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REVIEWS OPEN ACCESS

IODOTHYRONINE DEIODINASES: KEY ENZYMES BEHIND THE ACTION OF THYROID HORMONE

Hanny Cho Too[1, 2], Mitsuhiro Shibata[1], Masato Yayota[3] and Atsushi Iwasawa[3]

[1] *United Graduate School of Agricultural Science, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan*
[2] *Livestock Breeding and Veterinary Department, Ministry of Agriculture, Livestock and Irrigation, Naypyidaw, Myanmar*
[3] *Faculty of Applied Biological Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan*

ABSTRACT

Thyroid hormone acts virtually on every cell of the vertebrate body and regulates numerous cellular functions by binding to nuclear thyroid hormone receptors. Circulating concentration of thyroid hormone is under the control of thyroid-stimulating hormone (TSH) secreted from the pituitary gland. Thyroid hormone mainly secreted from the thyroid gland is thyroxine (T_4), while the nuclear thyroid hormone receptor prefers binding to triiodothyronine (T_3) about tenfold. Therefore, T_4 must be converted to T_3 mostly in extra-thyroidal tissues to exert its actions. Recently more researchers have paid attention to the fact that this conversion is carried out by members of iodothyronine deiodinases, enzymes that reside in the cellular membranes, thereby enabling cell-specific regulation of T_3/T_4 balance largely independent of their circulating concentrations. Three different deiodinases (D1, D2 and D3) are characterized in vertebrate species, none of which is under the control of circulating TSH. D2 catalyzes deiodination of less active T_4 to produce active T_3. D3 removes iodine from T_3 or T_4 to produce diiodothyronine (T_2) or reverse T3 (rT_3), respectively, both of which are inactive. On the contrary, D1 is an inefficient enzyme in that it is three orders of magnitude less efficient in catalyzing T_4 compared with D2 and D3. D1 may function like a futile enzyme, since it can both activate and inactivate T_4 with almost the same velocity. However, D1 catalyzes removal of iodine from rT_3 much more efficiently than from T_4, and hence its possible importance in recycling iodine, especially in iodine deficiency such as in certain hypothyroid patients and avian embryos in the confined eggshells. In addition, deiodinase homologs of non-vertebrate chordates such as amphioxi and ascidians characterized recently have provided useful information to gain deep insight into thyroid hormone signaling system from evolutionary aspect. This review briefly summarizes the present status of research and perspectives of studying iodothyronine deiodinases, key enzymes behind the thyroid hormone action.

Keywords: iodothyronine deiodinases; feedback control; thyroid hormones; vertebrate evolution

Introduction

Thyroid hormones, 3,5,3'-triiodothyronine (T_3) and 3,5,3' 5'-tetraiodothyronine (T_4 or thyroxine), are iodinated tyrosine derivatives that are synthesized only in the thyroid gland among all the organs of the body. They exert their hormonal action mainly by binding to nuclear thyroid hormone receptors that reside virtually in every cell throughout the body (Sinha and Yen, 2014). Thyroid hormones play indispensable roles in many biological pathways including metabolism and body temperature homeostasis in homeotherms such as mammals and birds (Hulbert, 2000; Silva, 2006; McNabb and Darras, 2015), as well as coordination of normal development of various vertebrates such as metamorphosis in amphibians (Tata, 2006; Brown and Cai, 2007) and neurogenesis of central nervous system in mammals and birds (Van Herck *et al*., 2013; Bernal, 2015).

It is frequently presented in basic biology textbooks that the secretion of thyroid hormones is under the control of the hypothalamo-pituitary-thyroid axis, where hypothalamic releasing hormones stimulate the pituitary gland to secrete the thyroid stimulating hormone (TSH) and TSH in turn acts on the thyroid gland to stimulate multiple steps of the secretion of thyroid hormones. Thyroid hormones inhibit the secretion of releasing hormones in the hypothalamus and TSH in the pituitary, thereby comprising a negative feedback loop (Nussey and Whitehead, 2001; McNabb and Darras, 2015).

Thyroid hormones include T_4 and T_3; when the iodine atom at 5' position of T_4 is replaced with a hydrogen atom, the molecule is called T_3. As far as a sufficient amount of iodide is available,

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the thyroid gland predominantly synthesizes and releases T_4 into circulation. For example, the thyroid gland of normal human subject releases T_4 about tenfold more than T_3 (Fisher *et al*., 1971). Binding of T_3 to nuclear thyroid hormone receptors of the target cells is the major mechanism of thyroid hormone action. Nuclear thyroid hormone receptors contain in their molecules a DNA binding domain that recognizes a specific sequence named thyroid hormone response element (TRE) in the promotor region of thyroid hormone-regulated genes, thereby acting as a transcription factor in the target cells (Sinha and Yen, 2014). However, the affinity of T_4 with the nuclear thyroid hormone receptor is about tenfold lower than that of T_3 (Sinha and Yen, 2014). Thus, T_3 is generally regarded as the active thyroid hormone and T_4 as a prohormone that has to be converted into T_3 in peripheral or extra-thyroidal tissues to exert thyroid hormone action (Schweizer *et al*., 2008).

Conversion between thyroid hormones is catalyzed by iodothyronine deiodinases; this fact is usually omitted in basic biology textbooks, but should be among the most indispensable factors for thyroid hormone action, if one considers the importance of regulating T_3 concentration in target tissues. Deiodinase family consists of three types of enzymes, namely D1, D2 and D3. This review focuses on how thyroid hormone activity is regulated by the three different deiodinases and also emphasizes the importance of viewing deiodinases from evolutionary angle.

Specific properties of deiodinases

Thyroid hormones have two rings in their molecules (Fig. 1A): outer or phenolic ring and inner or tyrosyl ring. Deiodinases remove iodine atom from either the 5' position of the outer ring or from the 5 position of the inner ring. The former is tagged as outer ring deiodination (ORD) and the latter is called as inner ring deiodination (IRD). Enzymes that catalyze ORD are referred to as the outer ring deiodinases that correspond to the Enzyme Commission (EC) number 1.21.99.4, whereas enzymes that catalyze IRD are referred to as the inner ring deiodinases (EC 1.21.99.3).

Of the three deiodinases, D1 is a multifunctional enzyme that can catalyze both ORD and IRD, with the values of Michaelis-Menten constant (Km) for ORD and IRD both within a micromolar range (Fekkes *et al*., 1982). The Km value corresponds to the substrate concentration when the velocity of the enzyme reaction is half-maximum; Higher the Km value, the enzyme is roughly considered less efficient in catalyzing the substrate (Johnson, 2013). D1 prefers reverse T_3 (rT_3) and rT_3 sulfate > T_2 sulfate >> T_4 as ORD substrates, whereas it prefers T_4 sulfate > T_3 sulfate >> T_3 and T_4 as IRD substrates (St Germain *et al*., 2009). D2 catalyzes only ORD and is considered the main T_4-activating enzyme, having a low Km value for T_4 (ca. 2 nM) compared with that of D1 (ca. 1 µM) (Arrojo e Drigo and Bianco, 2011). D2 prefers T_4 to rT_3 as a substrate (St Germain *et al*., 2009). D3 catalyzes only IRD with nanomolar Km values for both T_4 (ca. 30 nM) and T_3 (ca. 6 nM) and considered the main thyroid hormone-inactivating enzyme (Arrojo e Drigo and Bianco, 2011). As indicated by the Km values, D3 prefers T_3 to T_4 ca. 5-fold as its substrate.

These three types of deiodinases share a remarkable feature that they contain selenocysteine (SeCys) in their active site (Bianco *et al*., 2002). SeCys is a rare amino acid where cysteinyl sulfur is substituted with selenium. When SeCys is replaced with cysteine, catalytic efficiency of the deiodinases is markedly decreased (Berry *et al*., 1992; Buettner *et al*., 2000; Kuiper *et al*., 2003). Human D1 protein (GenBank gi 13195755) consists of 249 amino acids with SeCys at position 126. Likewise, human D2 protein (gi 1518542) consists of 273 amino acids with SeCys at position 133 and human D3 (gi 56103188) 278 amino acids with SeCys at 144. Sequences of the three deiodinases are identical at only 55 amino acids, i.e. 22% as of human D1 (Fig. 2), but 49 amino acids surrounding the active center containing SeCys are highly conserved bearing 77% identity (Orozco *et al*., 2012). All are integral membrane proteins; D1 and D3 reside at the plasma membrane and D2 resides at the endoplasmic reticulum, with their catalytic sites probably oriented toward the cytosol (Gereben *et al*., 2008a). This means thyroid hormones must enter the cells to be deiodinated.

Despite the earlier assumption that translocation of small, hydrophobic molecules like thyroid hormones across the lipid bilayer membrane occurs by simple diffusion, thyroid hormones do not readily cross the plasma membrane. The majority of cellular thyroid hormone uptake occurs via several transmembrane transporters belonging to different transporter families including members of the monocarboxylate transporters (MCTs), the organic anion transporters family (OATPs) and the L-type amino acid transporters (LATs) (Friesema *et al*., 2005; Visser *et al*., 2008).

Physiological roles of deiodinases

D1

D1 is not a very efficient enzyme for catalyzing T_4 as described above. However, since the human liver contains a large amount of D1 and the liver is one of the largest organs in the body, D1 in the liver is believed to be an important regulator of circulating T_3 concentrations (Schweizer *et al*., 2008). It has been estimated that in humans around 80% of the circulating T_3 production results from extra-thyroidal deiodination of T_4 by D1 and D2 (Engler and Burger, 1984), among which D1 supplies a significant fraction of the circulating T_3 of euthyroid humans and even more in the patients of hyperthyroidism (Bianco *et al*., 2002). However, transgenic and knockout mouse technologies have casted doubts on this alleged role of D1 and provided evidence that D1 is not so crucial for maintaining circulating T_3 level as believed (Schneider *et al*., 2006;

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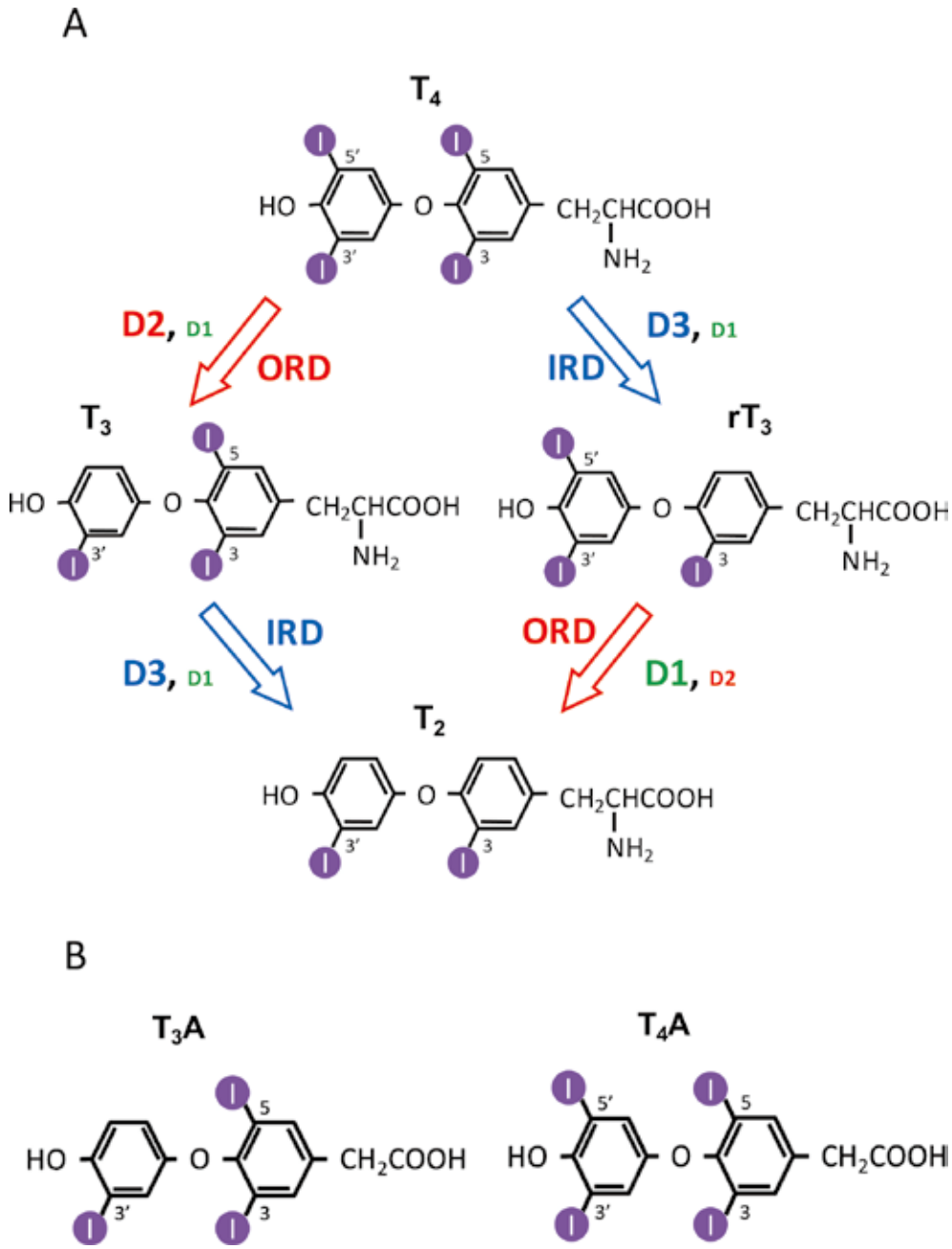

Fig.1. (A) Major pathways of thyroid hormone deiodination in vertebrates. Deiodinases (D1, D2 and D3) remove an iodine atom from the 5'-position of the outer ring or the 5-position of the inner ring of thyroid hormones. The former is called outer ring deiodination (ORD) and the latter is called inner ring deiodination (IRD). Removal of 5'-iodine or 5-iodine from T_4 leads to its activation to T_3 or inactivation to reverse T_3 (rT_3), respectively. The different font sizes of the deiodinase names correspond to their relative activity for the deiodination. T_2: 3,3'-diiodothyronine. (B) Specific substrates of an amphioxus deiodinase bfDy: 3,3',5-triiodothyroacetic acid (T_3A) and 3,3',5,5'-tetraiodothyroacetic acid (T_4A). Note their structural resemblance to T_3 and T_4, respectively.

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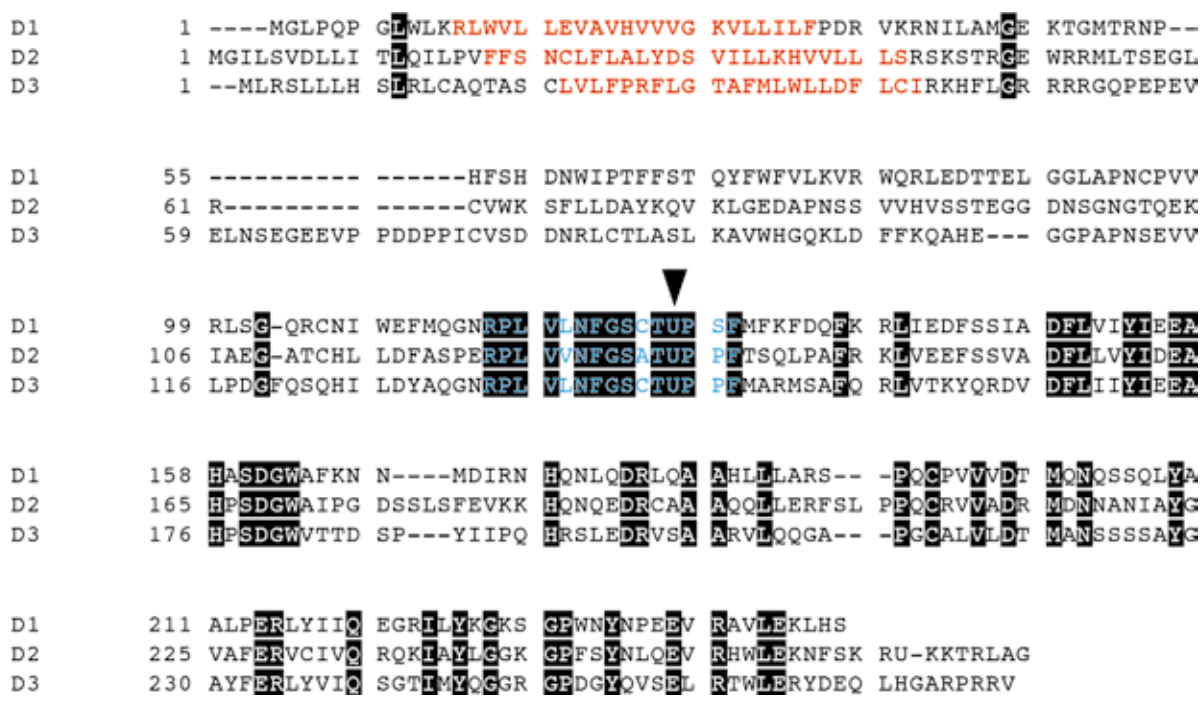

Fig.2. Alignment of the amino acid sequences of the three human iodothyronine deiodinases (D1, D2 and D3). Amino acids that are identical among three deiodinases are indicated in black square. Gaps are inserted for the best alignment. The code U indicated by an arrow head is selenocysteine where cysteinyl sulfur is substituted with selenium. Regions surrounding the selenocysteine where amino acids are colored in blue are predicted active sites of the enzymes and they are highly conserved among the three deiodinases. Amino acids colored in red comprise predicted transmembrane regions. Sequences were retrieved from the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/). GenBank gi: 13195755 for D1, 1518542 for D2 and 56103188 for D3. Multiple sequence alignment was performed using the online software clustalw provided by the DNA Data Bank of Japan (http://clustalw.ddbj.nig.ac.jp). Functional domains were predicted using the online software InterPro provided by the European Bioinformatics Institute (http://www.ebi.ac.uk/interpro).

Schiweizer *et al*., 2008). Since D1 catalyzes the deiodination of rT_3 and its sulfate much more efficiently than T_4, hepatic D1 is recently considered as a "scavenger" of these inactive iodothyronines from circulation (St Germain *et al*., 2009). This scavenger function seems of particular importance in the patients of hypothyroidism caused by iodine deficiency (Maia *et al*., 2011). But, D1 activity in the thyroid gland, where T_4 is supplied as substrate continuously, increases prominently in the patients of hyperthyroidism, which is considered the main cause of the elevation of circulating T_3 concentration (Maia *et al*., 2011). Taken together, the function of D1 may be different to some extent between physiological and pathological conditions.

In chickens, the yolk sac membrane is the heaviest organ throughout the embryonic development, much heavier than the embryonic liver (Fig. 3). For example, on day 17 of the 21-day incubation period, the wet weight of the yolk sac membrane (4.16 g) is ca. 8.9-fold heavier than the embryonic liver of the same day. The yolk, surrounded by the yolk sac membrane, contains large amounts of T_3 and T_4 of maternal i.e. hen origin that will be transferred to and used by the embryo for its appropriate development (McNabb and Wilson, 1997). We have found continuous expression of D1 mRNA in the yolk sac membrane from day 3 to the end of the 21-day incubation (Cho Too *et al*., unpublished data). In view of the large amount of thyroid hormones in the yolk, we speculate that yolk sac D1 contributes, rather than to the activation of thyroid hormone, to the recycling of iodine, which cannot be supplied from outside the egg, by deiodination of rT_3. In this regard, D1 in the chicken yolk sac membrane may act as a scavenger like D1 in the liver of the iodine-deficient patients.

D2 and D3

As described above, both D2 and D3 have low Km values for thyroid hormones, which enables a precise control of tissue-specific thyroid hormone action (Köhrle, 1999; Bianco and Kim, 2006; Gereben *et al*., 2008b). A well-known example is the control of

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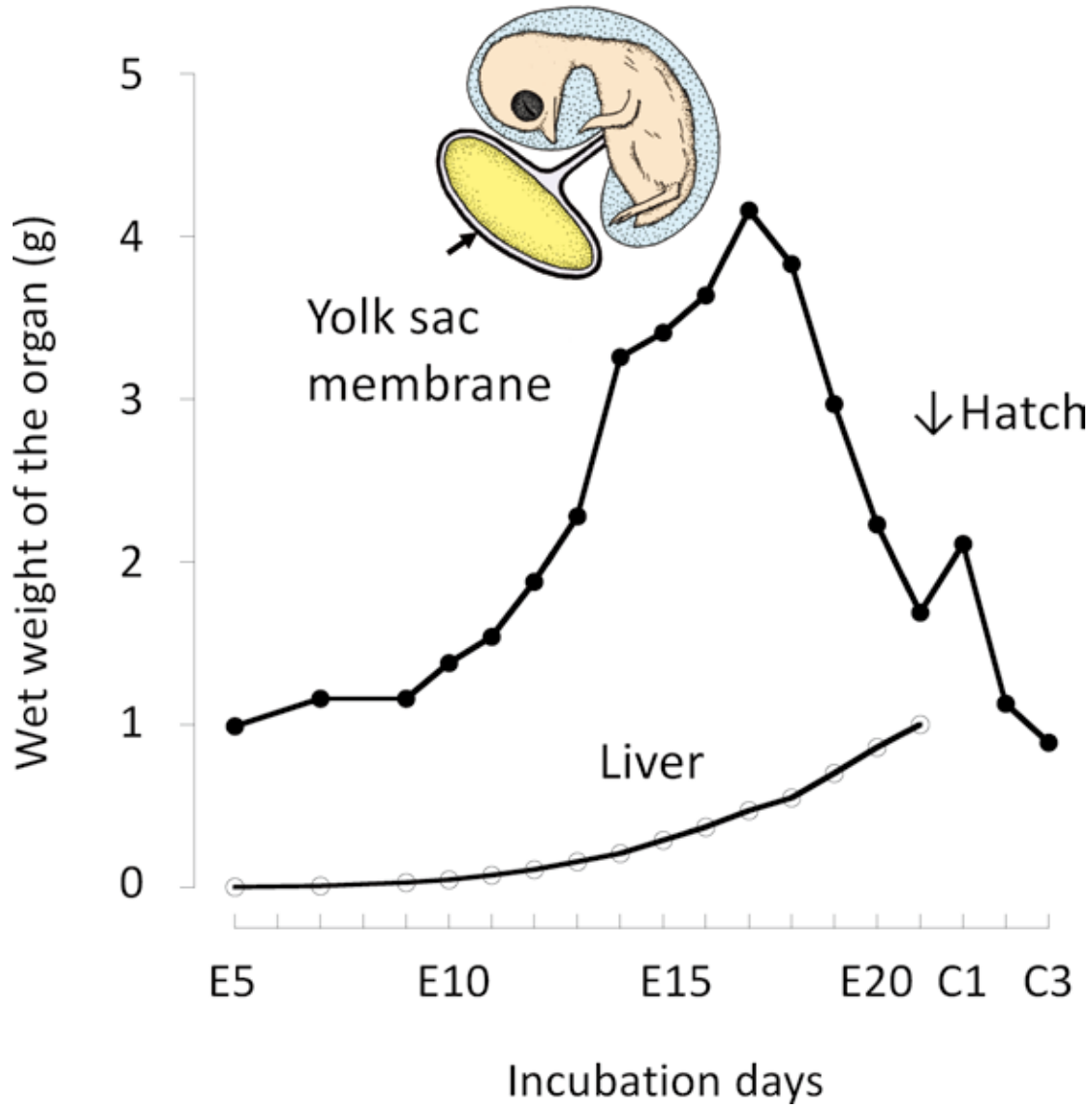

Fig.3. Changes in the weights of the embryonic liver (open circles) and the yolk sac membrane (closed circles) during 21-day incubation of the chicken (unpublished data of Cho Too *et al*., 2016). The yolk sac membrane was weighed until the third day post-hatch. E = embryo, C = chick. The illustration shows a chicken embryo of ca. E13 enclosed in amniotic sac and the yolk sac membrane, indicated by an arrow, that surrounds the yolk.

amphibian metamorphosis. Although metamorphosis of amphibian tadpoles is initiated and maintained obligatorily by T_3, different tissues such as limb, tail, intestine and gill respond to the same concentration of plasma thyroid hormones at different timing. This seems enabled in part by the fact that different tissues express D2 and D3 at different timing, thereby increasing and decreasing intracellular T_3 concentrations of specific cells largely independent of plasma thyroid hormone concentrations (Becker *et al*., 1997; Brown and Cai, 2007; Darras and Van Herck, 2012).

Another well-documented example of tissue-specific action of deiodinases is their differential localization in the brain (Fig. 4). In mammals, D2 is localized predominantly in the astrocyte, a glial cell of the brain, and D3 is expressed mainly in the neuron (Guadaño-Ferraz *et al*., 1997; Tu *et al*., 1999), which suggests a

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following coordination of astrocytes and neurons: T_4 taken up from the circulation by endothelial cells of the blood vessels in the brain is transported to the astrocytes surrounding the blood vessels. Then it is deiodinated by D2 in the astrocytes and the resulting T_3 is transferred to the neurons. Transferred T_3 is then used and eventually inactivated by D3 in the neurons (Courtin *et al*., 2005; Bernal, 2015). Recent studies of the embryonic brain of chickens revealed that D2 was expressed in the endothelial cells of the blood vessels, which might be favorable for a more direct supply of T_3 to the neurons (Van Herck *et al*., 2015). Localizations of deiodinases and cellular interaction of deiodinase-mediated thyroid hormone actions within an organ can thus be different between taxonomical groups of animals.

For another example of differential actions of deiodinases, we have evaluated mRNA expression of deiodinases in the chorioallantoic membrane of developing chicken embryo throughout its incubation period (Fig. 5). This membrane is originated from the combination of all three germ layers, i.e. ectoderm, endoderm and mesoderm, and gradually formed from around day 4 until day 12 of incubation by a fusion of the chorion and the allantois, both of which are extraembryonic membranes. Since chorioallantoic membrane is highly vascularized and spreading beneath the eggshell, it contributes to the gas exchange through the pores of the eggshell until the embryonic lung starts functioning just prior to hatch (Etches, 1990).

As shown in Fig. 5, all three deiodinases were expressed in this membrane. D2 expression was relatively high on days 11-12, days 15-16 and day 20. T_3 is known as a regulator of vascular formation (Ishizuya-Oka and Shi, 2005; Astorga and Carlsson, 2007), and thus especially the first peak of D2 may have a role in the vascular formation of the developing chorioallantoic membrane by increasing intracellular T_3 level. D3 expression tended to be relatively high when D2 expression was relatively low, which suggests coordinated action of D2 and D3 may be important for the function of this membrane. D1 expression was somewhat low with less characteristic changes throughout the incubation period. Is D1 less important for this membrane? Van der Geyten *et al*. (2002) pointed that if expression

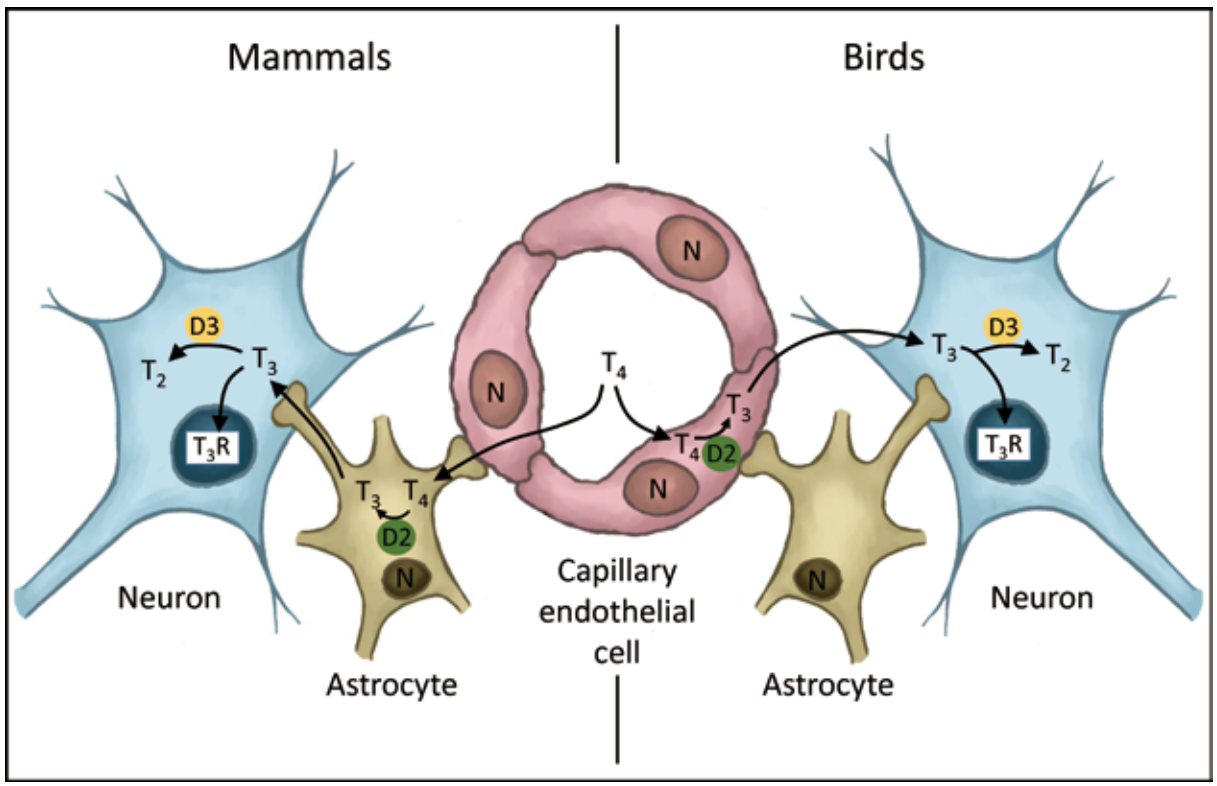

Fig.4. Thyroid hormone deiodination and interaction between neuron, astrocyte and capillary endothelial cell in the brain of mammals and birds. Transmembrane transporters, which are not depicted, are necessary for transport of thyroid hormones in and out of the cells. N = nucleus, T_3R = nuclear T_3 receptor. See text for more details.

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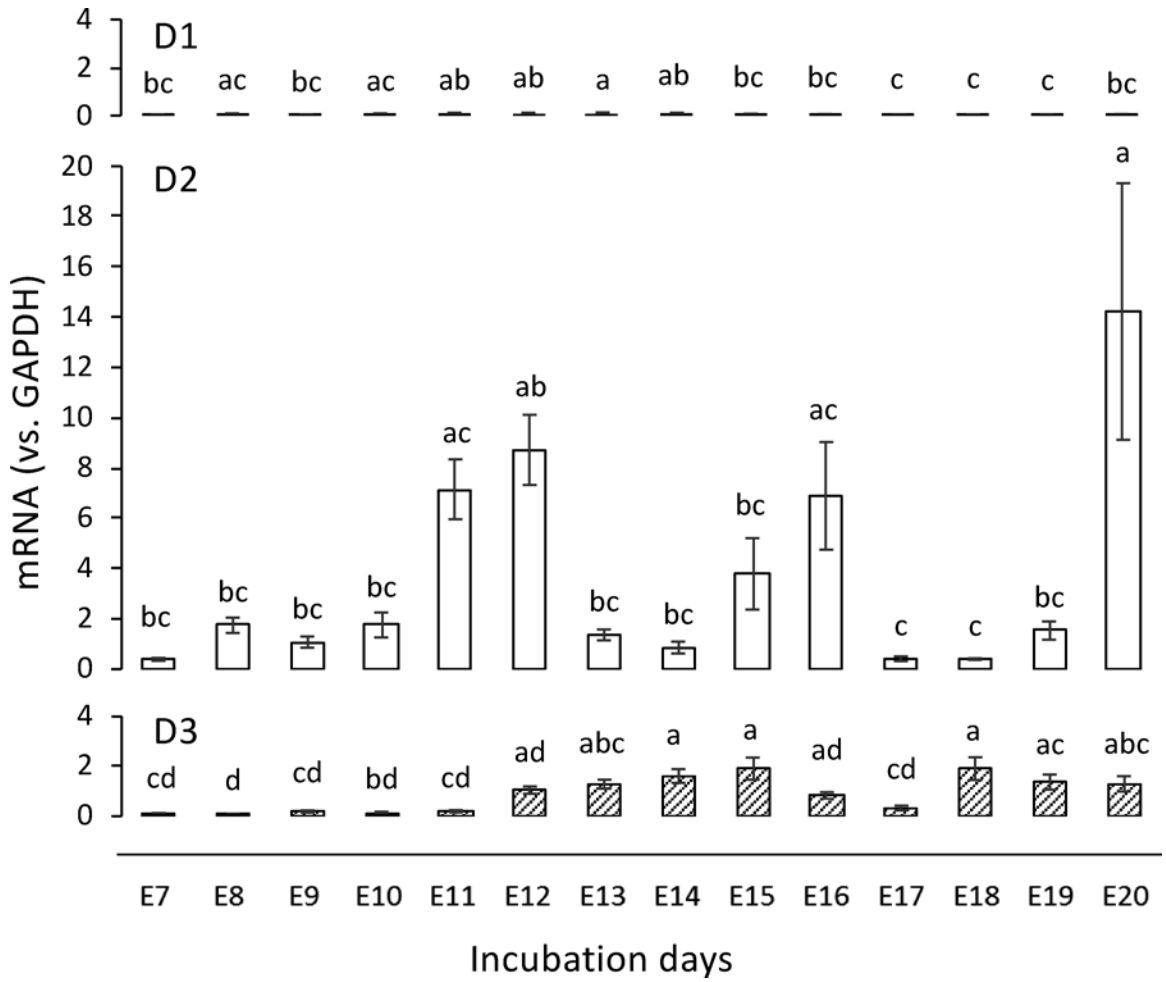

Fig.5. Expression of iodothyronine deiodinase (D1, D2 and D3) genes in the chorioallantoic membrane during embryonic development of chicken (unpublished data of Cho Too *et al*., 2016). Total RNA extracted from the chorioallantoic membrane was reverse transcribed into first strand cDNA using a random primer. The gene expression was determined by quantitative PCR performed in a Mx3000P Real-Time PCR System (Agilent Technologies, Tokyo, Japan) with a two-step standard cycling program for Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix (Agilent). Relative expression values were calculated with an installed software of the Mx3000P System based on the standard curve method using a serial dilution of pooled cDNA as standard. Primer sequences used for amplifying D2, D3 and GAPDH were reported by Van Herck *et al*. (2012). Those used for amplifying D1 were 3'-GCAGCACAATTTCTTCAGCA-5' (forward primer) and 3' -GTAATTCCAAGGCCCCACTC-5' (reverse primer). Each data represents mean ± SEM (n=7). Means without a common letter are significantly different (p<0.05 by Tukey-Kramer multiple comparison test). Y-axes represent expression of D1, D2 or D3 versus GAPDH, a housekeeping gene, in arbitrary units. E = embryo.

of a deiodinase is limited to a specific cell type in an organ, its expression level per whole organ would be low, but it might play a role in regulating intracellular thyroid hormones in that specific cell. Low expression level may not necessarily indicate low importance. We consider that it is necessary to specify cell types in the chorioallantoic membrane where the three deiodinases are expressed using, for example, *in situ* hybridization technique.

Furthermore, if a large amount of D2 or D3 is expressed in an organ with a large volume and high blood flow such as the liver, these deiodinase activities can affect circulating thyroid hormone concentrations. For example, D3 activity is very high in embryonic chicken liver, but it falls by 98% from day 17

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Thyroid Hormone Signaling Components	Reported Presence or Absence in Deuterostomes				
TRH or equivalent	–	–	–	–	+
TSH or equivalent	–	–	–	–	+
Thyroid hormones and receptors	+	?	+	+	+
Deiodinase or deiodinase-like protein	Deionidase-like*	?	Deionidase-like* (bfDx,bfDy,bfDt)	Deionidase-like* (hrDx,ciDx,ciDy, csDx, csDy)	D1, D2, D3

*homology in amino acid sequences

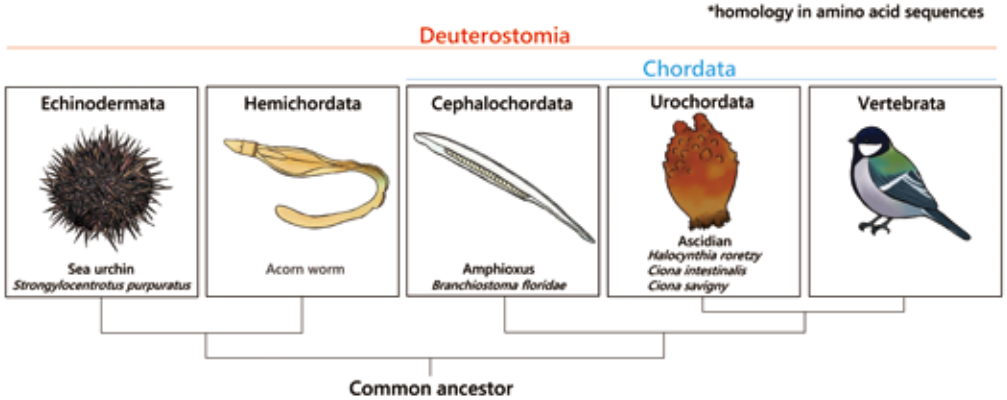

Fig.6. A schematic phylogenic tree of the members of deuterostomes with illustrations of representative species and a table showing presence (+), absence (-), or yet unknown (?) of thyroid hormone signaling components in each phylum and subphylum. Scientific names mentioned under the illustrations indicate that genes encoding deiodinases or deiodinase-like proteins are found and/or cDNAs are cloned in these species; Names of deiodinases from these organisms, such as bfDx from *Branchiostoma floridae*, are placed in the corresponding column of the table. TRH = thyrotropin-releasing hormone, which is the hypothalamic hormone in mammals that releases TSH from the pituitary gland.

toward hatching on day 21. This marked decrease in D3 activity is accompanied by a plasma T_3 surge, which suggests that the decrease in hepatic D3 activity leads to the elevation of plasma T_3 level (Van der Geyten *et al*., 1997). The increase in plasma T_3 has been found indispensable to advance a number of processes for normal hatching of chicken embryos (De Groef *et al*., 2013).

Deiodinases from evolutionary aspects

Characteristics of D1 reaction are somewhat enigmatic. As described above, D1 is a multifunctional enzyme that has both ORD and IRD activity. Although Km value for its substrate T_4 is high, enzymatic reaction can proceed significantly if a large amount of T_4, i.e. as large as its Km value, or a large amount of the enzyme itself exists. When D1 reacts with T_4, the Vmax (maximum enzyme velocity)/Km ratios for ORD (13 μM·min/pmol·mg protein) or IRD (9 μM·min/pmol·mg protein) are similar, suggesting that these reactions occur at almost equal rates (Bianco *et al*., 2002). Could it be a wise strategy for a cell to activate T_4 while inactivating it at the same time by the same enzyme?

Orozco *et al*. (2012) performed phylogenetic analysis of known amino acid sequences of deiodinases from ca. 20 vertebrate species including mammals, birds, amphibians and fish. The D1 sequence was the most variable of the three deiodinases, which suggested D1 had the longest evolutionary history. D2 and D3 sequences were close to each other and less variable than the D1 sequence, suggesting they appeared more recently in evolution. Since genes for D2 and D3 are located on the same chromosome, these authors suggested that D2 and D3 might have evolved by gene duplication (Orozco *et al*., 2012). D2 and D3 have similar, low Km values for their substrates; Km value of D2 for T_4 being ca. 2 nM while that of D3 for T_3 being ca. 6 nM, as described above. It is therefore considered that the emergence of D2 and D3 in evolution has made it possible to regulate intracellular and circulating concentrations of thyroid hormones

much more accurately than they had been regulated by D1, and thus has evolved significant roles in such biological functions as development, metamorphosis and metabolism.

Deiodinases have been analyzed not only in vertebrates but in non-vertebrate chordates (Fig. 6). The genome sequence of amphioxus, a cephalochordate, was already released (Putnam *et al*., 2008) and a set of proteins necessary for thyroid hormone production, secretion, circulation and cellular signaling have been revealed encoded in the amphioxus genome (Paris *et al*., 2008a). To date, an amphioxus *Branchiostoma floridae* deiodinase named bfDy has been biochemically characterized. The bfDy does not deiodinate either T_4, T_3 or rT_3, but it catalyzes specific, high-affinity IRD of 3,3',5-triiodothyroacetic acid and 3,3',5,5'-tetraiodothyroacetic acid (Fig. 1B), molecules that have the same outer and inner ring structures as T_3 and T_4, respectively (Klootwijk *et al*. 2011). Surprisingly, these molecules are produced endogenously in B. *floridae* from T_3 and T_4 and regulate metamorphosis in this species by binding to a specific, amphioxus thyroid hormone receptor (Paris *et al*., 2008b, 2010). Klootwijk *et al*. (2011) thus hypothesized that 3,3',5-triiodothyroacetic acid is a primordial bioactive thyroid hormone.

Recent studies have revealed that urochordates such as ascidians are evolutionarily more relevant to vertebrates than cephalochordates such as amphioxi (Delsuc *et al*., 2006). In the ascidian *Halocynthia roretzy*, a deiodinase named hrDx has been biochemically characterized. Indeed, this enzyme contains a SeCys residue like vertebrate deiodinases, and its catalytic activity resembles vertebrate D1 (Shepherdley *et al*., 2004). Furthermore, some authors (Paris *et al*. 2008a) have reported existence of deiodinase-like proteins in animals even more primitive than the cephalochordates such as the sea urchin *Strongylocentrotus purpuratus*, a non-chordate deuterostome, which deserves detailed studies including substrate specificity and other biochemical characterizations of this sea urchin protein. Taken together, although we do not have evidence yet that extant deiodinase of the amphioxus bfDy has close connection with ancestral D1, nor do we know to what extent the deiodinase-like protein of the sea urchin affects its thyroid hormone-signaling system, the peculiar substrate specificity of extant vertebrate D1 may be better interpreted in view of the history of the molecular evolution of thyroid hormones, thyroid hormone receptors and deiodinases.

Perspectives

Fig. 6 illustrates representative deuterostomes including vertebrates and the presence or absence of the components of thyroid hormone signaling system. In vertebrates, circulating thyroid hormone concentrations are maintained by the negative feedback loop, where increased circulating thyroid hormone inhibits secretion of releasing hormones from the hypothalamus and TSH from the pituitary gland, resulting in the decrease of thyroid hormone secretion from the thyroid gland. On the other hand, since non-vertebrate chordates such as cephalochordates and urochordates do not have the hypothalamus and the pituitary gland, regulation of thyroid hormone concentrations by the negative feedback loop as in vertebrates does not exist. In such species, deiodinases and factors regulating deiodinases should therefore be important for the maintenance of not only intracellular but circulating thyroid hormone concentrations. Various factors are reported to regulate vertebrate deiodinase expression and activity (Gereben *et al*., 2008b), among which are thyroid hormones themselves. Human gene for D1 is known to contain TRE where thyroid hormone receptor binds (Toyoda *et al*., 1995). No TREs have been found in either gene for D2 or D3 so far, but expression of D2 mRNA increases and that of D3 mRNA decreases in hypothyroidism and the opposite is also true in hyperthyroidism (Bianco *et al*., 2002), which indicates that all deiodinases are principally sensitive to thyroid hormones.

Most primordial thyroid hormone system thus seems likely that deiodinases regulate the balance of active/inactive thyroid hormones and thyroid hormones in turn regulate deiodinase activity and/or expression. In this regard, studies of thyroid hormone system in chordates like amphioxi and ascidians that do not have the hypothalamus or the pituitary gland and also in non-chordate deuterostomes such as sea urchins seems very attractive and promising to unravel vertebrate thyroid hormone system from evolutionary aspects.

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