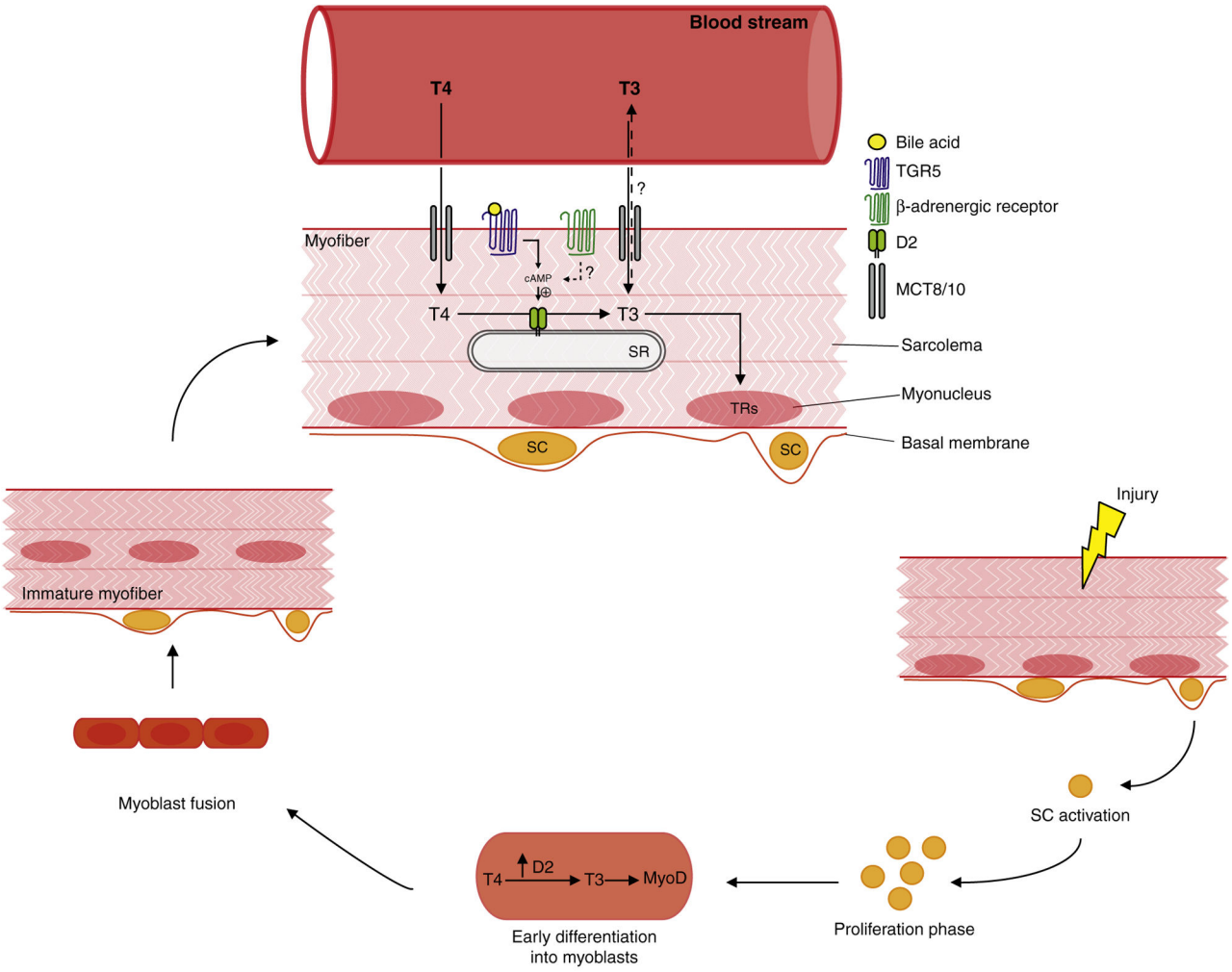

Fig. 3.

Schematic illustration of the D2 participation on the feedback system regulation of hypothalamic–pituitary axis (HPT). In the hypothalamus, D2 is strategically located on the lining floor and infralateral wall of the third ventricle (III), near TRH-expressing hypophysiotropic neurons located in the paraventricular nucleus (PVN). In the anterior pituitary gland, D2 is co-expressed with TSH in pituitary thyrotrophs. The drop in serum T3 is sensed by TRH-expressing hypophysiotropic neurons in the PVN and the pituitary TSH-secreting thyrotrophs, de-repressing the expression of TRH and TSH-β genes, respectively. In contrast, the drop in serum T4 can only de-repress TRH and TSH thanks to D2, which translates the serum T4 signal by locally converting it to T3 in the PVN and in the pituitary thyrothrophs, therefore establishing an important signaling pathway that controls thyroid hormone economy.

Table 1

Regulatory pathways of D2.

Tissue	Stimuli	Mechanism	Pathway/factor
BAT	Bile acids	Transcriptional	TGR5–cAMP [14]
	Chemical chaperones	Transcriptional	Unknown [40]
	Cold exposure	Transcriptional	β-Adrenergic receptor-cAMP [4,90]
		Post-transcriptional	Deubiquitination — USP33 [29]
Bone (*tibia growth plate*)	Indian hedgehog (Ihh)	Post-transcriptional	Ubiquitination — WSB1 [24]
Brain	LPS	Transcriptional	NF-kappaB [79]
	Thyroxine (T4)	Post-transcriptional	Ubiquitination [15,10] — TEB4 [27], WSB-1 [24]
Lung (*airway cells*)	LPS	Transcriptional	Injury [37,38]
	ER stress	Post-transcriptional	PERK-eIF2a [16]
	Chemical chaperones	Post-transcriptional	Reversal of ER stress [16]
Skeletal muscle	Bile acids	Transcriptional	TGR5–cAMP [14]
	Kaempferol (KPF)	Transcriptional	cAMP [43]
	Forskolin (FSK)	Transcriptional	cAMP [104]
	Cold exposure	Transcriptional	Adrenergic receptors [109]

The table describes the regulatory pathways and factors that affect D2 expression levels.