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Review

Cross-kingdom hormonal signaling: an insight from thyroid hormone functions in marine larvae

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Summary

Thyroid hormones (THs) are small, lipophilic signaling molecules built from tyrosine and iodine. TH action is well characterized in vertebrates, where these molecules play a fundamental role as regulators of development, metabolism, growth and differentiation. Increasing evidence suggests that THs also function in a variety of invertebrate species. Two alternative sources of hormone for animals are exogenous (from food items) and endogenous synthesis. We propose that exogenous THs can convey environmental information as well as regulate metabolism, revealing new communication avenues between organisms from different kingdoms. While such modes of cross-kingdom communication have been previously considered for fatty acid-based signaling and steroid hormones in plant–animal interactions, this is the first attempt to explore such a mode of action for TH signaling. We suggest that exogenous sources of TH (from food) may have been ancestral, while the ability to synthesize TH endogenously may have evolved independently in a variety of metazoans, resulting in a diversity of signaling pathways and, possibly, morphological structures involved in TH-signaling.

Key words: thyroid hormone, mollusc, echinoderm, iodine, nuclear hormone receptor, non-genomic action, sea urchin, *Aplysia*.

Introduction

Many structurally similar signaling molecules are shared between organisms from different kingdoms. For example, oxidation products of fatty acids such as oxylipins and eicosanoids play fundamental roles in plant and animal metabolism, respectively (Stanley, 1999; Stoka, 1999). As these molecules are common to both kingdoms, they can be exploited for plant–insect recognition and manipulation purposes (Schultz and Appel, 2004). Another group of signaling molecules that could be involved in such a cross-kingdom cross-talk (*sensu* Schultz and Appel, 2004) are ecdysteroids. Their role in regulating insect molting and metamorphosis is well known (Nijhout, 1994). Intriguingly the essential precursors for ecdysteroid synthesis in insects originate from their food, suggesting possibilities for their use in cross-kingdom communication (for further discussion, see Hodin, in press).

Thyroid hormones (THs) are critical metabolic regulators in all vertebrates (i.e. Hulbert, 2000; Valverde-R et al., 2004; Yen, 2001). Moreover THs are well known for orchestrating amphibian and lamprey metamorphoses (Manzon et al., 2001; Manzon and Youson, 1997; Shi et al., 1996; Yaoita and Brown, 1990; Youson, 2003). Recent observations suggest that THs and their metabolites are not restricted to the vertebrates but instead are widely distributed in the animal and plant kingdoms (Eales, 1997; Heyland et al., 2005). In fact, we have recently shown that these hormones can act *via* exogenous routes as environmental messengers in echinoderm larvae (Heyland and Hodin, 2004), in turn suggesting a possibility of cross-kingdom interaction.

Iodine is the essential component of THs (Fig. 1A). Two complementary routes of iodine and TH incorporation in plants and animals are illustrated in Fig. 1A. While marine invertebrate larvae may synthesize hormones endogenously from incorporated iodine (organification), it is also conceivable that their primary source of THs and their metabolites is marine phytoplankton (ingestion). These compounds, having accumulated in phytoplankton, would then be shuttled to marine invertebrate larvae that feed on algae, providing them with an enriched source of hormones and/or pre-hormones that can be more readily transformed into the active compounds. Thus, THs may be transferred through the food chain. Consequently, the utilization of iodine and its organic forms as signaling molecules would depend primarily on (a) the availability of iodine in the marine environment; (b) the recruitment of cellular machinery inside the organism capable of performing the necessary biochemical modifications of

organic iodine; and (c) the presence of receptors capable of decoding these signals.

Below, we trace the path of organic iodine from the diet to the targeted response. We outline the role of THs and other organic forms of iodine within animals and plants. Specifically, we explore the functions of TH-like molecules as algal chemical defence and as exogenous and endogenous signaling molecules in marine invertebrate larvae. We hypothesize that these small, lipophilic, vitamin-like molecules may have been exploited by a large number of taxa as regulators of development, specifically during metamorphosis and larval settlement (Fig. 1B).

Exogenous sources of iodine, tyrosine and thyroid hormones for animals

Iodine distribution is highly variable among habitats (Mairh et al., 1989). In seawater, it is part of a complex mixture dominated by iodate and iodide at concentrations of 40–60 parts per billion (Truesdale, 1994; Truesdale and Upstill-Goddard, 2003). Both macro- and microalgae accumulate significant amounts of iodine (Mairh et al., 1989; Saenko et al., 1978; Wong et al., 2002). Some marine algae such as kelp and microalgae contain up to 1% iodine; the actual content can vary substantially based on the season, water temperature and depth (Mairh et al., 1989; Saenko et al., 1978; Wong et al., 2002). The most common organic forms of iodine found in algae are iodomethane (CH_3I) and its derivatives, such as diiodomethane (CH_2I_2) and iodobutane (C_4H_9I) (Collen et al., 1994). Other organic forms of iodine, including iodotyrosines, are present in the diatom *Chaetoceras gracilis* (Chino et al., 1994). Using antibody-based detection methods, we have found that three species of unicellular algae, *Dunaliella tertiolecta*, *Isochrysis aff. galbana* (*T-ISO*) and *Rhodomonas lens*, also contain thyroxine (Fig. 2). Since many marine organisms feed on phytoplankton and algae that are rich in iodine, the diet is a likely source of this element. It has been shown that invertebrates and their larvae incorporate different forms of iodine from the seawater and some have been shown to contain various iodinated tyrosines such as T4 (L-thyroxine), T3 (3,3′′,5-triiodo-L-thyronine), rT3 (reverse 3,3′′,5-triiodo-L-thyronine), T2 (diiodotyrosine) and T1 (monoiodotyrosine) (Eales, 1997). TH precursors and active THs could be directly transferred from prey to predator. Yet, the mechanisms involved in iodine uptake, synthesis and the potential transfer of such compounds between organisms are poorly understood.

The other critical building block of THs is tyrosine. In animals tyrosine is synthesized from the essential dietary amino acid phenylalanine. However, alternative routes have been described for Cnidaria. In the sea anemone *Aiptasia pulchella* (Cnidaria), tyrosine and six other amino acids (histidine, isoleucine, leucine, lysine, phenylalanine and valine) are transferred directly from symbiotic algae (Wang and Douglas, 1999). Similarly, tyrosine and phenylalanine are abundant in dissolved organic matter (DOM; Yamashita and Tanoue, 2003). DOM includes organic compounds ranging from macromolecules to low molecular mass compounds such as simple organic acids and short-chain hydrocarbons, which are dissolved in water and may be directly incorporated by aquatic animals. Several lines of evidence suggest that DOM may serve as a nutrient source for invertebrate larvae in marine

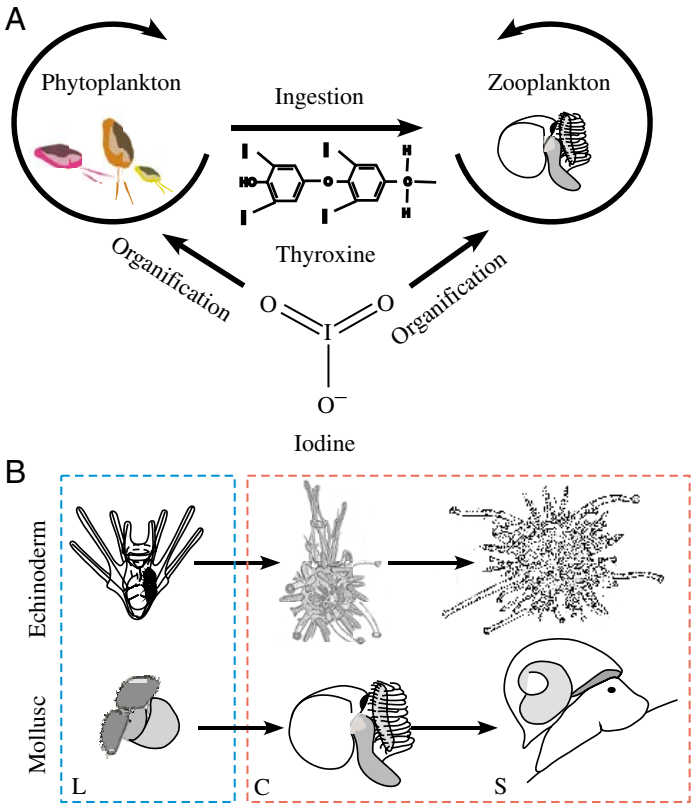

Fig. 1. (A) A hypothesis for iodine-based cross-kingdom communication in marine ecosystems. Marine organisms incorporate iodine into an organic matrix (organification) and synthesize thyroid hormone (TH)-related signaling molecules, which are then shuttled through the food chain (phytoplankton to zooplankton). This hypothesis is supported by previously published evidence and our own new data (see Fig. 2) of TH-like molecules being present in marine algae and various marine invertebrates and their larval forms. (B) One functional aspect of this cross-kingdom communication is the involvement of TH-related compounds in development to metamorphosis of various marine invertebrate larvae, represented schematically in B for molluscs (veliger larvae) and echinoderms (pluteus larvae). New evidence suggests that THs are used as developmental signals by larvae and that the primary source may be exogenous (Chino et al., 1994; Heyland and Hodin, 2004), although we were able to find evidence for endogenous synthesis as well (Heyland and Hodin, 2004). The role of THs in metamorphosis *per se* remains to be elucidated. The blue frame indicates larval development (L) and the red frame metamorphic development with metamorphic competence (C) and settlement (S). Please note that here we are using the term THs generically for thyroid-like hormones, since the specific chemical identification of THs in these lineages requires further confirmation using microanalytical methods (e.g. mass spectrometry and NMR). Images of echinoid larvae modified from Hyman (1995).

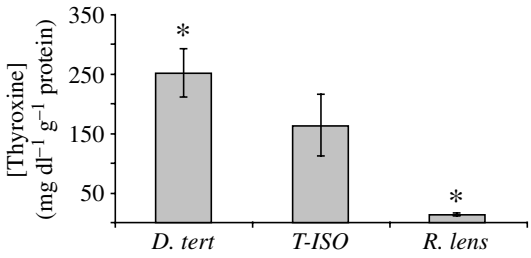

Fig. 2. Various algae species commonly used as larval nutrition in laboratory cultures contain thyroxine. We reared replicate samples of algae of three species [*Dunaliella tertiolecta* (*D. tert.*), *Isochrysis aff. galbana* (*T-ISO*) and *Rhodomonas lens* (*R. lens*)] at the coastal research center (WHOI) in Woods Hole (MA, USA) in summer 2002 in 25 l containers using protocols previously described by McEdward and Herrera (1999), although adapted for large-scale use. Sterile starters for all three algae species were obtained from Dr A. D. Anderson's laboratory (Woods Hole Oceanographic Institute, MA, USA). After collection of replicate samples we performed methanol:chloroform extractions and separated small molecular species using Amicon® (Bedford, MA, USA) Ultra-15 Centrifugal Filter Device (5 kDa) and then dried samples down in a Speed-Vac™. We re-dissolved pellets in 50 µl 0.01 mol l^{-1} NaOH and measured thyroxine using ELISA (Total Thyroxine (Total T4) ELISA Kit Alpha Diagnostics, San Antonio, TX, USA) following the manufacturer's instructions. We determined total protein content for samples using the Micro BCA™ Protein Assay Kit from Pierce (Rockford, IL, USA). Although *T-ISO* has an approximately 10 times smaller cell volume than *D. tert.* it contains approximately the same amount of thyroxine [T4 standardized by protein content (mg dl^{-1} g^{-1} protein)]. Differences in TH content of these algae may reflect differential effects of these algae on larval development and morphogenesis. Values are means ± 1 S.E.M. (N=3). Asterisks indicate significant difference in hormone content between samples using Student's t-test, P<0.05.

and freshwater ecosystems (Jaeckle and Manahan, 1992; Shilling and Manahan, 1994; Thomas, 1997). In summary, essential building blocks for TH biosynthesis are present in the marine environment. Moreover, algae contain iodinated tyrosines that could be transferred to marine invertebrate larvae *via* the food chain. Therefore both routes of TH metabolism outlined in Fig. 1 may occur in marine ecosystems.

Thyroid hormone metabolism and biosynthesis in vertebrates and invertebrates

TH synthesis requires iodine to be covalently bound to tyrosine residues. In vertebrates, ingested iodine is shuttled into the follicle cells of the thyroid gland by the sodium iodine symporter (NIS; Dai et al., 1996), and then into the follicular lumen for storage and further processing. Upon recruitment, the inorganic iodine is conjugated to thyroglobulin (TG). Thyroid peroxidase (TPO), a large (105 kDa) heme-containing glycoprotein, iodinates tyrosyl residues on TG (reviewed in Nunez and Pommier, 1982). Hydrogen peroxide (H_2O_2) is required for the proper transfer of iodine to the acceptor residue *via* a free radical intermediate. Thyroid NADPH oxidase appears to be the primary enzyme involved in regulated synthesis of H_2O_2. Two iodinated tyrosyls are then coupled to form THs (primarily T4). For activation of the hormone, TG is then shuttled out of the follicular lumen into the extracellular space on the basal side of the follicle cell *via* pinocytotic vesicles. Once these vesicles reach the extracellular space, active TH is cleaved off and released into the blood stream (for more details on these processes see Taurog, 2000).

Increasing evidence suggests that basal chordates (urochordates and cephalochordates) have the ability to synthesize THs in the endostyle, a specialized feeding organ associated with the pharynx (reviewed in Eales, 1997). Many authors homologize the endostyle of hemichordates, cephalochordates and urochordates with the thyroid gland of vertebrates using developmental molecular markers (TTF-1, TTF-2 and Pax-8, Mazet, 2002; Ogasawara et al., 1999b; Ogasawara and Satou, 2003; Ogasawara et al., 2001; Sasaki et al., 2003; Satake et al., 2004; Takacs et al., 2002; Valverde-R et al., 2004; Yu et al., 2002) and functional arguments (TPO, TG and TSH receptor, Ogasawara, 2000; Ogasawara et al., 1999a; Shepherdley et al., 2004; Valverde-R et al., 2004). However, both the endostyle and thyroid gland are present in such basal chordates as parasitic lampreys. The endostyle in lampreys is a larval structure, which transforms into a thyroid gland-like organ with follicular cells after metamorphosis (Wright and Youson, 1976). This suggests that the endostyles of urochordates, cephalochordates and lampreys are homologous to each other and that the thyroid gland evolved *de novo* within the vertebrate clade, therefore at best the vertebrate thyroid or lamprey endostyle–thyroid complex can only be homologized with the endostyles of urochordates and cephalochordates at a very general level, as an organ involved in TH synthesis. Interestingly, both the thyroid gland and endostyle are closely associated with the pharyngeal region of the digestive tract, suggesting a link between thyroid hormone function and food uptake.

It has been repeatedly suggested that invertebrates such as arthropods, annelids, echinoderms and molluscs have the ability to synthesize THs and TH-like compounds that affect the organism's physiology (reviewed in Eales, 1997; Heyland et al., 2005). However, no specific morphological structure has been associated with TH function and synthesis in these groups and the hormone effects appear to be extremely diverse, ranging from effects on calcium metabolism to effects on development and reproduction (reviewed in Eales, 1997). Thus we propose that THs were independently co-opted as signaling molecules in many marine invertebrates *via* various structures and pathways. Initially dietary sources of iodine and THs may have been dominant, later being replaced by endogenous synthesis in some clades.

Thyroid hormones as developmental signals in echinoids

In echinoids (sea urchins and sand dollars), two forms of THs (T4 and T3) regulate development to metamorphic competence (Chino et al., 1994; Heyland and Hodin, 2004). Recently we showed that thyroxine application is sufficient to change the

larval developmental mode of the sand dollar *Leodia sexiesperforata* from an obligatory feeder to a facultative feeder, which can complete metamorphosis and settle in the absence of food (Heyland et al., 2004). THs not only accelerate development in echinoids, but differentially affect larval and juvenile morphogenesis as well. For example, while larval development and growth are inhibited by THs, development of juvenile structures is accelerated (Heyland and Hodin, 2004).

These differential responses of larval and juvenile structures to TH in echinoids are strikingly similar to the adaptive phenotypically plastic response of these larvae to varying food concentrations (Heyland and Hodin, 2004), suggesting that ingested TH may be the plasticity cue in these larvae. These findings support the hypothesis that THs from algae (i.e. Fig. 2) provide nutrition-related signals to echinoid larvae that alone can regulate distinct physiological responses. While all vertebrates obtain iodine from their diet, the direct transfer of THs (T4 and T3) across the intestinal wall has also been observed (Wynn, 1961). For example, some amphibian tadpoles obtain THs from crustaceans that they prey on. Increased TH levels in these predatory tadpoles correlate with accelerated metamorphosis (Pfennig, 1992). This situation could lead to a feeding preference of tadpoles for crustaceans with high TH levels. These findings show that THs from exogenous sources physiologically affect development – an observation in favor of the cross-kingdom (cross-phyla for the amphibian example) communication hypothesis.

While plant-derived exogenous TH signaling may represent the ancestral mode of thyroid metabolism in animals, evidence from echinoids suggests that some evolved endogenous synthesis. Exposing sand dollar larvae to TH-synthesis inhibitors delays metamorphic competence, a process that is regulated by THs. Moreover, metamorphically inhibited larvae can be rescued with the application of exogenous T4 (Heyland and Hodin, 2004), supporting the hypothesis of endogenous synthesis in this group.

Endogenous TH synthesis can be advantageous because it leaves the organism independent from exogenous sources. On the other hand, it might be associated with high metabolic costs. If metabolic costs could be lowered when an existing pathway is co-opted for a novel function, it could potentially result in a wide diversity of enzymatic candidates and signaling pathways participating in TH metabolism in different organisms. In the next section we argue that candidates for endogenous TH synthesis are present in many marine invertebrate species and could have been co-opted many times independently for this function.

The role of peroxidases in thyroid hormone biosynthesis

Two peroxidases, thyroid oxidase (THOX) and thyroid peroxidase (TPO), are essential for TH synthesis in vertebrates. THOX catalyzes the production of H_2O_2, which then oxidizes the free TPO enzyme to the so called complex I. By binding iodide, complex I is oxidized to complex II, which reacts with tyrosyl residues of TG.

Both THOX and TPO share the primary structure of the active site involved in heterolytic cleavage of the iron linked O–O bond of hydrogen peroxide (Poulos, 1988; Poulos and Finzel, 1983) with other peroxidases found in protists, bacteria, plants, fungi and animals (Taurog, 1999). These sites are essential to dismutate hydrogen peroxide necessary for the oxidation of iodide (or any other halide). For example, chloroperoxidase from the mold *Caldariomyces fumago* can catalyze the synthesis of significant amounts of thyroxine from thyroglobulin and iodine (Taurog and Howells, 1966). Other members of the peroxidase superfamily (*sensu* Taurog, 1999) are the haloperoxidases found in marine algae, where they catalyze the oxidation of halogens, a process responsible for the synthesis of small, volatile halocarbons (Gribble, 2003).

Animal and plant peroxidases evolved from different ancestors (O'Brien, 2000). Their ability to catalyze the oxidation of halogens and the synthesis of H_2O_2 led to their use for various biological functions. We hypothesize that one such function is TH synthesis in various invertebrates. Our observations that thiourea and other TPO inhibitors block iodine uptake (A. Heyland, unpublished) and metamorphosis (Heyland and Hodin, 2004) in echinoid larvae directly support this hypothesis. However, isolation, biochemical and pharmacological characterization of enzymes responsible for TH synthesis in marine invertebrates will be required.

Organic forms of iodine may have been used by algae as defence against excessive predation, or to suppress the oxidative environment inside the cell by scavenging hydrogen peroxide and superoxide (Collen et al., 1994; Giese et al., 1999). Due to its high chemical reactivity, iodine is often rapidly neutralized to the less reactive iodide in cells and tissues, especially in the gut (Gosselin et al., 1984; Reynolds, 1989). It is conceivable that the aforementioned reactions involving peroxidases may have initially served as detoxification mechanisms; the signaling role of THs may have evolved secondarily. Under this scenario, the critical enzymes were recruited and selected for their ability to efficiently catalyze the subsequent reactions necessary for TH synthesis. An analogous hypothesis has been recently suggested for the evolution of juvenile hormones as signaling molecules in insects (Hodin, in press).

An ortholog of the vertebrate THOX enzyme has recently been cloned from the sea urchin *Lytechinus variegatus* (Wong et al., 2004), where its catalytic activity induces an oxidative burst at fertilization. However, functions in embryonic or larval development have not yet been investigated. We are currently identifying other peroxidases in sea urchin and mollusc larvae (A. Heyland and L. L. Moroz, unpublished data) that could be good candidates for TH synthesis or incorporation.

Receptors without ligands: the search for the thyroid hormone-related signal transduction pathways in marine invertebrates

The signaling potential of any molecule is regulated by its synthesis and transport, and its link to a specific transduction

pathway. In vertebrates, TH synthesis and release are tightly regulated *via* the hypothalamo–pituitary axis (HP-axis). At the target tissue, de-iodinases can regulate the availability of T3 by removing one iodine from T4 (Yen, 2001) One mode of signal transduction of THs in vertebrates that is well understood is *via* thyroid hormone receptors (TRs). Upon binding to its cognate TR, T3 turns on transcription *via* a complex cascade of intracellular events involving various other nuclear hormone receptors (NRs) and co-factors (Yen, 2001). TRs have a much higher affinity for T3 than other THs, which leads to the notion that T3 is the active hormone and T4 is the pre-hormone.

To date, there is no completely characterized invertebrate TR analog. Candidates for receptors such as CiNR1 (Carosa et al., 1998) failed to bind DNA. Other attempts to characterize TH binding proteins in ascidians remained ambiguous due to very low binding affinity to T3 (Fredriksson et al., 1993). New candidates such as putative TR from a trematode expressed sequence tags (EST) database (CD154489) and the TR identified from the sea urchin *Strongyolentrotus purpuratus* genome (GenBank, Accession number: XM_784395) remain to be characterized molecularly and physiologically before any statement about their identity can be made.

Structurally similar molecules can signal *via* radically different pathways. Terpenoids, for example, occur as signaling molecules in plants and animals: gibberellins (hormones regulating blooming cycle in plants) are diterpenoids, ecdysteroids (arthropod hormones) are triterpenes and juvenile hormones are sesquiterpenoids. Insects co-opted NRs for the signal transduction of ecdysteroids, while plants use a variety of alternative pathways (Thomas and Sun, 2004). The mechanistic basis for this flexibility in hormonal signal transduction is still poorly understood. Recent efforts in understanding how xenobiotics (environmental contaminants) can mimic hormonal effects in animals provide evidence that low affinity binding to NRs and receptor cross-talk between NRs is primarily involved in this physiological interference (Mclachlan, 2001).

It should not come as a surprise that although no NRs have been identified in plants, fungi and bacteria (Escriva et al., 2000), animal hormones could have relevant physiological effects in these groups. Furthermore, we should be prepared to consider alternative signal transduction pathways for TH action in vertebrates and invertebrates. For example, it has become clear that THs signal *via* non-genomic (also called non-nuclear or non-transcriptional) pathways in vertebrates. This mode is characterized by relatively fast signal transduction that does not necessarily involve protein synthesis, instead acting through a suite of membrane-signaling pathways that may involve kinases or calmodulin (Yen, 2001). Two major targets of non-genomic thyroid and steroid hormone action are the central nervous system and the vascular system. Some recent reviews provide excellent background information about this mode of signaling (Simoncini and Genazzani, 2003; Hulbert, 2000; Davis and Davis, 1996; Christ et al., 1999; Falkenstein et al., 2000; Schmidt et al., 2000).

Our knowledge about such alternative modes of signaling is still rudimentary, however, and dependent on molecular information and rigorous functional physiological manipulation of the organism, a task that is not yet easily accomplished in the majority of invertebrate species. Defining the signal transduction pathway(s) involved in TH signaling across different kingdoms may require us to broaden our view and distance ourselves from established schemes such as the signaling of THs *via* NR pathways.

Conclusion and perspectives

THs are generally thought of as vertebrate-specific hormones that signal *via* NR cascades. However, increasing evidence suggests that TH signaling is not restricted to the vertebrates or even chordates. TH plays a critical role in animals that do not possess a thyroid gland or an endostyle. Moreover, growing evidence suggests that THs generated by food items have physiological effects on the 'consumers'. This may help us understand how endogenous hormone synthesis evolved. We hypothesize that the process of TH synthesis may have evolved through a peroxidase-dependent defence mechanism, because these ubiquitous enzymes have the ability to efficiently oxidize iodine. Furthermore, nuclear hormone receptor signaling is just one of the many possible signal transduction pathways for THs; several alternatives may have been recruited by different metazoans. Both the omnipresence of iodinated ligands in the marine environment and the multitude of TH signal transduction pathways emphasize that TH signaling has most likely evolved independently many times among the Metazoa, and that many more TH transduction pathways remain to be discovered.

List of abbreviations

DOM	dissolved organic matter
HP	hypothalamo–pituitary
NIS	sodium iodine symporter
NR	nuclear receptor
rT3	reverse 3,3′′,5-triiodo-L-thyronine
T1	monoiodotyrosine
T2	diiodotyrosine
T3	3,3′′,5-triiodo-L-thyronine
T4	L-thyroxine
TG	thyroglobulin
TH	thyroid hormone
THOX	thyroid oxidase
TPO	thyroid peroxidase
TR	thyroid hormone receptor
TSH	thyroid stimulating hormone

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