REVIEW

Iodothyronine deiodinases: a functional and evolutionary perspective

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Abstract

From an evolutionary perspective, deiodinases may be considered pivotal players in the emergence and functional diversification of both thyroidal systems (TS) and their iodinated messengers. To better understand the evolutionary pathway and the concomitant functional diversification of vertebrate deiodinases, in the present review we summarized the highlights of the available information regarding this ubiquitous enzymatic component that represents the final, common physiological link of TS. The information reviewed here suggests that deiodination of tyrosine metabolites is an ancient feature of all chordates studied to date and consequently, that it precedes the integration of the TS that characterize vertebrates. Phylogenetic analysis presented here points to D1 as the oldest vertebrate deiodinase and to D2 as the most recent deiodinase gene, a hypothesis that agrees with the notion that D2 is the most specialized and finely regulated member of the family and plays a key role in vertebrate neurogenesis. Thus, deiodinases seem to be major participants in the evolution and functional expansion of the complex regulatory network of TS found in vertebrates.

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Introduction

Thyroidal systems (TS) occur exclusively in vertebrates and seem to have evolved by the selection and functional diversification of iodine, the scarcest and heaviest natural halogen known to be essential for living systems. In vertebrates, iodine is critical for the synthesis of iodothyronines or thyroid hormones (THs), which, according to the ontogenetic stage of the organism, regulate early and post-embryonic developmental processes and metabolic balance (Eales 1997, Valverde-R *et al.* 2004, Yun *et al.* 2005, Tata 2006). The major TH in circulation is thyroxine (T_4) whose synthesis is restricted to the thyroid gland. The main physiological role of T_4 is to serve as a pro-hormone that, through its organ-specific dehalogenation, can be tailored into a family of active or inactive iodinated compounds. Hence, orchestrated by the network of neuroendocrine signals that assemble the classic hypothalamic–pituitary–thyroid axis (HPT), true TS comprise a fourth target-cell enzymatic component. This important physiological feature of TS, in which the action of its iodine-containing chemical messengers is finely tuned at the local target-cell level, is subserved by a set of ubiquitous reductive dehalogenases generally named iodothyronine deiodinases (Ds). Indeed, iodothyronine deiodination is the essential first step in the pre-receptor control mechanism of TH action (Nobel *et al.* 2001). From an evolutionary perspective, Ds may be considered pivotal players in the emergence and functional diversification of both TS and their iodinated messengers. To better understand the evolutionary pathway and the concomitant functional diversification of Ds, in the present review we summarized the highlights of the available information regarding this enzymatic component that represents the final, common physiological link of TS.

Overview of the deiodinase family

Deiodinases are dimeric integral-membrane, thyroredoxin fold-containing selenoproteins that catalyze the stereo-specific and sequential removal of iodine atoms from the pro-hormone T_4, generating active and inactive isomers of both triiodothyronine (T_3) and diiodothyronine (T_2). This biotransformation of TH occurs in practically every tissue of the organism and is catalyzed by three distinct deiodinase isotypes: D1, D2, and D3, each with different catalytic properties and specific tissue and developmental expressions. Two deiodinases, D1 and D2, serve the activating or outer ring-deiodinating pathway (ORD) by converting T_4 to T_3. The inactivating or inner ring-deiodinating pathway (IRD) is

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catalyzed primarily by D3, which converts T_4 and T_3 into inactive metabolites (reverse T_3 (rT_3) and 3, 3′-T_2 respectively). Thus, peripheral Ds tightly regulate, in an organ-specific manner, both circulating levels and the local intracellular concentrations of active and inactive TH (reviewed by Gereben *et al.* (2008)).

In spite of their distinct functional roles in TH homeostasis, members of this enzyme family share a common structural organization, thus suggesting that they may have diverged from a common ancestral gene. Although from mammals to fish D1 and D2 are coded by single genes and the majority of vertebrates also have a single gene for D3, most studied fish species have two genes coding for different isoforms of D3 (Valverde-R *et al.* 2004, Klootwijk *et al.* 2011). The three deiodinases are dimeric integral-membrane proteins of ∼60 kDa anchored to cellular membranes through a single transmembrane (TM) segment within their first 16–40 amino-terminal residues. This membrane segment is partly responsible for the specific subcellular topology that characterizes this enzyme family: D1 and D3 reside at the plasma membrane and D2 at the endoplasmic reticulum, thus allowing the intracellular availability of active and inactive TH to be precisely regulated based on tissue-specific and functional demand (Fig. 1). Protein modeling of human Ds suggests that the three paralogs belong to the thioredoxin-fold protein family and share strong similarities with the active site of iduronidase, a member of the GH-A-fold of glycoside hydrolase family. Their proposed molecular arrangement consists of four functional domains known as: TM, hinge (H), linker (L), and globular or catalytic (G), with the TM and G domains being essential for protein dimerization and hence for its full catalytic activity (Callebaut *et al.* 2003). Of note is the fact that although its physiological significance remains unknown, there is a low level of heterodimerization between D3:D1 and D3:D2 (Curcio-Morelli *et al.* 2003, Sagar *et al.* 2008). In the three deiodinases, the G domain encompasses a highly conserved (77% identity) 'core' sequence of 49 amino acids (115–163; human D1 numbering) with the selenocysteine (SeCys) residue in positions 126, 133, and 144 in D1, D2, and D3 respectively (Fig. 2). This 'core' sequence includes a segment, which we will refer to as the 'signature string', that consists of nine highly conserved residues: FGS(C/A)(T/S)XP(P/S)F. Also in all Ds, the 'core' sequence includes a second, well-conserved group of 16 residues (148–163, D1 numbering), which has been implicated in the homodimerization of the protein and is known as the deiodinase dimerization domain (DDD). Of these 16, the key residues are (I/V)Y(I/L/V) (152–154) for D1, two subdomains F(L/V)LYI (153–157) and SDG (164–166) for D2, and (I/V)YI (170–172) for D3 (Leonard *et al.* 2005, Sagar *et al.* 2008). In this context, a notable feature of all members of the deiodinase family is that when aligned (Fig. 2), they can be divided into two distinct regions: a conserved region (CR) that comprises the G and DDD domains (115–249, human

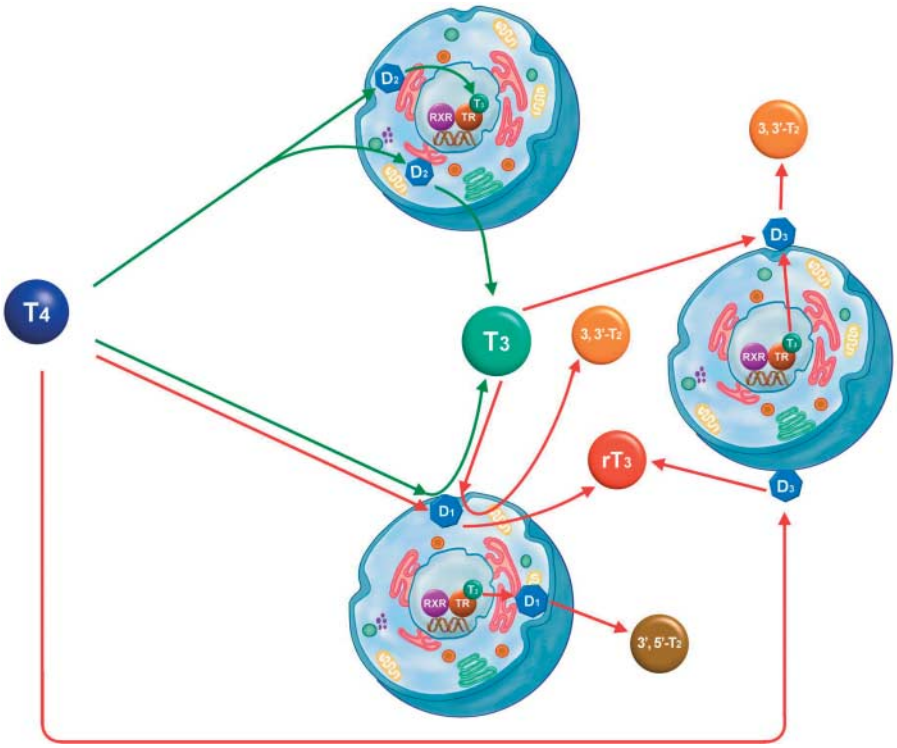

Figure 1 Subcellular topology and function of deiodinases. Green and red arrows show the activating and inactivating pathway respectively.

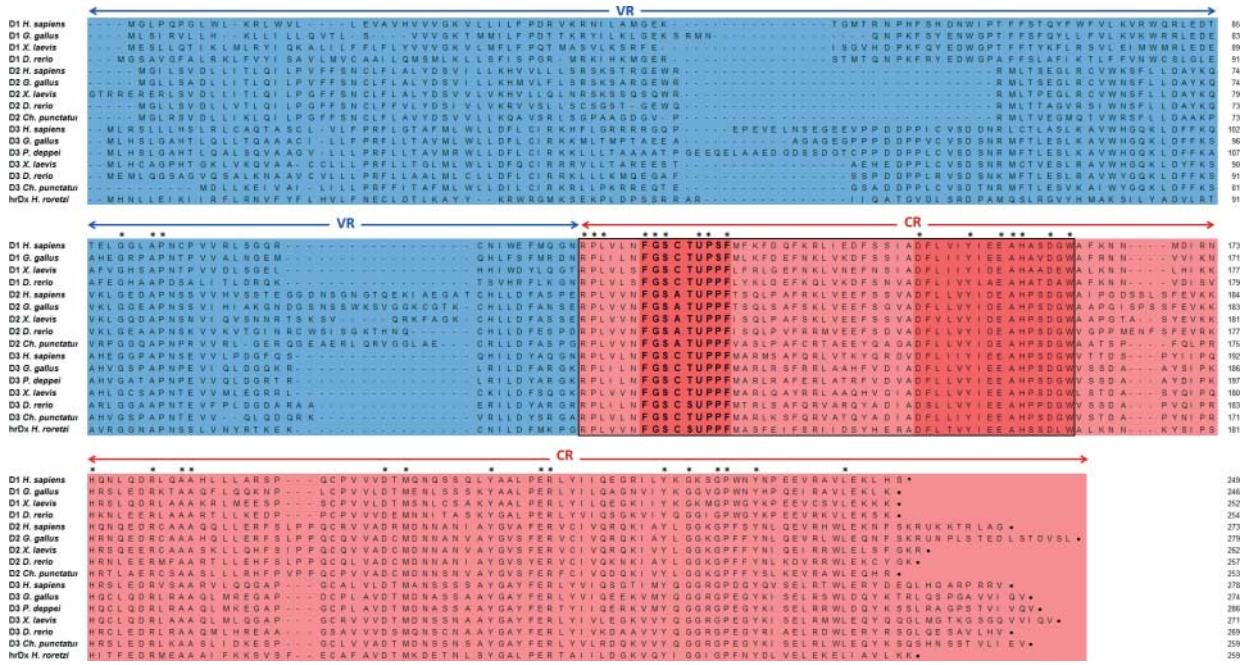

Figure 2 Sequence alignment of the three deiodinases in representative species. The arrows indicate the limits of the VR and CR in blue and red respectively. The 'core' sequence of 49 amino acids is contained in the box. In the VR, only three amino acids are well conserved between species, while the CR contains 31 conserved amino acids among species including the ascidian *Halocynthia roretzi*. The 'signature string' and a second well-conserved group are shaded dark red. The invariant amino acids are indicated with an asterisk. Black dots represent the COOH termini. Sequence information used for analysis: D1: *Bos taurus* gi, 169791016; *Chrysiptera cyanea* gi, 291170272; *Canis lupus familiaris* gi, 14717808; *Danio rerio* gi, 148277097; *Equus caballus* gi, 262050548; *Felis catus* gi, 57163803; *Fundulus heteroclitus* gi, 28144872; *Gallus gallus* gi, 148277086; *Homo sapiens* gi, 13195755; *Macaca mulatta* gi, 169790989; *Mus musculus* gi, 110825976; *Oryctolagus cuniculus* gi, 153791214; *Paralichthys olivaceus* gi, 171451963; *Pan troglodytes* gi, 169790987; *Rattus norvegicus* gi, 51988898; *Sparus aurata* gi, 116734190; *Suncus murinus* gi, 15128559; *Sus scrofa* gi, 48675925; *Takifugu rubripes* gi, 169659160; *Xenopus laevis* gi, 71841595. D2: *Bos taurus* gi, 58330899; *Chrysiptera cyanea* gi, 291170270; *Coturnix japonica* gi, 171948719; *Canis lupus familiaris* gi, 169790961; *Danio rerio* gi, 52630451; *Equus caballus* gi, 262050558; *Fundulus heteroclitus* gi, 14717797; *Gallus gallus* gi, 4927718; *Homo sapiens* gi, 1518542; *Mus musculus* gi, 6753638; *Neoceratodus forsteri* gi, 22128042; *Oryctolagus cuniculus* gi, 126572617; *Oryzias latipes* gi, 211904101; *Oncorhynchus mykiss* gi, 16550970; *Paralichthys olivaceus* gi, 71451965; *Rana catesbeiana* gi, 14717792; *Rattus norvegicus* gi, 132566529; *Siganus guttatus* gi, 283858738; *Sus scrofa* gi, 48675915; *Takifugu rubripes* gi, 169659162; *Xenopus laevis* gi, 13811437. D3: *Bos taurus* gi, 58330894; *Carassius auratus* gi, 145336998; *Chrysiptera cyanea* gi, 291170274; *Chiloscyllium punctatum* gi, 161328016; *Danio rerio* gi, 295789007; *Gallus gallus* gi, 169790979; *Homo sapiens* gi, 56103188; *Macaca mulatta* gi, 169790981; *Mus musculus* gi, 76827841; *Neoceratodus forsteri* gi, 37912833; *Ovis aries* gi, 169790985; *Oreochromis niloticus* gi, 5824106; *Pituophis deppei* gi, 270056392; *Paralichthys olivaceus* gi, 171451967; *Rana catesbeiana* gi, 14717804; *Sparus aurata* gi, 116734194; *Sus scrofa* gi, 48675921; *Takifugu rubripes* gi, 169659164; *Xenopus laevis* gi, 14717810; and *Xenopus tropicalis* gi, 169791009. Outgroup: *Halocynthia roretzi* gi, 38640513.

D1 numbering) and a variable region (VR) that includes the remaining three domains. As discussed later, the analysis of these two regions may provide important clues regarding the evolutionary path of this enzyme family. Of note is the fact that as we found minor discrepancies in the sequence data between different data bases, for the present review we only considered sequence information from GenBank.

All Ds contain at their active center (G domain) the modified amino acid SeCys, which is encoded by an in-frame UGA triplet that functions as a codon for the incorporation of the rare amino acid (Berry *et al.* 1991). At physiological pH, SeCys is ionized and becomes a potent electron donor, which favors an efficient deiodinating reaction (reviewed in Gereben *et al.* (2008)). Also, all Ds contain in their 3′ UTRs a selenocysteine insertion sequence (SECIS), which is a *cis*-acting signal required for the incorporation of SeCys into the protein during translation. SECIS elements form non-Watson–Crick base pairs and have been found to adopt two alternative hairpin loops, designated form 1 or form 2 (reviewed in Bianco *et al.* (2002)). The functional implications of this variation are unknown, as there is no clear evidence that the two forms of the SECIS element differ in their effects upon mRNA translation in other selenoproteins (Fagegaltier *et al.* 2000). Furthermore, deiodinase transcription requires an ancient and very complex *trans*-acting machinery; the mechanisms involved in this process were recently reviewed (Lu & Holmgren 2009, Palioura *et al.* 2009).

Deiodinase genes

Owing to the availability of whole-genome sequencing, Ds genes (*DIO1*, *DIO2*, and *DIO3*) have been identified in a number of vertebrate species including mammals, birds, amphibians, and fish; however, no genomic information on reptilian Ds is yet available. All *DIO1* genes described thus far comprise four to five exons from which one (chimpanzee, rat, and chicken), two (zebrafish), five (macaque), eight (mouse), or 14 (human) transcripts can be generated. The location of *Dio1* varies greatly among species; however, it is contained on chromosome 1 in all primate genomes analyzed. The best-studied promoter region of a *Dio1* is that of human, which contains two thyroid hormone responsive elements (TREs, Toyoda *et al*. 1995). *Dio2* and *Dio3* are co-localized on the same chromosome in most of the species with available sequenced genomes, i.e. human, macaque, chimpanzee, rat, mouse, dog, horse, pig, and chicken (chromosome 14, 7, 14, 6, 12, 8, 24, 7, and 5 respectively). The structure of *Dio2* comprises two exons spliced by a single intron. The size of the intron varies among species, the teleostean intron being the smallest (4·7 kb); however, all are located at the same position within the mRNA transcript. There is only one transcript from *Dio2*, with the exception of human and chimpanzee in which 12 and two transcripts of the gene have been found respectively. The physiological importance of these different forms of mRNA is still unknown. The promoter region of *Dio2* has been analyzed in only a few species. Some differences that could be of phylogenetic relevance may explain its differential expression observed in various vertebrate species. In contrast to the mammalian *Dio2*, no TATA or CCAAT boxes were found in the teleostean homolog (Orozco *et al*. 2002). Furthermore, the human, rat, and mouse genes contain a single, completely conserved canonical cAMP response element (CRE) around 70 bp upstream of the TATA box, but no CRE sequence is present within 1·3 kb upstream of the killifish gene. Additionally, only the human *DIO2* is stimulated by thyroid transcription factor 1 (TTF1; Gereben *et al*. 2001). Unlike *Dio1* and *Dio2*, no introns are present in the *Dio3* genes analyzed thus far (human, macaque, chimpanzee, rat, mouse, chicken, and zebrafish). Thus, *Dio3* has been included among the rare genes in the eukaryotic kingdom (6% of total genome) that have no introns (Hernandez *et al*. 1999). Mouse *Dio3* is imprinted and preferentially expressed from the paternal allele (Hernandez *et al*. 2002).

Deiodinase mRNAs

The number of identified deiodinase mRNAs has greatly increased in the last few years. In the present review, we have included only the available full-length (open reading frame) sequences that have been characterized biochemically and/or molecularly. Among these sequences, D1, D2, and D3 are highly conserved as judged by the 68, 75, and 69% identity, respectively, at the amino acid level. At variance with the other deiodinases, D3 mRNAs have been identified in all five classes of vertebrates including chondrichthyans and reptiles, and snake D3 is the longest so far reported (Martínez *et al*. 2008, Villalobos *et al*. 2010). All known deiodinase mRNAs include a SeCys-encoding TGA codon as well as a SECIS element in the 3′-UTR. SECIS elements have been found to adopt two alternative hairpin loops, designated form 1 or form 2. In form 1 structures, the essential AA(A) nucleotides are contained in a single open loop. By contrast, in form 2 the adenosines are located in a second bulged region. The predicted secondary structure of the D1 SECIS resembles that of form 1, with the single exception of the killifish, which resembles form 2 (Orozco *et al*. 2003). In all D2 and D3 mRNAs, the predicted secondary structure closely resembles that of SECIS form 2. A unique feature of D2 mRNAs is the presence of a second, in-frame TGA codon that located four to eight amino acids from the C-terminus of the protein; however, this site is not critical for deiodination (St Germain & Galton 1997, Orozco *et al*. 2002).

Deiodinase protein

Subcellular topology

Deiodinases are differentially localized at the subcellular level. D1 is a plasma membrane protein with its catalytic globular domain facing the cytosol, whereas the TM domain of D2 is anchored to the endoplasmic reticulum and its globular domain, including the active site, faces the perinuclear cytosol. D3 is anchored in the plasma membrane, with most of the molecule, including the active center, facing the extracellular space. D3 undergoes an unusual bidirectional recycling between plasma membrane and early endosomes. Indeed, recent studies have shown that plasma membrane D3 recycles rapidly to the early endosomal compartment, and apparently only a minute fraction progresses to late endosomes and to lysosomal proteolysis. The rest of the endosomal D3 pool is potentially recyclable, which could constitute a mechanism to reexpose the selenium-containing active center of the enzyme at the cell surface. Retention of internalized D3 in early endosomes could explain its long half-life (∼12 h) and allows for the possibility that an appropriate signal could lead to its rapid relocalization to the cell surface with a consequent acute inactivation of circulating T_4 and T_3 (Fig. 1; reviewed in Gereben *et al*. (2008)).

Biochemical properties

The pattern of deiodinase expression varies somewhat between species (Table 1). Although initially elusive in amphibians, D1 orthologs have been found recently in the genomes of *Xenopus laevis* and *Xenopus tropicalis* (Kuiper *et al*. 2006). The difficulty in identifying amphibian D1 activity

Table 1 Main regulators of the activity and expression of deiodinases. References: Fish: García-G *et al.* (2004, 2007), Orozco & Valverde-R (2005), Walpita *et al.* (2007), Isorna *et al.* (2009), Johnson & Lema (2011), Li *et al.* (2011*a*), Noyes *et al.* (2011), Wambiji *et al.* (2011) and Marlatt *et al.* (2012); Amphibian: Brown (2005), Morvan Dubois *et al.* (2006), Croteau *et al.* (2009), Lorenz *et al.* (2009), Bonett *et al.* (2010), Duarte-Guterman & Trudeau (2010), Cheng *et al.* (2011) and Langlois *et al.* (2011); Reptiles: Shepherdley *et al.* (2002*a*,*b*) and Villalobos *et al.* (2010); Birds: Gereben *et al.* (1999), Yoshimura *et al.* (2003), Darras *et al.* (2006), Watanabe *et al.* (2007), Elnagar *et al.* (2010), Egloff *et al.* (2011), Li *et al.* (2011*b*); Mammals: Kalsbeek *et al.* (2005), Revel *et al.* (2006), Barrett *et al.* (2007), Watanabe *et al.* (2007), Yasuo *et al.* (2007), Gereben *et al.* (2008) and Yasuo & Yoshisura (2009). Modified from Valverde-R *et al.* (2004)

	D1					D2					D3				
	Fish	Amphibians	Reptiles	Birds	Mammals	Fish	Amphibians	Reptiles	Birds	Mammals	Fish	Amphibians	Reptiles	Birds	Mammals
Main tissue distribution	Liver Kidney Gills Brain Gonads	Liver Kidney Brain	Liver	Liver Kidney Gut Lungs bursa	Liver Kidney Thyroid Pituitary	Liver Retina Brain Gonads	Brain Skin Gut Tail	Gut Lung Heart	Brain HG Thyroid Lung	Pituitary Brain Tanicytes BAT Adrenals	Liver Skin Gill Brain	Liver Gut Kidney	Liver Heart Gut Muscle Brain	Brain Liver Kidney Gut bursa	Placenta Skin Brain P-uterus
Embryo			Kidney Pancreas Lung Gut heart					Liver Kidney	Liver	Liver					
Substrate regulation															
Hyperthyroidism	↓↓ mRNA	No effect	↑	No effect	↑↑ mRNA ↓↓protein	↓↓ mRNA ↓ protein	↑↑ mRNA	↑↑	No data	↓ mRNA ↓↓protein (ubiquitin)	↑	↑↑mRNA	No effect	↑	↑↑mRNA
Hypothyroidism	↑↑	No data	↓	↑	↓	↑	No data	No data	↑	↑	No effect	↓	No data	↑(liver) ↓(brain)	↓
Other regulators															
Up-regulators	• BDE-209 (embryo)	No data	No data	• Leptin • PRL GH TRH GC DBDPE (embryonic liver)	• RA GH • cAMP TSH (thyroid)	• BDE-209 (embryo) • GC (liver)	•CORT (M tail) • GC (brain) • PFOS	• GC (liver)	• GC (embryonic brain)	• cAMP GC • Insulin glucagon CA bile acids (BAT)	No data	• finsateride (brain) • UVB exposure (tail)	No data	No data	• RA aFGF bFGF EGF
Down-regulators	• Acetochlor (larvae) • TDF (embryo)	No data	• GC CG +T_3 PP (embryonic kidney)	• DEX ACTH (liver)	• GC cytokines Se- deficiency	• GC (short-term) (liver) • Melatonin (brain) • Acetochlor (Larvae)	• GC (M liver) • UVB exposure (tail)	• GC (embryonic liver and kidney)	No data	• GH • Melatonin (brain)	• GC (liver and gill) • Estrogen (liver)	• GC (liver) • DEX and GC (brain)	• GC GC +T_3 (embryonic liver)	• GC GH Leptin (embryonic liver • BTBPE	• GH
Physiological demands															
Up-regulators	• Hypo-OE • Pro-M to EC	• Late EG	• EG (liver, kidney)	• EG (liver) • cold-stress (kidney)	• Lactation (MG)	• Hypo-OE (liver) • PM (lamprey gut) • M (brain, muscle) • Fasting (brain) • L:D (liver, skin, brain, heart, gonads)	• M • PM	• EG (brain, liver, kidney)	• L:D (brain) • EG: 1/3 and 3/3 (brain)	• Fasting cold-stress • (BAT and adrenal) • L:D (brain)	• Sm (brain) • M+L:D	• M	• EG (liver)	• Fasting 2/3 EG (liver) • cold-stress	• Gestation (uterus and placenta) • Hypoxia • L:D (short days: HT)
Down-regulators	• Hyper-OE (liver)	• Post-M	EG gut, lung)	• HS (liver)	• Fasting (liver)	• Fasting (liver) • M (lamprey gut) • Hyper- OE (liver)	No data	No data	• EG: 2/3 (brain: synaptogenesis)	No data	• M, mid-late	• M	No data	• Fasting (kidney) • Late EG (liver) • L:D (long days)	No data

B. frabicii, *Bursa frabicii*; BAT, brown adipose tissue; MG, mammary gland; p-uterus, pregnant uterus; HG, Harderian gland; HT, hypothalamus; EG, embryogenesis; M, metamorphosis/metamorphic; PM, pre-metamorphic; Pro-M, pro-metamorphic; Post-M, post-metamorphic; EC, early climax; Sm, smoltification; L:D, light:dark cycle; HS, heat stress; OE, osmotic environment; DEX, dexamethazone; CORT, corticosterone; GC, glucocorticoids; CA, catecholamines; PP, propranolol; PRL, prolactin; TRH, thyrotropin-releasing hormone; RA, retinoic acid; FGF, fibroblast growth factor; EGF, epidermal growth factor; BDE-209, decabromodiphenyl ether; DBDPE, decabromodiphenyl ethane; BTBPE, 1,2-*bis*(2,4,6-tribromophenoxy) ethane; TDF, triadimefon; PFOS, perfluorooctane sulfonate

resulted from the biochemical properties of the enzyme. Indeed, a critically important characteristic of D1-catalyzed deiodination is its remarkable sensitivity to inhibition by 6-*n*-propyl-2-thiouracil (PTU), with the exception of fish (Orozco *et al.* 1997, 2000, Sanders *et al.* 1997) and amphibian D1 (Kuiper *et al.* 2006), which are less sensitive to this inhibitor. In fact, PTU sensitivity has been used to demonstrate the specificity of T_4 to T_3 conversion and to distinguish D1 from the other Ds. The amino acids in the active center of D1 are highly conserved in various species (Fig. 2). The only known exceptions are fish and frog, in which proline replaces serine at position 128 and 132 respectively (Sanders *et al.* 1997, Orozco *et al.* 2003, Kuiper *et al.* 2006). Of note is the fact that the D2 and D3 enzymes, which are PTU-insensitive, also contain the proline residue at the corresponding position. Interestingly, site-directed mutagenesis has shown that the proline/serine change can explain frog (Kuiper *et al.* 2006), but not fish, D1 PTU insensitivity (Sanders *et al.* 1997, Orozco *et al.* 2003). This differential response to PTU between mammalian D1 and the amphibian and teleostean orthologs probably reflects a difference in the function of the enzyme in these species. In mammals and chicken, D1 is usually highly expressed in the liver, which plays an important role in regulating plasma T_3 levels (reviewed in Darras *et al.* (2006) and Gereben *et al.* (2008)). By contrast, D1 function in teleostean and amphibian T_3 plasma regulation is less clear (Finnson *et al.* 1999, Kuiper *et al.* 2006). Another indication for a different function might be that sulfated iodothyronines (rT_3S, T_3S, and T_4S) are very good substrates for mammalian D1 enzymes, but not for the teleostean or amphibian orthologs (Sanders *et al.* 1997, Finnson *et al.* 1999).

Aside from the peculiar PTU insensitivity, some, but not all, teleostean D1s exhibit yet another distinct feature related to their use of cofactors in the deiodinase reaction. The catalytic cycle of all Ds depends on a reducing thiol co-substrate that regenerates the selenoenzyme to its native state. Although no endogenous co-substrate has been identified, dithiothreitol (DTT) is commonly used to activate the enzymes *in vitro*. Both gilthead seabream kidney (Klaren *et al.* 2005) and killifish gill (Orozco *et al.* 2000) D1s have been shown to be inhibited by DTT. This kinetic characteristic seems be tissue specific.

The physiological role of deiodinases

D1 is the only member of the family that catalyzes both ORD and IRD of various iodothyronine derivatives. This complex dual catalytic activity suggests that D1 could have more than one function. Indeed, besides contributing to the circulating T_3 pool, the enzyme recycles iodine and operates as a scavenger, clearing plasma rT_3 and other inactive sulfated iodometabolites (Fig. 1; Schneider *et al.* 2006, Gereben *et al.* 2008). Models of deiodinase deficiency have helped to understand the physiological role of Ds. In this context, D1 knockout (KO) alone (Schneider *et al.* 2006) and combined D1/D2-KO (Galton *et al.* 2009) have shown that D1 may also play an important role in limiting the detrimental effects of conditions that alter normal thyroid function, like hyperthyroidism and iodine deficiency. This notion could apply to other vertebrates, as zebrafish D1 knockdown is only detrimental (developmental delay and dysmorphologies) when combined with a D2 knockdown, suggesting that D1 is only crucial in a depleted thyroidal status (Walpita *et al.* 2010). Thus, the D1 contribution to the circulating T_3 pool could be important during specific stages of high demand for TH such as embryogenesis and metamorphosis of fish and amphibian, as demonstrated by its upregulation during these processes in several species (i.e. Shepherdley *et al.* (2002*b*), Morvan Dubois *et al.* (2006), Campinho *et al.* (2010) and Itoh *et al.* (2010)).

D2 is an obligate ORD selenodeiodinase, which mainly catalyzes the conversion of T_4 to T_3 and T_3 to 3,5-T_2 (Fig. 1). The enzyme is considered to be the critical homeostatic T_3-generating deiodinase due to its substantial physiological plasticity. A number of transcriptional and posttranscriptional mechanisms have evolved to ensure limited expression and tight control of the D2 protein level, which is critical for its homeostatic function (see below). D2 is expressed in the mammalian brain, especially in glial cells; astrocytes and the ependymoglial cells known as tanycytes that line the walls and floor of the third ventricle are particularly important in functional terms as they produce more than 75% of the nuclear T_3 in the rat cerebral cortex (Guadaño-Ferraz *et al.* 1997, Rodríguez *et al.* 2010, Moháacsik *et al.* 2011). Another conspicuous functional distinction between D1/D3 and D2 is the fact that the latter exhibits a remarkable circadian rhythm entrained by the light/dark cycle. This rhythmicity has been documented in teleosts (García-G *et al.* 2004, Isorna *et al.* 2009), birds (Yoshimura *et al.* 2003), and mammals (Luna *et al.* 1995, reviewed in Gereben *et al.* (2008)) in several neuroendocrine structures, such as the hypothalamus, pituitary, pineal, and adrenal glands, as well as in brown adipose tissue, liver, Harderian gland, and cerebral cortex. Furthermore, recent studies support the notion that D2 expression in hypothalamic tanycytes is an important factor in regulation of seasonal reproduction both in mammals and birds (Williams & Duncan Bassett 2011, Ikegami & Yoshimura 2012). Surprisingly, the D2-KO mouse exhibited a very mild phenotype, showing an unimpaired reproductive capacity, small and transient growth abnormalities, and no loss in mobility. The most conspicuous features observed in this KO model were increased T_4 and TSH serum levels, accompanied by a resistance of pituitary TSH to T_4. Hence, D2 seems to be critical in the pituitary/thyroid feedback regulation of TSH secretion, at least in mammals (Schneider *et al.* 2001). Furthermore, D2-KOs exhibit retarded postnatal development of the cochlea, which resulted in severely impaired auditory function in the adult (Ng *et al.* 2004). Possible defects in other crucial TH-dependent neurodevelopmental functions, such as vision, learning, and memory, are currently unknown.

D3 has exclusively IRD activity and catalyzes the conversion of T_4 to rT_3 and T_3 to 3,3′-T_2, both of which are biologically inactive (Fig. 1). D3 is the predominant deiodinase expressed during embryonic life, and its activity is much higher than that found in adult tissues. Consequently, the enzyme is thought to control TH homeostasis by protecting tissues from an excess of TH during the species-specific ontogenetic programs. Indeed, although hepatic D3 expression is limited to embryonic life in most vertebrates, during adulthood the enzyme is also expressed in the liver of those omnivorous species that devour whole pray. This finding, which is in accord with the protective role of D3 during embryogenesis, has led to the proposal that hepatic D3 helps to prevent an inappropriate systemic overload of exogenous T_3 after feeding (Martínez *et al.* 2008, Villalobos *et al.* 2010). In the context of the protective role of D3, it is interesting that expression of this enzyme resumes during critical illness and different hypoxic–ischemic conditions such as myocardial infarction and chronic inflammation (reviewed in Huang & Bianco (2008), Mebis & Van den Berghe (2009), Warner & Beckett (2010) and Solís-S *et al.* (2011)). Developmental programing of the thyroid axis is markedly perturbed in D3-deficient mice, resulting in a persistent congenital hypothyroidism and causing partial neonatal lethality, growth retardation, and impaired fertility in D3-KOs (Hernandez *et al.* 2006, St Germain *et al.* 2009).

Regulation of deiodinases

As summarized in Table 1, depending on the organ and the species, different hormonal, nutritional, and developmental signals, as well as physiological demands, modulate the expression and activity of Ds. However, TH availability is the most potent and well-studied regulator. In most vertebrates, hyperthyroidism increases D1 activity and transcription, whereas hypothyroidism exerts the opposite effects. In humans, but not in rodents, the presence of two canonical TREs in the 5′ flanking region of *DIO1* explains the observed responses to substrate. However, fish D1 exhibits a distinct down-regulatory response. In fish, long- and short-term T_4- or T_3-hyperthyroidism does not alter hepatic D1 activity, but D1 mRNA levels do decrease. Furthermore, long-term hypothyroidism acutely increases hepatic D1 activity and levels of mRNA (reviewed in Orozco & Valverde-R (2005)).

Hyperthyroidism suppresses D2 activity and the expression of its mRNA in most studied tissues and species, whereas hypothyroidism increases them. Thus, at least in mammals, the very short half-life (<1 h) of D2 is further shortened in cells exposed to physiological concentrations of its substrate, T_4, and in experimental situations, to rT_3 or even T_3. This down-regulation of D2 activity by substrate is a rapid and potent regulatory feedback loop that efficiently controls T_3 production and intracellular T_3 concentration based on the availability of T_4. In this regard, experimental data suggest that

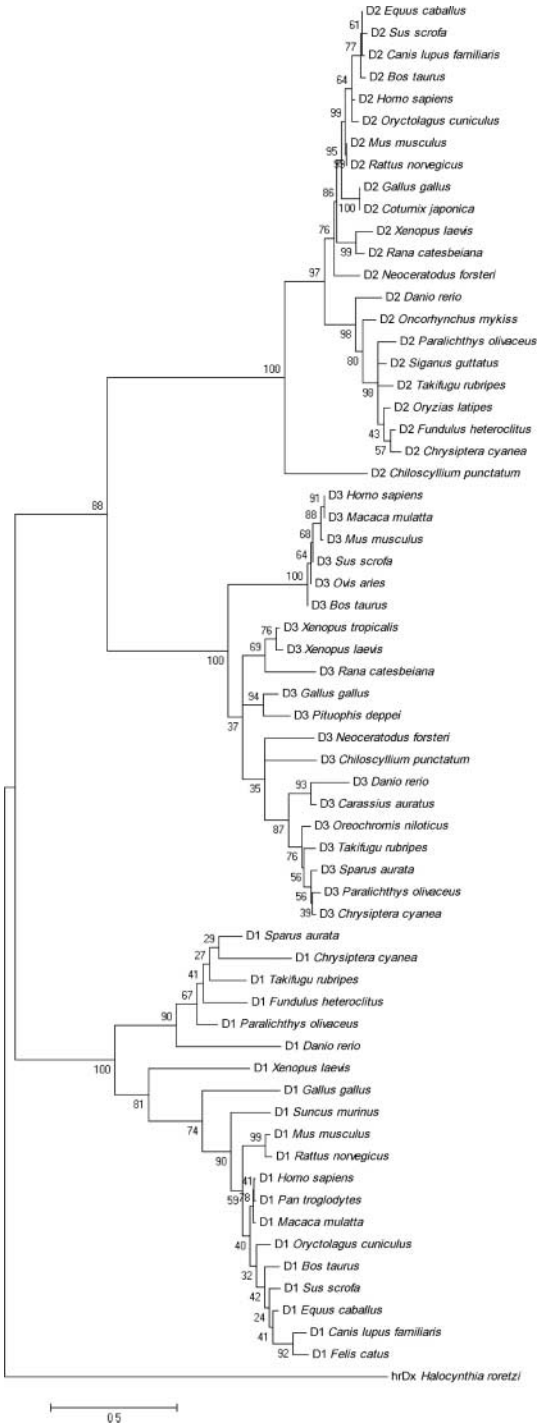

Figure 3 Phylogenetic reconstruction of vertebrate deiodinases. The outgroup represented by hrDx is the basal branch of the tree. Ds are grouped into three clusters, the D1 cluster being the most variable, suggesting that this paralog is the oldest. D2 and D3 clusters are close to each other and exhibit less variability than the D1 cluster; its appearance is more recent. The evolutionary distance scale is the number of amino acid substitutions per site, and the number of nodes represents the bootstrap statistic.

enzyme–substrate interaction induces selective degradation of the complex in the proteasome, which is initiated by conjugation to ubiquitin. Interestingly, the ubiquitinated D2 can then be either degraded in the proteasome complex or deubiquitinated to its unconjugated form, which reactivates the enzyme. These complex posttranscriptional mechanisms have been extensively studied and reviewed elsewhere (Gereben *et al.* 2008). By contrast, the molecular mechanisms that explain the pre-transcriptional regulation of D2 by substrate are not yet understood in any vertebrate species. In this context, the presence of a negative TRE in the human *DIO2* 5′-FR has been inferred, although it has not yet been identified (Gereben *et al.* 2008).

It has been reported that in teleost liver, 3,5-T_2 regulates the activity and expression of D2 (García-G *et al.* 2004, 2007). Little is known about the kinetics of 3,5-T_2 in vertebrates; nevertheless, the fact that this iodothyronine clearly regulates D2 suggests that it could play a physiological role in teleostean TS.

As previously mentioned, changes in the activity of D3 modulate both circulating and tissue thyroidal status by accelerating or retarding T_3 inactivation to maintain homeostasis. In agreement, thyroidal status parallels D3 activity in several species, increasing during hyperthyroidism and decreasing during hypothyroidism in the CNS (reviewed in Gereben *et al.* (2008)). This pattern has also been observed in other physiological situations such as in amphibian development, in which the pre-metamorphic surge of T_3 rapidly stimulates D3 in frog tadpoles (reviewed in Brown (2005)). The mechanisms of this regulation are still far from clear. Although a dramatic increase of D3 mRNA was observed after short-term (8 days) T_3 treatment, it is still not known whether this is a consequence of gene transcription, mRNA stabilization, or a combination of the two factors. Furthermore, the promoter analysis conducted in the rat and human *Dio3* showed a positive but modest regulation by T_3 (Gereben *et al.* 2008).

It is important to note that lately, an important effort has been made to analyze the impact that anthropogenic endocrine disruptors have upon deiodinase activity. In fact, D mRNA levels, at least in fish and amphibians, are sensitive to thyroid-disrupting chemicals and may provide useful molecular markers for exposure to them (i.e. Picard-Aitken *et al.* (2007), Croteau *et al.* (2009), and Li *et al.* (2009, 2011*a*)). Examples of the regulatory effects of some thyroid disruptors on deiodinase expression are also included in Table 1.

Phylogeny of deiodinases

The synthesis and metabolism of iodine-containing informational molecules, particularly the iodinated tyrosine messengers that characterize vertebrate TS, seem to have originated at the base of deuterostomes and evolved for endocrine function by the exploitation and diversification

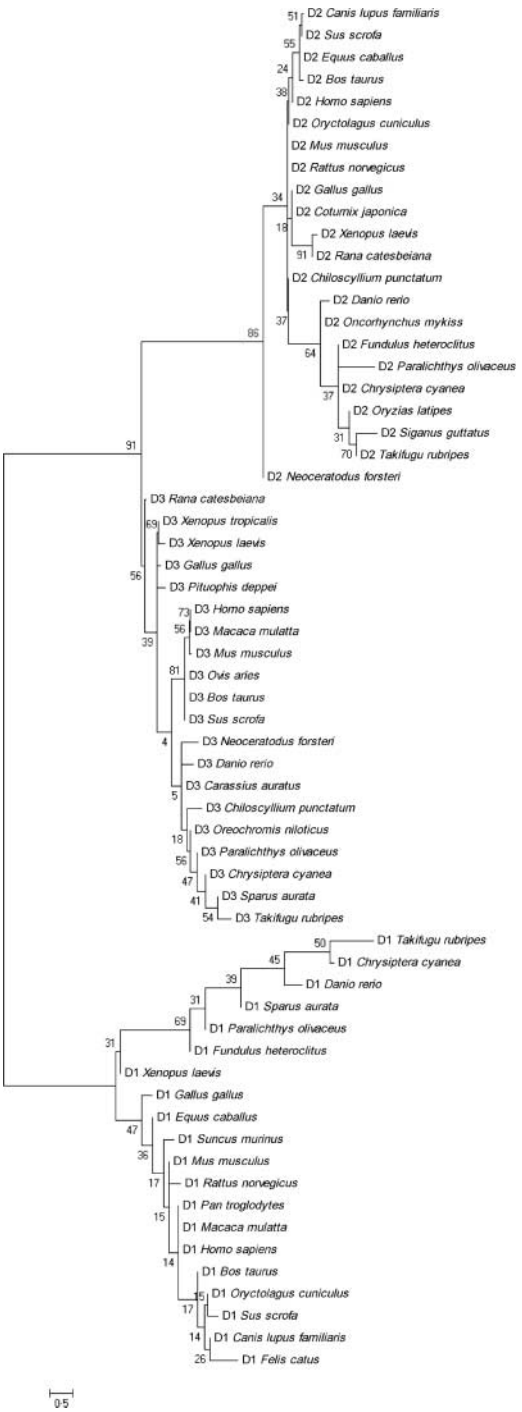

Figure 4 Phylogenetic reconstruction of VR vertebrate deiodinases. The topology of the tree shows similarity with the tree of complete sequences. Among the three paralogs, the largest variability (length of branches) corresponds to D1, particularly in fish and amphibian orthologs. The VR shows more amino acid replacements over time (compare with Fig. 5), suggesting that this region is the target of evolutionary innovation. The evolutionary distance scale is the number of amino acid substitutions per site, and the number of nodes represents the bootstrap statistic.

of the complementary enzymatic dyad of halogenation/dehalogenation (for review Eales (1997) and Valverde-R *et al.* (2004)). Indeed, current information supports the notion that the vertebrate thyroid gland, as well as the endostyle and subpharyngeal gland in invertebrate chordates, may have evolved from a common ancestor (for review Kluge *et al.* (2005) and Paris *et al.* (2008)). Thus, the genomes of cephalochordates (amphioxus) and urochordates (tunicates) contain orthologs of the main genes involved in thyroid hormonogenesis and the TH-signaling pathway (Na/I symporter, thyroid peroxidase, deiodinase, and TH receptor), but they lack the components for neuroendocrine control of the thyroid (for review: Holland *et al.* (2008) and Paris *et al.* (2008)). In the ascidian *Halocynthia roretzy*, at least one deiodinase homolog (hrDx) has been biochemically characterized. The enzyme presents a *bona fide* SECIS and conserves the signature string that characterizes vertebrate deiodinases. The catalytic activity of hrDx resembles a vertebrate D1 enzyme as it shows ORD activity, ping–pong kinetics, and prefers rT_3 as substrate. However like fish and amphibian D1s, hrDx is PTU-insensitive and contains proline instead of serine at the corresponding site (see Section Deiodinase protein; Shepherdley *et al.* 2004). In the amphioxus, *Branchiostoma floridae*, three deiodinase homologs have recently been cloned: at their catalytic site, two have SeCys. The only one that has been characterized, bfDy, has a Cys residue; does not deiodinate T_4, T_3, or rT_3; is not inhibited by PTU; and specifically catalyzes the IRD of thyroacetic acid metabolites of T_4 and T_3 (TA4 and TA3 respectively; Klootwijk *et al.* 2011). Furthermore, these acidic metabolites are the endogenous ligands of *B. floridae* TH receptors and they control metamorphosis in this species, supporting the notion that even in invertebrate chordates, this distinct, inactivating deiodinative pathway is physiologically relevant (Paris *et al.* 2008, 2010). Interestingly, these invertebrate chordate deiodinase homologs could have an even more ancient origin, and there is evidence that invertebrates like the scallop (*Chlamys farreri*; Wu *et al.* 2012) and the echinoderm (*Strongylocentrotus purpuratus*; Heyland *et al.* 2006) may express putative deiodinases. Thus, deiodinase functional diversity in extant vertebrates seems to stem from a common ancestral molecular scaffold that could have already been present in the ancient metazoa. Even though there is no information regarding the catalytic activities of the remaining two SeCys-containing, putative *B. floridae* homologs, the singular substrate selectivity of bfDy poses the intriguing question of whether ancestral deiodinases played a protective role, as does the extant vertebrate IRD, and/or fulfilled multiple roles that were later divided among several enzymes.

With the aim to trace the evolutionary history of Ds, we compared and analyzed (neighbor-joining) available D1, D2, and D3 peptide sequences retrieved from 33 species ranging from chondrichthyan and teleost fishes to mammals, as well as the urochordate hrDx, all of which have been expressed and/or characterized in functional terms. As depicted in Fig. 2 and advanced in the 'Deiodinase overview' section, the

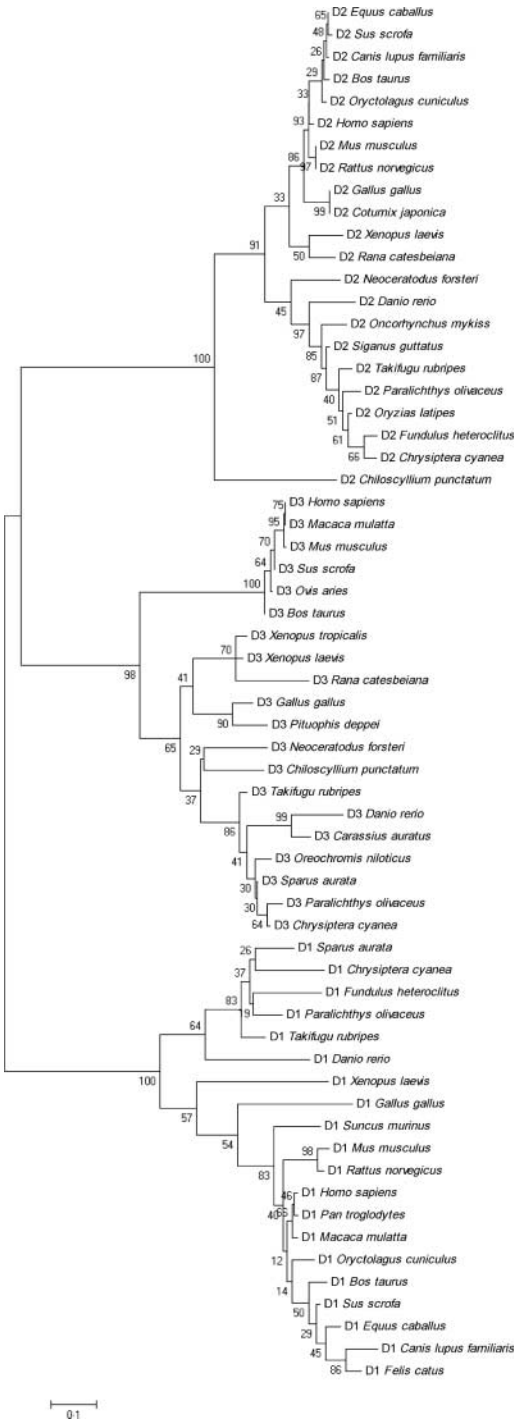

Figure 5 Phylogenetic reconstruction of CR vertebrate deiodinases. The topology of the tree shows that this region exhibits an important degree of conservation, which is more evident in the case of mammalian D2. This homogeneity shows that this region has been under a tight selective pressure maintaining its structure highly conserved among species. The evolutionary distance scale is the number of amino acid substitutions per site, and the number of nodes represents the bootstrap statistic.

initial alignment of the three vertebrate deiodinases (20 D1, 22 D2, and 20 D3 sequences) revealed a 45% identity among paralogs and 68, 75, and 69% among orthologs respectively. We then separately analyzed the protein sequences of Ds in two major regions: the first half or 'VR' includes the TM, H, and L domains, while the second half corresponds to the 'CR' and contains the G domain. Our results revealed that although the G domains are very similar (60% identity), the remaining domains (TM, H, and L) are the most variable (20% identity) among paralogs but, interestingly, are relatively conserved domains among orthologs (D1, 50%; D2, 55%; and D3, 60% identity). In hrDx, only the G domain is highly conserved, and it contains the nine amino acid signature string FGS(C/A)(T/S)XP(P/S)F (Fig. 2).

As expected and depicted in Fig. 2, the phylogenetic analysis resolves the different orthologs into three distinct clusters whose branches represent the evolutionary distance between deiodinase paralogs within the vertebrate phyla. For this analysis, we used hrDx as the out-group, in contrast to bfDy, as it is a SeCys-containing deiodinase. As observed (Fig. 3), hrDx is resolved at the base of the tree placing it as the most ancient. Between vertebrate deiodinases, the D1 cluster appears before that of D2 and D3, and it exhibits longer branches, which represent a high rate of evolutionary change; thus, this analysis suggests that it is the oldest of the three deiodinases. D2 and D3 share a node that represents an immediate common ancestor that could have resembled D1 in that it contained both activating and inactivating activities; by duplication, the two resulting genes acquired specialized functions.

As shown in Fig. 4, the phylogenetic analysis of the VR of the three deiodinase paralogs reveals that this region displays the greatest rate of evolutionary change. Indeed, the evolutionary distance scale is longer than that of the CR phylogenetic tree (0·5 vs 0·1 respectively). This suggests that the VR has been under a more relaxed selective pressure with the consequent gain of change and physiologic novelty. Furthermore, due in part to the reduced number and/or the lack of sequences from the three paralogs covering the entire vertebrate phyla, these changes are more evident when comparing fishes and mammals (Fig. 3), the vertebrate classes with the largest number of available D sequences (6–10 and 6–12 respectively). However, note that the amino acid substitutions among orthologs are not random but biochemically equivalent, thus maintaining the hydrophobic clusters in relatively constant same positions in the corresponding VR. This bioequivalence between orthologs could explain their shared features. With these caveats in mind, it is nevertheless important to notice that subtle differences among orthologs could be due to environmental and/or physiological species-specific demands.

In D1, variability was also highest in the VR. This, together with the topology of the VR phylogenetic tree, is consistent with our suggestion that D1 is the oldest vertebrate deiodinase. This suggestion is based on the notion that the longer the enzyme exists, the higher the opportunity for variation, expressed as mutations. In this context, the facts that D3 in most studied fish species is encoded by two genes and that *Dio2* and *Dio3* co-localize on the same chromosome suggest that the two enzymes may have evolved by gene duplication. This event is believed to play a major role in evolution because the additional copy would be relatively free from selective pressure, thus providing a source of genetic material for mutation, drift, and selection to act upon, making new evolutionary opportunities possible. In this context, the phylogenetic trees are presented here to point D2 as the most recent deiodinase gene. This hypothesis agrees with the notion that D2 is the most specialized and finely regulated member of the family and plays a key role in vertebrate neurogenesis.

The highly conserved identity of the CR is a consequence of the low rate of evolutionary change, as judged by the homogeneous distances of the phylogenetic tree branches (Fig. 5). Under an evolutionary scenario, it is reasonably valid to assume that the G domain in deiodinases has been under a tight selective pressure, maintaining a structure that is highly conserved among species. In fact, the G domain seems to be conserved in all enzymes so far identified (Dx, hrDx, and bfDy) that metabolize iodinated tyrosine compounds, supporting the idea that these enzymes are central components of an ancient and preserved homeostatic strategy. This notion is worthy of further attention and represents a frontier of knowledge in thyroid physiology.

Concluding remarks

In general, the ligand/receptor couple has been considered central for the understanding of the origin and functional diversification of endocrine systems. However, in those systems in which, as in the case of TH, ligand activity depends on its enzymatic biotransformation at the pre-receptor level, a third player must be considered to fully understand the evolution and functional expansion of the system. By selectively removing iodine atoms from one or another of the two tyrosine rings of the halogen-containing messenger, deiodinases represent a sophisticated, tissue-specific on/off switch for regulating TH activity. Indeed, the information reviewed here suggests that deiodination of tyrosine metabolites is an ancient feature of all chordates studied to date and consequently, that it precedes the integration of the TS proper that characterizes vertebrates. In fact, like their vertebrate counterparts, the non-SeCys deiodinase homologs in cephalochordates and urochordates are instrumental in regulating metamorphosis, a transitional stage of development conserved in all chordates and in which TH and/or some of its iodine derivatives are the key physiological regulators (Paris *et al.* 2008, 2010, Laudet 2011). Thus, deiodinases seem to be major players in the evolution and functional expansion of the complex regulatory network of TS found in vertebrates. As previously stated, the comparative approach in the study of TS is unavoidable, because, according to Dobzhansky (1973), 'Nothing in biology makes sense, except in the light of evolution'.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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References

Barrett P, Ebling FJ, Schuhler S, Wilson D, Ross AW, Warner A, Jethwa P, Boelen A, Visser TJ, Ozanne DM *et al*. 2007 Hypothalamic thyroid hormone catabolism acts as a gatekeeper for the seasonal control of body weight and reproduction. *Endocrinology* **148** 3608–3617. (doi:10.1210/en.2007-0316)

Berry MJ, Banu L & Larsen PR 1991 Type I iodothyronine deiodinase is a selenocysteine-containing enzyme. *Nature* **349** 438–440. (doi:10.1038/349438a0)

Bianco AC, Salvatore D, Gereben B, Berry MJ & Larsen PR 2002 Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocrine Reviews* **23** 38–89. (doi:10.1210/er.23.1.38)

Bonett RM, Hoopfer ED & Denver RJ 2010 Molecular mechanisms of corticosteroid synergy with thyroid hormone during tadpole metamorphosis. *General and Comparative Endocrinology* **168** 209–219. (doi:10.1016/j.ygcen.2010.03.014)

Brown DD 2005 Role of deiodinase in amphibian metamorphosis. *Thyroid* **15** 815–821. (doi:10.1089/thy.2005.15.815)

Callebaut I, Curcio-Morelli C, Mornon JP, Gereben B, Buettner C, Huang S, Castro B, Fonseca TL, Harney JW, Larsen PR *et al*. 2003 The iodothyronine selenodeiodinases are thioredoxin-fold family proteins containing a glycoside hydrolase clan GH-A-like structure. *Journal of Biological Chemistry* **278** 36887–36896. (doi:10.1074/jbc.M305725200)

Campinho MA, Galay-Burgos M, Sweeney GE & Power DM 2010 Coordination of deiodinase and thyroid hormone receptor expression during the larval to juvenile transition in sea bream (*Sparus aurata*, Linnaeus). *General and Comparative Endocrinology* **165** 181–194. (doi:10.1016/j.ygcen.2009.06.020)

Cheng Y, Cui Y, Chen HM & Xie WP 2011 Thyroid disruption effects of environmental level perfluorooctane sulfonates (PFOS) in *Xenopus laevis*. *Ecotoxicology* **20** 2069–2078. (doi:10.1007/s10646-011-0749-3)

Croteau MC, Davidson M, Duarte-Guterman P, Wade M, Popesku JT, Wiens S, Lean DR & Trudeau VL 2009 Assessment of thyroid system disruption in *Rana pipiens* tadpoles chronically exposed to UVB radiation and 4-tert-octylphenol. *Aquatic Toxicology* **95** 81–92. (doi:10.1016/j.aquatox.2009.05.013)

Curcio-Morelli C, Gereben B, Zavacki AM, Kim BW, Huang S, Harney JW, Larsen PR & Bianco AC 2003 *In vivo* dimerization of types 1, 2, and 3 iodothyronine selenodeiodinases. *Endocrinology* **144** 937–946. (doi:10.1210/en.2002-220960)

Darras VM, Verhoelst CHJ, Reyns GE, Kühn ER & Van der Geyten S 2006 Thyroid hormone deiodination in birds. *Thyroid* **16** 25–35. (doi:10.1089/thy.2006.16.25)

Dobzhansky T 1973 Nothing in biology makes sense except in the light of evolution. *American Biology Teacher* **35** 125–129.

Duarte-Guterman P & Trudeau VL 2010 Regulation of thyroid hormone-, oestrogen- and androgen-related genes by triiodothyronine in the brain of *Silurana tropicalis*. *Journal of Neuroendocrinology* **22** 1023–1031. (doi:10.1111/j.1365-2826.2010.02047.x)

Eales JG 1997 Iodine metabolism and thyroid-related functions in organisms lacking thyroid follicles: are thyroid also vitamins? *Proceedings of the Society for Experimental Biology and Medicine* **214** 302–317. (doi:10.3181/00379727-214-44098)

Egloff C, Crump D, Chiu S, Manning G, McLaren KK, Cassone CG, Letcher RJ, Gauthier LT & Kennedy SW 2011 *In vitro* and *in ovo* effects of four brominated flame retardants on toxicity and hepatic mRNA expression in chicken embryos. *Toxicology Letters* **207** 25–33. (doi:10.1016/j.toxlet.2011.08.015)

Elnagar SA, Scheideler SE & Beck MM 2010 Reproductive hormones, hepatic deiodinase messenger ribonucleic acid, and vasoactive intestinal polypeptide-immunoreactive cells in hypothalamus in the heat stress-induced or chemically induced hypothyroid laying hen. *Poultry Science* **89** 2001–2009. (doi:10.3382/ps.2010-00728)

Fagegaltier D, Lescure A, Walczak R, Carbon P & Krol A 2000 Structural analysis of new local features in SECIS RNA hairpins. *Nucleic Acids Research* **15** 2679–2689. (doi:10.1093/nar/28.14.2679)

Finnson KW, McLeese JM & Eales JG 1999 Deiodination and deconjugation of thyroid hormone conjugates and type I deiodination in liver of rainbow trout, *Oncorhynchus mykiss*. *General and Comparative Endocrinology* **115** 387–397. (doi:10.1006/gcen.1999.7326)

Galton VA, Schneider MJ, Clark AS & St Germain DL 2009 Life without thyroxine to 3,5,3′-triiodothyronine conversion: studies in mice devoid of the 5′-deiodinases. *Endocrinology* **150** 2957–2963. (doi:10.1210/en.2008-1572)

García-GC, Jeziorski MC, Valverde-RC & Orozco A 2004 Effects of iodothyronines on the hepatic thyroid hormone activating pathway in killifish. *General and Comparative Endocrinology* **135** 201–209. (doi:10.1016/j.ygcen.2003.09.010)

García GC, López-Bojorquez L, Nuñez J, Valverde RC & Orozco A 2007 3,5-Diiodothyronine in vivo maintains euthyroidal expression of type 2 iodothyronine deiodinase, growth hormone, and thyroid hormone receptor beta1 in the killifish. *American Journal of Physiology. Regulatory and Integral Comparative Physiology* **29** R877–R883. (doi:10.1152/ajpregu.00101.2007)

Gereben B, Bartha T, Tu HM, Harney JW, Rudas P & Larsen PR 1999 Cloning and expression of the chicken type 2 iodothyronine 5′-deiodinase. *Journal of Biological Chemistry* **274** 13768–13776. (doi:10.1074/jbc.274.20.13768)

Gereben B, Salvatore D, Harney JW, Tu HM & Larsen PR 2001 The human, but not rat, dio2 gene is stimulated by thyroid transcription factor-1 (TTF-1). *Molecular Endocrinology* **15** 112–124. (doi:10.1210/me.15.1.112)

Gereben B, Zavacki AM, Ribich S, Kim BW, Huang SA, Simonides WS, Zeöld A & Bianco AC 2008 Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. *Endocrine Reviews* **29** 898–938. (doi:10.1210/er.2008-0019)

Guadaño-Ferraz A, Obregon MJ, St Germain D & Bernal J 1997 The type 2 iodothyronine deiodinase is expressed primarily in glial cells in the neonatal rat brain. *PNAS* **94** 10391–11036. (doi:10.1073/pnas.94.19.10391)

Hernandez A, Lyon GJ, Schneider MJ & St Germain DL 1999 Isolation and characterization of the mouse gene for the type 3 iodothyronine deiodinase. *Endocrinology* **140** 124–130. (doi:10.1210/en.140.1.124)

Hernandez A, Fiering S, Martinez E, Galton VA & St Germain D 2002 The gene locus encoding iodothyronine deiodinase type 3 (Dio3) is imprinted in the fetus and expresses antisense transcripts. *Endocrinology* **143** 4483–4486. (doi:10.1210/en.2002-220800)

Hernandez A, Martinez ME, Fiering S, Galton VA & St Germain D 2006 Type 3 deiodinase is critical for the maturation and function of the thyroid axis. *Journal of Clinical Investigation* **116** 476–484. (doi:10.1172/JCI26240)

Heyland A, Price DA, Bodnarova-Buganova M & Moroz LL 2006 Thyroid hormone metabolism and peroxidase function in two non-chordate animals. *Journal of Experimental Zoology. Part B, Molecular and Developmental Evolution* **306** 551–566. (doi:10.1002/jez.b.21113)

Holland LZ, Albalat R, Azumi K, Benito-Gutiérrez E, Blow MJ, Bronner-Fraser M, Brunet F, Butts T, Candiani S, Dishaw LJ *et al.* 2008 The amphioxus genome illuminates vertebrate origins and cephalochordate biology. *Genome Research* **18** 1100–1111. (doi:10.1101/gr.073676.107)

Huang SA & Bianco AC 2008 Reawakened interest in type III iodothyronine deiodinase in critical illness and injury. *Nature Clinical Practice. Endocrinology & Metabolism* **4** 148–155. (doi:10.1038/ncpendmet0727)

Ikegami K & Yoshimura T 2012 Circadian clocks and the measurement of daylength in seasonal reproduction. *Molecular and Cellular Endocrinology* **349** 76–81. (doi:10.1016/j.mce.2011.06.040)

Isorna E, Obregon MJ, Calvo RM, Vázquez R, Pendón C, Falcón J & Muñoz-Cueto JA 2009 Iodothyronine deiodinases and thyroid hormone receptors regulation during flatfish (*Solea senegalensis*) metamorphosis. *Journal of Experimental Zoology. Part B, Molecular and Developmental Evolution* **312B** 231–246. (doi:10.1002/jez.b.21285)

Itoh K, Watanabe K, Wu X & Suzuki T 2010 Three members of the iodothyronine deiodinase family, dio1, dio2 and dio3, are expressed in spatially and temporally specific patterns during metamorphosis of the flounder, *Paralichthys olivaceus*. *Zoological Science* **27** 574–580. (doi:10.2108/zsj.27.574)

Johnson KM & Lema SC 2011 Tissue-specific thyroid hormone regulation of gene transcripts encoding iodothyronine deiodinases and thyroid hormone receptors in striped parrotfish (*Scarus iseri*). *General and Comparative Endocrinology* **172** 505–517. (doi:10.1016/j.ygcen.2011.04.022)

Kalsbeek A, Buijs RM, van Schaik R, Kaptein E, Visser TJ, Doulabi BZ & Fliers E 2005 Daily variations in type II iodothyronine deiodinase activity in the rat brain as controlled by the biological clock. *Endocrinology* **146** 1418–1427. (doi:10.1210/en.2004-0763)

Klaren PH, Haasdijk R, Metz JR, Nitsch LM, Darras VM, Van der Geyten S & Flik G 2005 Characterization of an iodothyronine 5′-deiodinase in gilthead seabream (*Sparus auratus*) that is inhibited by dithiothreitol. *Endocrinology* **146** 5621–5630. (doi:10.1210/en.2005-0050)

Klootwijk W, Friesema ECH & Visser TJ 2011 A nonselenoprotein from amphioxus deiodinates triac but not T_3: is triac the primordial bioactive thyroid hormone? *Endocrinology* **152** 3259–3267. (doi:10.1210/en.2010-1408)

Kluge B, Renault N & Rohr KB 2005 Anatomical and molecular reinvestigation of lamprey endostyle development provides new insight into thyroid gland evolution. *Development Genes and Evolution* **215** 32–40. (doi:10.1007/s00427-004-0450-0)

Kuiper GG, Klootwijk W, Morvan Dubois G, Destree O, Darras VM, Van der Geyten S, Demeneix B & Visser TJ 2006 Characterization of recombinant *Xenopus laevis* type I iodothyronine deiodinase: substitution of a proline residue in the catalytic center by serine (Pro132Ser) restores sensitivity to 6-propyl-2-thiouracil. *Endocrinology* **147** 3519–3529. (doi:10.1210/en.2005-0711)

Langlois VS, Duarte-Guterman P & Trudeau VL 2011 Expression profiles of reproduction- and thyroid hormone-related transcripts in the brains of chemically-induced intersex frogs. *Sexual Development: Genetics, Molecular Biology, Evolution, Endocrinology, Embryology, and Pathology of Sex Determination and Differentiation* **5** 26–32. (doi:10.1159/000322875)

Laudet V 2011 The origins and evolution of vertebrate metamorphosis. *Current Biology* **21** R726–R737. (doi:10.1016/j.cub.2011.07.030)

Leonard JL, Simpson G & Leonard DM 2005 Characterization of the protein dimerization domain responsible for assembly of functional selenodeiodinases. *Journal of Biological Chemistry* **280** 11093–11100. (doi:10.1074/jbc.M500011200)

Li W, Zha J, Li Z, Yang L & Wang Z 2009 Effects of exposure to acetochlor on the expression of thyroid hormone related genes in larval and adult rare minnow (*Gobiocypris rarus*). *Aquatic Toxicology* **31** 87–93. (doi:10.1016/j.aquatox.2009.06.002)

Li W, Zha J, Yang L, Li Z & Wang Z 2011*a* Regulation of thyroid hormone related genes mRNA expression by exogenous T_3 in larvae and adult Chinese rare minnow (*Gobiocypris rarus*). *Environmental Toxicology and Pharmacology* **31** 189–197. (doi:10.1016/j.etap.2010.10.007)

Li R, Hu Y, Ni Y, Xia D, Grossmann R & Zhao R 2011*b* Leptin stimulates hepatic activation of thyroid hormones and promotes early posthatch growth in the chicken. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology* **160** 200–206. (doi:10.1016/j.cbpa.2011.06.001)

Lorenz C, Opitz R, Lutz I & Kloas W 2009 Corticosteroids disrupt amphibian metamorphosis by complex modes of action including increased prolactin expression. *Comparative Biochemistry and Physiology. Toxicology & Pharmacology* **150** 14–21. (doi:10.1016/j.cbpc.2009.05.013)

Lu J & Holmgren A 2009 Selenoproteins. *Journal of Biological Chemistry* **284** 723–727. (doi:10.1074/jbc.R800045200)

Luna M, Guzmán G, Navarro L, de la Peña SS & Valverde-R C 1995 Circadian rhythm of type II 5′deiodinase activity in the rat hypothalamic–pituitary–adrenal axis. *Endocrine* **8** 597–601. (doi:10.1007/BF02953025)

Marlatt VL, Gerrie E, Wiens S, Jackson F, Moon TW & Trudeau VL 2012 Estradiol and triiodothyronine differentially modulate reproductive and thyroidal genes in male goldfish. *Fish Physiology and Biochemistry* **38** 283–296. (doi:10.1007/s10695-011-9506-z)

Martínez LM, Orozco A, Villalobos P & Valverde-R C 2008 Cloning and characterization of a type 3 iodothyronine deiodinase (D3) in the liver of the chondrichtyan *Chiloscyllium punctatum*. *General and Comparative Endocrinology* **156** 464–469. (doi:10.1016/j.ygcen.2008.02.012)

Mebis L & Van den Berghe G 2009 The hypothalamus–pituitary thyroid axis in critical illness. *Netherlands Journal of Medicine* **67** 332–340.

Mohácsik P, Zeöld A, Bianco AC & Gereben B 2011 Thyroid hormone and the neuroglia: both source and target. *Journal of Thyroid Research* **2011** 215718.

Morvan Dubois G, Sebillot A, Kuiper GG, Verhoelst CH, Darras VM, Visser TJ & Demeneix BA 2006 Deiodinase activity is present in *Xenopus laevis* during early embryogenesis. *Endocrinology* **147** 4941–4949. (doi:10.1210/en.2006-0609)

Ng L, Goodyear RJ, Woods CA, Schneider MJ, Diamond E, Richardson GP, Kelley MW, St Germain DL, Galton VA & Forrest D 2004 Hearing loss and retarded cochlear development in mice lacking type 2 iodothyronine deiodinase. *PNAS* **101** 3474–3479. (doi:10.1073/pnas.0307402101)

Nobel S, Abrahmsen L & Opperman U 2001 Metabolic conversion as a pre-receptor control machanism for lipophilic hormones. *European Journal of Biochemistry* **268** 4113–4125. (doi:10.1046/j.1432-1327.2001.02359.x)

Noyes PD, Hinton DE & Stapleton HM 2011 Accumulation and debromination of decabromodiphenyl ether (BDE-209) in juvenile fathead minnows (*Pimephales promelas*) induces thyroid disruption and liver alterations. *Toxicological Sciences* **122** 265–274. (doi:10.1093/toxsci/kfr105)

Orozco A & Valverde-R C 2005 Thyroid hormone deiodination in fish (review). *Thyroid* **15** 799–813. (doi:10.1089/thy.2005.15.799)

Orozco A, Silva JE & Valverde-R C 1997 Rainbow trout liver expresses two iodothyronine phenolic deiodinase pathways with the characteristics of mammalian types I and II 5′deiodinases. *Endocrinology* **138** 254–258. (doi:10.1210/en.138.1.254)

Orozco A, Linser PJ & Valverde-R C 2000 Kinetic characterization of outer-ring deiodinase activity (ORD) in the liver, gill and retina of *Fundulus heteroclitus*. *Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology* **126** 283–290. (doi:10.1016/S0305-0491(00)00186-3)

Orozco A, Jeziorski MC, Linser PJ, Greenberg RM & Valverde-R C 2002 Cloning of the gene and complete cDNA encoding a type 2 deiodinase in *Fundulus heteroclitus*. *General and Comparative Endocrinology* **128** 162–167. (doi:10.1016/S0016-6480(02)00071-0)

Orozco A, Villalobos P, Jeziorski MC & Valverde-R C 2003 The liver of *Fundulus heteroclitus* expresses deiodinase type 1 mRNA. *General and Comparative Endocrinology* **130** 84–91. (doi:10.1016/S0016-6480(02)00570-1)

Palioura S, Sherrer RL, Steitz TA, Söll D & Simonovic M 2009 The human SepSecS–tRNASec complex reveals the mechanism of selenocysteine formation. *Science* **325** 321–325. (doi:10.1126/science.1173755)

Paris M, Brunet F, Markov GV, Schubert M & Laudet V 2008 The amphioxus genome enlightens the evolution of the thyroid hormone signaling pathway. *Development Genes and Evolution* **218** 667–680. (doi:10.1007/s00427-008-0255-7)

Paris M, Hillenweck A, Bertrand S, Delous G, Escriva H, Salko D, Cravedi J-P & Laudet V 2010 Active metabolism of thyroid hormone during metamorphosis in amphioxus. *Integrative and Comparative Biology* **50** 63–74. (doi:10.1093/icb/icq052)

Picard-Aitken M, Fournier H, Pariseau R, Marcogliese DJ & Cyr DG 2007 Thyroid disruption in walleye (*Sander vitreus*) exposed to environmental contaminants: cloning and use of iodothyronine deiodinases as molecular biomarkers. *Aquatic Toxicology* **83** 200–211. (doi:10.1016/j.aquatox.2007.04.004)

Revel FG, Saboureau M, Pévet P, Mikkelsen JD & Simonneaux V 2006 Melatonin regulates type 2 deiodinase gene expression in the Syrian hamster. *Endocrinology* **147** 4680–4687. (doi:10.1210/en.2006-0606)

Rodríguez EM, Blázquez JL & Guerra M 2010 The design of barriers in the hypothalamus allows the median eminence and the arcuate nucleus to enjoy private milieus: the former opens to the portal blood and the latter to the cerebrospinal fluid. *Peptides* **31** 757–776. (doi:10.1016/j.peptides.2010.01.003)

Sagar GD, Gereben B, Callebaut I, Mornon JP, Zeöld A, Curcio-Morelli C, Harney JW, Luongo C, Mulcahey MA, Larsen PR *et al*. 2008 The thyroid hormone-inactivating deiodinase functions as a homodimer. *Molecular Endocrinology* **22** 1382–1393. (doi:10.1210/me.2007-0490)

Sanders JP, van der Geyten S, Kaptein E, Darras VM, Kühn ER, Leonard JL & Visser TJ 1997 Characterization of a propilthiouracil-insensitive type I iodothyronine deiodinase. *Endocrinology* **138** 5153–5160. (doi:10.1210/en.138.12.5153)

Schneider MJ, Fiering SN, Pallud SE, Parlow AF, St Germain DL & Galton VA 2001 Targeted disruption of the type 2 selenodeiodinase gene (*DIO2*) results in a phenotype of pituitary resistance to T_4. *Molecular Endocrinology* **15** 2137–2148. (doi:10.1210/me.15.12.2137)

Schneider M, Fiering S, Thai B, Wu SY, St Germain E, Parlow AF, St Germain D & Galton A 2006 Targeted disruption of the type 1 selenodeiodinase gene (*Dio1*) results in marked changes in thyroid hormone economy in mice. *Endocrinology* **147** 580–589. (doi:10.1210/en.2005-0739)

Shepherdley CA, Daniels CB, Orgeig S, Richardson SJ, Evans BK & Darras VM 2002*a* Glucocorticoids, thyroid hormones, and iodothyronine deiodinases in embryonic saltwater crocodiles. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **283** R1155–R1163. (doi:10.1152/ajpregu.00015.2002)

Shepherdley CA, Richardson SJ, Evans BK, Kühn ER & Darras VM 2002*b* Thyroid hormone deiodinases during embryonic development of the saltwater crocodile (*Crocodylus porosus*). *General and Comparative Endocrinology* **126** 153–164. (doi:10.1006/gcen.2002.7786)

Shepherdley CA, Klootwijk W, Makabe KW, Visser TJ & Kuiper GG 2004 An ascidian homolog of vertebrate iodothyronine deiodinases. *Endocrinology* **145** 1268. (doi:10.1210/en.2003-1248)

Solís-S JC, Orozco A, García-G C, Robles-Osorio L & Valverde-R C 2011 Bioactivity of thyroid hormones. Clinical significance of membrane transporters, deiodinases and nuclear receptors. *Revista de Investigación Clínica* **63** 287–308.

St Germain D & Galton VA 1997 The deiodinase family of selenoproteins. *Thyroid* **7** 655–668. (doi:10.1089/thy.1997.7.655)

St Germain DL, Galton VA & Hernandez A 2009 Minireview: defining the roles of the iodothyronine deiodinases: current concepts and challenges. *Endocrinology* **150** 1097–1107. (doi:10.1210/en.2008-1588)

Tata JR 2006 Amphibian metamorphosis as a model for the developmental actions of thyroid hormone. *Molecular and Cellular Endocrinology* **246** 10–20. (doi:10.1016/j.mce.2005.11.024)

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

Valverde-R C, Orozco A, Becerra A, Jeziorski MC, Villalobos P & Solis-S JC 2004 Halometabolites and cellular dehalogenase systems. An evolutionary perspective. *International Reviews of Cytology* **234** 143–199. (doi:10.1016/S0074-7696(04)34004-0)

Villalobos P, Orozco A & Valverde-R C 2010 Molecular cloning and characterization of a type 3 iodothyronine deiodinase in the pine snake *Pituophis deppei*. *General and Comparative Endocrinology* **169** 167–173. (doi:10.1016/j.ygcen.2010.08.001)

Walpita CN, Grommen SV, Darras VM & Van der Geyten S 2007 The influence of stress on thyroid hormone production and peripheral deiodination in the Nile tilapia (*Oreochromis niloticus*). *General and Comparative Endocrinology* **150** 18–25. (doi:10.1016/j.ygcen.2006.07.002)

Walpita CN, Crawford AD & Darras VM 2010 Combined antisense knockdown of type 1 and type 2 iodothyronine deiodinases disrupts embryonic development in zebrafish (*Danio rerio*). *General and Comparative Endocrinology* **166** 134–141. (doi:10.1016/j.ygcen.2009.09.011)

Wambiji N, Park YJ, Kim SJ, Hur SP, Takeuchi Y & Takemura A 2011 Expression of type II iodothyronine deiodinase gene in the brain of a tropical spinefoot, *Siganus guttatus*. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology* **160** 447–452. (doi:10.1016/j.cbpa.2011.03.023)

Warner MH & Beckett GJ 2010 Mechanisms behind the nonthyroidal illness syndrome: an update. *Journal of Endocrinology* **205** 1–13. (doi:10.1677/JOE-09-0412)

Watanabe T, Yamamura T, Watanabe M, Yasuo S, Nakao N, Dawson A, Ebihara S & Yoshimura T 2007 Hypothalamic expression of thyroid hormone-activating and -inactivating enzyme genes in relation to photorefractoriness in birds and mammals. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **292** R568–R572. (doi:10.1152/ajpregu.00521.2006)

Williams GR & Duncan Bassett JH 2011 Deiodinases: the balance of thyroid hormone. Local control of thyroid hormone action: role of type 2 deiodinase. *Journal of Endocrinology* **209** 261–272. (doi:10.1530/JOE-10-0448)

Wu T, Shi X, Zhou Z, Wang L, Wang M, Wang L, Huang M, Yang C & Song L 2012 An iodothyronine deiodinase from *Chlamys farreri* and its induced mRNA expression after LPS stimulation. *Fish & Shellfish Immunology* **33** 286–293. (doi:10.1016/j.fsi.2012.05.011)

Yasuo S & Yoshisura T 2009 Comparative analysis of the molecular basis of photoperiodic signal transduction in vertebrates. *Integrative and Comparative Biology* **49** 507–518. (doi:10.1093/icb/icp011)

Yasuo S, Yoshimura T, Ebihara S & Korf HW 2007 Temporal dynamics of type 2 deiodinase expression after melatonin injections in Syrian hamsters. *Endocrinology* **148** 4385–4392. (doi:10.1210/en.2007-0497)

Yoshimura T, Yasuo S, Watanabe M, Iigo M, Yamamura T, Hirunagi K & Ebihara S 2003 Light-induced hormone conversion of T_4 to T_3 regulates photoperiodic response of gonads in birds. *Nature* **426** 178–181. (doi:10.1038/nature02117)

Yun A, Lee PY, Bazar KA, Daniel SM & Doux JD 2005 The incorporation of iodine in thyroid hormone may stem from its role as a prehistoric signal of ecologic opportunity: an evolutionary perspective and implications for modern diseases. *Medical Hypotheses* **65** 804–810. (doi:10.1016/j.mehy.2005.02.007)

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