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.


Introduction

Thyroidal systems (TS) occur exclusively in vertebrates and seem to have evolved by the selection and functional diversification of iodine, the rarest 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 (T4) whose synthesis is restricted to the thyroid gland. The main physiological role of T4 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 stereospecific and sequential removal of iodine atoms from the pro-hormone T4, generating active and inactive isomers of both triiodothyronine (T3) and diiodothyronine (T2). 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 T4 to T3. The inactivating or inner ring-deiodinating pathway (IRD) is
catalyzed primarily by D3, which converts T4 and T3 into inactive metabolites (reverse T3 (rT3) and 3,3',5'-T2 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.

**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. 1991). Furthermore, deiodinase transcription requires an ancient and very complex trans-acting machinery; the mechanisms involved in this process were recently reviewed (Lu & Holmgren 2009, Paloura 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 SecCys-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 all 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 relocation to the cell surface with a consequent acute inactivation of circulating T4 and T3 (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

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<th>Function and evolution of deiodinases</th>
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<td>Physiological demands</td>
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<tr>
<td>Hyper-OE</td>
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<td>EG (liver, kidney)</td>
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<td>Hypo-OE (liver)</td>
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<td>Fasting (liver)</td>
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<td>PM</td>
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<td>GC (M liver)</td>
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<td>UVA exposure (tail)</td>
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<td>GC (liver)</td>
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<td>GA (embryonic liver and kidney)</td>
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<td>GH</td>
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<td>Fasting (brain)</td>
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<td>2/3 EG (liver)</td>
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<td>Fasting</td>
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Substrate regulation

Hyperthyroidism

Down-regulators | GC, GC + T3, PRL (embryonic kidney) | T4, TSH (thyroid) | DEX (brain) | DEX (liver) |
| No data | No data | No data | No data | No data |

Hypothyroidism

No data | No data | No data | No data | No data |

Other regulators

Up-regulators | BDE-209 (embryo) | BDE-209 (embryo) | T4, TSH (thyroid) | T4, TSH (thyroid) |
| No data | No data | No data | No data |

Down-regulators | DEX (liver) | DEX (liver) | DEX (liver) | DEX (liver) |
| No data | No data | No data | No data |

Other regulators

Up-regulators | Leptin | RA | GH | GH |
| No data | No data | No data | No data |

Down-regulators | Acetichlor (larvae) | Acetichlor (larvae) | Acetichlor (larvae) | Acetichlor (larvae) |
| No data | No data | No data | No data |

Physiological demands

Up-regulators | Hypo-OE | Late EG | Pro-M to EC | Pro-M to EC |
| No data | No data | No data | No data |

Down-regulators | Hyper-OE (liver) | Post-M | Hyper-OE (liver) | Hyper-OE (liver) |
| No data | No data | No data | No data |

Down-regulators | Hypo-OE (liver) | Hypo-OE (liver) | Hypo-OE (liver) | Hypo-OE (liver) |
| No data | No data | No data | No data |

Function and evolution of deiodinases - A. Orozco and others
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 T3 to T3 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 T3 levels (reviewed in Darras et al. 2006 and Gereben et al. 2008). By contrast, D1 function in teleostean and amphibian T3 plasma regulation is less clear (Finnson et al. 1999, Kuiper et al. 2006). Another indication for a different function might be that sulfated iodothyronines (rT3S, T3S, and T4S) 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 gillhead 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 to 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 T3 pool, the enzyme recycles iodine and operates as a scavenger, clearing plasma rT3 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 hyper-thyroidism 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 T3 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. Shephardley et al. 2002b, 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 T4 to T3 and T3 to 3,5-T2 (Fig. 1). The enzyme is considered to be the critical homeostatic T3-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 tanyocytes 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 T3 in the rat cerebral cortex (Guadiano-Ferraz et al. 1997, Rodríguez et al. 2010, Mohaácsik 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 tanyocytes 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 T4 and TSH serum levels, accompanied by a resistance of pituitary TSH to T4. 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 T4 to rT3 and T3 to 3,3'-T2, 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 prey. 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 T3 after feeding (Martı́nez 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 Solı́s-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 T4- or T3-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, T4, and in experimental situations, to rT3 or even T3. This down-regulation of D2 activity by substrate is a rapid and potent regulatory feedback loop that efficiently controls T3 production and intracellular T3 concentration based on the availability of T4. In this regard, experimental data suggest that
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-T2 regulates the activity and expression of D2 (García-G et al. 2004, 2007). Little is known about the kinetics of 3,5-T2 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 T3 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 T3 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) T3 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 T3 (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, 2011a)). 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 of the D enzymes. 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).

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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, 2011a)). Examples of the regulatory effects of some thyroid disruptors on deiodinase expression are also included in Table 1.

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 roretzi, 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 rT₃ 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 T₄, T₃, or rT₃; is not inhibited by PTU; and specifically catalyzes the IRD of thyroacetic acid metabolites of T₄ and T₃ (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
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, 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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