

## REVIEW

# Iodothyronine deiodinase structure and function: from ascidians to humans

Veerle M Darras and Stijn L J Van Herck

Animal Physiology and Neurobiology Section, Department of Biology, Laboratory of Comparative Endocrinology, KU Leuven, Naamsestraat 61, PO Box 2464, B-3000 Leuven, Belgium

(Correspondence should be addressed to V M Darras; Email: veerle.darras@bio.kuleuven.be)

### Abstract

Iodothyronine deiodinases are important mediators of thyroid hormone (TH) action. They are present in tissues throughout the body where they catalyse 3,5,3'-triiodothyronine (T<sub>3</sub>) production and degradation via, respectively, outer and inner ring deiodination. Three different types of iodothyronine deiodinases (D1, D2 and D3) have been identified in vertebrates from fish to mammals. They share several common characteristics, including a selenocysteine residue in their catalytic centre, but show also some type-specific differences. These specific characteristics seem very well conserved for D2 and D3, while D1 shows more evolutionary diversity related to its *K<sub>m</sub>*, 6-*n*-propyl-2-thiouracil sensitivity and dependence on dithiothreitol as a cofactor *in vitro*. The three deiodinase types have an impact on systemic T<sub>3</sub> levels and they all contribute directly or indirectly to intracellular T<sub>3</sub> availability in different tissues. The relative contribution

of each of them, however, varies amongst species, developmental stages and tissues. This is especially true for amphibians, where the impact of D1 may be minimal. D2 and D3 expression and activity respond to thyroid status in an opposite and conserved way, while the response of D1 is variable, especially in fish. Recently, a number of deiodinases have been cloned from lower chordates. Both urochordates and cephalochordates possess selenodeiodinases, although they cannot be classified in one of the three vertebrate types. In addition, the cephalochordate amphioxus also expresses a non-selenodeiodinase. Finally, deiodinase-like sequences have been identified in the genome of non-deuterostome organisms, suggesting that deiodination of externally derived THs may even be functionally relevant in a wide variety of invertebrates.

*Journal of Endocrinology* (2012) **215**, 189–206

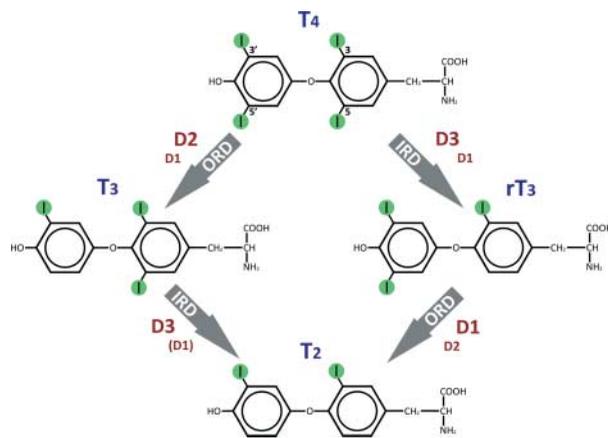
### Introduction

Thyroid hormones (THs) are involved in a wide variety of processes in developing as well as adult vertebrates. The majority of these actions are mediated by nuclear TH receptors, which are ligand-dependent transcription factors. TH receptors have a high affinity for 3,5,3'-triiodothyronine (T<sub>3</sub>) and a lower affinity for 3,5,3',5'-tetraiodothyronine or thyroxine (T<sub>4</sub>), which is the major TH secreted by the thyroid gland. T<sub>4</sub> can be converted into T<sub>3</sub> by a process called (mono-) deiodination, occurring predominantly in extra-thyroidal tissues. Evidence for this type of peripheral conversion was found already in 1950 following injection of radiolabelled T<sub>4</sub> into the blood stream of rats (Gross & Leblond 1950).

It took until the 1970s to study the reaction mechanisms of deiodination in more detail. These studies, performed mainly *in vitro* on rat tissues, eventually led to the characterisation of three different types of activity and the corresponding

enzymes were defined as type 1, type 2 and type 3 iodothyronine deiodinase (D1, D2 and D3). Two of these enzyme types are selective: D2 only catalyses outer ring deiodination (ORD) while D3 only catalyses inner ring deiodination (IRD). The D1 enzyme is non-selective and catalyses both ORD and IRD (Fig. 1). Additional properties distinguishing D1 from D2 and D3 are its sensitivity to inhibition by 6-*n*-propyl-2-thiouracil (PTU), the relatively high *K<sub>m</sub>* for its preferred substrate T<sub>3</sub> (3,5,3'-triiodothyronine) and reverse T<sub>3</sub> or rT<sub>3</sub> (3,3',5'-triiodothyronine) and the ping-pong kinetics of the reaction (Leonard & Visser 1986).

In the following years, a multitude of kinetic studies confirmed the presence of more or less similar types of deiodinating activities in other mammals as well as in birds and reptiles (Rudas 1986, Galton & Hiebert 1987, Rudas *et al.* 1993, Hugenberger & Licht 1999, Fenton & Valverde 2000, Shepherdley *et al.* 2002). The situation in amphibians and fish seemed to be different as no high *K<sub>m</sub>* D1-like



**Figure 1** Major pathways of thyroid hormone deiodination. D1, D2, D3, deiodinase types 1, 2 and 3.

activity could be detected in amphibians and most fishes studied at that time, with the exception of tilapia. Tilapia kidney catalysed ORD of rT<sub>3</sub> with a relatively high *K<sub>m</sub>*, but the activity did not seem to be inhibited by PTU (Mol *et al.* 1993).

Differences were also observed in the temperature dependence of the deiodinating activities between endotherm and exotherm vertebrates. The optimum for T<sub>4</sub> and/or rT<sub>3</sub> ORD was rather constant and high (around 37 °C) for mammals and birds but varied in fish from low (25–30 °C) for rainbow trout and turbot, living in cold water, up to high (37 °C) for tilapia and the catfish *Clarias garipepinus*, living in warm water. The optimum for IRD of T<sub>3</sub> was more uniform around 30–37 °C (Mol *et al.* 1997, 1998). ORD activities in the liver of reptiles remained stable over a broad temperature range, as found in the turtle *Trachemys scripta* (28–37 °C) and the lizard *Scleroponus grammicus* (15–42 °C) (Hugenberger & Licht 1999, Fenton & Valverde 2000).

It took until 1991 before the first iodothyronine deiodinase, rat D1, was cloned and identified as a selenocysteine (Sec)-containing protein (Berry *et al.* 1991). From that time onwards, a variety of deiodinases from different species have been cloned and some recombinant proteins have been functionally characterised. These data provide more insight into the evolution of the structure and function of this group of enzymes and have challenged part of our initial view on the differences between the three types of deiodinases. Although they can be found in all vertebrate classes, their structure and maybe more importantly their tissue distribution show some interesting variations that have an impact on their biological function. In the present review we will provide a comparative view on the structure and function of the iodothyronine deiodinases known so far. For clarity, we will use throughout the text *dio1*, *dio2* and *dio3* for the vertebrate deiodinase genes (always with lower case letters when referring to multiple species) and D1, D2 and D3 for the deiodinase proteins.

## Structure and activity of deiodinases

### Common characteristics of the three types of vertebrate deiodinases

All vertebrate deiodinases that have been cloned so far are selenoproteins with a Sec in their catalytic centre. Sec is encoded by UGA which normally functions as a stop codon. Therefore, all deiodinases contain a selenocysteine insertion sequence (SECIS) element in the 3'-UTR of the mRNA. The SECIS element forms a stem-loop structure necessary to interact with components of the translational machinery (selenocysteyl-tRNA and elongation factor; Berry *et al.* 1993). The structure and function of the SECIS elements present in deiodinases are discussed in more detail in other reviews, e.g. Bianco *et al.* (2002) and Gereben *et al.* (2008).

The three types of deiodinases belong to the thioredoxin fold superfamily and share a similar structural organisation. They are integral membrane proteins that function as homodimers, although probably only one monomer partner is required for catalytic activity (Bianco & Larsen 2005). The hydrophobic transmembrane region of the enzymes is located in the N-terminal domain. Their active site is oriented towards the cytoplasm and its core sequence is very well conserved in all known deiodinases (Gereben *et al.* 2008). A second highly conserved 16 amino acid (AA) region located in the C-terminal half of the protein, containing one of the two conserved His residues, seems to be important for the formation of the homodimers (Leonard *et al.* 2005). The Sec residue in the catalytic centre plays an essential role in deiodinase activity. Substitution of this Sec by Cys results in a drop of catalytic activity by two or three orders of magnitude, whereas substitution by Leu or Ala completely abolishes activity (Gereben *et al.* 2008). Information on the three-dimensional conformation of deiodinases is limited due to the lack of soluble, catalytically active protein for crystallisation purposes, but hydrophobic cluster analysis of the deiodinase sequences compared with other members of the thioredoxin superfamily allowed to propose a general model (Callebaut *et al.* 2003). It shows a protein composed of a single aminoterminal-anchoring segment, a short hinge region, and a thioredoxin fold-containing globular domain as described in more detail elsewhere (Callebaut *et al.* 2003, Bianco & Larsen 2005, Bianco & Kim 2006).

### Structure and activity of D1

The biochemical characteristics of the D1 enzyme were originally determined using rat liver homogenates and microsomes. D1 was described as a high *K<sub>m</sub>* non-selective enzyme that catalysed both ORD and IRD reactions. Its efficiency for ORD of rT<sub>3</sub> was more than 100-fold higher than for ORD of T<sub>4</sub>, while sulphation of T<sub>4</sub> and T<sub>3</sub> strongly increased IRD efficiency (Leonard & Visser 1986). *In vitro* deiodination depended on the presence of a reducing cofactor such as dithiothreitol (DTT). The enzyme showed ping-pong kinetics and was strongly inhibited by PTU (100% by

0.05 mM PTU). This inhibition was uncompetitive with substrate and competitive with cofactor. Enzyme activity was also inhibited by iodoacetate (IAc) and iopanoic acid (IOP) (Kobayashi *et al.* 1985, Leonard & Visser 1986).

Since then, D1 has been cloned from a wide variety of vertebrate species and presently information of partial/complete nucleotide coding sequences for D1 from ~70 vertebrate species is available from different databases. The structure of the gene encoding D1, *dio1*, has been described for mouse (Maia *et al.* 1995a) and human (Toyoda *et al.* 1996, Jakobs *et al.* 1997a). The gene consists of four exons, which seems to be the case for all species available in the Gene database at the NCBI website. The TGA (Sec) codon is located in exon 2, while the TAG (stop) codon and the SECIS element are located in exon 4. The 5'-flanking region (5'-FR) of the mouse and human *dio1* genes is GC rich and contains no TATA or CAAT boxes (Maia *et al.* 1995a, Jakobs *et al.* 1997b). The human gene also contains two thyroid hormone response elements (TREs), but, although mouse and rat D1 mRNAs are also increased by T<sub>3</sub>, canonical TREs have not yet been identified in the 5'-FR of these genes (Bianco *et al.* 2002). Splice variants have been described for rat D1 mRNA differing in the length of the 3'-UTR (Navarro *et al.* 1997). Quite recently, a D1 mRNA splice variant has also been found in a fish, the walleye (*Sander vitreus*), containing an 11-bp insert downstream of the codon for Sec. The resulting protein, if translated, would however not be homologous to other D1s due to a frame shift (Picard-Aitken *et al.* 2007).

Comparison of the full coding sequence of different D1s shows that the D1 protein is fairly constant in length consisting of 244–257 AAs (Table 1). The Sec is situated around position 121–130. Functional characterisation of the recombinant D1 protein has only been done for a limited number of species. The data available for mammals show that the Km for ORD of rT<sub>3</sub> is up to two orders of magnitude higher in dog and cat compared with human, rat, pig and house shrew (Table 1). In contrast to the lower efficiency for ORD of rT<sub>3</sub>, the efficiency of these enzymes for deiodination of T<sub>4</sub> and T<sub>3</sub> is quite similar to that in human or rat (Toyoda *et al.* 1997, Kuiper *et al.* 2003).

Sequence comparison of the proteins from human, rat, dog and cat shows that various differences are concentrated in the region between AA residues 40 and 70. Both in dog and cat the AA residues present at position 48–52, in human and rat D1s are missing. Other differences are found in the AA residues corresponding to positions 45–46 and 65–66 in the human sequence (Fig. 2). Mutagenesis of the dog and cat sequences showed that the insertion of Thr-Gly-Met-Thr-Arg (human 48–52) alone did not improve deiodination of rT<sub>3</sub>. For the cat enzyme, a combination of mutations including the insertion of those five AA as well as AA substitutions placing Gly-Glu and Phe at the positions 45–46 and 65 was necessary to improve the ORD of rT<sub>3</sub> to the level typical for human D1. In dog, the most efficient mutation was the replacement of Leu-Tyr by Phe-Phe at the position

corresponding to 65–66 in the human sequence. Opposite mutations in the human sequence indicated that deletion of the 48–52 sequence increased the Km for rT<sub>3</sub> ORD only twofold, while changing Phe at position 65 into Leu increased the Km tenfold (Toyoda *et al.* 1994, Kuiper *et al.* 2003). The conclusion of the comparative study of these enzymes was that the Phe at position 65 found in most D1 sequences (Fig. 2) is particularly important for ORD of rT<sub>3</sub> but not for ORD of iodothyronines with two iodines on the inner ring, possibly due to interaction of the aromatic ring of Phe (or Tyr in killifish) with the mono-substituted inner ring of rT<sub>3</sub> (Toyoda *et al.* 1997).

Only one full D1 coding sequence has been cloned from birds and none from reptiles. The chicken AA sequence shows 61% homology with the human sequence (Fig. 2) and functional studies of the recombinant protein in cell culture revealed identical substrate specificity and sensitivity to inhibitors as the human and rat enzyme (Van der Geysen *et al.* 1997).

The cloning of the first fish D1 from tilapia (*Oreochromis niloticus*) in 1997 was an important milestone as it showed that one of the major characteristics originally defined for D1, namely its high sensitivity to inhibition by PTU, is not a conserved feature (Sanders *et al.* 1997). Tilapia D1 shows 47% AA sequence homology with human D1 (Fig. 2); it also has a Sec in its active site and has a similar substrate preference. It is, however, insensitive to PTU (only 5% inhibition by 1 mM PTU) and also approximately tenfold less sensitive to IAc and aurothioglucose (ATG) than rat and human D1 (Sanders *et al.* 1997). A few years later, killifish (*Fundulus heteroclitus*) D1 was cloned and expressed, showing also low sensitivity to PTU (only 10% inhibition by 1 mM PTU; Orozco *et al.* 2003).

Characterisation of fish D1 proteins has led to more surprises. Sea bream (*Sparus auratus*) kidney and liver contain high Km rT<sub>3</sub> ORD activity that is inhibited by DTT, the artificial cofactor that stimulates *in vitro* activity of all other deiodinases characterised so far. In the absence of DTT, the Km for rT<sub>3</sub> is 5 µM, which is close to the 2 µM described for tilapia in the presence of DTT (Klaren *et al.* 2005). The cDNA cloned from sea bream kidney confirmed the presence of a D1 protein with a catalytic centre identical to that of tilapia. The Phe at position 65 and the Gly-Glu at position 45–46, important for ORD activity in mammals, are also conserved. It was suggested that the substitution of some conserved positively charged AA residues from the N-terminal hydrophobic domain of mammalian D1 by uncharged polar or non-polar residues in sea bream D1 may be linked to its divergent interaction with DTT (Klaren *et al.* 2005), but this hypothesis remains to be tested. Interestingly, the low level of rT<sub>3</sub> ORD measured in sea bream kidney homogenates in the presence of DTT is almost insensitive to PTU, while the higher activity level in the absence of DTT is about 68% inhibited by 0.1 mM PTU. This finding shows some similarity with the fact that PTU is only efficient in blocking mammalian or chicken D1 activity when there

**Table 1** Vertebrate species for which the full coding sequence of D1 is available in GenBank. Information on PTU sensitivity and  $K_m$  for ORD of  $rT_3$  by the recombinant proteins has been taken from 1, Toyoda *et al.* (1994); 2, Berry *et al.* (1991); 3, Wassen *et al.* (2004); 4, Kuiper *et al.* (2003); 5, Rogatcheva *et al.* (2002); 6, Van der Geyten *et al.* (1997); 7, Kuiper *et al.* (2006); 8, Klaren *et al.* (2005); 9, Sanders *et al.* (1997); and 10, Orozco *et al.* (2003)

| Species                       | Common name         | GenBank ID (mRNA) | Number of AAs | PTU sensitive | $K_m$ (ORD $rT_3$ )        |
|-------------------------------|---------------------|-------------------|---------------|---------------|----------------------------|
| Mammals                       |                     |                   |               |               |                            |
| <i>Homo sapiens</i>           | Human               | NM_000792         | 249           | +             | 0.32 $\mu$ M (1)           |
| <i>Pan troglodytes</i>        | Chimpanzee          | NM_001122651      | 249           | +             |                            |
| <i>Pongo abelii</i>           | Orangutan           | XM_002810800      | 249           | +             |                            |
| <i>Macaca mulatta</i>         | Rhesus monkey       | NM_001122652      | 249           | +             |                            |
| <i>Oryctolagus cuniculus</i>  | Rabbit              | NM_001099958      | 249           | +             |                            |
| <i>Cavia porcellus</i>        | Guinea pig          | NM_001257974      | 249           | +             |                            |
| <i>Mus musculus</i>           | Mouse               | NM_007860         | 257           | +             |                            |
| <i>Rattus norvegicus</i>      | Rat                 | NM_021653         | 257           | +             | 0.13 $\mu$ M (2)           |
| <i>Cricetulus griseus</i>     | Chinese hamster     | NM_001256759      | 257           | +             |                            |
| <i>Sus scrofa</i>             | Pig                 | NM_001001627      | 249           | +             | 0.17 $\mu$ M (3)           |
| <i>Bos taurus</i>             | Cattle              | NM_001122593      | 249           | +             |                            |
| <i>Bubalus bubalis</i>        | Water buffalo       | JQ791197          | 249           | +             |                            |
| <i>Equus caballus</i>         | Horse               | NM_001166452      | 244           | +             |                            |
| <i>Canis familiaris</i>       | Dog                 | NM_001007126      | 244           | +             | 9 $\mu$ M (1)              |
| <i>Felis catus</i>            | Cat                 | NM_001009267      | 244           | +             | 11 $\mu$ M (4)             |
| <i>Suncus murinus</i>         | Asian house shrew   | AB055517          | 257           | +             | 0.14 $\mu$ M (5)           |
| Birds                         |                     |                   |               |               |                            |
| <i>Gallus gallus</i>          | Chicken             | NM_001097614      | 246           | +             | 0.26 $\mu$ M (6)           |
| Amphibians                    |                     |                   |               |               |                            |
| <i>Xenopus tropicalis</i>     | Western clawed frog | NM_001256297      | 252           | –             |                            |
| <i>Xenopus laevis</i>         | African clawed frog | NM_001095667      | 252           | –             | 0.3 $\mu$ M (7)            |
| Teleost fishes                |                     |                   |               |               |                            |
| <i>Paralichthys olivaceus</i> | Japanese flounder   | AB362421          | 248           | –             |                            |
| <i>Sparus aurata</i>          | Gilthead sea bream  | AJ619717          | 248           | ±             | 5 $\mu$ M (8) <sup>a</sup> |
| <i>Chrysiptera cyanea</i>     | Sapphire devil      | GU583740          | 248           | –             |                            |
| <i>Oreochromis niloticus</i>  | Nile tilapia        | XM_003439801      | 248           | –             | 2 $\mu$ M (9)              |
| <i>Takifugu rubripes</i>      | Japanese pufferfish | NM_001136144      | 248           | –             |                            |
| <i>Fundulus heteroclitus</i>  | Killifish/mummichog | AY184803          | 248           | –             | 0.12 $\mu$ M (10)          |
| <i>Danio rerio</i>            | Zebrafish           | NM_001007283      | 254           | –             |                            |

<sup>a</sup> $K_m$  for native protein.

is sufficient substrate turnover (Goswami & Rosenberg (1988) and own observations). It could mean that sea bream D1 can be classified as mildly PTU-sensitive, although less sensitive than mammalian D1s.

Although it had long been accepted that amphibians do not have D1, a D1 cDNA was finally cloned from the frog *Xenopus laevis* (Kuiper *et al.* 2006). The AA sequence shows 51% homology with the human D1 (Fig. 2), and the recombinant protein is a high  $K_m$  enzyme with ORD and IRD activity. In contrast to the mammalian D1s, *Xenopus* D1 catalyses ORD of  $rT_3$  and  $T_4$  with equal efficiency, and sulphation does not stimulate the IRD of  $T_3$  or the ORD of  $rT_3$ . Similar to tilapia D1, the enzyme is approximately tenfold less sensitive to inhibition by IAc and ATG than rat and human D1 and quite insensitive to PTU (only 15% inhibition by 1 mM PTU).

Several research groups have used site-directed mutagenesis in search of the factors determining PTU sensitivity. Comparison of the active site of the PTU-insensitive D1s from tilapia, killifish and *Xenopus* with that of PTU-sensitive D1s in mammals and chicken showed two consistent

substitutions: Pro instead of Ser two positions downstream of Sec, and Ser instead of Asn six positions upstream from Sec (Fig. 2). A Pro128Ser mutation of tilapia D1 showed decreased ORD activity but remained resistant to PTU (Sanders *et al.* 1997). A Ser124Asn mutation in *Xenopus* D1 also did not influence PTU sensitivity, but in this species the Pro132Ser mutation strongly increased inhibition by PTU up to the level typical for mammalian enzymes. At the same time, this mutation increased the  $K_m$  for ORD of  $T_4$  and  $rT_3$  about tenfold (Kuiper *et al.* 2006). Using the opposite approach, PTU resistance could be induced by the Ser128Pro substitution in human D1 without changing the  $K_m$  for  $rT_3$  or  $T_4$  ORD (Callebaut *et al.* 2003). Taken together, these results strongly suggest that the Ser two positions downstream of Sec plays a role in the PTU sensitivity of D1. However, the above-mentioned results for sea bream D1, showing partial PTU resistance despite the presence of Pro at this position, suggest that rather than the type of AA *per se*, differences in substrate turnover rate could also be a determining factor, a possibility already discussed for the *Xenopus* Pro132Ser mutant protein (Kuiper *et al.* 2006).



### Structure and activity of D2

D2 was originally characterised from rat brain tissue as an obligate ORD enzyme (Fig. 1) with a  $K_m$  for its preferred substrate  $T_4$  in the low nanomolar range (Kaplan & Yaskoski 1980, Visser *et al.* 1981). The enzyme showed a sequential reaction mechanism and had a high need for DTT as a cofactor *in vitro*. The activity was not inhibited by PTU, weakly inhibited by IAc and strongly inhibited by IOP (Leonard & Visser 1986).

The first D2 was cloned in 1995 from the bullfrog *Rana catesbeiana*. The mRNA sequence contained an in-frame UGA codon and a putative SECIS element in the 3'-UTR. Expression of capped RNA transcripts in *Xenopus* oocytes resulted in ORD activity with typical D2 characteristics (Davey *et al.* 1995). The open reading frame of the partial rat and human D2 cDNAs cloned 1 year later showed the presence of two in-frame TGA codons, one located in a region very similar to the active site sequence of other known deiodinases, while the other one was located not far from an unambiguous TAG stop codon (Croteau *et al.* 1996). The structure of the *dio2* gene has been described for human (Celi *et al.* 1998), mouse (Davey *et al.* 1999), killifish (Orozco *et al.* 2002) and trout (Sambroni *et al.* 2001). The *dio2* gene typically consists of two exons, separated by a single intron. Exon 2 contains the TGA(Sec) codon located at the active site and the SECIS element (Valverde *et al.* 2004). Except for bullfrog, D2 mRNA is rather long, ranging from 4.7 kb in killifish up to 8.0 kb in mouse. This is due to an extended 3'-UTR with the SECIS element positioned close to the poly(A) tail (Valverde *et al.* 2004). The human, mouse and rat *dio2* 5'-FR all contain consensus TATA and CAAT elements and a functional CRE, but only human *DIO2* has thyroid transcription factor 1 (TTF1) binding sites (Bianco *et al.* 2002). In contrast to mammals, the killifish 5'-FR does not contain TATA or CAAT boxes and no CRE sequence is present within 1.3 kb upstream of the transcription start site, which could be phylogenetically relevant for the control of *dio2* gene transcription (Valverde *et al.* 2004). The rainbow trout *dio2* gene, on the other hand, has a CAAT box and two putative TATA boxes in the promoter region, suggesting that the transcription of the *dio2* gene might be differentially controlled amongst fish species (Sambroni *et al.* 2001).

The existence of multiple splice variants has been described for human D2 mRNA (Bartha *et al.* 2000, Ohba *et al.* 2001). The human gene possibly codes for four different proteins. One is the typical deiodinase (variant a) while a longer deiodinase (variant b), containing three Sec residues, may be generated by splicing in of an additional exon located between the two typical ones. Two other variants (c and d) are truncated proteins. The long D2 variant with three Sec residues may also be present in chimpanzee, orangutan, rhesus monkey and marmoset. D2 mRNA splice variants have also been described in chicken (Gereben *et al.* 2002) and in the Australian lungfish (Sutija *et al.* 2003). The chicken splice variant cannot express a functional protein due to a shift in the

reading frame, while the lungfish splice variants only differ in their 3'-UTR and therefore would lead to identical proteins.

By now full or partial cDNA sequence information is available for ~80 vertebrate species. The length of the D2 protein typically varies between 257 and 279 AAs (Table 2), although additional longer forms may exist in primates as discussed earlier. The sequence alignment in Fig. 3A clearly illustrates that the protein structure has been highly conserved from fish up to human. One striking difference is found at the C-terminus where some sequences contain a second Sec while others do not. A more detailed comparison of the available C-terminal coding sequences clearly separates the teleost fish from the other vertebrates (Fig. 3B). At the position where the sequences of the other vertebrates (except pig and bullfrog) encode a Sec, the sequences of teleost fish have an unambiguous stop codon. This should, however, not influence their function because mutation of the second Sec codon in human D2 to a Cys codon or an unambiguous stop codon did not change the catalytic activity of the enzyme (Salvatore *et al.* 1999). It can therefore not be excluded that the primary function of the second UGA in the D2 sequence is to function as a stop codon has been recently suggested (Mariotti *et al.* 2012).

### Structure and activity of D3

D3 was first characterised from rat brain tissue and human placenta as an obligate IRD enzyme (Fig. 1; Kaplan & Yaskoski 1980, Roti *et al.* 1981). D3 showed a substrate preference for  $T_3$  in the low nanomolar range. Its *in vitro* activity required relatively high DTT concentrations, although very high concentrations (>200 mM) seemed to be detrimental. The enzyme showed sequential type reaction kinetics and was resistant to inhibition by PTU, while activity was inhibited by IAc and IOP (Leonard & Visser 1986).

The first D3 cDNA was cloned in 1994 from *X. laevis* tadpole tail (St Germain *et al.* 1994). The clone contained an in-frame Sec codon and had a SECIS element in the 3'-UTR. Expression of capped RNA transcripts in *Xenopus* oocytes confirmed that the protein catalysed IRD of  $T_3$  with a  $K_m$  of 2 nM and was resistant to PTU as well as to ATG (St Germain *et al.* 1994). One year later, two mammalian D3 cDNAs were identified from rat neonatal skin and human placenta (Croteau *et al.* 1995, Salvatore *et al.* 1995). By now partial or complete cDNA sequences are available for around 50 D3s, coding for proteins with variable length.

The AA sequence of D3 has been well conserved throughout vertebrate evolution, particularly around the active site containing the Sec (Fig. 4). The only striking difference is found at the N-terminus where most predicted mammalian D3 proteins are longer than those of non-mammalian vertebrates (Table 3). This difference is linked to the fact that the mammalian genomic *dio3* sequences include two putative transcription start sites. This may lead to proteins of respectively 304 or 278 AA long, the last one corresponding more closely to the length in non-mammalian

**Table 2** Vertebrate species for which the full coding sequence of D2 is available in GenBank. Information on the *K<sub>m</sub>* for ORD of T<sub>4</sub> by the recombinant proteins has been taken from 1, Croteau *et al.* (1996); 2, Wassen *et al.* (2004); 3, Gereben *et al.* (1999); and 4, Orozco *et al.* (2002)

| Species                       | Common name               | GenBank ID (mRNA) | Number of AAs   | <i>K<sub>m</sub></i> (ORD T <sub>4</sub> ) |
|-------------------------------|---------------------------|-------------------|-----------------|--|
| <b>Mammals</b>                |                           |                   |                 |  |
| <i>Homo sapiens</i>           | Human                     | NM_000793         | 273 (isoform a) | 1·2 nM (1)                                 |
| <i>Pan troglodytes</i>        | Chimpanzee                | NM_001122638      | 273 (isoform 2) |  |
| <i>Pongo abelii</i>           | Orangutan                 | XM_002824993      | 309             | 0·7 nM (1)                                 |
| <i>Macaca mulatta</i>         | Rhesus monkey             | NM_001159294      | 309             |  |
| <i>Callithrix jacchus</i>     | White-tufted-ear marmoset | XM_003734052      | 309             |  |
| <i>Oryctolagus cuniculus</i>  | Rabbit                    | NM_001256300      | 269             |  |
| <i>Cavia porcellus</i>        | Guinea pig                | NM_001257977      | 269             |  |
| <i>Mus musculus</i>           | Mouse                     | NM_010050         | 266             |  |
| <i>Rattus norvegicus</i>      | Rat                       | NM_031720         | 266             |  |
| <i>Cricetulus griseus</i>     | Chinese hamster           | NM_001256762      | 266             |  |
| <i>Sus scrofa</i>             | Pig                       | NM_001001626      | 265             |  |
| <i>Bos taurus</i>             | Cattle                    | NM_001010992      | 269             |  |
| <i>Equus caballus</i>         | Horse                     | NM_001166455      | 269             | 8 nM (2)                                   |
| <i>Ailuropoda melanoleuca</i> | Giant panda               | XM_002919047      | 272             |  |
| <i>Canis lupus familiaris</i> | Dog                       | NM_001122645      | 269             | 1 nM (3)                                   |
| <i>Monodelphis domestica</i>  | Grey short-tailed opossum | XM_003339485      | 268             |  |
| <b>Birds</b>                  |                           |                   |                 |  |
| <i>Gallus gallus</i>          | Chicken                   | NM_204114         | 279             | 1 nM (3)                                   |
| <i>Meleagris gallopavo</i>    | Turkey                    | XM_003206645      | 264             |  |
| <b>Reptiles</b>               |                           |                   |                 |  |
| <i>Anolis carolinensis</i>    | Green anole               | XM_003214362      | 266             |  |
| <b>Amphibia</b>               |                           |                   |                 |  |
| <i>Rana catesbeiana</i>       | Bullfrog                  | L42815            | 264             | 0·64 nM (4)                                |
| <i>Xenopus tropicalis</i>     | Western clawed frog       | NM_001197232      | 258             |  |
| <i>Xenopus laevis</i>         | African clawed frog       | AF354707          | 262             |  |
| <b>Lung fishes</b>            |                           |                   |                 |  |
| <i>Neoceratodus forsteri</i>  | Australian lungfish       | AF327438          | 269             |  |
| <b>Teleost fishes</b>         |                           |                   |                 |  |
| <i>Oncorhynchus mykiss</i>    | Rainbow trout             | NM_001124268      | 264             | 0·64 nM (4)                                |
| <i>Paralichthys olivaceus</i> | Japanese flounder         | AB362422          | 271             |  |
| <i>Siganus guttatus</i>       | Goldlined spinefoot       | GU372962          | 270             |  |
| <i>Chrysiptera cyanea</i>     | Sapphire devil            | GU583739          | 271             |  |
| <i>Takifugu rubripes</i>      | Japanese pufferfish       | NM_001136145      | 270             |  |
| <i>Fundulus heteroclitus</i>  | Mummichog/killifish       | U70869            | 266             |  |
| <i>Oryzias latipes</i>        | Japanese medaka           | NM_001136521      | 277             |  |
| <i>Danio rerio</i>            | Zebrafish                 | NM_212789         | 257             |  |

vertebrates. So far it remains unclear whether one or both protein variants occur *in vivo*. Interestingly, the sequence of wallaby, the only marsupial for which a full coding sequence is available, only shows a long variant but with a very different N-terminus compared with placental mammals (Fig. 4). Only a few recombinant D3 proteins have so far been expressed, yielding PTU-resistant, low *K<sub>m</sub>* IRD enzymes with very similar characteristics (Table 3).

All known *dio3* genes consist of a single exon, a characteristic that is rather uncommon in the eukaryotic kingdom (Hernandez *et al.* 1998, 1999). According to the NCBI Gene database, the rabbit *dio3* gene is an exception, having two putative exons, although this seems doubtful given the identity of the putative intron sequence with the missing part of the cDNA of other mammalian *DIO3* sequences. The promoter region of the mouse and human *dio3* gene contains consensus TATA, CAAT and GC elements (Hernandez *et al.* 1999). The *Dio3* gene seems to give rise to

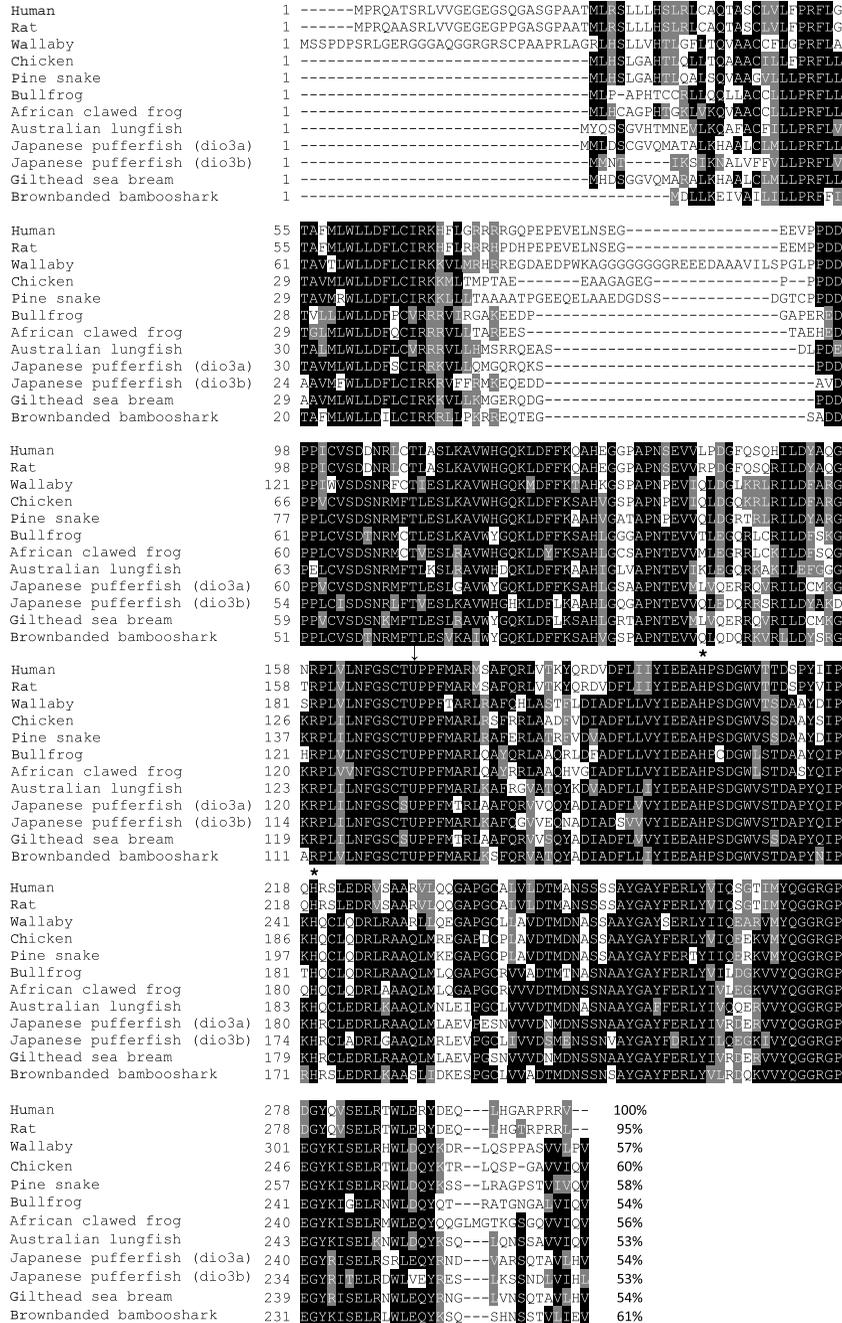
mRNA variants of different length in rat (Tu *et al.* 1999). Multiple D3 mRNA variants are also found in fish such as tilapia (Sanders *et al.* 1999) and trout (Bres *et al.* 2006), but in these species the variants may derive from two different genes. The genome databases of the pufferfishes *Takifugu* and *Tetraodon* each contain two *dio3* orthologues (Itoh *et al.* 2010) and this is probably also the case for other teleosts (Mariotti *et al.* 2012). Many genes are found to be duplicated in teleosts, a phenomenon that can be linked to a whole genome duplication that occurred in fish before the teleost radiation (Amores *et al.* 1998, Taylor *et al.* 2003).

An important discovery made in 2002 was that mouse *Dio3* is an imprinted gene (Hernandez *et al.* 2002). Genomic imprinting is an epigenetic process that causes genes to be expressed according to their parental origin. In vertebrates, its emergence is associated with the evolution of the placenta and therefore it does not occur in oviparous species (Edwards *et al.* 2008). The *dio3* gene is located in



(*Branchiostoma floridae*) and the urochordate ascidians *Ciona intestinalis*, *Ciona savignyi* and *Halocynthia roretzi*. Only one ascidian deiodinase has been fully cloned and characterised (Shepherdley *et al.* 2004). Its sequence contains a region very similar to the active centre of D1, D2 and D3, including a

UGA codon, and a SECIS element is present in the 3'-UTR. The recombinant selenoprotein (259 AA long) catalysed ORD of rT<sub>3</sub> and T<sub>4</sub> with an apparent Km around 3 µM but showed minimal IRD activity. The enzyme activity followed ping-pong type reaction kinetics, was stimulated by DTT and



**Figure 4** AA sequence alignment for some representative vertebrate D3 proteins. Identical AAs are indicated in black, similar AAs in grey. The catalytic Sec residue is indicated by the arrow and the two conserved His residues by the asterisks. Protein sequences were obtained from GenBank and aligned by ClustalW; the nucleotide accession numbers as well as genus and species names are shown in Table 3. The numbers at the end of each sequence indicate the percentage of AA identical to the human sequence.

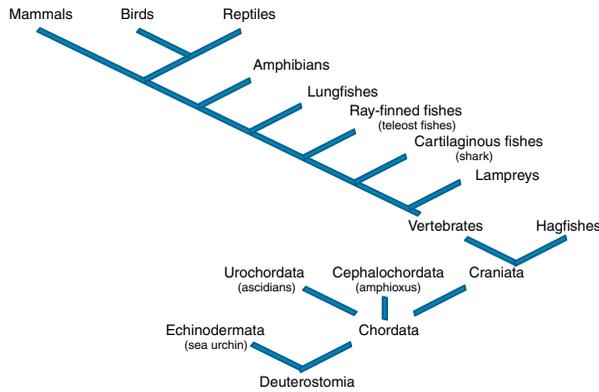
**Table 3** Vertebrate species for which the full coding sequence of D3 is available in GenBank. The two values for the number of AAs in most mammalian proteins are related to the presence of two putative translation start sites. The first number is the total length as indicated presently by GenBank and the shorter one (between brackets) the length if the translation starts at the position similar to the translation start site in non-mammalian species. Information on the  $K_m$  for IRD of  $T_3$  by the recombinant proteins has been taken from 1, Salvatore *et al.* (1995); 2, Croteau *et al.* (1995); 3, Villalobos *et al.* (2010); 4, St Germain *et al.* (1994); 5, Sanders *et al.* (1999); 6, Martinez *et al.* (2008); and 10, Orozco *et al.* (2003)

| Species                         | Common name               | GenBank ID (mRNA) | Number of AAs | $K_m$ (IRD $T_3$ ) |           |
|---------------------------------|---------------------------|-------------------|---------------|--------------------|-----------|
| <b>Mammals</b>                  |                           |                   |               |                    |           |
| <i>Homo sapiens</i>             | Human                     | NM_001362         | 304 (278)     | 1·2 nM (1)         |           |
| <i>Pongo abelii</i>             | Orangutan                 | XM_002825118      | 304 (278)     |                    |           |
| <i>Macaca mulatta</i>           | Rhesus monkey             | NM_001122649      | 304 (278)     | 1 nM (2)           |           |
| <i>Callithrix jacchus</i>       | White-tufted-ear marmoset | XM_003734077      | 304 (278)     |                    |           |
| <i>Oryctolagus cuniculus</i>    | Rabbit                    | XM_002721708      | 281 (255)     |                    |           |
| <i>Mus musculus</i>             | Mouse                     | NM_172119         | 304 (278)     |                    |           |
| <i>Rattus norvegicus</i>        | Rat                       | NM_017210         | 304 (278)     |                    |           |
| <i>Cricetulus griseus</i>       | Chinese hamster           | NM_001256784      | 304 (278)     |                    |           |
| <i>Sus scrofa</i>               | Pig                       | NM_001001625      | 305 (278)     |                    |           |
| <i>Bos taurus</i>               | Cattle                    | NM_001010993      | 301 (278)     |                    |           |
| <i>Ovis aries</i>               | Sheep                     | NM_001122650      | 278           |                    |           |
| <i>Ailuropoda melanoleuca</i>   | Giant panda               | XM_002926734      | 304 (278)     |                    |           |
| <i>Canis lupus familiaris</i>   | Dog                       | NM_001164188      | 304 (278)     | 2 nM (4)           |           |
| <i>Macropus eugenii</i>         | Wallaby                   | EU919199          | 330           |                    |           |
| <i>Ornithorhynchus anatinus</i> | Platypus                  | EU919198          | 298           |                    |           |
| <b>Birds</b>                    |                           |                   |               |                    |           |
| <i>Gallus gallus</i>            | Chicken                   | NM_001122648      | 274           | 11 nM (3)          |           |
| <i>Pituophis deppei</i>         | Pine snake                | GQ862344          | 286           |                    |           |
| <b>Reptiles</b>                 |                           |                   |               |                    |           |
| <b>Amphibians</b>               |                           |                   |               |                    |           |
| <i>Rana catesbeiana</i>         | Bullfrog                  | L41731            | 269           | 2 nM (4)           |           |
| <i>Xenopus tropicalis</i>       | Western clawed frog       | NM_001113667      | 271           |                    |           |
| <i>Xenopus laevis</i>           | African clawed frog       | NM_001087863      | 271           |                    |           |
| <b>Lung fishes</b>              |                           |                   |               |                    |           |
| <i>Neoceratodus forsteri</i>    | Australian lungfish       | AY339982          | 271           | 20 nM (5)          |           |
| <b>Teleost fishes</b>           |                           |                   |               |                    |           |
| <i>Paralichthys olivaceus</i>   | Japanese flounder         | AB362423          | 259           |                    |           |
| <i>Siganus guttatus</i>         | Goldlined spinefoot       | GU385469          | 268           |                    |           |
| <i>Sparus aurata</i>            | Gilthead sea bream        | DQ888896          | 267           |                    |           |
| <i>Halichoeres trimaculatus</i> | Three-spot wrasse         | GU385468          | 248           |                    |           |
| <i>Chrysiptera cyanea</i>       | Sapphire devil            | GU583741          | 267           |                    |           |
| <i>Oreochromis niloticus</i>    | Nile tilapia              | Y11111            | 267           |                    |           |
| <i>Takifugu rubripes</i>        | Japanese pufferfish       | NM_001136146      | 268 (Dio3a)   |                    |           |
|                                 |                           | NM_001136147      | 262 (Dio3b)   |                    |           |
| <i>Carassius auratus</i>        | Goldfish                  | EF190704          | 274           |                    |           |
| <i>Danio rerio</i>              | Zebrafish                 | NM_001177935      | 269 (dio3)    |                    |           |
|                                 |                           | NM_001256003      | 264 (dio3a)   |                    |           |
| <b>Cartilaginous fishes</b>     |                           |                   |               |                    |           |
| <i>Chiloscyllium punctatum</i>  | Brownbanded bamboo shark  | EU275162          | 259           |                    | 10 nM (6) |

was insensitive to inhibition by PTU up to 1 mM. This mixture of characteristics does not agree with one of the three known vertebrate deiodinases, and the sequence was named hrDx. The genomes of *C. intestinalis* and *C. savignyi* both contain a partial sequence homologous to this hrDx (ciDx/csDx), as well as a second homologous sequence that was named ciDy/csDy and is not present in *H. roretzi* (Shepherdley *et al.* 2004).

Several of the deiodinase-like sequences identified from the amphioxus (*B. floridae*) genome contain a UGA codon as well as putative SECIS elements, suggesting that amphioxus expresses several selenodeiodinases (Paris *et al.* 2008a). Recently three partial amphioxus deiodinase-like sequences

were compared, showing that one of them (bfDy) contained a UGC(Cys) codon instead of the UGA(Sec) codon present in bfDt and bfDx (Klootwijk *et al.* 2011). The full cDNA for this bfDy has been cloned. No SECIS element was found in the 3'-UTR, and UGA functions as a true stop codon at the end of the coding sequence. The resulting enzyme (266 AA long) did not deiodinate  $T_4$  or  $T_3$  but, surprisingly, catalysed IRD of 3,5,3'-triiodothyroacetic acid (Triac) and 3,5,3',5'-tetraiodothyroacetic acid (Tetrac) with a  $K_m$  of respectively 6·8 and 68 nM. The enzyme was stimulated by DTT, was not inhibited by PTU or IAc and was weakly inhibited by IOP (Klootwijk *et al.* 2011). Although the enzyme did not deiodinate  $T_4$  and  $T_3$ , both hormones can probably bind to its



**Figure 5** Evolutionary relationship between the major groups of Deuterostomia based on information from the taxonomy page of NCBI (<http://www.ncbi.nlm.nih.gov/taxonomy>). The position of some specific species/subdivisions discussed in this review is shown between brackets.

active centre as they were capable of inhibiting IRD of Triac. This is different from the situation in the sea squirt *H. roretzi* where neither Triac nor Tetrac could compete for the ORD of  $rT_3$  (Shepherdley *et al.* 2004). The high affinity of bfDy for Triac is very interesting from an evolutionary point of view, as it has been shown that the amphioxus homologue of the vertebrate TH receptors is stimulated by Triac and not by  $T_3$ , suggesting that Triac could be an ancient bioactive TH (Paris *et al.* 2008b, Klootwijk *et al.* 2011).

Comparison of the deduced AA sequence of the deiodinase-like sequences of amphioxus and the ascidians with that of deiodinases from some representative vertebrate species shows that the urochordate sequences cluster together in a group, clearly distinct from the three clusters of typical vertebrate D1, D2 and D3, as well as distinct from the cephalochordate sequence (Fig. 6A). Despite this clear difference in overall sequence, the core AA sequence is well conserved (Fig. 6B).

Some recent papers also report the existence of deiodinase-like sequences in non-chordate animals. One of them is a deuterostome (Fig. 5), the sea urchin *Strongylocentrotus purpuratus*, where one sequence was found containing the catalytic domain with a putative Sec (Paris *et al.* 2008a). An evolutionary study of eukaryotic selenoproteomes even identified a selenodeiodinase homologue in the unicellular slime mold *Dictyostelium discoideum* (Lobanov *et al.* 2007). Sequences possibly coding for non-selenodeiodinases have been identified in the genome of the sea anemone *Nematostella vectensis* (Klootwijk *et al.* 2011). The complete cloning and functional characterisation of these proteins, as well as of possible other non-chordate deiodinase-like proteins, will hopefully help to clarify the possible existence and function of (iodothyronine) deiodinases in these animals. It has been shown that several non-chordate invertebrates contain THs, probably from exogenous food sources, and this allows one to hypothesise that TH signalling may not be

restricted to chordates but could indeed occur in a wide variety of animals (Heyland & Moroz 2005).

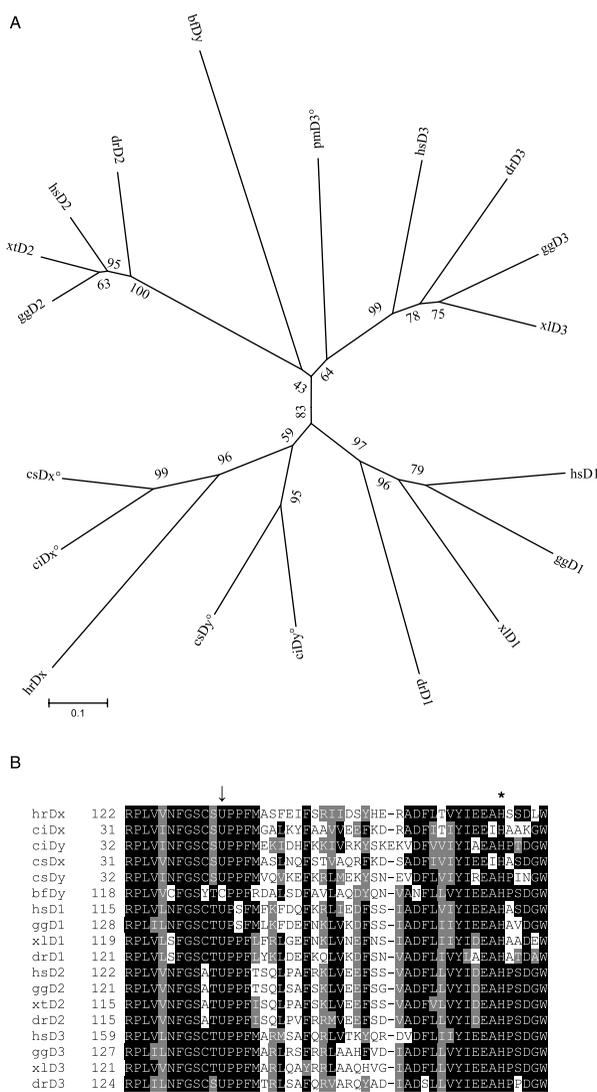
### Localisation and biological role of the deiodinases

The fact that mammalian liver and kidney predominantly expressed D1, while D2 expression was mainly found in brain has originally favoured the hypothesis that D1 is a  $T_3$  'exporting' enzyme that is responsible for most of the circulating  $T_3$ , while D2 is responsible for the production of  $T_3$  that is used in the cells where it is produced. Early data on the distribution of D3 in mammalian tissues led to the initial idea that this enzyme has little impact in adult life and is only important during development, to protect the embryo/foetus from premature  $T_3$  exposure. However, several decades of more profound and comparative research have substantially increased our insight into the distribution and functioning of the three types of deiodinases in vertebrates, and have led to a far more refined view on their biological role. It also showed that several functions have been conserved, while others apparently have changed during vertebrate evolution.

#### Control of circulating $T_3$ levels

Several of the more recent reviews suggest that the major role of D1 may be to clear  $rT_3$  and sulphated iodothyronines from the circulation. As such, it functions as a scavenger enzyme to remove inactive iodothyronines and recycle iodine within the organism (Bianco *et al.* 2002, St Germain *et al.* 2009, Maia *et al.* 2011). This does not, of course, exclude a role for D1 in the peripheral production of circulating  $T_3$ . In postnatal mammals, D1 is typically the major deiodinating activity present in the liver and kidney (Bates *et al.* 1999, Wassen *et al.* 2004), two organs that have a high blood perfusion rate. Subcellular localisation studies have shown that D1 is integrated in the plasma membrane in contrast to D2, which is localised in the endoplasmic reticulum (Baqui *et al.* 2000). This localisation could certainly contribute to the rapid equilibration of the  $T_3$  produced by D1 in liver and kidney with plasma  $T_3$ . Recently, some polymorphisms in the *DIO1* gene in humans were also found to be correlated with changes in plasma  $T_3$  (de Jong *et al.* 2007). However, there are also arguments against a predominant role of D1 in peripheral  $T_3$  production. The major one is that D1 is extremely inefficient in converting  $T_4$  into  $T_3$  when compared with D2, which has a 700-fold greater catalytic efficiency (Maia *et al.* 2011).

It is clear by now that in mammals both D1 and D2 contribute to the production of plasma  $T_3$ , but their relative contribution varies amongst species. In euthyroid rat, where ~50% of plasma  $T_3$  derives from extrathyroidal  $T_4$  to  $T_3$  conversion (Chanoine *et al.* 1993), D1 (mainly from liver and kidney) and D2 (mainly from skeletal muscle) may equally contribute to peripheral  $T_3$  production (Nguyen *et al.* 1998). In euthyroid man, where as much as 80% of  $T_3$  is produced



**Figure 6** (A) Phylogenetic tree of invertebrate and representative vertebrate deiodinases AA sequence information using the Neighbour-joining method with 1000 bootstrap replications. The percentage of replicate trees in which the associated taxa are clustered together is indicated next to the branches. The tree presented is unrooted. Sequences marked by 'open circle' are only partially known sequences. (B) Alignment of the core amino acid sequences of invertebrate and representative vertebrate deiodinases. Identical AAs are indicated in black, similar AAs in grey. The catalytic Sec residue is indicated by the arrow and the conserved His residue by the asterisk. Vertebrate sequence information was obtained from GenBank, invertebrate sequences are available in literature (Shepherdley *et al.* 2004, Klootwijk *et al.* 2011). Bf, *Branchiostoma floridae*; ci, *Ciona intestinalis*; cs, *Ciona savignyi*; dr, *Danio rerio*; gg, *Gallus gallus*; hs, *Homo sapiens*; hr, *Halocynthia roretzi*; pm, *Petromyzon marinus*; xl, *Xenopus laevis*; xt, *Xenopus tropicalis*.

peripherally (Engler & Burger 1984), D2 from skeletal muscle could be the major source of plasma  $T_3$  (Maia *et al.* 2005).

In several birds and reptiles, high D1 activity is found in liver and kidney, while some peripheral organs such as liver, lung, and intestine express low levels of D2 (Freeman & McNabb 1991, Darras *et al.* 1992, Gereben *et al.* 1999, Fenton & Valverde 2000, Shepherdley *et al.* 2002). It can therefore be assumed that in these vertebrate classes too, both enzymes are important for the peripheral production of the  $T_3$  that is released into the circulation. By contrast, D1 does not seem to play a role for plasma  $T_3$  in amphibians. D1 mRNA is present in *Xenopus* tadpoles, mainly in the head region (Morvan Dubois *et al.* 2006), but so far D1 activity has not been detected in any adult amphibian, where peripheral tissues only show D2 and D3 activity (Galton 1988, Darras *et al.* 2002). In the case of fish it does not seem possible to generalise. Substantial D2 expression/activity has been found in peripheral organs, including liver and kidney, but the amount of D1 expression/activity varies widely amongst species (Leatherland *et al.* 1990, VanPutte *et al.* 2001, Orozco *et al.* 2003, Picard-Aitken *et al.* 2007). In euthyroid tilapia, D1 activity is found in kidney but not in liver (Mol *et al.* 1993), and fasting and re-feeding experiments suggested that D2 is the major enzyme regulating circulating  $T_3$  levels (Van der Geysen *et al.* 1998).

The amount of  $T_3$  in plasma is controlled not only by  $T_3$  production but also by  $T_3$  degradation. D3 plays a major role in this process, especially during development, and this role seems to be conserved throughout vertebrate evolution. Inverse correlations between decreasing D3 activity and increasing plasma/whole body  $T_3$  levels have been observed in developing vertebrates (Galton & Hiebert 1988, Darras *et al.* 1992). In chicken embryos, an acute decrease in hepatic D3 expression following growth hormone or glucocorticoid injection rapidly increased circulating  $T_3$  concentrations (Van der Geysen *et al.* 2001a). D3 also contributes to the control of plasma  $T_3$  in adult life. This is most evident in situations where peripheral D3 expression is increased, such as illness and food deficiency. Partial food restriction strongly increased D3 activity in liver of chicken and rat, resulting in a drop in circulating  $T_3$  without concomitant change in hepatic D1 activity (Darras *et al.* 1995). Increased D3 activity in liver and skeletal muscle has also been linked to the reduction in plasma  $T_3$  in critically ill humans and rabbits (Peeters *et al.* 2003, Debaveye *et al.* 2005).

#### Control of intracellular $T_3$ availability

Part of the  $T_3$  present in cells is taken up directly from the circulation while another part is produced within the cell. The ratio of plasma derived vs locally produced  $T_3$  varies amongst different tissues. Early studies in rat injected with radiolabelled  $T_4$  and  $T_3$  showed that the percentage of locally produced  $T_3$  bound to the nuclei was low for tissues with predominant D1 activity (14% in kidney and 28% in liver) and high for tissues with predominant D2 activity (55% in brown

adipose tissue and 65–75% in cerebral cortex; Silva *et al.* 1978, Crantz *et al.* 1982, van Doorn *et al.* 1985, Bianco & Silva 1987). Together with the finding that D2 is located in the endoplasmic reticulum (Baqui *et al.* 2000) this led to the idea that the  $T_3$  generated by D2 in a given cell stays within that cell and binds to the nuclear receptors, unless it is degraded by D3. Later evidence, however, showed that this is not necessarily the case. The  $T_3$  produced by D2 in peripheral tissues of amphibians and fish is an important source of plasma  $T_3$  and a study in tilapia suggested that the type of tissue expressing ORD activity (in that case liver) and not necessarily the type of deiodinase *per se* (in that case D2) determines whether the  $T_3$  is released into the plasma (Van der Geyten *et al.* 1998).

The combined presence of D2 and D3 in many tissues and the coordinated changes in their activities during ontogeny allow these tissues to control intracellular  $T_3$  availability in part independently from the level in circulation. Perhaps the best example is found during amphibian metamorphosis, where at a given stage different tissues undergo opposite changes. The expression patterns of D2 and D3 found in metamorphosing *R. catesbeiana* tadpoles were clearly tissue-specific and linked to the different timing of metamorphic changes. Interestingly, in tissues where both genes were expressed, the expression profiles changed in a parallel way, indicating a potential for really tight control of intracellular  $T_3$  levels (Becker *et al.* 1997). This does not, however, imply that D2 and D3 are necessarily expressed within the same cell. This has become very clear from studies in brain, where the cellular distribution pattern of D2 and D3 has been studied at the mRNA and protein level. The general picture emerging from these studies in rat, mouse and human is that D2 expression is restricted to glial cells while neurons mainly express D3 (Guadano-Ferraz *et al.* 1997, Tu *et al.* 1999). Neurons therefore cannot convert  $T_4$  into  $T_3$  and depend on  $T_3$  produced by neighbouring glial cells or taken up into the brain via the cerebrospinal fluid or the blood capillaries present throughout the brain. As cellular uptake and efflux of THs are regulated by a variety of TH transporters (Visser *et al.* 2008, 2011), it is now clear that intracellular  $T_3$  availability in neurons (and any other cell type) is tightly controlled by the combined action of TH transporters and deiodinases (Horn & Heuer 2010). D2 and D3 are present in all non-mammalian vertebrate brains studied and can therefore work together in controlling intracellular  $T_3$  levels. D2 is clearly expressed in several glial cell types of chicken brain and D3 has been found in neurons (Gereben *et al.* 2004, Verhoelst *et al.* 2005). Interestingly, an immunohistochemical study in embryonic chicken cerebellum suggested that both D2 and D3 are transiently expressed in Purkinje cells, although not at the same time (Verhoelst *et al.* 2005). More detailed data on the cellular distribution of both deiodinases during the ontogeny of different species are certainly needed to draw general conclusions on the presence or absence of D2 in vertebrate neurons.

It remains unclear whether D1 plays a role in regulating intracellular  $T_3$  availability in brain. D1 mRNA has been

detected in brain of several mammals, birds and fish, and even in *Xenopus* tadpoles (Bates *et al.* 1999, Chan *et al.* 2002, Morvan Dubois *et al.* 2006, Johnson & Lema 2011, Van Herck *et al.* 2012). This mRNA is translated at least in part into D1 protein as shown in chicken, where the amount of D1 protein in cerebellum was influenced by thyroid status (Verhoelst *et al.* 2004). However, only minimal D1 activity could be found in brain tissue so far.

#### Response of deiodinases to thyroid status

The exact molecular mechanisms by which deiodinase expression is regulated at the transcriptional level remains unclear, because only the human *DIO1* gene has been shown to contain TREs in the 5'-FR (Toyoda *et al.* 1995). It is nevertheless not surprising that all three deiodinases are highly responsive to thyroid status given their important role in regulating intracellular  $T_3$  availability. Hypothyroidism increases D2 mRNA expression and decreases D3 mRNA expression, while hyperthyroidism has the opposite effects. These typical responses occur in different tissues and have been demonstrated in a wide variety of vertebrates (St Germain *et al.* 1994, Bianco *et al.* 2002, Orozco & Valverde 2005, Rudas *et al.* 2005, Johnson & Lema 2011). In addition to transcriptional regulation, D2 is also strongly regulated by  $T_4$  at the posttranslational level via a process of ubiquitination (Gereben *et al.* 2008, Arrojo & Bianco 2011).

The TH-dependent regulation of D1 seems to be more complex. It has been known for a long time that D1 activity in liver and kidney is increased in hyperthyroid rat and decreased in hypothyroid rat and this was confirmed later at the mRNA level (Kaplan & Utiger 1978, Berry *et al.* 1990). Similar changes have been observed in mouse and human (Maia *et al.* 1995b, Zhang *et al.* 1998, Friedrichsen *et al.* 2003). A study in pig with an anti-D1 antiserum showed that the amount of D1 protein was strongly reduced in microsomes prepared from hypothyroid liver or kidney, while there was no or only a slight increase of D1 in microsomes from hyperthyroid animals (Wassen *et al.* 2004). In house musk shrew, hypothyroidism did not reduce hepatic D1 mRNA and activity, while administration of a high dose of  $T_3$  resulted in a modest but significant decrease in D1 mRNA and activity (Rogatcheva *et al.* 2002).

The few data available from birds (chicken) show some variation, but in most studies hyperthyroidism seemed to increase hepatic D1 activity, while hypothyroidism decreased hepatic D1 activity and also lowered D1 protein in brain (Decuyper *et al.* 1987, Verhoelst *et al.* 2004, Darras *et al.* 2006). Data from fish studies show a different response of D1 to thyroid status. A 24-h exposure to  $T_4$ ,  $T_3$  or 3,5- $T_2$  decreased D1 mRNA levels in killifish liver but without affecting enzyme activity (Garcia *et al.* 2004). Long-term  $T_3$  supplementation in the food in rainbow trout also did not change hepatic D1 activity (Finnson & Eales 1999). In tilapia, long-term  $T_3$  supplementation had no impact on hepatic or renal D1 activity, while  $T_4$  supplementation strongly

increased hepatic D1 activity (Van der Geyten *et al.* 2005). Long-term hypothyroidism strongly induced D1 mRNA and activity in tilapia liver, while D1 activity in kidney was reduced or remained unchanged (Mol *et al.* 1999, Van der Geyten *et al.* 2001b). By contrast, hypothyroidism increased D1 activity in kidney of hypothyroid rainbow trout (Burel *et al.* 2000). In striped parrotfish, neither T<sub>3</sub> nor methimazole exposure changed D1 mRNA levels in liver, brain or gonads (Johnson & Lema 2011). Taken together, these data suggest that the divergence in D1 response in fish might be related not only to species or tissue differences but also to the way hypo- or hyperthyroidism is induced.

### Conclusion

The presence of iodothyronine deiodinases in animals as diverse as ascidians and humans suggests that this type of enzyme was already present in the common ancestor of all chordates and possibly even earlier in evolution. The comparative study amongst vertebrates has shown that the structure of each of the three typical types of deiodinases (D1, D2 and D3) has been highly conserved. This is also true for most of their biochemical properties, with the clear exception of the PTU sensitivity of D1 and possibly also its DTT dependence. Although many full coding sequences have been identified by now, only a limited number of D1 and even fewer D2 and D3 cDNAs have been translated into recombinant proteins for functional characterisation. More efforts in this direction, combined with site-directed mutagenesis, would certainly help to further unravel the specific interactions between the enzyme and different substrates/inhibitors that are responsible for the typical differences in activity between the three enzyme types. A more detailed elucidation of the 3D structure of deiodinases would also be very helpful in this context. Another remaining challenge for the future is the identification of the natural cofactor(s) for these enzymes as their functional characteristics, typically determined in the presence of the artificial reductant DTT, may certainly differ *in vivo*.

Studies from fish to mammals have shown that D2 and D3 are expressed and active in all investigated species and react to thyroid status in a consistent way. The presence of D1 activity seems more variable and its response to thyroid status more divergent. As a result, the relative contributions of D1 and D2 to circulating T<sub>3</sub> levels also show considerable fluctuations. Comparative studies of the 5'-FR of the deiodinase genes from a wider variety of vertebrates, as well as further research on possible posttranscriptional regulation, are needed to increase our understanding of the molecular mechanisms controlling deiodinase expression.

### 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.

### Funding

This review did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

### References

- Amores A, Force A, Yan YL, Joly L, Amemiya C, Fritz A, Ho RK, Langeland J, Prince V, Wang YL *et al.* 1998 Zebrafish *hox* clusters and vertebrate genome evolution. *Science* **282** 1711–1714. (doi:10.1126/science.282.5394.1711)
- Arrojo EDR & Bianco AC 2011 Type 2 deiodinase at the crossroads of thyroid hormone action. *International Journal of Biochemistry & Cell Biology* **43** 1432–1441. (doi:10.1016/j.biocel.2011.05.016)
- Baqui MM, Gereben B, Harney JW, Larsen PR & Bianco AC 2000 Distinct subcellular localization of transiently expressed types 1 and 2 iodothyronine deiodinases as determined by immunofluorescence confocal microscopy. *Endocrinology* **141** 4309–4312. (doi:10.1210/en.141.11.4309)
- Bartha T, Kim SW, Salvatore D, Gereben B, Tu HM, Harney JW, Rudas P & Larsen PR 2000 Characterization of the 5'-flanking and 5'-untranslated regions of the cyclic adenosine 3',5'-monophosphate-responsive human type 2 iodothyronine deiodinase gene. *Endocrinology* **141** 229–237. (doi:10.1210/en.141.1.229)
- Bates JM, St Germain DL & Galton VA 1999 Expression profiles of the three iodothyronine deiodinases, D1, D2, and D3, in the developing rat. *Endocrinology* **140** 844–851. (doi:10.1210/en.140.2.844)
- Becker KB, Stephens KC, Davey JC, Schneider MJ & Galton VA 1997 The type 2 and type 3 iodothyronine deiodinases play important roles in coordinating development in *Rana catesbeiana* tadpoles. *Endocrinology* **138** 2989–2997. (doi:10.1210/en.138.7.2989)
- Berry MJ, Kates AL & Larsen PR 1990 Thyroid hormone regulates type I deiodinase messenger RNA in rat liver. *Molecular Endocrinology* **4** 743–748. (doi:10.1210/mend-4-5-743)
- Berry MJ, Banu L & Larsen PR 1991 Type I iodothyronine deiodinase is a selenocysteine-containing enzyme. *Nature* **349** 438–440. (doi:10.1038/349438a0)
- Berry MJ, Banu L, Harney JW & Larsen PR 1993 Functional characterization of the eukaryotic SECIS elements which direct selenocysteine insertion at UGA codons. *EMBO Journal* **12** 3315–3322.
- Bianco AC & Silva JE 1987 Nuclear 3,5,3'-triiodothyronine (T<sub>3</sub>) in brown adipose tissue: receptor occupancy and sources of T<sub>3</sub> as determined by *in vivo* techniques. *Endocrinology* **120** 55–62. (doi:10.1210/endo-120-1-55)
- Bianco AC & Larsen PR 2005 Cellular and structural biology of the deiodinases. *Thyroid* **15** 777–786. (doi:10.1089/thy.2005.15.777)
- Bianco AC & Kim BW 2006 Deiodinases: implications of the local control of thyroid hormone action. *Journal of Clinical Investigation* **116** 2571–2579. (doi:10.1172/JCI29812)
- 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)
- Bres O, Plohman JC & Eales JG 2006 A cDNA for a putative type III deiodinase in the trout (*Oncorhynchus mykiss*): influence of holding conditions and thyroid hormone treatment on its hepatic expression. *General and Comparative Endocrinology* **145** 92–100. (doi:10.1016/j.ygcen.2005.07.008)
- Burel C, Boujard T, Escaffre AM, Kaushik SJ, Boeuf G, Mol KA, Van der Geyten S & Kuhn ER 2000 Dietary low-glucosinolate rapeseed meal affects thyroid status and nutrient using in rainbow trout (*Oncorhynchus mykiss*). *British Journal of Nutrition* **83** 653–664. (doi:10.1017/S0007114500000830)
- 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)

- Celi FS, Canettieri G, Yarnall DP, Burns DK, Andreoli M, Shuldiner AR & Centanni M 1998 Genomic characterization of the coding region of the human type II 5'-deiodinase gene. *Molecular and Cellular Endocrinology* **141** 49–52. (doi:10.1016/S0303-7207(98)00093-8)
- Chan S, Kachilele S, McCabe CJ, Tannahill LA, Boelaert K, Gittoes NJ, Visser TJ, Franklyn JA & Kilby MD 2002 Early expression of thyroid hormone deiodinases and receptors in human fetal cerebral cortex. *Brain Research. Developmental Brain Research* **138** 109–116. (doi:10.1016/S0165-3806(02)00459-5)
- Chanoine JP, Braverman LE, Farwell AP, Safran M, Alex S, Dubord S & Leonard JL 1993 The thyroid gland is a major source of circulating T<sub>3</sub> in the rat. *Journal of Clinical Investigation* **91** 2709–2713. (doi:10.1172/JCI116510)
- Charalambous M & Hernandez A 2012 Genomic imprinting of the type 3 thyroid hormone deiodinase gene: regulation and developmental implications. *Biochimica et Biophysica Acta*. In press. (doi:10.1016/j.bbagen.2012.03.015)
- Charalambous M, Ferron SR, da Rocha ST, Murray AJ, Rowland T, Ito M, Schuster-Gossler K, Hernandez A & Ferguson-Smith AC 2012 Imprinted gene dosage is critical for the transition to independent life. *Cell Metabolism* **15** 209–221. (doi:10.1016/j.cmet.2012.01.006)
- Crantz FR, Silva JE & Larsen PR 1982 An analysis of the sources and quantity of 3,5,3'-triiodothyronine specifically bound to nuclear receptors in rat cerebral cortex and cerebellum. *Endocrinology* **110** 367–375. (doi:10.1210/endo-110-2-367)
- Croteau W, Whittemore SL, Schneider MJ & St Germain DL 1995 Cloning and expression of a cDNA for a mammalian type III iodothyronine deiodinase. *Journal of Biological Chemistry* **270** 16569–16575. (doi:10.1074/jbc.270.28.16569)
- Croteau W, Davey JC, Galton VA & St Germain DL 1996 Cloning of the mammalian type II iodothyronine deiodinase. A selenoprotein differentially expressed and regulated in human and rat brain and other tissues. *Journal of Clinical Investigation* **98** 405–417. (doi:10.1172/JCI118806)
- Darras VM, Visser TJ, Berghman LR & Kuhn ER 1992 Ontogeny of type I and type III deiodinase activities in embryonic and posthatch chicks: relationship with changes in plasma triiodothyronine and growth hormone levels. *Comparative Biochemistry and Physiology. A, Comparative Physiology* **103** 131–136. (doi:10.1016/0300-9629(92)90252-L)
- Darras VM, Cokelaere M, Dewil E, Arnouts S, Decuyper E & Kuhn ER 1995 Partial food restriction increases hepatic inner ring deiodinating activity in the chicken and the rat. *General and Comparative Endocrinology* **100** 334–338. (doi:10.1006/gcen.1995.1164)
- Darras VM, Van der Geyten S, Cox C, Segers IB, De Groef B & Kuhn ER 2002 Effects of dexamethasone treatment on iodothyronine deiodinase activities and on metamorphosis-related morphological changes in the axolotl (*Ambystoma mexicanum*). *General and Comparative Endocrinology* **127** 157–164. (doi:10.1016/S0016-6480(02)00038-2)
- Darras VM, Verhoelst CH, Reyens GE, Kuhn ER & Van der Geyten S 2006 Thyroid hormone deiodination in birds. *Thyroid* **16** 25–35. (doi:10.1089/thy.2006.16.25)
- Davey JC, Becker KB, Schneider MJ, St Germain DL & Galton VA 1995 Cloning of a cDNA for the type II iodothyronine deiodinase. *Journal of Biological Chemistry* **270** 26786–26789. (doi:10.1074/jbc.270.45.26786)
- Davey JC, Schneider MJ, Becker KB & Galton VA 1999 Cloning of a 5·8 kb cDNA for a mouse type 2 deiodinase. *Endocrinology* **140** 1022–1025. (doi:10.1210/en.140.2.1022)
- Debaveye Y, Ellger B, Mebis L, Van Herck E, Coopmans W, Darras V & Van den Berghe G 2005 Tissue deiodinase activity during prolonged critical illness: effects of exogenous thyrotropin-releasing hormone and its combination with growth hormone-releasing peptide-2. *Endocrinology* **146** 5604–5611. (doi:10.1210/en.2005-0963)
- Decuyper E, Buyse J, Scanes CG, Huybrechts L & Kuhn ER 1987 Effects of hyper- or hypothyroid status on growth, adiposity and levels of growth hormone, somatomedin C and thyroid metabolism in broiler chickens. *Reproduction, Nutrition, Development* **27** 555–565. (doi:10.1051/rnd:19870414)
- van Doorn J, Roelfsema F & van der Heide D 1985 Concentrations of thyroxine and 3,5,3'-triiodothyronine at 34 different sites in euthyroid rats as determined by an isotopic equilibrium technique. *Endocrinology* **117** 1201–1208. (doi:10.1210/endo-117-3-1201)
- Eales JG, Holmes JA, McLeese JM & Youson JH 1997 Thyroid hormone deiodination in various tissues of larval and upstream-migrant sea lampreys, *Petromyzon marinus*. *General and Comparative Endocrinology* **106** 202–210. (doi:10.1006/gcen.1996.6865)
- Eales JG, McLeese JM, Holmes JA & Youson JH 2000 Changes in intestinal and hepatic thyroid hormone deiodination during spontaneous metamorphosis of the sea lamprey, *Petromyzon marinus*. *Journal of Experimental Zoology* **286** 305–312. (doi:10.1002/(SICI)1097-010X(20000215)286:3<305::AID-JEZ9>3.0.CO;2-5)
- Edwards CA, Mungall AJ, Matthews L, Ryder E, Gray DJ, Pask AJ, Shaw G, Graves JA, Rogers J, Dunham I *et al.* 2008 The evolution of the DLK1–DIO3 imprinted domain in mammals. *PLoS Biology* **6** e135. (doi:10.1371/journal.pbio.0060135)
- Engler D & Burger AG 1984 The deiodination of the iodothyronines and of their derivatives in man. *Endocrine Reviews* **5** 151–184. (doi:10.1210/edrv-5-2-151)
- Fenton B & Valverde RC 2000 Hepatic outer-ring deiodinase in a Mexican endemic lizard (*Sceloporus grammicus*). *General and Comparative Endocrinology* **117** 77–88. (doi:10.1006/gcen.1999.7384)
- Finsson KW & Eales JG 1999 Effect of T-3 treatment and food ration on hepatic deiodination and conjugation of thyroid hormones in rainbow trout, *Oncorhynchus mykiss*. *General and Comparative Endocrinology* **115** 379–386. (doi:10.1006/gcen.1999.7325)
- Freeman TB & McNabb FM 1991 Hepatic 5'-deiodinase activity of Japanese quail using reverse-T<sub>3</sub> as substrate: assay validation, characterization, and developmental studies. *Journal of Experimental Zoology* **258** 212–220. (doi:10.1002/jez.1402580211)
- Friedrichsen S, Christ S, Heuer H, Schafer MK, Mansouri A, Bauer K & Visser TJ 2003 Regulation of iodothyronine deiodinases in the Pax8 – / – mouse model of congenital hypothyroidism. *Endocrinology* **144** 777–784. (doi:10.1210/en.2002-220715)
- Galton VA 1988 Iodothyronine 5'-deiodinase activity in the amphibian *Rana catesbeiana* at different stages of the life cycle. *Endocrinology* **122** 1746–1750. (doi:10.1210/endo-122-5-1746)
- Galton VA & Hiebert A 1987 The ontogeny of the enzyme systems for the 5'- and 5-deiodination of thyroid hormones in chick embryo liver. *Endocrinology* **120** 2604–2610. (doi:10.1210/endo-120-6-2604)
- Galton VA & Hiebert A 1988 The ontogeny of iodothyronine 5'-monodeiodinase activity in *Rana catesbeiana* tadpoles. *Endocrinology* **122** 640–645. (doi:10.1210/endo-122-2-640)
- Garcia GC, Jeziorski MC, Valverde RC & Orozco A 2004 Effects of iodothyronines on the hepatic outer-ring deiodinating pathway in killifish. *General and Comparative Endocrinology* **135** 201–209. (doi:10.1016/j.ygcen.2003.09.010)
- 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, Kollar A, Harney JW & Larsen PR 2002 The mRNA structure has potent regulatory effects on type 2 iodothyronine deiodinase expression. *Molecular Endocrinology* **16** 1667–1679. (doi:10.1210/me.16.7.1667)
- Gereben B, Pachucki J, Kollar A, Liposits Z & Fekete C 2004 Ontogenic redistribution of type 2 deiodinase messenger ribonucleic acid in the brain of chicken. *Endocrinology* **145** 3619–3625. (doi:10.1210/en.2004-0229)
- Gereben B, Zavacki AM, Ribich S, Kim BW, Huang SA, Simonides WS, Zeold 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)
- Goswami A & Rosenberg IN 1988 Iodothyronine 5'-deiodination in rat kidney microsomes: sensitivity to propylthiouracil. *Endocrinology* **123** 2774–2781. (doi:10.1210/endo-123-6-2774)
- Gross J & Leblond CP 1950 Metabolism of the thyroid hormone in the rat as shown by physiological doses of labeled thyroxine. *Journal of Biological Chemistry* **184** 489–500.

- Guadano-Ferraz A, Obregon MJ, St Germain DL & Bernal J 1997 The type 2 iodothyronine deiodinase is expressed primarily in glial cells in the neonatal rat brain. *PNAS* **94** 10391–10396. (doi:10.1073/pnas.94.19.10391)
- Hernandez A, Park JP, Lyon GJ, Mohandas TK & St Germain DL 1998 Localization of the type 3 iodothyronine deiodinase (DIO3) gene to human chromosome 14q32 and mouse chromosome 12F1. *Genomics* **53** 119–121. (doi:10.1006/geno.1998.5505)
- 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)
- Heyland A & Moroz LL 2005 Cross-kingdom hormonal signaling: an insight from thyroid hormone functions in marine larvae. *Journal of Experimental Biology* **208** 4355–4361. (doi:10.1242/jeb.01877)
- Horn S & Heuer H 2010 Thyroid hormone action during brain development: more questions than answers. *Molecular and Cellular Endocrinology* **315** 19–26. (doi:10.1016/j.mce.2009.09.008)
- Hugenberger JL & Licht P 1999 Characterization of thyroid hormone 5'-monodeiodinase activity in the turtle (*Trachemys scripta*). *General and Comparative Endocrinology* **113** 343–359. (doi:10.1006/gcen.1998.7197)
- 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)
- Jakobs TC, Koehler MR, Schmutzler C, Glaser F, Schmid M & Kohrle J 1997a Structure of the human type I iodothyronine 5'-deiodinase gene and localization to chromosome 1p32–p33. *Genomics* **42** 361–363. (doi:10.1006/geno.1997.4736)
- Jakobs TC, Schmutzler C, Meissner J & Kohrle J 1997b The promoter of the human type I 5'-deiodinase gene – mapping of the transcription start site and identification of a DR + 4 thyroid-hormone-responsive element. *European Journal of Biochemistry* **247** 288–297. (doi:10.1111/j.1432-1033.1997.00288.x)
- 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)
- de Jong FJ, Peeters RP, den Heijer T, van der Deure WM, Hofman A, Uitterlinden AG, Visser TJ & Breteler MM 2007 The association of polymorphisms in the type 1 and 2 deiodinase genes with circulating thyroid hormone parameters and atrophy of the medial temporal lobe. *Journal of Clinical Endocrinology and Metabolism* **92** 636–640. (doi:10.1210/jc.2006-1331)
- Kaplan MM & Utiger RD 1978 Iodothyronine metabolism in liver and kidney homogenates from hyperthyroid and hypothyroid rats. *Endocrinology* **103** 156–161. (doi:10.1210/endo-103-1-156)
- Kaplan MM & Yaskoski KA 1980 Phenolic and tyrosyl ring deiodination of iodothyronines in rat brain homogenates. *Journal of Clinical Investigation* **66** 551–562. (doi:10.1172/JCI109887)
- 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 EC & Visser TJ 2011 A nonselenoprotein from amphioxus deiodinates triac but not T<sub>3</sub>: is triac the primordial bioactive thyroid hormone? *Endocrinology* **152** 3259–3267. (doi:10.1210/en.2010-1408)
- Kobayashi S, Gao Y & Pittman CS 1985 The substrate specificity, tissue specificity and regulation of the 5' deiodination systems in rat liver and kidney tissues. *Endocrinologia Japonica* **32** 781–792. (doi:10.1507/endoctrj1954.32.781)
- Kuiper GG, Wassen F, Klootwijk W, Van Toor H, Kaptein E & Visser TJ 2003 Molecular basis for the substrate selectivity of cat type I iodothyronine deiodinase. *Endocrinology* **144** 5411–5421. (doi:10.1210/en.2003-0728)
- 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)
- Leatherland JF, Reddy PK, Yong AN, Leatherland A & Lam TJ 1990 Hepatic 5'-monodeiodinase activity in teleosts invitro – a survey of 33 species. *Fish Physiology and Biochemistry* **8** 1–10. (doi:10.1007/BF00004426)
- Leloup J & Seugnet I 1989 *In vivo* thyroxine monodeiodination in the Ascidian, *Phallusia mamillata*. *General and Comparative Endocrinology* **74** 276–277.
- Leonard JL & Visser TJ 1986 Biochemistry of deiodination. In *Thyroid Hormone Metabolism*, pp 189–229. Ed. G Hennemann. New York & Basel: Marcel Dekker Inc.
- 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)
- Lobanov AV, Fomenko DE, Zhang Y, Sengupta A, Hatfield DL & Gladyshev VN 2007 Evolutionary dynamics of eukaryotic selenoproteomes: large selenoproteomes may associate with aquatic life and small with terrestrial life. *Genome Biology* **8** R198. (doi:10.1186/gb-2007-8-9-r198)
- Maia AL, Berry MJ, Sabbag R, Harney JW & Larsen PR 1995a Structural and functional differences in the dio1 gene in mice with inherited type 1 deiodinase deficiency. *Molecular Endocrinology* **9** 969–980. (doi:10.1210/me.9.8.969)
- Maia AL, Kieffer JD, Harney JW & Larsen PR 1995b Effect of 3,5,3'-triiodothyronine (T<sub>3</sub>) administration on dio1 gene expression and T<sub>3</sub> metabolism in normal and type 1 deiodinase-deficient mice. *Endocrinology* **136** 4842–4849. (doi:10.1210/en.136.11.4842)
- Maia AL, Kim BW, Huang SA, Harney JW & Larsen PR 2005 Type 2 iodothyronine deiodinase is the major source of plasma T<sub>3</sub> in euthyroid humans. *Journal of Clinical Investigation* **115** 2524–2533. (doi:10.1172/JCI25083)
- Maia AL, Goemann IM, Meyer EL & Wajner SM 2011 Deiodinases: the balance of thyroid hormone: type 1 iodothyronine deiodinase in human physiology and disease. *Journal of Endocrinology* **209** 283–297. (doi:10.1530/JOE-10-0481)
- Mariotti M, Ridge PG, Zhang Y, Lobanov AV, Pringle TH, Guigo R, Hatfield DL & Gladyshev VN 2012 Composition and evolution of the vertebrate and Mammalian selenoproteomes. *PLoS ONE* **7** e33066. (doi:10.1371/journal.pone.0033066)
- Martinez LM, Orozco A, Villalobos P & Valverde RC 2008 Cloning and characterization of a type 3 iodothyronine deiodinase (D3) in the liver of the chondrichthyan *Chiloscyllium punctatum*. *General and Comparative Endocrinology* **156** 464–469. (doi:10.1016/j.ygcen.2008.02.012)
- McLeese JM, Wright GM, Youson JH & Eales JG 2000 Deiodination activity in extrathyroidal tissues of the Atlantic haggfish, *Myxine glutinosa*. *Journal of Experimental Zoology* **287** 445–452. (doi:10.1002/1097-010X(20001101)287:6<445::AID-JEZ6>3.0.CO;2-A)
- Mol K, Kaptein E, Darras VM, de Greef WJ, Kuhn ER & Visser TJ 1993 Different thyroid hormone-deiodinating enzymes in tilapia (*Oreochromis niloticus*) liver and kidney. *FEBS Letters* **321** 140–144. (doi:10.1016/0014-5793(93)80095-C)
- Mol KA, Van Der Geyten S, Darras VM, Visser TJ & Kuhn ER 1997 Characterization of iodothyronine outer ring and inner ring deiodinase activities in the blue tilapia, *Oreochromis aureus*. *Endocrinology* **138** 1787–1793. (doi:10.1210/en.138.5.1787)
- Mol KA, Van der Geyten S, Burel C, Kuhn ER, Boujard T & Darras VM 1998 Comparative study of iodothyronine outer ring and inner ring deiodinase activities in five teleostean fishes. *Fish Physiology and Biochemistry* **18** 253–266. (doi:10.1023/A:1007722812697)
- Mol KA, Van der Geyten S, Kuhn ER & Darras VM 1999 Effects of experimental hypo- and hyperthyroidism on iodothyronine deiodinases in Nile tilapia, *Oreochromis niloticus*. *Fish Physiology and Biochemistry* **20** 201–207. (doi:10.1023/A:1007739431710)

- 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)
- Navarro L, Landa A, Valverde RC & Aceves C 1997 Mammary gland type I iodothyronine deiodinase is encoded by a short messenger ribonucleic acid. *Endocrinology* **138** 4248–4254. (doi:10.1210/en.138.10.4248)
- Nguyen TT, Chapa F & DiStefano JJ III 1998 Direct measurement of the contributions of type I and type II 5'-deiodinases to whole body steady state 3,5,3'-triiodothyronine production from thyroxine in the rat. *Endocrinology* **139** 4626–4633. (doi:10.1210/en.139.11.4626)
- Ogasawara M, Di Lauro R & Satoh N 1999 Ascidian homologs of mammalian thyroid peroxidase genes are expressed in the thyroid-equivalent region of the endostyle. *Journal of Experimental Zoology* **285** 158–169. (doi:10.1002/(SICI)1097-010X(19990815)285:2<158::AID-JEZ8>3.0.CO;2-0)
- Ohba K, Yoshioka T & Muraki T 2001 Identification of two novel splicing variants of human type II iodothyronine deiodinase mRNA. *Molecular and Cellular Endocrinology* **172** 169–175. (doi:10.1016/S0303-7207(00)00368-3)
- Orozco A & Valverde RC 2005 Thyroid hormone deiodination in fish. *Thyroid* **15** 799–813. (doi:10.1089/thy.2005.15.799)
- Orozco A, Jeziorski MC, Linser PJ, Greenberg RM & Valverde RC 2002 Cloning of the gene and complete cDNA encoding a type 2 deiodinase from *Fundulus heteroclitus*. *General and Comparative Endocrinology* **128** 162–167. (doi:10.1016/S0016-6480(02)00071-0)
- Orozco A, Villalobos P, Jeziorski MC & Valverde RC 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)
- Paris M, Brunet F, Markov GV, Schubert M & Laudet V 2008a 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, Escriva H, Schubert M, Brunet F, Brtko J, Ciesielski F, Roecklin D, Vivat-Hannah V, Jamin EL, Cravedi JP *et al.* 2008b Amphioxus postembryonic development reveals the homology of chordate metamorphosis. *Current Biology* **18** 825–830. (doi:10.1016/j.cub.2008.04.078)
- Peeters RP, Wouters PJ, Kaptein E, van Toor H, Visser TJ & Van den Berghe G 2003 Reduced activation and increased inactivation of thyroid hormone in tissues of critically ill patients. *Journal of Clinical Endocrinology and Metabolism* **88** 3202–3211. (doi:10.1210/jc.2002-022013)
- 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)
- da Rocha ST, Edwards CA, Ito M, Ogata T & Ferguson-Smith AC 2008 Genomic imprinting at the mammalian Dlk1–Dio3 domain. *Trends in Genetics* **24** 306–316. (doi:10.1016/j.tig.2008.03.011)
- Rogatcheva M, Hayashi Y, Oda S, Seo H, Cua K, Refetoff S, Murakami M, Mori M & Murata Y 2002 Type 1 iodothyronine deiodinase in the house musk shrew (*Suncus murinus*, Insectivora: Soricidae): cloning and characterization of complementary DNA, unique tissue distribution and regulation by T(3). *General and Comparative Endocrinology* **127** 48–58. (doi:10.1016/S0016-6480(02)00021-7)
- Roti E, Fang SL, Green K, Emerson CH & Braverman LE 1981 Human placenta is an active site of thyroxine and 3,3',5'-triiodothyronine tyrosyl ring deiodination. *Journal of Clinical Endocrinology and Metabolism* **53** 498–501. (doi:10.1210/jcem-53-3-498)
- Rudas P 1986 Comparison of type I 5'-deiodination of thyroxine and of reverse-triiodothyronine in rat and chicken liver homogenates. *General and Comparative Endocrinology* **63** 400–407. (doi:10.1016/0016-6480(86)90139-5)
- Rudas P, Bartha T & Frenyo LV 1993 Thyroid hormone deiodination in the brain of young chickens acutely adapts to changes in thyroid status. *Acta Veterinaria Hungarica* **41** 381–393.
- Rudas P, Ronai Z & Bartha T 2005 Thyroid hormone metabolism in the brain of domestic animals. *Domestic Animal Endocrinology* **29** 88–96. (doi:10.1016/j.domaniend.2005.02.032)
- Salvatore D, Harney JW & Larsen PR 1999 Mutation of the Secys residue 266 in human type 2 selenodeiodinase alters 75Se incorporation without affecting its biochemical properties. *Biochimie* **81** 535–538. (doi:10.1016/S0300-9084(99)80106-0)
- Salvatore D, Low SC, Berry M, Maia AL, Harney JW, Croteau W, St Germain DL & Larsen PR 1995 Type 3 iodothyronine deiodinase: cloning, *in vitro* expression, and functional analysis of the placental selenoenzyme. *Journal of Clinical Investigation* **96** 2421–2430. (doi:10.1172/JCI118299)
- Sambroni E, Gutierrez S, Cauty C, Guiguen Y, Breton B & Lareyre JJ 2001 Type II iodothyronine deiodinase is preferentially expressed in rainbow trout (*Oncorhynchus mykiss*) liver and gonads. *Molecular Reproduction and Development* **60** 338–350. (doi:10.1002/mrd.1096)
- Sanders JP, Van der Geyten S, Kaptein E, Darras VM, Kuhn ER, Leonard JL & Visser TJ 1997 Characterization of a propylthiouracil-insensitive type I iodothyronine deiodinase. *Endocrinology* **138** 5153–5160. (doi:10.1210/en.138.12.5153)
- Sanders JP, Van der Geyten S, Kaptein E, Darras VM, Kuhn ER, Leonard JL & Visser TJ 1999 Cloning and characterization of type III iodothyronine deiodinase from the fish *Oreochromis niloticus*. *Endocrinology* **140** 3666–3673. (doi:10.1210/en.140.8.3666)
- Shepherdley CA, Richardson SJ, Evans BK, Kuhn ER & Darras VM 2002 Characterization of outer ring iodothyronine deiodinases in tissues of the saltwater crocodile (*Crocodylus porosus*). *General and Comparative Endocrinology* **125** 387–398. (doi:10.1006/gcen.2001.7764)
- Shepherdley CA, Klootwijk W, Makabe KW, Visser TJ & Kuiper GG 2004 An ascidian homolog of vertebrate iodothyronine deiodinases. *Endocrinology* **145** 1255–1268. (doi:10.1210/en.2003-1248)
- Silva JE, Dick TE & Larsen PR 1978 The contribution of local tissue thyroxine monodeiodination to the nuclear 3,5,3'-triiodothyronine in pituitary, liver, and kidney of euthyroid rats. *Endocrinology* **103** 1196–1207. (doi:10.1210/endo-103-4-1196)
- St Germain DL, Schwartzman RA, Croteau W, Kanamori A, Wang Z, Brown DD & Galton VA 1994 A thyroid hormone-regulated gene in *Xenopus laevis* encodes a type III iodothyronine 5-deiodinase. *PNAS* **91** 7767–7771. (doi:10.1073/pnas.91.16.7767)
- 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)
- Sutija M, Longhurst TJ & Joss JM 2003 Deiodinase type II and tissue specific mRNA alternative splicing in the Australian lungfish, *Neoceratodus forsteri*. *General and Comparative Endocrinology* **132** 409–417. (doi:10.1016/S0016-6480(03)00115-1)
- Taylor JS, Braasch I, Frickey T, Meyer A & Van de Peer Y 2003 Genome duplication, a trait shared by 22000 species of ray-finned fish. *Genome Research* **13** 382–390. (doi:10.1101/gr.640303)
- Toyoda N, Harney JW, Berry MJ & Larsen PR 1994 Identification of critical amino acids for 3,5,3'-triiodothyronine deiodination by human type 1 deiodinase based on comparative functional-structural analyses of the human, dog, and rat enzymes. *Journal of Biological Chemistry* **269** 20329–20334.
- 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.
- Toyoda N, Kleinhaus N & Larsen PR 1996 The structure of the coding and 5'-flanking region of the type 1 iodothyronine deiodinase (dio1) gene is normal in a patient with suspected congenital dio1 deficiency. *Journal of Clinical Endocrinology and Metabolism* **81** 2121–2124. (doi:10.1210/jc.81.6.2121)
- Toyoda N, Kaptein E, Berry MJ, Harney JW, Larsen PR & Visser TJ 1997 Structure-activity relationships for thyroid hormone deiodination by mammalian type I iodothyronine deiodinases. *Endocrinology* **138** 213–219. (doi:10.1210/en.138.1.213)

- Tu HM, Legradi G, Bartha T, Salvatore D, Lechan RM & Larsen PR 1999 Regional expression of the type 3 iodothyronine deiodinase messenger ribonucleic acid in the rat central nervous system and its regulation by thyroid hormone. *Endocrinology* **140** 784–790. (doi:10.1210/en.140.2.784)
- Valverde C, Orozco A, Becerra A, Jeziorski MC, Villalobos P & Solis JC 2004 Halometabolites and cellular dehalogenase systems: an evolutionary perspective. *International Review of Cytology* **234** 143–199. (doi:10.1016/S0074-7696(04)34004-0)
- Van der Geyten S, Sanders JP, Kaptein E, Darras VM, Kuhn ER, Leonard JL & Visser TJ 1997 Expression of chicken hepatic type I and type III iodothyronine deiodinases during embryonic development. *Endocrinology* **138** 5144–5152. (doi:10.1210/en.138.12.5144)
- Van der Geyten S, Mol KA, Plumeyers W, Kuhn ER & Darras VM 1998 Changes in plasma T-3 during fasting/refeeding in tilapia (*Oreochromis niloticus*) are mainly regulated through changes in hepatic type II iodothyronine deiodinase. *Fish Physiology and Biochemistry* **19** 135–143. (doi:10.1023/A:1007790527748)
- Van der Geyten S, Segers I, Gereben B, Bartha T, Rudas P, Larsen PR, Kuhn ER & Darras VM 2001a Transcriptional regulation of iodothyronine deiodinases during embryonic development. *Molecular and Cellular Endocrinology* **183** 1–9. (doi:10.1016/S0303-7207(01)00644-X)
- Van der Geyten S, Toguyeni A, Baroiller JF, Fauconneau B, Fostier A, Sanders JP, Visser TJ, Kuhn ER & Darras VM 2001b Hypothyroidism induces type I iodothyronine deiodinase expression in tilapia liver. *General and Comparative Endocrinology* **124** 333–342. (doi:10.1006/gcen.2001.7722)
- Van der Geyten S, Byamungu N, Reyns GE, Kuhn ER & Darras VM 2005 Iodothyronine deiodinases and the control of plasma and tissue thyroid hormone levels in hyperthyroid tilapia (*Oreochromis niloticus*). *Journal of Endocrinology* **184** 467–479. (doi:10.1677/joe.1.05986)
- Van Herck SL, Geysens S, Delbaere J, Tylzanowski P & Darras VM 2012 Expression profile and thyroid hormone responsiveness of transporters and deiodinases in early embryonic chicken brain development. *Molecular and Cellular Endocrinology* **349** 289–297. (doi:10.1016/j.mce.2011.11.012)
- VanPutte CL, MacKenzie DS & Eales JG 2001 Characterization of hepatic low-K(m) outer-ring deiodination in red drum (*Sciaenops ocellatus*). *Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology* **128** 413–423. (doi:10.1016/S1096-4959(00)00348-1)
- Verhoelst CH, Darras VM, Doulabi BZ, Reyns G, Kuhn ER & Van der Geyten S 2004 Type I iodothyronine deiodinase in euthyroid and hypothyroid chicken cerebellum. *Molecular and Cellular Endocrinology* **214** 97–105. (doi:10.1016/j.mce.2003.10.074)
- Verhoelst CH, Roelens SA & Darras VM 2005 Role of spatiotemporal expression of iodothyronine deiodinase proteins in cerebellar cell organization. *Brain Research Bulletin* **67** 196–202. (doi:10.1016/j.brainres-bull.2005.06.030)
- Villalobos P, Orozco A & Valverde RC 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)
- Visser TJ, Leonard JL, Kaplan MM & Larsen PR 1981 Different pathways of iodothyronine 5'-deiodination in rat cerebral cortex. *Biochemical and Biophysical Research Communications* **101** 1297–1304. (doi:10.1016/0006-291X(81)91588-6)
- Visser WE, Friesema EC, Jansen J & Visser TJ 2008 Thyroid hormone transport in and out of cells. *Trends in Endocrinology and Metabolism* **19** 50–56. (doi:10.1016/j.tem.2007.11.003)
- Visser WE, Friesema EC & Visser TJ 2011 Minireview: thyroid hormone transporters: the knowns and the unknowns. *Molecular Endocrinology* **25** 1–14. (doi:10.1210/me.2010-0095)
- Wassen FW, Klootwijk W, Kaptein E, Duncker DJ, Visser TJ & Kuiper GG 2004 Characteristics and thyroid state-dependent regulation of iodothyronine deiodinases in pigs. *Endocrinology* **145** 4251–4263. (doi:10.1210/en.2004-0356)
- Zhang CY, Kim S, Harney JW & Larsen PR 1998 Further characterization of thyroid hormone response elements in the human type 1 iodothyronine deiodinase gene. *Endocrinology* **139** 1156–1163. (doi:10.1210/en.139.3.1156)

Received in final form 19 July 2012

Accepted 23 July 2012

Made available online as an Accepted Preprint  
23 July 2012