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The deiodinase family: selenoenzymes regulating thyroid hormone availability and action

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Abstract. Thyroid hormones control growth, development, differentiation and metabolism in vertebrates. Most of the actions of the active thyroid hormone T3 (3,5,3′-triiodo-L-thyronine) are exerted via ligand-activated nuclear T3 receptors. Activation of the secretory product of the thyroid gland, L-thyroxine (3,3′,5,5′-tetraiodo-L-thyronine), or T4, is catalyzed by two enzymes, iodothyronine-5′-deiodinases type I and type II. Inactivation of T4 and T3 occurs via type III iodothyronine-5-deiodinase and to some extent by type I 5′-deiodinase. Complementary DNAs (cDNAs) encoding the substrate-binding selenocysteine-containing subunits of the deiodinases were cloned, though some controversy still exists on the type II 5′-deiodinase subunits. Characterization of tissue-specific expression patterns indicates that these selenium-dependent enzymes exert tight control on local and systemic availability of active T3. Thus, deiodinases are envisaged as guardians to the gate of thyroid hormone action mediated by T3 receptors.

Key words. Thyroid hormone metabolism; selenoenzymes; deiodination; thyroxine; triiodothyronine; development; comparative endocrinology; receptor.

Introduction

Thyroid hormones control growth, development, differentiation and many aspects of intermediary and structural metabolism in amphibia, fish, most higher vertebrates and mammals. Most of the actions of the active thyroid hormone T3 (3,5,3′-triiodo-L-thyronine) are exerted via members of the c-erbA family of nuclear receptors, acting as ligand-modulated transcription factors (TRα1, TRβ1, TRβ2) either in concert with closely related ligand-independent transcription factors (TRα2 or rev-erbA) or by formation of heterodimers with retinoid receptors (RXRs or RARs) or other members of the c-erbA family [1, 2]. Recently, functional T3 receptors were also identified in mitochondria [3]. In addition, strong experimental evidence has also accumulated for rapid, direct thyroid hormone action at the plasma membrane or other subcellular organelles [4–7]. The latter effects are also exerted by L-thyroxine (T4), reverse T3 (3,3′,5′-triiodo-L-thyronine, rT3) or 3,5-T2 (3,5-diiodo-L-thyronine) and probably do not involve (nuclear) T3 receptors. These so far unknown binding sites or thyroid hormone-binding proteins exhibit structure-activity relationships different from those of nuclear T3 receptors.

Synthesis of thyroid hormone occurs exclusively in the thyroid gland, whose main secretory product is T4 and to some degree also T3. Enzymic production of partially deiodinated products (T3, rT3, and the T2s) mainly occurs in extrathyroidal tissues but to a minor extent in the thyroid gland [8]. Modifications of the alanine side chain of the amino acid T4 and its less iodinated derivatives as well as sulfation or glucuronidation reactions of the acidic 4′-phenolate group of the iodothyronines are of minor importance in thyroid hormone metabolism [9]. As thyroid hormones are highly hydrophobic, several proteins are involved in binding, distribution and transport through the vascular, interstitial and cellular compartments. These proteins control free hormone concentrations, restrict bioavailability and prevent elimination of the iodothyronines [10].

The deiodinase isozymes

At least three deiodinase enzymes have been identified during the last few years and have been cloned and characterized at the molecular level [8]. The existence of deiodinases was first postulated immediately after the discovery of T3 in 1952 by Pitt-Rivers and colleagues [11]. The unequivocal proof of extrathyroidal production of T3 was obtained in 1970 after administration of T4 to athyreotic patients [12]. The initial description of enzymatic in vitro production of T3 from T4 by 5′-deiodinase (now type I) in perfused livers, intact hepatocytes and subcellular fractions was enabled in 1975 with the development of highly sensitive radioimunoassays for T35 [13, 14]. Soon after, a second enzyme forming rT3 from T4 and degrading T3 to 3,3′-T2, the so-called 5-deiodinase (now type III), was postulated. Deiodination experiments in brain tissues of eu- and hypothyroid rats using the potent inhibitor of type I 5′-deiodinase, 6-*n*-propyl-2-thiouracil (PTU), led to the discovery of the type II 5′-deiodinase in 1981 [15]. T4 is also deiodinated at the tyrosyl ring in the 5-position by the 5-deiodinase (type III). These three deiodinase enzymes, types I, II and III, show rather different biochemical and regulatory characteristics, exhibit different tissue distribution and developmental patterns of expression, and respond differently to deiodinase inhibitors (see table 1). Recently, cDNAs encoding subunits of these deiodinase enzymes have been cloned, functionally expressed and characterized in several cell types, tissues and species [8, 16]. Current experimental evidence suggests that the three deiodinases comprise a family of selenoproteins encoded by different genes. However, some controversy still exists on the exact biochemical nature of the type II 5′-deiodinase (see below).

Gene structure, chromosomal location and biochemical characteristics of deiodinases

Type I 5′deiodinase 5′DI, mainly expressed in liver, kidney, thyroid and euthyroid anterior pituitary, functions as a homodimer of a 27-kDa subunit encoded by 2.1-kb messenger RNA (mRNA) [17, 18]. The human 5′DI gene located on the short arm of the human chromosome 1p32–33 (corresponding to mouse chromosome 4), is 17.5 kb long, and is composed of four exons [19]. Several regulatory elements of the promoter of the human 5′DI gene have been functionally characterized. Like house-keeping genes, it does not contain a CAAT or TATA box [20, 21]. The reaction product of the enzyme, T3 and retinoids induce expression of the 5′DI gene via two complex thyroid hormone response retinoic acid response (TRE/RARE) elements located in

Table 1. Deiodinase isoenzymes, biochemical characteristics, regulation and function.

Property	Type I 5′-deiodinase	Type II 5′-deiodinase	Type III 5-deiodinase
Function	systemic >local T3 production, degradation of rT3 and sulfated iodothyronines	local >systemic T3 production	inactivation of T4 and T3
Expression	liver, kidney, thyroid, pituitary, heart, brown adipose tissue in sheep	(hypothyroid) pituitary, brain, brown adipose tissue, skin, placenta; thymus, pineal and harderian glands; glial cells and tanycytes	placenta, brain; many tissues; not pituitary, thyroid, kidney, adult liver
Cosubstrate	DTT or DTE in vitro; not glutathione or thioredoxin in vivo	DTT or DTE in vitro; higher concentrations than for 5′DI	DTT or DTE in vitro; higher concentrations than for 5′DI
Subcellular location	endoplasmic reticulum in liver, inner plasma membrane in kidney and thyroid	inner plasma membrane; p29 subunit associated with F-actin and perinuclear vesicles respectively	endoplasmic reticulum
Cloned in species	human, rat, mouse, dog, chicken; not expressed in *Rana catesbeiana*, *Oreochromis niloticus* (tilapia), rainbow trout	human, rat, mouse, chicken, *Rana catesbeiana*, *Fundulus heteroclitus* (teleost), rainbow trout	human, rat, mouse, chicken, *Rana catesbeiana*, *Xenopus laevis*
Essential amino acid residues	histidine, selenocysteine, cysteine, phenylalanine	Selenocysteine (?)	selenocysteine
Induction	T3, retinoids; TSH and cAMP in thyroid only; testosterone (liver), carbohydrate	cAMP; FGF; phorbolesters via PKC; ANP and CNP via cyclic GMP in glial cells	T3, FGF, EGF
Repression	Ca^{2+}-PI pathway in thyroid; dexamethasone	T3	
Inhibition	PTU, iodoacetate, aurothioglucose, iopanoate	T4, rT3, iopanoate	iopanoate

FGF: fibroblast growth factor. PKC: protein kinase C. ANP: atrial natriuretic peptide. CNP: C-type natriuretic peptide. EGF: epidermal growth factor.

the proximal promoter/enhancer region 5′ to the transcriptional start site at $-23/-24$ bp. 5′DI prefers rT3 as substrate and shows rather low affinity for T4 (K_{Mapp}, 2 μM). The type I enzyme also deiodinates 4′-*O*-sulfates of T4 and T3 at the tyrosyl ring by 5-deiodination under certain reaction conditions and thus shows limited reaction specificity due to some wobble in its active site [8, 22]. 5′DI cDNAs have been cloned from several species (human, rat, mouse, dog, chicken and tilapia) [16, 23–26].

The type II 5′-deiodinase acts as a heterotrimeric complex of ~200 kDa containing a 29-kDa subunit that interacts with filamentous actin. In rat astrocytes, ligand binding to this subunit leads to alteration of the polymerization state of the actin cytoskeleton associated with substrate binding and deiodination [27]. In contrast to 5′DI, the type II 5′D prefers T4 over rT3 as substrate and is strictly a phenolic ring 5′-deiodinase with a nanomolar apparent K_M for T4. The human gene encoding 5′DII has been mapped to chromosome 14q24.2–q24.3 and consists of two exons and a 7.4-kb intron [28, 29]. So far no detailed information on its promoter or regulation is available. 5′DII is mainly expressed in the central nervous system (CNS), the hypothyroid pituitary and the brown adipose tissue of rodents, as well as in some other tissues. In contrast to 5′DI, 5′DII is rapidly inactivated by its substrate T4 and rT3, but not T3, by mechanisms involving internalization associated with polymerization of the actin cytoskeleton as well as preoteasomal degradation [4, 30]. Recent evidence suggests that 5′DII activity is repressed by T3 via transcriptional mechanisms [31]. Several agents such as growth factors, cyclic AMP (cAMP) and corticosteroids and others stimulate 5′DII activity [22] (table 1).

In addition to intracellular redistribution via the F-actin microfilament network [4], inactivation of 5′DII in pituitary tumor cell lines via the proteasome pathway exists. Substrate (rT3) can accelerate degradation and reduce the half-life of 5′DII activity from ~1 h to 20 min [30]. Oxidation of the selenoyl intermediate of 5′DII after completion of the first half reaction of the 5′-deiodination of the substrate rT3 or T4 might provide the signal for accelerated degradation of the 5′DII (subunit) via the proteasome pathway. This hypothesis is compatible with a previous proposal of mine in that deiodinases might not act as enzymes that are regenerated to perform several reaction cycles but rather as 'vectorial catalysts', which inactivate substrate. Thereby deiodinase both provides a signal to the cell via alteration of polymerization state of F-actin microfilaments and eliminates the messengers T4 and T3 combined with termination of the signal by irreversible proteolytic degradation of (part of the) 5′DII enzyme [8, 32]. Neosynthesis of 5′DII after stimulation of gene expression by low-circulating thyroid hormone levels or other agents (cAMP) [33] and growth factors known to induce 5′DII expression might allow rapid regulation of the availabilty of local (and systemic) T3 supply for homeostatic control of growth, differentiation, development and basal metabolism.

The type III 5-deiodinase enyzme inactivates T4 and its metabolites by removal of iodine atoms at the tyrosyl ring [8]. The holoenzyme structure of this enzyme, containing a 32-kDa substrate binding subunit, is not yet known. The gene encoding 5D is located on the human chromosome 14q32 (corresponding to mouse chromosome 12F1) [34]. The gene contains a single 1.8-kb exon. A putative 526-nucleotide promoter fragment contains consensus TATA, CAAT and GC elements and drives luciferase reporter gene constructs after transfection into several cell types. 5D is expressed in many tissues, but not adult liver, kidney, thyroid and pituitary. It shows a different developmental profile compared with the two other activating deiodinases, and is thought to prevent accumulation of T4 or its active metabolite T3 in cells and tissues at inappropriate times and concentrations, especially during development but also in adult organisms [8]. High expression of 5DIII in human fetal and neonatal liver, adipose tissue and skeletal muscle is thought to account for thyroid hormone degradation in these immature organs during development [35, 36]. Recently, overexpression of 5DIII in tadpoles was shown to arrest metamorphosis by preventing resorption of tails and gills, indicating the importance of coordinated control of cellular T3 levels both at the level of synthesis and degradation [37]. In the turtle, a 5DIII form similar to that of birds and rodents has recently been characterized and found to have a much wider tissue distribution (highest expression in kidney, and much lower levels in liver, pancreas, heart, ovary and brain) and enzyme characteristics distinct from 5DIII that has been characterized in other species thus far [38]. Expression of mammalian 5DIII is stimulated by T3 and retinoids (similar to 5′DI), growth factors and phorbol esters; the latter agents apparently act via stimulation of the MEK/ERK signalling cascade [39]. cDNAs for 5DIII have been cloned for human, rat, chicken, *Xenopus laevis*, *Rana catesbeiana* and *tilapia* [8, 16, 40].

The selenoenzyme nature of the deiodinase isoenzymes

So far the exact mechanism of reductive deiodination of iodothyronines and the cleavage of the aromatic carbon-iodine bond of iodothyronines is not understood. Thus the identification of the deiodinase family as selenoenzymes provided some progress in understanding this unusual monodeiodination reaction, which occurs

in sequential steps. The physiological cofactor of this reaction has not yet been identified (or may not even exist). In vitro, reduced dithiols with strong reduction potentials such as DTT or DTE support the reaction and act as cosubstrates with millimolar apparent K_M in this two-substrate deiodination reaction [8, 22]. Whereas 5′DI exhibits a two-substrate ping-pong mechanism of reaction with the formation of an oxidized enzyme selenoyl-iodide intermediate, the 5′DII and 5DIII reaction proceeds with a two-substrate sequential mechanism of reaction without formation of an oxidized enzyme selenoyl-iodide intermediate. This difference might contribute to the observation that PTU and aurothioglucose, which are potent inhibitors of 5′DI, are less effective in the type II 5′D and the type III 5D reaction [8, 16]. Nevertheless, a type I selenoenyzme deiodinase in tilapia is less sensitive to these agents, and site-directed mutagenesis experiments point to the contribution of other active site residues as well as to differences in substrate and ligand preferences [25, 41].

Using a combination of affinity labelling methods with a substrate analogue [17, 42] and metabolic 75-selenite labelling of membranes exhibiting different 5′DI activity in selenium-depleted and -repleted animals [43], an unequivocal identification of 5′DI as a selenoenzyme containing selenocysteine in its active site was independently achieved in 1990 by two groups [43, 44]. Shortly after, the expression cloning of the rat 5′DI cDNA in *Xenopus laevis* oocytes confirmed these findings and identified the UGA-codon directing cotranslational selenocysteine insertion into the 27-kDa 5′DI subunit in the context of a hairpin-loop structure in the 3′-untranslated region, the SECIS element [18]. Replacement of selenocysteine by cysteine or other amino acids leads to a marked decrease or loss in enzyme activity similar to observations for other selenoenzymes [e.g. gluthathione peroxidase (GPx)]. Deletion of the SECIS element of the mRNA produces a truncated protein where the base triplet UGA is interpreted as an amber stop codon. Heterologous SECIS elements from other mRNAs encoding for selenoproteins can substitute for the 5′DI SECIS element, but with different efficiency [45]. In contrast to procaryotic SECIS elements that are located immediately downstream of the UGA codon and form part of the hairpin stem structure, the SECIS elements of eukaryotic mRNAs may act over considerable distances or even in trans position. However, cotranslational selenocysteine insertion efficiency markedly decreases if the SECIS element is located too far downstream (see below, type II 5′-deiodinase) [46].

Soon after 5′DI, 5DIII was identified as selenoenzyme using reverse-transcriptase polymerase chain reaction (RT-PCR)-based homology cloning and degenerated oligonucleotide screening methods, based on the assumption of homologies between the active sites of the individual deiodinase isoenzymes [34, 40, 47]. Similar to 5′DI, the selenocysteine residue is encoded by UGA in the context of a SECIS element in the 3′ untranslated region (UTR) of the 5DIII mRNA. Substitution of selenocysteine by cysteine or another amino acid again resulted in marked reduction or inactivation of the 5DIII enzyme activity.

Some difficulties were encountered during the cloning and identification of the type II 5′deiodinase. Using RT-PCR and degenerated oligonucleotide homology cloning, a putative rat and human 5′DII cDNA was identified, which hybridized to a 7–8-kb mRNA, much larger than the mRNAs of 5′DI and 5DIII that are in the 2-kb range [16, 48–54]. Furthermore, the tissue distribution of this heterogenous 5′DII mRNA did not fully correspond to the distribution of the 5′DII enzyme activity. Some tissues such as kidney, where no 5'DII activity can be measured, exhibit strong 5′DII hybridization signals, whereas no 5′DII hybridization signal could be found in some tissues clearly expressing 5′DII activity. Furthermore, initially no SECIS element could be located in the 3′UTR at a distance so far found in other selenocysteine-containing proteins such as the 5′DI or 5DIII, the 5′DII in *Rana catesbeiana* [48], GPx isozymes, selenoprotein P or W or others. Recently, using PCR-cloning methods, full-length cDNA clones were assembled for the human, mouse, chicken and *Fundulus heteroclitus* 5′DII gene, all of which contain a SECIS element far downstream (4–7 kb) of the UGA codon [46, 52, 53]. The efficiency of these SECIS elements to incorporate selenocysteine into the 5′DII subunit protein is extremely poor, but positioning this SECIS element closer to the UGA codon allows selenocysteine incorporation also in heterologous context. Functional 5′DII activity and incorporation of selenium was shown for the proteins encoded by these artificial or assembled cDNA constructs. In contrast to the mammalian 5′DII gene, the 5′DII cDNA isolated from *Rana catesbeiana* is more closely related to the 5′DI and 5DIII gene structure and hybridizes to a 1.5-kb mRNA [48]. Here, a convincing demonstration of experimental data on the selenoprotein nature of the enzmye has been presented. In contrast to the human and rat cDNA, which contain a second UGA codon close to the 3′ end of the coding region, the *Rana* and *Fundulus* cDNA have only one UGA codon in a domain highly similar to that of the UGA codons of the 5′DI and 5DIII and the first in-frame UGA codon of mammalian 5′DII [51, 55]. Apparently, the translation of the downstream UGA codon and incorporation of selenocysteine into the in vitro translated protein encoded by chimeric 5′DII cDNA-SECIS hybrids is not required to generate 5′DII activity [56]. In contrast to all other 5′DII enzymes, the *Fundulus* 5′DII also catalyzes 5′-deiodination

of T3. Whether this represents a phylogenetic precursor of the other deiodinases or a teleost-specific evolutionary event remains to be studied in further species [55].

Currently, not enough comparative sequence data is available to allow evolutionary considerations explaining the divergence of the mammalian 5′DII gene organization, expression and function compared with those of amphibia. However, the marked similarity of the 5′DII cDNAs among the mammalian species together with biological evidence on function and expression in various vertebrates supports the hypothesis that the 5′DII cDNAs so far identified might encode at least a subunit of the functional 5′DII enzyme. Contrary to this interpretation, a recent report raises some doubts on the nature of this 5′DII cDNA and its function as 5′DII subunit [54]. Immunoprecipitation experiments, cellular antisense approaches, hybridization selection and comparative expression and modulation of the p29 protein, supposed to represent a substrate-binding subunit with biochemical and cell biological properties remarkably congruent to that of functional 5′DII activity at least in rat astrocyte cultures, suggest that the putative 5′DII cDNA protein or its fragment, terminated at the UGA codon, cannot fully account for the attributes of a 5′DII subunit or functional protein. Several lines of evidence support the identification of the p29 protein as the substrate-binding subunit of 5′DII, such as proportionality of affinity labelling of p29 and concomitant inactivation of 5′DII, tissue distribution of p29 and 5′DII activity, cAMP stimulation of 5′DII activity and redistribution of p29 from the perinuclear to the plasmamembrane space in astrocytes [4, 33, 57]. Further studies, including identification of the other so far unknown 5′DII subunit(s) or regulatory properties, are required to resolve this discrepancy.

Whereas 5′DI and 5DIII contain only one UGA codon, the 5′DII cDNA contains two UGA codons, one in a position close to the C-terminus, a situation similar to mammalian thioredoxin reductases (TrxR). In these enzymes this penultimate SeC residue is essential for enzyme activity. The selenoprotein nature of TrxR was not recognized for years, and this UGA was misinterpreted as stop codon [58]. Site-directed mutagenesis experiments, eliminating the second SeC residue in the 5′DII cDNA, revealed that this residue is not essential for the deiodinase activity [56], whereas replacements of the conserved SeC residue, located in a position similar to that of other deiodinases, markedly reduces or even destroys funtional activity.

Initial assumptions that the different PTU sensitivity of the type I and type II 5′-deiodinase, used for experimental operative distinction between the two isoenzymes in parallel to their different reaction characteristics, might be associated with the presence or absence of selenocysteine in the active site of the two enzymes were not substantiated and were even contradicted. Whereas replacement of the selenocysteine by cysteine in the 5′DI caused both a marked loss in enzyme activity and substrate turnover and led to a sequential mechanism of reaction similar to the 5′DII enzyme, recent data on type I 5′D isozmyes in other species revealed low PTU sensitivity despite the selenoprotein character and other biochemical characteristics typical for 5′DI enzymes [16, 25, 41, 59, 60]. Site-directed mutagenesis studies on several deiodinase isozmyes now identified a series of amino acid residues essential for substrate, cosubstrate and inhibitor binding as well as variations in the complex two-substrate mechansim of reaction (ping-pong, ordered, sequential) and confirmed previous biochemical evidence on active site residues involved in deiodination [8, 61, 62].

Selenium-dependent expression of 5′DI

Initial evidence from animal experimental, clinical and in vitro cell culture studies suggested a clear selenium-dependent expression of 5′DI varying with selenium availability [32, 63, 64]. In contrast, no clear directly selenium-dependent expression could initially be shown for 5′DII [65–70]. Some data indicated that under conditions of severe selenium deficiency, expression of type III 5-deiodinase decreases, supporting its identification as a selenoprotein [68, 71].

Is there evidence that selenium status affects expression of human type I 5′-deiodinase and thus alters T3 production?

So far no clear demonstration of selenium-dependent expression of 5′DI activity in vivo with subsequent decrease in T3 production has been shown in human (patho-)physiology [8]. Some alterations of serum thyroid hormone levels are associated with altered selenium concentrations in the blood. However, no evidence for a cause-effect relationship has been presented. In contrast, selenium supplementation in septic patients known to present with the syndrome of nonthyroidal illness and low serum T3 levels improved the clinical outcome of the patients, but this was not correlated to recovery of T3 levels to normal [72]. Moreover, in one study even a decrease in serum T3 levels and T3/T4 ratio has been suggested to be correlated to very high selenium intake [73]. In elderly patients associations between trends of lower serum T3 levels and decreased plasma selenium values were reported [74, 75]. Variations of serum thyroid hormone levels were also found after selenium supplementations were administered in children suffering from cystic fibrosis or living on protein-free diets due to phenylketonuria or patients on

long-term parenteral nutrition [76, 77]. However, these alterations might be caused by changes in thyroid function itself or selenium-induced alterations in thyroid hormone homeostasis and not directly by altered expression of type I 5′-deiodinase.

In an experimental study, we analyzed the selenium content of human thyroid tissue and the levels of functional expression of the two selenoenzymes type I 5′D and glutathione peroxidase [8, 78]. We found no correlation between selenium tissue content and expression of either of the two functional selenoenzymes in the same specimen of normal, pathological or cancerous thyroid tissue, indicating that other regulatory factors override control of enzyme expression by the selenium content (at least in the human thyroid).

In contrast to these observation in humans, animal experimental evidence clearly supports regulation of 5′DI expression by the selenium status [16, 32, 43, 44, 63, 66, 68, 79, 80]. However, these models allow much more pronounced manipulations of selenium status either by withdrawal or replenishment to extents not found in human nutrition. Especially in rats, clear dose-response relationships for selenium control of 5′DI epxression in various tissues have been presented. Remarkable is the finding that different tissues expressing 5′DI activity respond differently and in an hierarchical or well-regulated manner to manipulations of selenium status. Furthermore, various selenoproteins studied show marked tissue-specific expression differences in the hierarchy of selenium depletion and repletion [66, 79–83]. Generally, 5DI is ranked higher in the preference of selenium supply than the cytosolic GPx (cGPx), whereas phospholipid hydroperoxide GPx (PHGPx) and SeP appear to be ranked at the same level or even higher in the selenium hierarchy than 5′D, at least in those tissues where all three selenoenzymes are expressed.

Whereas expression of liver and kidney 5′DI rapidly responds to selenium, variation thyroid 5′DI shows only minor response to selenium manipulations [32, 68, 84–89]. This might be due to the fact that thyroid, similar to other organs of the endocrine system (adrenals, gonads, pancreas) as well as the central nervous system, either retains or accumulates selenium stores during depletion, whereas the major body stores such as muscle, skin, liver and so on are rapidly depleted after selenium withdrawal and repleted after the other tissue stores are filled [8, 90]. The mechanism underlying this preferential mobilization and redistribution of tissue selenium is still unclear. It might be speculated that vital organs required for survival, systemic regulation and reproduction of the organism are protected from selenium deficiency.

In an animal model, iodine-deficient heifers and their offspring, a marked 10–12-fold increase in the activity of the selenoenzyme type I 5′-deiodinase accompanied by a 2–4-fold increase in cGPx was found by iodine deficiency [85]. However, these changes were only observed in the thyroid, not in liver, pituitary or brain, indicating both tissue-specific regulation and adaption of expression of selenoenzymes to physiological demand. The increased thyroidal T3 production at the local level, as well as the individual and distinct regulation of individual selenoproteins in the same tissue, is independent from selenium supply.

In brains of rats, selenium- and iodine-deficient in the second generation, expression and activities of 5′DI, cGPx and PHGPx remained relatively constant, whereas serum thyroid hormone levels and expression of these enzymes showed marked alterations in livers and thyroids of these animals [68, 69, 88, 91]. Independent of selenium status, iodine deficiency increases 5′DII activity in brain and pituitary, consistent with decreased thyroid hormone levels in this constellation [31, 68, 85, 92–94]. These findings confirm a minor effect of manipulation of (peripheral) selenium status on expression of selenoproteins in the brain even after prolonged selenium deficiency [68, 69, 95] and suggest an efficient retention or redistribution of limited selenium sources to tissues and selenoproteins involved in coordination of critical and essential vital functions.

Is there an hierarchy of selenium incorporation into different selenocysteine-containing proteins?

In cell culture experiments, using both primary culture or established immortalized or tumor cell lines, a clear dependence of 5′DI expression from selenium supply in the medium can be shown. In nontransformed porcine kidney cells, 5′DI activity and mRNA levels rapidly increase if selenium concentration in the culture medium reaches nanomolar levels. A plateau of activity and expression is reached above 200 nM, and concentrations higher than 1000 nM lead to decreases in 5′DI activity, probably a sign of toxicity of high selenite concentration in these (and other epithelial) cells [80]. Both transcriptional and posttranscriptional mechanisms contribute to this selenite-induced expression of 5′DI. However, at low selenite concentrations, 5′DI mRNA (similar to those of other mRNAs coding for selenocysteine-containing proteins) is stabilized by a yet unknown mechanism [82]. Probably mRNA is protected by ribosomes arresting at UGA codons in the context of SECIS elements in the 3′UTR region due to lack of the other selenium-dependent factors required to translate UGA codons. Alternatively, specific proteins binding to and protecting SECIS structures in the absence of selenium might be involved in the observed maintainance of significant basal steady-state transcript

levels of 5′DI even at very low selenite concentrations in the culture medium [45, 82, 96–98].

A further observation of these experiments using cell cultures might contribute to explain the observed hierarchy of selenium incorporation into different selenoproteins. During the selenite depletion phase we observed a transient decrease followed by an increase in 5′DI expression in LLC-PK1 cells, whereas cGPx continued to decrease during further depletion periods [80]. Probably selenium mobilized by turnover of the more abundant cGPx, which also has a shorter half-life in these cells than 5′DI is shifted and incorporated into the very low abundance 5′DI enzyme, thus maintaining the potential to synthesize the essential hormone T3 from T4. The mechanisms behind these observations are not understood, because the mammalian homologue of SelB is still missing. On the other hand, comparative analysis of the efficiency of various SECIS structures identified so far revealed a different potency and efficiency of these structures in directing selenocysteine incorporation into the same coding frame fused to different SECIS elements [45, 99–102]. This observation might also be part of the explanation for the cell- and tissue-specific hierarchy of selenium incorporation into eukaryotic selenoproteins in the intact organisms.

Similar to kidney and liver cell culture studies, selenium-dependent expression of selenoproteins can also be shown in thyroid cell lines. In the immortalized nontransformed rat thyroid cell line FRTL5, selenium depletion reduces activity and mRNA levels of cGPX, whereas minor alterations are observed for expression and mRNA abundance of both 5′DI and PHGPx [32, 63, 64, 78, 81, 86]. Induction of 5′DI by TSH requires the presence of selenium in the culture medium [81, 86]. In contrast to FRTL5 cells, only minor variations of 5′DI expression are observed after manipulation of selenium supply in the culture medium, whereas cGPx expression is doubled at selenium concentrations exceeding 100 nM [64, 78]. These findings indicate that regulation of expression of the selenoenzyme 5′DI depends on factors other than selenium availability, but presence of sufficient selenium is essential and a factor in exerting this expression.

Selenium-dependent regulation of expression of 5′DII?

In animal experiments demonstrating clear selenium-dependent expression of 5′DI (and later on 5DIII), no direct evidence had been found indicating alterations of 5′DII in the brain, pituitary, placenta, skin, brown adipose tissues or other organs expressing 5′DII activity. This led to the interpretation that 5′DII is not a selenoprotein, in contrast to 5′DI, an assumption compatible with the low sensitivity of 5′DII against the inhibitors PTU or thiogold-glucose, which are potent agents against selenoproteins (see above). Also, several attempts to provoke altered 5′DII expression by manipulation of selenium availability in cell culture models initially failed [65, 67], providing even more support for the hypothesis that 5′DII might be a cysteine homologue of the selenoenzyme 5′DII. 5′DII rapidly responds to alterations of thyroid hormone status. Therefore, rapid effects exerted by manipulation of selenium status on thyroid hormone synthesis and secretion as well as on 5′DI activity might indirectly lead to thyroid hormone (especially T4 or rT3)-induced alteration of 5′DII expression, obscuring or overriding direct regulation of 5′DII activity or expression by selenium status. Recently, some experimental evidence in cell culture models and in vitro using chimeric 5′DII constructs or mutants of the putative 5′DII cDNA has been brought forward to indicate selenium regulation of 5′DII [49, 70]. However, due to the short half-life of 5′DII, its regulation by many other factors including its own substrate and the already mentioned difficulties expressing enough functional enzyme in vitro, further studies are needed to clearly establish direct selenium regulation of 5′DII expression not only in cell lines or animal experimental models but also in vivo.

Selenium-dependent regulation of 5DIII

Similar to 5′DII, expression and function of 5DIII shows limited response to natural or experimental variations of selenium supply [68, 103]. However, several studies could convincingly demonstrate, at least in cell culture experiments, clear selenium-dependent stimulation of 5DIII expression [40, 71]. As already suggested for 5′DII, the SECIS element of 5DIII appears to be more efficient than that of 5′DI, at least in those chimeric constructs tested so far. Currently no conditions are known where physiological or pathophysiological variations of selenium status in human beings directly alter transcription, expression or function of any of the three deiodinases to an extent that modulates thyroid hormone homeostasis or action. This statement does not exclude the possibility of such effects during development and maturation or under specific circumstances in some tissues, organs or cells. Animal experimental data also indicate that nondeiodinative metabolism of conjugated (sulfated) thyroid hormone metabolites is altered by variations of selenium status, leading to increased half-life of active thyroid hormones under conditions of selenium deficiency and disturbed thyroid hormone status [104].

Deiodinases in development

Recently, the crucial role of development- and tissue-specific expression of the deiodinase isoenzymes for strict and timely control of available thyroid hormone levels in various organs including the brain has been lucidly demonstrated in the traditional models of thyroid hormone- controlled metamorphosis of amphibia [105]. With the molecular identification and characterization of both isoforms of deiodinase enzymes and T3 receptors, these sensitive techniques and analytical tools made available hereby revealed a clear pattern of T3-regulated control of gene expression, mediated by T3 receptors, during the complex remodelling and differentiation programs associated with tadpole metamorphosis [37, 106–109]. Stage- and tissue-specific sequential regulation of thyroid hormone receptors and 5′DII and 5DIII during metamorphosis in *R. catesbeiana* and *X. laevis* tadpoles allows for ordered progress of timely differentiation and resorption processes independent of circulating systemic thyroid hormone levels. Subsequent high expression of 5DIII in tissues that have completed metamorphosis would render them resistant to further inappropriate action of thyroid hormone [107]. Similarly, complex patterns of expression of deiodinase isoenyzmes and T3-receptor forms are also reported for developing rat and chicken models [23, 110, 111]. No information is available whether and to what extent selenium availability regulates developmental expression of deiodinase isoenzymes. In summary, deiodinases are envisaged as guardians to the gate of thyroid hormone action mediated by ligand-activated nuclear and mitochondrial T3 receptors, and other cellular or systemic targets of iodothyronine metabolites.

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