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Forum Review

Peroxides and Peroxide-Degrading Enzymes in the Thyroid

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Abstract

Iodination of thyroglobulin is the key step of thyroid hormone biosynthesis. It is catalyzed by thyroid peroxidase and occurs within the follicular space at the apical plasma membrane. Hydrogen peroxide produced by thyrocytes as an oxidant for iodide may compromise cellular and genomic integrity of the surrounding cells, unless these are sufficiently protected by peroxidases. Thus, peroxidases play two opposing roles in thyroid biology. Both aspects of peroxide biology in the thyroid are separated in space and time and respond to the different physiological states of the thyrocytes. Redox-protective peroxidases in the thyroid are peroxiredoxins, glutathione peroxidases, and catalase. Glutathione peroxidases are selenoenzymes, whereas selenium-independent peroxiredoxins are functionally linked to the selenoenzymes of the thioredoxin reductase family through their thioredoxin cofactors. Thus, selenium impacts directly and indirectly on protective enzymes in the thyroid, a link that has been supported by animal experiments and clinical observations. In view of this relationship, it is remarkable that rather little is known about selenoprotein expression and their potential functional roles in the thyroid. Moreover, selenium-dependent and -independent peroxidases have rarely been examined in the same studies. Therefore, we review the relevant literature and present expression data of both selenium-dependent and -independent peroxidases in the murine thyroid. *Antioxid. Redox Signal*. 10, 1577–1592.

Iodination of Thyroglobulin Requires the Production of Hydrogen Peroxide by Duox

The active thyroid hormone 3,3′,5-triiodo-L-thyronine (T3) binds as specific ligand to T3-receptors (TR), which act as transcription factors and regulate gene expression in a permissive manner. T3 can be secreted directly by the thyroid gland or is formed from the prohormone thyroxine (T4, 3,3′,5,5′-tetraiodo-L-thyronine) by iodothyronine 5′-deiodinases. Thyroid hormones control development, growth, differentiation, and basal metabolic rate, and influence virtually all anabolic and catabolic pathways of intermediary and structural metabolism. Thyroidal biosynthesis of iodothyronine hormones has evolved concomitant with the functional unit of the thyroid, the follicle, and represents a key step in the evolution of vertebrates (50) . Thyroid follicles are formed by a monolayer of highly polarized epithelial cuboidal cells, the thyrocytes (Fig. 1a), that are the only cells in vertebrates known to synthesize thyroid hormones by iodination of specific tyrosyl residues of thyroglobulin (Tg) using hydrogen peroxide (H_2O_2) as oxidizing agent for the co-substrate iodide. H_2O_2 is generated by thyroxidases (also called ThOx), the Ca^{2+} and NADPH-dependent dual function oxidases (DUOX1, DUOX2). The multifunctional hemoprotein thyroperoxidase (TPO) catalyzes both the iodination of tyrosyl residues of Tg by oxidation of iodide under reduction of H_2O_2 and the subsequent, again H_2O_2-dependent, head-to-tail coupling of iodinated tyrosyl residues, forming the diphenylether bond of iodothyronines that are still part of the polypeptide chain of Tg. The complete sequence of reactions of thyroid hormone biosynthesis occurs extracellularly at the apical inner surface of the follicular lumen. Iodinated Tg is stored there as polymerized colloid, until proteolysis by cathepsins liberates T3 and T4 molecules from the Tg protein backbone and the hormones are released to the blood stream (Fig 1b). Synthesis and secretion of thyroid hormones and functional organization of the intact follicular structure, which is essential for thyroid hormone synthesis, is controlled by the pituitary hormones thyrotropin (TSH) and thyrostimulin, and during pregnancy by the structurally related placental glycoproteohormone human chorionic gonadotropin (hCG). These hormones address a G-protein coupled receptor (GPCR), the TSH receptor (TSHR), which transduces its signals both via the Gs_α, adenylate cy-

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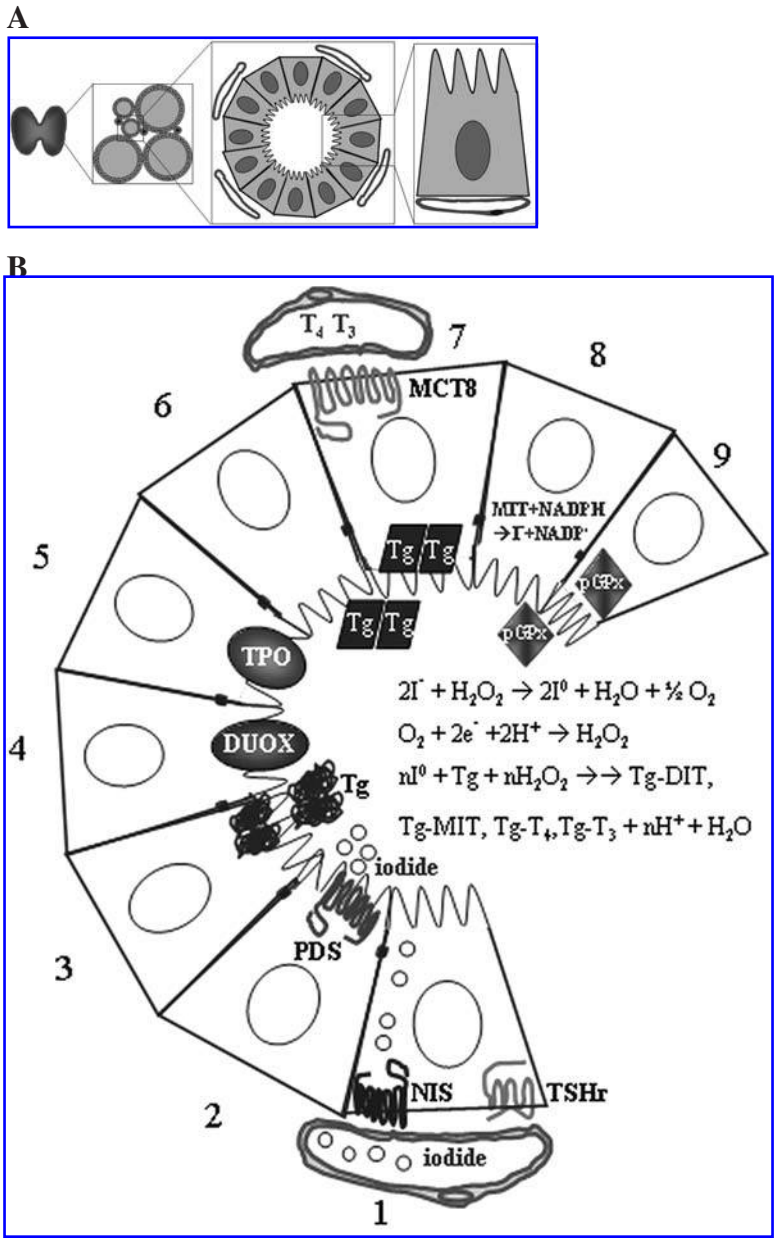

FIG. 1. Schematic illustration of the organization of thyroid follicles, thyroid hormone synthesis, storage, and secretion. (**A**) The highly vascularized thyroid gland is composed of follicles formed by a polarized tight epithelial monolayer of thyrocytes that enclose the colloid. Each follicle and its thyrocytes have access to the microcapillary network for accumulation of the essential trace element iodide and nutrients from the blood and secretion of thyroid hormones T4 and T3 by thyrocytes into the circulation. (**B**) Schematic illustration of the individual steps of iodide uptake and transport, thyroid hormone biosynthesis and secretion under control of the pituitary hormone TSH, that acts via a G-protein coupled receptor. 1. Basolateral iodide uptake by NIS; 2. apical export by pendrin (PDS); 3. synthesis and apical secretion of thyroglobulin (Tg); 4. synthesis and apical insertion of thyroperoxidase (TPO) and dual oxidase (DUOX) NADPH-dependent production of H_2O_2 by DUOX; 5. iodide oxidation, iodination of Tg-tyrosyl residues, and coupling of Tg iodtyrosine residues to iodothyronines is catalyzed by TPO using H_2O_2 as cosubstrate; 6. polymerization and deposition of iodinated Tg in colloid; 7. micropinocytosis, reduction, and cathepsin-catalyzed proteoloysis of Tg in secondary lysosomes release of thyroid hormones T4 and T3 into the blood by the transporter MCT8; 8. dehalogenation of DIT and MIT and reutilization of iodide for thyroid hormone biosynthesis; 9. secretion of pGPx (GPx-3) into the colloidal space for degradation of excess H_2O_2.

clase, protein kinase A pathway, and via G_q/G_{11}, phospholipase C, inositol phosphate Ca^{2+} signaling. Iodide uptake into thyrocytes against a concentration gradient is mediated by the basolateral sodium-iodide symporter (NIS), whose expression and activity is the most sensitive TSH-dependent process in the thyrocytes coupled to Gs_α signaling of TSH. The same signaling cascade also regulates stimulation of cell differentiation to functional thyrocytes expressing TPO and Tg, and at elevated TSH levels stimulates hypertrophy of thyrocytes. Mutations of the human TSHr, as well as thyrocyte-specific genetic inactivation of specific G proteins in mice, revealed that DUOX-dependent H_2O_2 production, Tg iodination, and iodothyronine coupling, macropinocytosis of iodinated Tg, thyroid hormone release, apical secretion of glutathione peroxidase 3 (GPx3) into the colloid space, hyperplasia and proliferative response to goitrogens, as well as vascularization of follicles is under TSH control via the G_q/G_{11}-PLC-IP-Ca^{2+} signaling pathway (15, 36, 44, 47). These observations indicate a key role of G_q/G_{11} signaling for thyroid hormone formation and release, including the TSH-dependent adaptive growth of the gland in iodine deficiency during exposure to goitrogens or due to genetic or functional defects in the regulation of the thyroid axis. In contrast, the adenylate cyclase–cAMP–PKA pathway of TSHR signaling appears to control iodide uptake, expression of NIS, Tg, TPO, and maintenance of the differentiated state of thyrocytes and follicular structure. These dual TSHr-dependent regulation pathways warrant an efficient and fine tuned balance between appropriate thyrocyte function and necessary proliferation under the life-long influence of continuous production of the cytotoxic agent H_2O_2, which is essential for thyroid hormone biosynthesis.

In the following, the processes involved in H_2O_2 generation by DUOX and its utilization by TPO for iodide oxidation, tyrosyl residue iodination, and coupling will be discussed. H_2O_2 availability is the rate limiting step in thyroid

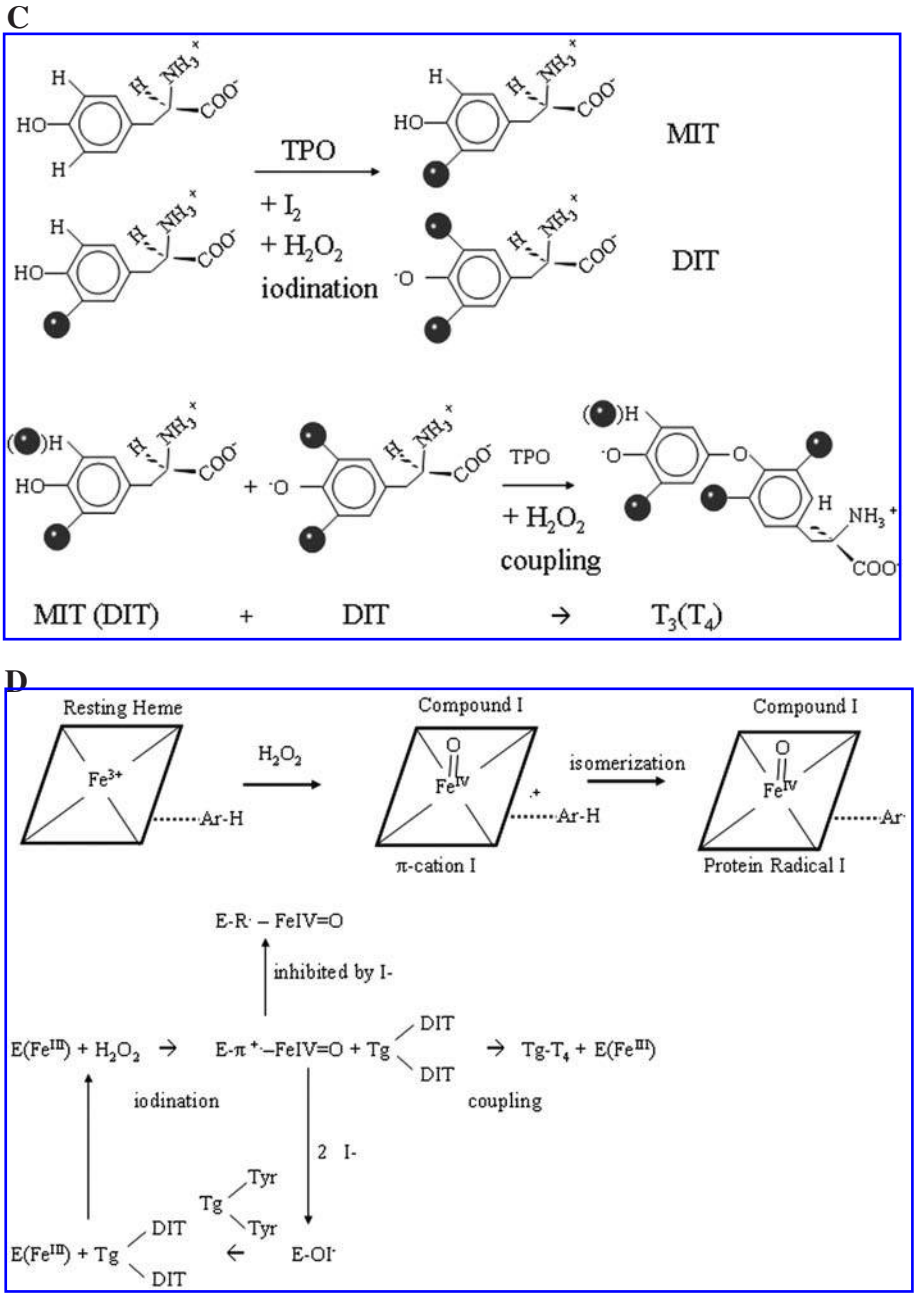

FIG. 1. (C) The hemoprotein thyroperoxidase (TPO) catalyzes the iodination of tyrosyl residues of the thyroglobulin (Tg) polypeptide chain and coupling of MIT and DIT residues to the iodothyronnines T4 and T3. H_2O_2 formed by the NADPH-dependent dual oxidase (Duox) acts as cosubstrate in the iodination and coupling step of the TPO reaction. (**D**) Proposed reaction schemes for the hemoprotein thyroperoxidase (TPO) during H_2O_2-dependent iodination and coupling reactions [modified after Taurog *et al*. (84)].

hormone biosynthesis (16). NADPH-dependent DUOX enzymes are Ca^{2+}-regulated via their cytosolic located EF-hand motive and attain activity only after their integration into the apical membrane with their active site and H_2O_2 release oriented towards the luminal colloid space. The DUOX flavoprotein enzymes 1 and 2 belong to the NOX family of NADPH-dependent oxidases and have a C-terminal domain related to the $gp91^{Phox}$ protein of leukocyte NADPH oxidase. The TPO-like extracellular N-terminal domain of DUOX probably mediates their association with TPO already along the post-translational maturation and trans-Golgi transport towards the apical thyrocyte membrane. There they remain located in enzymatically inactive submembraneous vesicular structures as a granule pool until TSH-induced Ca^{2+} signaling activates DUOX to its fully glycosylated form competent to build the H_2O_2 generating "thyroxisome" (81). This occurs by vesicle fusion with the apical plasma membrane of thyrocytes integrating mature DUOX into a functional domain, which also contains the DUOX-specific quality controlling maturation chaperones DUOXA1 and DUOXA2. These are five transmembrane helix *N*-glycosylated proteins allowing rapid ER exit of correctly folded DUOX into the trans-Golgi network (36). This apical functional domain also harbors TPO, caveolin, and a further thioredoxin-related EFP1 DUOX associated protein (81, 87). This DUOX activation and translocation mechanism warrants both that no intracellular generation of H_2O_2 occurs, that might be cytotoxic for the thyrocytes, and that H_2O_2 generated by DUOX is released directly "hand to mouth" (76) to the active TPO reducing it for iodide oxidation, tyrosyl iodination, and iodophenoxy–ether coupling. This relay-type of reaction might keep H_2O_2 concentrations as low as possible and as

effective as required for thyroid hormone biosynthesis. Currently available information of regulation of expression and function of DUOX and TPO would indicate that TPO expression and thus activity is mainly regulated at the transcriptional level, whereas DUOX activation and thus activity of the H_2O_2 reducing "thyroxisome" are under rapid TSHr-PLC-IP-Ca^{2+} control of DUOX maturation and less by transcriptional control of DUOX expression. The exact mechanism of Ca^{2+} and NADPH-dependent generation of H_2O_2 by DUOX is not yet known. Either direct H_2O_2 formation from O_2 or reduction of O_2 into superoxide anion $O_2^{\cdot -}$ and its further metabolism by superoxide dismutase to yield H_2O_2 have been proposed (18, 71).

Song *et al*. very recently reviewed the implications of their "thyroxisome" concept (76), which would explain cellular logistics and efficient control of extracellular H_2O_2 generation, protection, and life-long maintenance of long-lived thyrocytes, their intact epithelial follicular structures, thyroid hormone formation, storage, and secretion on demand, but also the inhibitory actions of iodide excess or intoxication via the "Wolff–Chaikoff mechanism." This mechanism is the physiological response to excess iodide, leading to transient inhibition of NIS-mediated basolateral uptake of iodide, inhibition of DUOX, and block of thyroid hormone synthesis and release, probably mediated by intracellular iodolipid species such as iodolactone or iodohexadecanal (84). Cellular accumulation of iodolipids might in addition impair TPO function and lead to "cellular stress" and other side reactions, especially if cellular Se-dependent redox active and antioxidative defense reactions (*e.g*., catalyzed by TrxR, GPx, see below) are not adequate due to insufficient Se supply of the gland, goitrogen exposure, or concomitant excessive stimulation by TSH or TSHr-stimulating antibodies. Of clinical and epidemiological relevance is also the NO/cGMP signaling-induced inhibition of TPO and Tg expression and thus thyroid hormone biosynthesis. The latter effect is probably causative for the adverse effects of smoking, known to be the major risk factor contributing to autoimmune thyroid disease, especially Graves' disease, apart from direct stimulatory NO effects on production of proinflammatory cytokines (4, 24, 30). Defects in Tg, TPO, DUOX, and DUOXA2 genes are known to impair thyroid hormone biosynthesis and H_2O_2 metabolism, and are involved in goitrogenesis, development of congenital hypothyroidism, as well as benign and malignant thyroid diseases.

In the absence of iodide, TPO appears to have catalase-like H_2O_2 degrading activity (60, 81), but can also oxidize several goitrogens and other oxidizable pharmaceuticals interfering with thyroid hormone biosynthesis via inhibition of TPO, that can be relieved by increasing iodide supply to the apical luminal surface.

Thyroperoxidase Is a Multifunctional Enzyme Essential for Iodination and Ring Coupling

TPO, the key enzyme of thyroid hormone biosynthesis, has been identified for a long time (84), but still the mechanisms of reactions involved in the three steps of its catalytic activity remain unclear. TPO mutations lead to goitrogenesis, TPO inhibition is the key principle of pharmacological treatment of hyperthyroidism by thiourea compounds, and "microsomal" autoantibodies directed against TPO represent the diagnostic parameter for Hashimoto's thyroiditis. This destructive autoimmune thyroid disease preferentially affects females in their reproductive age and later on. Treatment requires life-long thyroid hormone substitution, the main cause for thyroid hormone prescriptions world wide. TPO belongs to the myelo/lactoperoxidase family of hemoproteins and occurs in two forms of 105/110 kDa as a strictly thyrocyte-specific integral membrane protein of the apical membrane. TPO enzyme activity in the "thyroxisome" essentially depends on the presence of its prosthetic ferriprotoporphyrin IX group covalently bound to a Glu and an Asp residue in a histidine-rich domain of the apoprotein, facing the luminal colloid space of the follicle (84). The C-terminal sequence represents a membrane anchor. TPO gene expression is regulated by the same thyrocyte-specific combination of the three transcription factors TTF1, TTF2 (Nkx2.1), and Pax8 as Tg expression. For its catalytic activity, TPO requires H_2O_2 produced by DUOX after TSH-Ca^{2+}-dependent stimulation.

At least three schemes for iodide oxidation, iodination, and coupling (Fig. 1c) have been proposed, either involving one or two electron transfers, a free radical mechanism, a version where the iodonium cation I+ is the iodinating intermediate, and one mechanism with hypoiodite (IO-) as iodinating intermediate. In hypoiodite, the oxidation state of iodine is the same as in the iodonium cation. Missing information on the true chemical nature of the iodinating species X-I and a lacking consensus whether one electron or two electron mechanisms are involved, still complicate our picture of thyroid hormone biosynthesis (84).

Figure 1d summarizes the most favored mechanism where TPO reacts with one equivalent of H_2O_2 yielding compound **I**, a porphyrin π-cation radical containing oxyferryl (FeIV = O). In this two-electron oxidation/reduction reaction, H_2O_2 is reduced to water and TPO is oxidized. Compound **I** oxidizes the substrate A to a radical (·AH). A second one-electron oxidation reaction results in the neutral compound **II**. This oxyferryl center is coordinated to the porphyrin ligand. The native ferric state of TPO is regenerated from compound **II** in a one-electron substrate oxidation.

In the initially proposed free radical mechanism, two substrates interacting with the compound I form of TPO undergo one-electron oxidations to yield the radicals I· and Tyr·, which then readily form MIT, and in similar way DIT. The concept for I^+ as iodinating agent is compatible with the favored two-electron transfer of the reaction between iodide and compound I of TPO, generating TPO-I^+ as intermediate. The hypoiodite (IO^-) mechanism also involves a two-electron transfer for oxidation of iodide to hypoiodite bound to the compound I state of TPO, with $[EOI]^-$ as iodinating species:

$$E + H_2O_2 \rightarrow EO + H_2O$$

$$EO + I^- \rightarrow [EOI]^-$$

$$[EOI]^- + H_2O_2 \rightarrow O_2 + H_2O + I^- + E$$

Apart from TPO, lactoperoxidase and myeloperoxidase also catalyze this type of iodination reactions, which might explain occurrence of monoiodotyrosine residues in proteins other than Tg in nonthyrocyte cell and tissues. However, the

highly efficient follicular thyroid hormone biosynthesis requires both the specialized hormonogenic sites in Tg and the unique spatial organization of the H_2O_2-generating DUOX and the H_2O_2-consuming TPO on the apical extracellular surface of thyrocytes.

Protection of Thyrocytes by Antioxidative Enzymes and Their Regulation

Thyrocytes are long-lived cells and proliferation of normal thyroid cells is normally a rare event with three to five cell divisions per life time in humans (81). Thus, unlike other ROS-releasing cell types (*e.g.*, neutrophils), thyrocytes need protective mechanisms that limit the oxidative damage by life-long hydrogen peroxide production to cellular proteins, lipids, or nucleic acids (5, 53). Here, we provide a synopsis of the major redox-controlling enzyme systems in the thyroid in health and disease. For the purpose of this review and in order to simplify the presentation, we will sometimes call the enzymes discussed here "redox-protective enzymes," knowing that this term is an oversimplification. After a compilation of expression data, we will summarize what is known on the regulation of redox-protective enzymes in thyroid physiology and pathology.

Peroxiredoxin expression in the thyroid

In the peroxiredoxin (Prdx) family, three classes of enzymes are distinguished based on differences in structure and catalytic mechanism. Typical Prdxs are homodimeric and contain two catalytic Cys residues (Prdx 1,2, and 3). Atypical Prdx 4 is a monomer, and Prdx 5 and 6 contain only one reactive cystein (1-Cys Prdx). With time, Prdxs have accumulated a plethora of alternative names, complicating literature searches. Therefore, we have included in Table 1 all alternative names of Prdx and stay with the current systematic nomenclature. Table 1 also summarizes what we found on the expression of Prdxs in mammalian thyroid, with a focus on human, mouse, and rat. Often there are only data on mRNA expression obtained by Northern blot analysis or real time-PCR. Evidence of protein expression, such as Western blot analysis or activity measurements, is rather rare for the thyroid. In general, Prdx expression is reported ubiquitous and probably owing to redundancy of function among Prdxs, knockout mice for single Prdx genes have often mild phenotypes, sometimes resulting, however, in hemolytic anemia. On the contrary, specific roles for single Prdxs have been identified, for example, Prdx 2 associates with PDGF receptor and regulates signaling downstream of the receptor (14). We are not aware of a report on thyroid phenotypes in Prdx knockout mice. In an attempt to provide a systematic analysis of Prdx expression in the thyroid, we selected the mouse as a popular and genetically modifiable model organism and tested for Prdx expression by RT-PCR and Western blot (Fig. 2). The related glutaredoxins (Grx, Glrx) are subsumed in this paragraph, since we were not able to find any data on their function in the thyroid. In general, Glrxs are small thiol transferases that are involved in the regulation of proteins through glutathionylation (32), displaying strong anti-apoptotic activity *in vitro* (17, 58) and *in vivo* (45, 46). There exists a Glrx1 knockout mouse, the phenotype of which has not been reported (75).

Selenoprotein expression in the thyroid

During their catalytic cycle, Prdxs are reduced by thioredoxins (Txn), and in the case of Prdx6, also by πGST-catalyzed glutathionylation (62). Cytosolic Txn1 is reduced by thioredoxin reductase (TrxR1, TR1, Txnrd1), and mitochondrial Txn2 is reduced by Txnrd2 (57). Both Txn–Txnrd pairs are ubiquitously expressed in the thyroid (Table 2, Fig. 3). Unlike bacterial TrxR, mammalian Txnrds are selenoenzymes [*i.e.*, they contain the rare amino acid selenocysteine (Sec) in their polypeptide chain]. The Sec residues are located at the penultimate positions in Txnrds and are required for catalysis (37–39). Thus, ultimately "selenium-independent" Prdxs are dependent on the expression of selenoproteins. The first mammalian selenoprotein discovered was cytosolic glutathione peroxidase (GPx1, (25, 76)). Upon cloning of its cDNA, a TGA codon was found at the position occupied by Sec. How the STOP codon TGA was interpreted as a Sec codon during translation, became clear after the identification and cloning of the next mammalian selenoprotein, type I iodothyronine deiodinase (Dio1) (1, 6, 8).

A hairpin structure in the 3′-UTR of the Dio1 mRNA essential for Sec incorporation at the UGA codon was identified and named selenocysteine insertion sequence [SECIS element; (8)]. Later more biosynthetic cofactors for selenoprotein translation were identified (41).

GPx1 is a ubiquitous enzyme capable of degrading hydrogen peroxide in a GSH-dependent reaction. Its mRNA, protein, and activity have been found in the thyroid and expression depends on dietary selenium status (7, 68) (Table 2, Fig. 3). GPx2 is the gastrointestinal GPx that was not detected in the thyroid. GPx3 is also known as plasma GPx or extracellular GPx. As a secreted protein expressed by epithelia, it may play a role in the protection of these epithelia against oxidative damage (2). GPx3 mRNA is highly expressed in the thyroid (78) and induced by TSH *in vitro* (44). Recently, GPx3 protein has been demonstrated in colloid isolated from human thyroid (78). Since oxidative iodination and coupling of thyroglobulin takes place in the follicular lumen, GPx3 may play a role in returning the follicular lumen back to a more reducing environment in which cysteine proteinases of the cathepsin family are required for the degradation of Tg and liberation of T4 and T3 (28). GPx4 is called the phospholipid hypdroperoxide-specific GPx. It can reduce lipid hydroperoxides and exerts anti-apoptotic effects in cultured cells (77) and *in vivo* (89). Its role in the thyroid is currently under investigation (Chiu and Schweizer, unpublished work). GPx5 and GPx6 have not been demonstrated in the thyroid. The iodothyronine deiodinases are central to thyroid hormone metabolism (52). Although they probably do not play a role in the redox protection of the thyroid, they are discussed here owing to their dependence on selenium, since any manipulation of thyroidal selenium content not only compromises GPx1 expression, but may also impact on other selenoproteins, including Dios (1, 6). In rodents, only Dio1 has been demonstrated in the thyroid; in human thyroid both Dio1 and Dio2 have been found (9). Comparative modeling of Dio structures suggest that these enzymes contain a thioredoxin fold motive which might be involved in the reductive deiodination of thyroid hormones (12). Se-dependent Dio activity of thyrocytes contributes to local T3

TABLE 1. EXPRESSION OF PEROXIDASES IN THE THYROID

Enzyme (official symbol)		*Other known names*	*Reports about enzyme expression and/or regulation in thyroid gland of different species*
Peroxiredoxins			
Prdx1		PAG, proliferation-associated gene; PrxI; TDX2; MSP23, macrophage stress protein 23kDa; NKEFA, natural killer-enhancing factor A; OSF-3, osteoblast specific factor 3; TPx-A; Trx dependent peroxide reductase 2 (Tdpx2)	(a) Rat: mRNA expression by Northern blot (48) (b) Human: Prdx1 expression was found higher in samples of patients with different thyroidal diseases when compared with the corresponding contralateral healthy lobes (88). (c) Mouse: mRNA expression (by qRT-PCR*) was studied in order to detect transient changes in response to iodine deficient diet. No changes in mRNA expression were found with respect to controls after 2 or 3 months of treatment (61). (d) Mouse: this report, mRNA and protein expression in adult WT† mouse.
Prdx2		Calpromotin; PRP, protector protein; TPx; TSA, thiol-specific antioxidant, TDX1; NkefB, natural killer-enhancing factor B; PrxII; TPx-B; Tdpx1, thioredoxin peroxidase 1, Torin; Band-8; AL022839	(a) Reported as ubiquitously expressed in adult mouse and developing embryos by Northern blot, thyroid not included in the analysis (59). (b) In FRTL-5 cells, expression of Thiol-specific antioxidant (TSA) mRNA (by Northern blot) after treatment with TSH (at concentrations $>10^{-9}$ M) was increased within 6 h following treatment, and peaked at 8 h. Effects on TSA gene expression were specific to TSH (other growth factors did not alter TSA expression (49). (c) Prdx2 has been found underexpressed in human papillary thyroid carcinoma and proposed as biomarker for this tumor variant (11).
Prdx3	Thioredoxin as electron donor. Scavengers for H_2O_2 and other alkyl hydroperoxides; act in intracellular signalling.	Aop1, anti-oxidant protein 1; Ef2l; Mer5; Prx3; SP22; TDXM; D0Tohi1; AW822249	(a) Mouse: a significant transient increase in PRDX3 mRNA (qRT-PCR) was observed in mice treated for 8 weeks with iodine deficient diets (61). (b) Mouse: this report, mRNA and protein expression in adult WT mouse. Human tissue: PRX-IV mRNA expression was measured (by qRT-PCR) in thyroidal biopsies of healthy subjects and patients affected by MNG†. No differences were detected between affected and unaffected samples (33).
Prdx4		AOE372, antioxidant enzyme, human; TRANK	(a) Human: AOEB166 mRNa levels were quantified in a human master dot blot. High level of expression was observed in the thyroid gland (51). (b) Human: expression in human healthy and sick thyroidal specimens was examined by immunohistochemistry and immunobloting. In healthy

			follicles, signal was heterogeneous, being stronger in active follicular cells. The protein was detected in the cytoplasm, in inclusions that most likely corresponded to mitochondria. In MNG†, hot nodules, and Graves', Prdx5 cytosolic labelling was enhanced compared to the controls and signal was also detected in some nuclei. By immunoblot (comparison by densitometry), the signal was significantly increased in the samples of patients with Graves' disease compared with MNG‡ (31). (c) Mouse: a significant transient increase in PRDX5 mRNA levels (qRT-PCR) was observed in BalbC mice treated for 8 weeks with iodine deficient diets (61).
Prdx5		AOPP; PrxV; Pmp20, peroxisomal membrane protein 20; AOEB166	(a) Mouse: reported as ubiquitously expressed, expecially in epithelia. The enzyme was found in all tissues tested by (qRT-PCR and immunoblot); thyroid was not included in the analysis (70). (b) Mouse: this report, mRNA and protein expression in adult WT mouse.
Prdx6	Uses GSH and πGST as reductant (glutathionylation); acts as antioxidant	Non-seleno; Aop2, anti-oxidant protein 2; Ltw-4; ORF06; Brp-12; Lvtw-4; aiPLA2, acidic calcium-independent phospholipase A2; 1-cysPrx; AA690119; Aop2-rs3; KIAA0106; Prdx6-rs3; mKIAA0106; 9430088D19Rik	(a) Human: CAT activity was significantly lower in thyroid specimens from patients with Graves' disease and follicular thyroid adenoma than in the normal thyroid (63). (b) Human: tissue samples from thyroid tumors or normal thyroid tissues (opposite healthy lobe) were compared regarding CAT mRNA expression levels (qRT-PCR). Decreased expression of Cat were observed in anaplastic carcinomas compared with normal thyroid tissues (4). (c) Mouse: this report, mRNA and protein expression in adult WT mouse.
Catalase		Cas1; Cs-1; Cas-1; 2210418N07	(a) Human tissue: activity of several peroxidases in normal and neoplastic human cell lines was measured. For comparison, enzymes were also analyzed in human tissues, thyroid included (65). (b) Mouse: administration of TSH in young female mice results in augmentation of SOD activity in the thyroid gland, with a concomitant loss of SOD activity in the adrenal gland (86).
Superoxide dismutases SOD-1		Soluble SOD; Ipo1; SODC; Ipo-1; Sod-1; CuZnSOD; Cu/Zn-SOD; MGC107553; B430204E11Rik.	(c) Human: The concentration of Cu/Zn-SOD was significantly lower in thyroid specimens from patients with follicular adenoma and papillary carcinoma than in normal thyroid tissue (63).

(*continued*)

Table 1. Expression of Peroxidases in the Thyroid (Cont'd)

Enzyme (official symbol)	*Other known names*	*Reports about enzyme expression and/or regulation in thyroid gland of different species*
		(d) Avian: exogenous administration of TSH induced thyroidal SOD activity with a simultaneous burst in superoxide anion radical levels. The peak level of SOD activity in the thyroid gland correlated very well with the peak level of thyroxine concentrations in pigeon serum (80). (e) Human: expression levels of Cu/Zn SOD were measured in thyroid tumors or normal thyroid tissues (opposite healthy lobe). Decreased expression levels of Cu/Zn SOD mRNA were found in all thyroid tumors compared with normal samples (40). (f) Mouse: this report, mRNA and protein expression in adult WT mouse.
SOD-2	Mitochondrial SOD, MnSOD; Sod-2; MGC6144	(a) Human tissue: activity of several peroxidases in normal and neoplastic human cell lines was measured. For comparison, enzymes were also analyzed in normal human tissues, thyroid included. The content of MnSOD was found to be more variable than the content of Cu/Zn SOD enzyme (65). (b) Human: Mn-SOD concentration was significantly higher in thyroid specimens from patients with papillary thyroid carcinoma than those in normal thyroid tissue (63). (c) Rat: Mn-SOD content was found to increase in thyroid tissues of rats administered with TSH and in thyrocytes cultured in medium supplemented with TSH. In the thyroid glands of rats whose serum TSH level was elevated by inhibiting the synthesis of T3 and T4 by 6-methyl-2-thiouracil, the Mn-SOD increased as the TSH concentration increased (73). (d) Human tissue: the expression levels of catalase were measured (mRNA, qRT-PCR) in benign and malignant thyroid tissues. Expression levels of Mn-SOD were decreased only in differentiated tumors and not in anaplastic carcinomas compared with normal thyroids (opposite healthy lobe) (40). (e) Mouse: this report, mRNA and protein expression in adult WT mouse.
SOD-3	EC-SOD (extracellular SOD); AI314465; MGC13799	(a) The occurrence of EC SOD was determined in various human tissues (thyroid included) and several human cell lines (64). (b) Transient elevation of SOD3 mRNA (qRT-PCR) was observed in mice treated for 2 and 3 months with iodine deficient diets (61).

*qRT-PCR, quantitative real time PCR; †WT, wild type; ‡MNG, multinodular goiter.

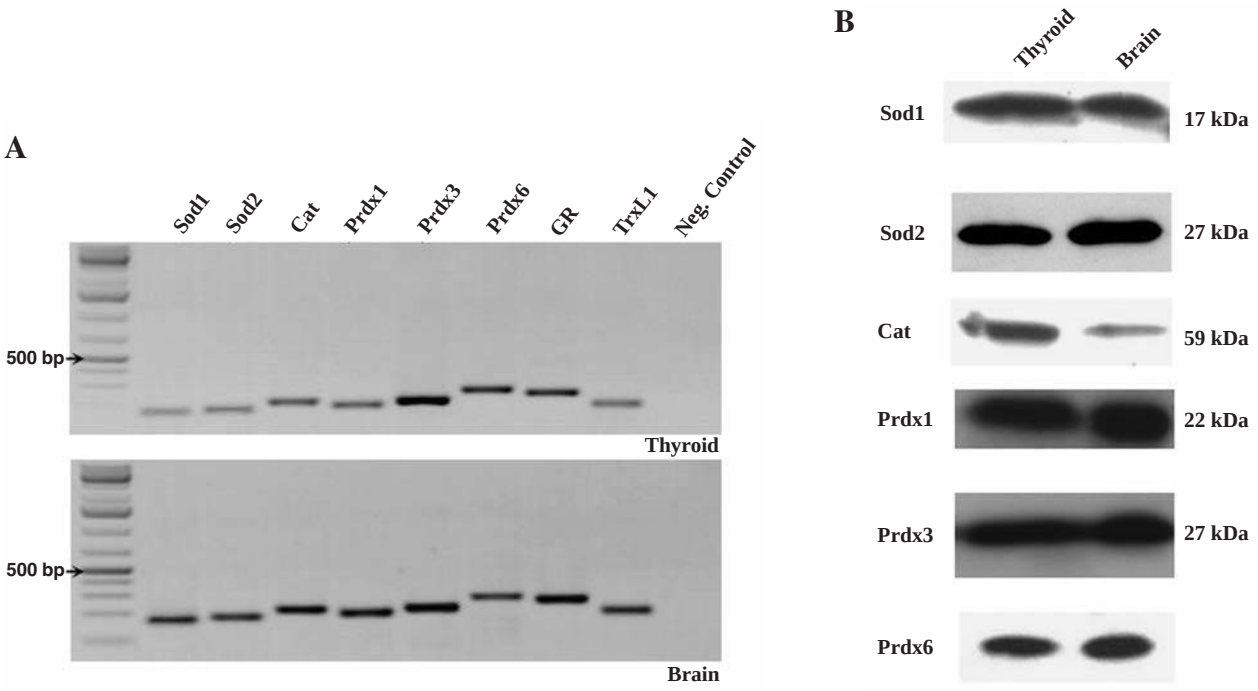

FIG. 2. Expression of Se-independent redox systems in the thyroid gland of WT mice. (**A**) Total RNA preparations from single murine thyroid glands and murine brain were reverse transcribed and used as templates for PCR amplification with specific primers for superoxide dismutases 1 and 2 (Sod1and Sod2), catalase (Cat), peroxiredoxins (Prdx1, Prdx3, Prdx6), glutathione reductase (GR), and thioredoxin-like 1 (TrxL1); the products were separated by agarose-gel electrophoresis. (**B**) Immunoblots were performed using protein homogenates of single thyroid glands or brain from C57BL/6 adult male mice. Proteins were resolved by SDS-polyacrylamide gel electrophoresis and targets were detected with anti Sod1, Sod2, Cat, Prdx1, Prdx3, and Prdx6 antibodies.

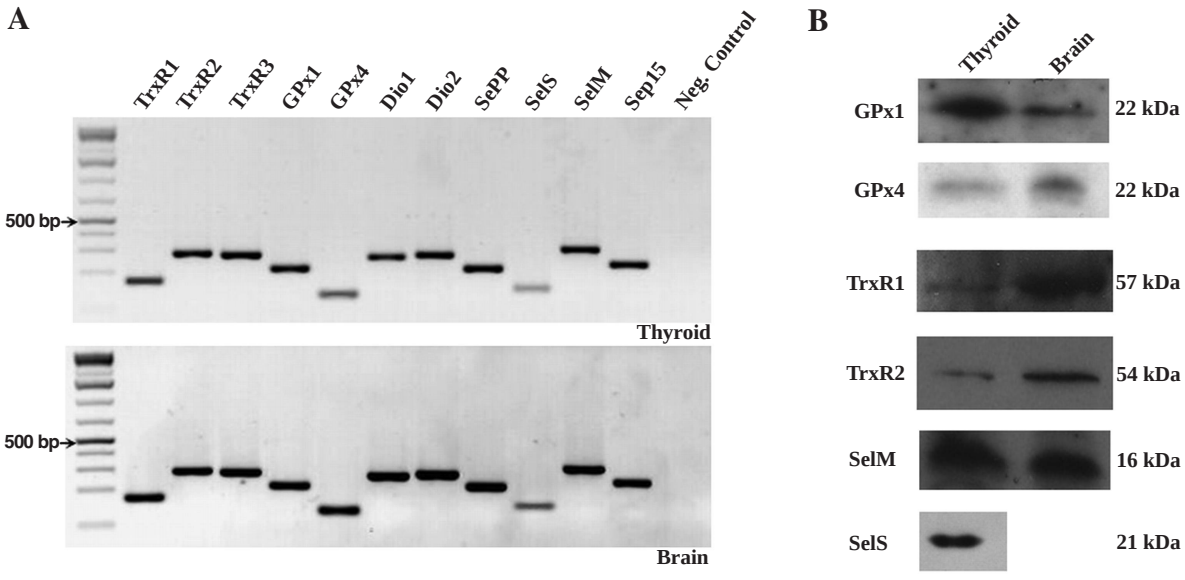

FIG. 3. Expression of selenoproteins in the thyroid gland of WT mice. (**A**) Total RNA preparations from single murine thyroid glands and murine brain were reverse transcribed and used as templates for PCR amplification with primers specific for thioredoxin reductases 1, 2, and 3 (TrxR1, TrxR2, TrxR3), glutathione peroxidases 1 and 4 (GPx1, Gpx4), deiodinases 1 and 2 (Dio1, Dio2), selenoproteins P, S, M (SePP, SelS, SelM) and Sep15. The products were separated by agarose-gel electrophoresis. (**B**) Western blot analysis was performed using protein homogenates of single thyroid glands or brain from C57BL/6 adult male mice. Proteins were resolved by SDS-polyacrylamide gel electrophoresis and targets were detected with anti GPx1, Gpx4, SelM, SelS, TrxnR1 and TrxnR2 specific mono- and polyclonal antibodies.

Table 2. Expression of Redox-Relevant Selenoproteins in the Thyroid Gland

Protein	*Other known names*	*Specie/brief description*
Glutathione peroxidases		
GPx1	cGPx; GPx-1; GSHPx-1; AI195024; AL033363	(a) FRTL-5 cells and porcine thyroid cells: when cultured in the presence of TSH or forskolin, the GSH peroxidase (GPx) activity in porcine thyroid cell cultures did not show any dependence on TSH. In contrast, in FRTL-5 cells a much higher GPx activity was observed in the presence of TSH than those cells cultured without TSH. However, effect of GPx on H_2O_2 degradation in this study was marked only at low H_2O_2 concentrations (0.1 mM or below). The study suggests that at higher H_2O_2 concentrations, Catalase activity is more important for H_2O_2 degradation than GPx. As total GPx activity is reported it could also represent GPx3 activity (10). (b) Selenoenzyme activity and expression were investigated in the thyroid and liver of second generation selenium- and/or iodine-deficient rats. Selenoprotein mRNA concentrations were maintained at control levels in thyroid glands from the selenium-deficient rat pups, but a differential effect was observed in selenoenzyme activities: cGSHPx activity was decreased to 45% and phGSHPx to 29% of that in selenium-adequate controls. In iodine-deficient thyroid glands, mRNA levels were increased 5.0 times for cGSHPx and 2.8 times for phGSHPx. In agreement with this, cGSHPx enzyme activity was also increased but phGSHPx activity was decreased despite the high mRNA abundance. Thyroid selenoprotein mRNA levels were also increased in combined selenium and iodine deficiency but again, effects on enzyme activities did not corresponded to messenger levels cGSHPx activity was unchanged and phGSHPx decreased (68). (c) Human and pig thyrocytes: Se-dependent activity of GPx1 was measured to analyse its participation in the intracellular H_2O_2 degradation and protection of thyrocytes from H_2O_2, iodide, EGF and TGF-b induced apoptosis (19, 23, 56). (d) Thyroidal cytosolic glutathione peroxidase activity was significantly decreased (>75%) by combined selenium and iodine deficiency in 11-day-old rats (69). (e) Human: GPx1 activity was measured in biopsies of goitrous and nongoitrus high school children (15–18 years old) with severe or moderate iodine deficiency. Activity was significantly lower in goitrous children than in control groups. Selenium (Se) status was also lower in those children with goiter (34, 35). (f) Mouse: GPx1 mRNA expression (by Northern blot) and total GPx activities in thyroid gland were compared between WT, heterozygous or homozygous Selenoprotein P (SePP) KO adult mice. No differences were found (79). (g) This report, WT* adult mouse.
GPx3	GPx; GSHPx-3; GSHPx-P; AA960521	(a) Human: quiescent primary human thyrocytes were found to synthesize and secrete the extracellular glutathione peroxidase. Under stimulation by TSH (and hence increased H_2O_2 production), secretion of GPx3 is reduced and the enzyme remains within the cell instead of been secreted. Protection of thyrocytes from peroxidative damage is discussed (44). (b) Human samples and thyroid cell lines: Se dependent expression of selenoproteins was analyzed in HTC† human samples, XTC.UC1‡ cells and other thyroid cell lines. Immunoblot analysis demonstrated the presence GPx3 in conditioned media of XTC.UC1 cells. Reduced levels of GPx3 mRNA (by in situ hybridization) were detected in HTC samples (67). (c) Human: in normal thyroid tissue, GPx3 mRNA was found at high levels exclusively in the thyrocytes (by situ hybridization), nevertheless, the protein was also extracted from the colloid by incubation with 0.5% sodium duodecyl sulfate. In thyroid carcinoma, the expression of GPx3 was found downregulated compared to matched normal thyroid tissue. As the follicular structure was disrupted in these samples, GPx3 signal was dispersed. (78) (d) Decreased expression of GPx3 in thyroid cancer tissue as analyzed by SAGE and cancer tissue profiling arrays (11, 40). (e) This report, WT adult mouse.

GPx4	PHGPx; snGPx; mtPHGPx; MGC103187; MGC118087; 1700027009Rik	(a) Rat: regulation of GSH-Px, PHGSH-Px and type-I iodothyronine 5′-deiodinase was investigated in liver, thyroid and heart of rats fed on Se-adequate and Se-deficient diets. In the thyroid, PHGSH-Px activity was unaffected by Se depletion but its mRNA increased. In contrast, in the thyroid there was no significant change in GSH-Px mRNA, but the activity decreased 50% in severe Se deficiency (7). (b) Human: mRNA detected by in situ hybridization and Northern blot (78).
Thioredoxin reductases		
TrxR1	TR; TR1; TrxR1, Txnrd1	(a) Human: A 57-kDa selenoprotein identified as thioredoxin reductase was found in human thyrocytes. The calcium-phosphoinositol second messenger cascade that controls hydrogen peroxide generation in human thyrocytes, is reported as an important stimulator of TR expression (43). (b) Human samples and thyroid cell lines: Se dependent expression of selenoproteins was analyzed in HTC† human samples, XTC.UC1‡ cells and other thyroid cell lines. Thioredoxin reductase and Glutathione peroxidase activity were found both in cell lysates and conditioned media of XTC.UC1‡ cells and were increased by Na_2SeO_3 (67). (c) This report, WT adult mouse.
TrxR2	TGR, Tr3, Trxr2, Txnrd2, TRXR2, SELZ, TR, TR-BETA, AA118373, ESTM573010.	(d) This report, WT adult mouse.
Other Selenoproteins		
SePP	Se-P; selp; AU018766; D15Ucla1	(a) HTC human samples and thyroid cell lines: Description of SePP expression is discussed (by immunoblot) in cultures of XTC.UC1‡ cells (contidioned and Se supplemented media) and by in situ hybridization in HTC samples (67). (b) Mouse: expression of SePP in thyroid (by Northern analysis) (79).
SelM	Selm; Sepm; A230103K18; 1500040L08Rik	(a) Human: Identification of a new eukaryotic selenoprotein, designated selenoprotein M. Expression of human SelM mRNA (by Northern blot) is shown in normal and tumor matched tissues, thyroid gland included (54). (b) This report, WT adult mouse.
Sep15	Sep15; 9430015P09Rik	(a) Human: Expression of human Sep15 mRNA (by Northern blot) in normal and tumor matched tissues, thyroid was included (54). (b) Human: SeP15 mRNA was detected in low levels by in situ hybridization. Signal was distributed over all cell types without a specific expression pattern (78).
SelW	Sepw1	The influence of deficient, adequate and excessive levels of dietary selenium (Se) on the selenoprotein W content and GPx activity was investigated in different rat tissues, thyroid included (82).
SelS	H47, VIMP	This report, WT adult mouse.

*WT, wild type; †HTC, Hürthle cell carcinoma; ‡XTC.UC1 cells, model of Hürthle cell carcinoma.

formation, which is required for hormonal regulation of their function and differentiation, and is in involved in systemic production of T3 secreted into the blood (48).

Based on structural similarity around the Sec residues and since many selenoproteins have Cys homologs, it has been assumed that selenoproteins likely are redox enzymes (26, 27, 55) that may potentially protect the cells from oxidative damage. However, of the 24 rodent selenoproteins, many are expressed in the thyroid, but the functions of many of those are not known (Table 2).

Catalase and superoxide dismutases

These enzymes are all expressed in human and rodent thyroid (Table 1, Fig. 2). Catalase (Cat) is a hydrogen peroxide-degrading enzyme located in peroxisomes. Since the biochemical pathways of peroxisomes are of lesser importance in the thyroid, Cat is rather weakly expressed in this organ, but stronger than in brain. The K_M value of Cat for H_2O_2 is much higher than intracellular H_2O_2 concentrations reported for thyrocytes, and H_2O_2 degradation in the thyroid appears to be handled primarily by the GPx family (81). Superoxide dismutases (Sod1–3) are not reducing, but rather producing, hydrogen peroxide from the more reactive superoxide radical. Cat has been knocked out in mice, but no defects were reported in their thyroids, although they were also not excluded (42). In humans, a catalase-deficient condition, acatalasemia, exists, but no thyroid phenotype has been demonstrated (see Ref. 81). While Sod1-deficient mice do not show an overt phenotype until at least 6 months of age (74), Sod2-deficient mice die from dilated heart failure (66). In both models, thyroid pathology has not been reported. Sod3-deficient mice are apparently healthy under normal conditions until at least 14 months, likely excluding major pathology of the thyroid (13).

Regulation of Redox-Protective Enzymes in Different States of Thyroid Physiology and Pathology

A survey of expression changes of redox protective enzymes in the thyroid under different conditions is, at times, confusing, since conceptually different physiological states (*e.g.*, primary, secondary, or pharmacologically induced hypo- or hyperthyroidism) are sometimes lumped together in the literature. For example, hyperthyroidism may result from increased endogenous TSH signaling, representing increased thyroidal activity. In contrast, animals are sometimes called hyperthyroid after application of thyroid hormones, although then the activity of the thyroid (as well as TSH) are suppressed. With respect to "peripheral" organs such as the liver, heart, gastrointestinal tract, or bone, it may not matter whether thyroidal activity is induced or repressed, as long as there is increased thyroid hormone action in the tissue under investigation. However, it should be clear from this discussion that there is a big difference between both models with regard to the thyroid. Thus, one has to be very careful interpreting expression data of redox protective enzymes in hyper- or hypothyroidism. What we found on the regulation of redox protective enzymes in summarized in Tables 1 and 2.

Hyperthyroidism/increased TSH signaling/Graves' disease/low iodine diet

GPx activity is induced by TSH in cultured FRTL-5 cells, but not in cultured porcine follicular cells (10). In these cells, Prdx2 was also induced by TSH, suggesting that increased peroxidase-mediated protection may be required to cope with TSH-induced thyroid hormone synthesis (49). Similarly, low iodine diet stimulated mRNA expression of Prxn 3 and 5 (61). In fact, increased Prdx 5 expression was also shown in Graves' disease by immunohistochemistry and Western blot analysis (31). Both Sod1 and Sod2 are induced by TSH (73, 80, 86). Unexpectedly, catalase activity was reduced in Graves' disease (63). Accordingly, selenium supplementation may be beneficial in Graves' disease as an adjuvant therapy (3). Secreted Sod 3 mRNA was elevated transiently in mice maintained on Sod 3 iodine-deficient diet for 2–3 months (61).

Hashimoto's thyroiditis, Graves disease, and inflammatory thyroid disease

M. Hashimoto is characterized by continuous progressive autoimmune destruction of thyroid follicles and thyrocytes. TPO autoantibodies are widely used in diagnostics and may be involved in pathology. With progressive destruction of their thyroids, patients become hypothyroid and require lifelong T4 replacement therapy. Recently, several prospective, double-blind controlled clinical studies reported beneficial effects of administration of various selenium compounds (selenite, selenomethionine, selenium yeast), as illustrated by decreased autoantibody titers and improved clinical scores of treated patients (22, 29, 85). However, the molecular mechanisms of selenium action in this disease is not known (21) and, apart from improved selenium status as indicated by blood Se or GPx activity, no data are available on altered selenoprotein expression in the thyroid gland of affected patients. Since beneficial effects of Se treatment have also been found in postpartum thyroiditis (72) and De Quervain's thyroiditis (20), the pertinent question is whether the therapeutic principle is altered selenoprotein expression in thyrocytes, stromal, endothelial, or immune cells infiltrating the thyroid gland, or combinations of these. Whether Se treatment is also beneficial in Graves' disease of the thyroid, caused by TSHr stimulating autoimmune antibodies, is currently under investigation in multicentric clinical studies.

Thyroid adenoma and cancer

The number of studies in this subject is too small to provide a basis for the separate treatment of adenoma and follicular, anaplastic, or papillary thyroid cancer. Thus, we will refer to the exact type of cancer only in the tables. Prdx1 expression was increased in thyroid cancer (88). Whether this is a common feature of Prdxs is not known, since they have not been systematically analyzed in thyroid cancers. Similarly, there is too little data on the regulation of selenoproteins in thyroid cancers: Schmutzler demonstrated that GPx3 mRNA was downregulated in thyroid cancer samples, as compared to matched normal controls. In addition, *in situ* hybridization revealed that GPx3 mRNA was exclusively localized to the thyrocytes, and Northern blot analysis showed the highest expression level for GPx3 among selenoproteins in normal thyroid tissue. Moreover, GPx3 mRNA was abundant in a human multiple tissue expression array, and a cancer profiling array indicated decreased transcript levels (78). GPx-3, downregulated in thyroid cancer tissue compared to normal paired samples, has thus been proposed as a biomarker for papillary thyroid cancer (11, 75, 83). Similarly,

two reports demonstrated a decrease of catalase in thyroid cancer (40, 63). Depending on the type of cancer, Sod1 activity is either unchanged or decreased (40, 63). In contrast, Sod2 was either unchanged or increased (40, 63) This first descriptive information on expression of redox-related proteins in thyroid cancer does not yet allow to draw conclusions on their pathogenic implications, and more studies are clearly needed.

Summary

There is good evidence that stimulation of the thyroid by TSH or TSHr stimulating antibodies induces the production of hydrogen peroxide, which is at the same time counteracted by increased expression of redox-protective enzymes. However, "at the same time" may be a premature conclusion. Maybe protection follows hormone biosynthesis with a lag phase allowing for efficient iodination. We know that not all thyroid follicles are simultaneously active, and even within one follicle the functional states of individual thyrocytes differ. The fact that the thyroid is a highly perfused and vascularized tissue containing probably more endothelial cells than thyrocytes is usually neglected, as is its regulation by innervation. It may thus be that the values of enzymatic activity or gene expression data of redox-protective enzymes determined by grinding up the whole gland may not exactly reflect the cell biology of individual thyroid cells, but rather represent a crude average over many follicles and different cell types. In addition, we have to admit that much more, and ultimately systematic, research is needed to assess fully the roles of redox-protective enzymes in the thyroid, and appreciate their interplay with peroxide-generating systems. Moreover, there is some evidence both from animal and clinical studies that demonstrate beneficial effects of selenium supplementation in settings of autoimmune inflammatory thyroid disease. To date, these effects are still tentatively explained by increased GPx expression, which implies the traditional concept of antioxidative protection against oxidative stress, an interpretation probably falling too short. However, even this hypothesis is highly speculative and based only on association, since selenoprotein knockout animals, with the exception of selenoprotein P, have not been analyzed in this respect. Given the high prevalence of thyroid disorders in the population and lacking rational therapeutic alternatives, it may be worth exploring this topic, perhaps with approaches considering the cell biology and functional diversity of the thyroid gland. Such systematic studies, combining expression analyses and genetic tools may thus ultimately help produce the solid ground of data required for designing models and devising therapeutic strategies.

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Abbreviations

Cat, catalase; Dio, deiodinases; GPx, glutathione peroxidase; GR, glutathione reductase; Prdx, peroxiredoxin; SelM, selenoprotein M; SelS, selenoprotein S; Sep15, selenoprotein 15; SePP, selenoprotein P; Sod, superoxide dismutase; TPO, thyroperoxidase; TrxL1, thioredoxin-like 1; TrxR, thioredoxin reductase.

References

1. Arthur JR, Nicol F, and Beckett GJ. Hepatic iodothyronine 5′-deiodinase. The role of selenium. *Biochem J* 272: 537–540, 1990.
2. Avissar N, Finkelstein JN, Horowitz S, Willey JC, Coy E, Frampton MW, Watkins RH, Khullar P, Xu YL, and Cohen HJ. Extracellular glutathione peroxidase in human lung epithelial lining fluid and in lung cells. *Am J Physiol* 270: L173–182, 1996.
3. Bacic Vrca V, Skreb F, Cepelak I, and Mayer L. Supplementation with antioxidants in the treatment of Graves' disease: the effect on the extracellular antioxidative parameters. *Acta Pharm* 54: 79–89, 2004.
4. Bazzara LG, Velez ML, Costamagna ME, Cabanillas AM, Fozzatti L, Lucero AM, Pellizas CG, and Masini–Repiso AM. Nitric oxide/cGMP signaling inhibits TSH-stimulated iodide uptake and expression of thyroid peroxidase and thyroglobulin mRNA in FRTL-5 thyroid cells. *Thyroid* 17: 717–727, 2007.
5. Beckett GJ and Arthur JR. Selenium and endocrine systems. *J Endocrinol* 184: 455–465, 2005.
6. Behne D, Kyriakopoulos A, Meinhold H, and Kohrle J. Identification of type I iodothyronine 5′-deiodinase as a selenoenzyme. *Biochem Biophys Res Commun* 173: 1143–1149, 1990.
7. Bermano G, Nicol F, Dyer JA, Sunde RA, Beckett GJ, Arthur JR, and Hesketh JE. Tissue-specific regulation of selenoenzyme gene expression during selenium deficiency in rats. *Biochem J* 311: 425–430, 1995.
8. Berry MJ, Banu L, and Larsen PR. Type I iodothyronine deiodinase is a selenocysteine-containing enzyme. *Nature* 349: 438–440, 1991.
9. Bianco AC, Salvatore D, Gereben B, Berry MJ, and Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr Rev* 23: 38–89, 2002.
10. Bjorkman U and Ekholm R. Hydrogen peroxide degradation and glutathione peroxidase activity in cultures of thyroid cells. *Mol Cell Endocrinol* 111: 99–107, 1995.
11. Brown LM, Helmke SM, Hunsucker SW, Netea–Maier RT, Chiang SA, Heinz DE, Shroyer KR, Duncan MW, and Haugen BR. Quantitative and qualitative differences in protein expression between papillary thyroid carcinoma and normal thyroid tissue. *Mol Carcinog* 45: 613–626, 2006.
12. Callebaut I, Curcio–Morelli C, Mornon JP, Gereben B, Buettner C, Huang S, Castro B, Fonseca TL, Harney JW, Larsen PR, and Bianco AC. The iodothyronine selenodeiodinases are thioredoxin-fold family proteins containing a glycoside hydrolase clan GH-A-like structure. *J Biol Chem* 278: 36887–36896, 2003.
13. Carlsson LM, Jonsson J, Edlund T, and Marklund SL. Mice lacking extracellular superoxide dismutase are more sensitive to hyperoxia. *Proc Natl Acad Sci USA* 92: 6264–6268, 1995.
14. Choi MH, Lee IK, Kim GW, Kim BU, Han YH, Yu DY, Park HS, Kim KY, Lee JS, Choi C, Bae YS, Lee BI, Rhee SG, and Kang SW. Regulation of PDGF signaling and vascular remodelling by peroxiredoxin II. *Nature* 435: 347–353, 2005.
15. Claus M, Jaeschke H, Kleinau G, Neumann S, Krause G, and Paschke R. A hydrophobic cluster in the center of the third extracellular loop is important for thyrotropin receptor signaling. *Endocrinology* 146: 5197–5203, 2005.

16. Corvilain B, van Sande J, Laurent E, and Dumont JE. The H2O2-generating system modulates protein iodination and the activity of the pentose phosphate pathway in dog thyroid. *Endocrinology* 128: 779–785, 1991.
17. Daily D, Vlamis–Gardikas A, Offen D, Mittelman L, Melamed E, Holmgren A, and Barzilai A. Glutaredoxin protects cerebellar granule neurons from dopamine-induced apoptosis by activating NF-kappa B via Ref-1. *J Biol Chem* 276: 1335–1344, 2001.
18. De Deken X, Wang D, Dumont JE, and Miot F. Characterization of ThOX proteins as components of the thyroid H(2)O(2)-generating system. *Exp Cell Res* 273: 187–196, 2002.
19. Demelash A, Karlsson JO, Nilsson M, and Bjorkman U. Selenium has a protective role in caspase-3-dependent apoptosis induced by H2O2 in primary cultured pig thyrocytes. *Eur J Endocrinol* 150: 841–849, 2004.
20. Duntas L, Loukari E, Mantzou E, Grab–Duntas B, and Pitsouni E. Selenomethionin adminstration may attenuate proinflammation in De Quervain's subacute thyroiditis. *Horm Res* 68: 17 Abstract, 2007.
21. Duntas LH. The role of selenium in thyroid autoimmunity and cancer. *Thyroid* 16: 455–460, 2006.
22. Duntas LH, Mantzou E, and Koutras DA. Effects of a six month treatment with selenomethionine in patients with autoimmune thyroiditis. *Eur J Endocrinol* 148: 389–393, 2003.
23. Ekholm R and Bjorkman U. Glutathione peroxidase degrades intracellular hydrogen peroxide and thereby inhibits intracellular protein iodination in thyroid epithelium. *Endocrinology* 138: 2871–2878, 1997.
24. Esteves RZ, van Sande J, and Dumont JE. Nitric oxide as a signal in thyroid. *Mol Cell Endocrinol* 90: R1–3, 1992.
25. Flohe L, Gunzler WA, and Schock HH. Glutathione peroxidase: a selenoenzyme. *FEBS Lett* 32: 132–134, 1973.
26. Fomenko DE and Gladyshev VN. Identity and functions of CxxC-derived motifs. *Biochemistry* 42: 11214–11225, 2003.
27. Fomenko DE, Xing W, Adair BM, Thomas DJ, and Gladyshev VN. High-throughput identification of catalytic redox-active cysteine residues. *Science* 315: 387–389, 2007.
28. Friedrichs B, Tepel C, Reinheckel T, Deussing J, von Figura K, Herzog V, Peters C, Saftig P, and Brix K. Thyroid functions of mouse cathepsins B, K, and L. *J Clin Invest* 111: 1733–1745, 2003.
29. Gartner R, Gasnier BC, Dietrich JW, Krebs B, and Angstwurm MW. Selenium supplementation in patients with autoimmune thyroiditis decreases thyroid peroxidase antibodies concentrations. *J Clin Endocrinol Metab* 87: 1687–1691, 2002.
30. Gerard AC, Boucquey M, van den Hove MF, and Colin IM. Expression of TPO and ThOXs in human thyrocytes is down-regulated by IL-1alpha/IFN-gamma, an effect partially mediated by nitric oxide. *Am J Physiol Endocrinol Metab* 291: E242–253, 2006.
31. Gerard AC, Many MC, Daumerie C, Knoops B, and Colin IM. Peroxiredoxin 5 expression in the human thyroid gland. *Thyroid* 15: 205–209, 2005.
32. Ghezzi P. Regulation of protein function by glutathionylation. *Free Radic Res* 39: 573–580, 2005.
33. Giardina E, Capon F, D'Apice MR, Amati F, Arturi F, Filetti S, Bonifazi E, Pucci S, Conte C, and Novelli G. Mutational analysis of Peroxiredoxin IV: exclusion of a positional candidate for multinodular goitre. *BMC Med Genet* 3: 5, 2002.
34. Giray B and Hincal F. Oxidative DNA base damage, antioxidant enzyme activities and selenium status in highly iodine-deficient goitrous children. *Free Radic Res* 36: 55–62, 2002.
35. Giray B, Hincal F, Tezic T, Okten A, and Gedik Y. Status of selenium and antioxidant enzymes of goitrous children is lower than healthy controls and nongoitrous children with high iodine deficiency. *Biol Trace Elem Res* 82: 35–52, 2001.
36. Grasberger H, Van Sande J, Hag–Dahood Mahameed A, Tenenbaum–Rakover Y, and Refetoff S. A familial thyrotropin (TSH) receptor mutation provides in vivo evidence that the inositol phosphates/Ca2+ cascade mediates TSH action on thyroid hormone synthesis. *J Clin Endocrinol Metab* 92: 2816–2820, 2007.
37. Gromer S, Johansson L, Bauer H, Arscott LD, Rauch S, Ballou DP, Williams CH, Jr., Schirmer RH, and Arner ES. Active sites of thioredoxin reductases: why selenoproteins? *Proc Natl Acad Sci USA* 100: 12618–12623, 2003.
38. Gromer S, Wessjohann LA, Eubel J, and Brandt W. Mutational studies confirm the catalytic triad in the human selenoenzyme thioredoxin reductase predicted by molecular modeling. *Chembiochem* 7: 1649–1652, 2006.
39. Gromer S, Wissing J, Behne D, Ashman K, Schirmer RH, Flohe L, and Becker K. A hypothesis on the catalytic mechanism of the selenoenzyme thioredoxin reductase. *Biochem J* 332: 591–592, 1998.
40. Hasegawa Y, Takano T, Miyauchi A, Matsuzuka F, Yoshida H, Kuma K, and Amino N. Decreased expression of catalase mRNA in thyroid anaplastic carcinoma. *Jpn J Clin Oncol* 33: 6–9, 2003.
41. Hatfield DL and Gladyshev VN. How selenium has altered our understanding of the genetic code. *Mol Cell Biol* 22: 3565–3576, 2002.
42. Ho YS, Xiong Y, Ma W, Spector A, and Ho DS. Mice lacking catalase develop normally but show differential sensitivity to oxidant tissue injury. *J Biol Chem* 279: 32804–32812, 2004.
43. Howie AF, Arthur JR, Nicol F, Walker SW, Beech SG, and Beckett GJ. Identification of a 57-kilodalton selenoprotein in human thyrocytes as thioredoxin reductase and evidence that its expression is regulated through the calcium-phosphoinositol signaling pathway. *J Clin Endocrinol Metab* 83: 2052–2058, 1998.
44. Howie AF, Walker SW, Akesson B, Arthur JR, and Beckett GJ. Thyroidal extracellular glutathione peroxidase: a potential regulator of thyroid-hormone synthesis. *Biochem J* 308: 713–717, 1995.
45. Kenchappa RS, Diwakar L, Annepu J, and Ravindranath V. Estrogen and neuroprotection: higher constitutive expression of glutaredoxin in female mice offers protection against MPTP-mediated neurodegeneration. *FASEB J* 18: 1102–1104, 2004.
46. Kenchappa RS and Ravindranath V. Glutaredoxin is essential for maintenance of brain mitochondrial complex I: studies with MPTP. *FASEB J* 17: 717–719, 2003.
47. Kero J, Ahmed K, Wettschureck N, Tunaru S, Wintermantel T, Greiner E, Schutz G, and Offermanns S. Thyrocyte-specific G(q)/G(11) deficiency impairs thyroid function and prevents goiter development. *J Clin Invest* 117: 2399–2407, 2007.
48. Kim H, Lee TH, Park ES, Suh JM, Park SJ, Chung HK, Kwon OY, Kim YK, Ro HK, and Shong M. Role of peroxiredoxins in regulating intracellular hydrogen peroxide and hydrogen peroxide-induced apoptosis in thyroid cells. *J Biol Chem* 275: 18266–18270, 2000.
49. Kim H, Park S, Suh JM, Chung HK, Shong M, and Kwon OY. Thyroid-stimulating hormone transcriptionally regulates the thiol-specific antioxidant gene. *Cell Physiol Biochem* 11: 247–252, 2001.

50. Kluge B, Renault N, and Rohr KB. Anatomical and molecular reinvestigation of lamprey endostyle development provides new insight into thyroid gland evolution. *Dev Genes Evol* 215: 32–40, 2005.
51. Knoops B, Clippe A, Bogard C, Arsalane K, Wattiez R, Hermans C, Duconseille E, Falmagne P, and Bernard A. Cloning and characterization of AOEB166, a novel mammalian antioxidant enzyme of the peroxiredoxin family. *J Biol Chem* 274: 30451–30458, 1999.
52. Kohrle J. The deiodinase family: selenoenzymes regulating thyroid hormone availability and action. *Cell Mol Life Sci* 57: 1853–1863, 2000.
53. Kohrle J, Jakob F, Contempre B, and Dumont JE. Selenium, the thyroid, and the endocrine system. *Endocr Rev* 26: 944–984, 2005.
54. Korotkov KV, Novoselov SV, Hatfield DL, and Gladyshev VN. Mammalian selenoprotein in which selenocysteine (Sec) incorporation is supported by a new form of Sec insertion sequence element. *Mol Cell Biol* 22: 1402–1411, 2002.
55. Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigo R, and Gladyshev VN. Characterization of mammalian selenoproteomes. *Science* 300: 1439–1443, 2003.
56. Lehmann P, Rank P, Hallfeldt KL, Krebs B, and Gartner R. Dose-related influence of sodium selenite on apoptosis in human thyroid follicles in vitro induced by iodine, EGF, TGF-beta, and H2O2. *Biol Trace Elem Res* 112: 119–130, 2006.
57. Lillig CH and Holmgren A. Thioredoxin and related molecules—from biology to health and disease. *Antioxid Redox Signal* 9: 25–47, 2007.
58. Lillig CH, Lonn ME, Enoksson M, Fernandes AP, and Holmgren A. Short interfering RNA-mediated silencing of glutaredoxin 2 increases the sensitivity of HeLa cells toward doxorubicin and phenylarsine oxide. *Proc Natl Acad Sci USA* 101: 13227–13232, 2004.
59. Lim MJ, Chae HZ, Rhee SG, Yu DY, Lee KK, and Yeom YI. The type II peroxiredoxin gene family of the mouse: molecular structure, expression and evolution. *Gene* 216: 197–205, 1998.
60. Magnusson RP, Taurog A, and Dorris ML. Mechanisms of thyroid peroxidase- and lactoperoxidase-catalyzed reactions involving iodide. *J Biol Chem* 259: 13783–13790, 1984.
61. Maier J, van Steeg H, van Oostrom C, Paschke R, Weiss RE, and Krohn K. Iodine deficiency activates antioxidant genes and causes DNA damage in the thyroid gland of rats and mice. *Biochim Biophys Acta* 1773: 990–999, 2007.
62. Manevich Y and Fisher AB. Peroxiredoxin 6, a 1-Cys peroxiredoxin, functions in antioxidant defense and lung phospholipid metabolism. *Free Radic Biol Med* 38: 1422–1432, 2005.
63. Mano T, Shinohara R, Iwase K, Kotake M, Hamada M, Uchimuro K, Hayakawa N, Hayashi R, Nakai A, Ishizuki Y, and Nagasaka A. Changes in free radical scavengers and lipid peroxide in thyroid glands of various thyroid disorders. *Horm Metab Res* 29: 351–354, 1997.
64. Marklund SL. Extracellular superoxide dismutase in human tissues and human cell lines. *J Clin Invest* 74: 1398–1403, 1984.
65. Marklund SL, Westman NG, Lundgren E, and Roos G. Copper- and zinc-containing superoxide dismutase, manganese-containing superoxide dismutase, catalase, and glutathione peroxidase in normal and neoplastic human cell lines and normal human tissues. *Cancer Res* 42: 1955–1961, 1982.
66. Melov S, Schneider JA, Day BJ, Hinerfeld D, Coskun P, Mirra SS, Crapo JD, and Wallace DC. A novel neurological phenotype in mice lacking mitochondrial manganese superoxide dismutase. *Nat Genet* 18: 159–163, 1998.
67. Menth M, Schmutzler C, Mentrup B, Hoang–Vu C, Takahashi K, Honjoh T, and Kohrle J. Selenoprotein expression in Hurthle cell carcinomas and in the human Hurthle cell carcinoma line XTC.UC1. *Thyroid* 15: 405–416, 2005.
68. Mitchell JH, Nicol F, Beckett GJ, and Arthur JR. Selenoenzyme expression in thyroid and liver of second generation selenium- and iodine-deficient rats. *J Mol Endocrinol* 16: 259–267, 1996.
69. Mitchell JH, Nicol F, Beckett GJ, and Arthur JR. Selenoprotein expression and brain development in preweanling selenium- and iodine-deficient rats. *J Mol Endocrinol* 20: 203–210, 1998.
70. Mo Y, Feinstein SI, Manevich Y, Zhang Q, Lu L, Ho YS, and Fisher AB. 1-Cys peroxiredoxin knock-out mice express mRNA but not protein for a highly related intronless gene. *FEBS Lett* 555: 192–198, 2003.
71. Nakamura Y, Ohtaki S, Makino R, Tanaka T, and Ishimura Y. Superoxide anion is the initial product in the hydrogen peroxide formation catalyzed by NADPH oxidase in porcine thyroid plasma membrane. *J Biol Chem* 264: 4759–4761, 1989.
72. Negro R, Greco G, Mangieri T, Pezzarossa A, Dazzi D, and Hassan H. The influence of selenium supplementation on postpartum thyroid status in pregnant women with thyroid peroxidase autoantibodies. *J Clin Endocrinol Metab* 92: 1263–1268, 2007.
73. Nishida S, Nakano T, Kimoto S, Kusunoki T, Suzuki K, Taniguchi N, Murata K, and Tomura TT. Induction of manganese superoxide dismutase by thyroid stimulating hormone in rat thyroid cells. *FEBS Lett* 416: 69–71, 1997.
74. Reaume AG, Elliott JL, Hoffman EK, Kowall NW, Ferrante RJ, Siwek DF, Wilcox HM, Flood DG, Beal MF, Brown RH, Jr., Scott RW, and Snider WD. Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury. *Nat Genet* 13: 43–47, 1996.
75. Reynaert NL, van der Vliet A, Guala AS, McGovern T, Hristova M, Pantano C, Heintz NH, Heim J, Ho YS, Matthews DE, Wouters EF, and Janssen–Heininger YM. Dynamic redox control of NF-kappaB through glutaredoxin-regulated S-glutathionylation of inhibitory kappaB kinase beta. *Proc Natl Acad Sci USA* 103: 13086–13091, 2006.
76. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, and Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. *Science* 179: 588–590, 1973.
77. Savaskan NE, Borchert A, Brauer AU, and Kuhn H. Role for glutathione peroxidase-4 in brain development and neuronal apoptosis: specific induction of enzyme expression in reactive astrocytes following brain injury. *Free Radic Biol Med* 43: 191–201, 2007.
78. Schmutzler C, Mentrup B, Schomburg L, Hoang–Vu C, Herzog V, and Köhrle J. Selenoproteins of the thyroid gland. *Biol Chem* 388: 11053–1059, 2007.
79. Schomburg L, Riese C, Michaelis M, Griebert E, Klein MO, Sapin R, Schweizer U, and Kohrle J. Synthesis and metabolism of thyroid hormones is preferentially maintained in selenium-deficient transgenic mice. *Endocrinology* 147: 1306–1313, 2006.
80. Singh PP, Kumar P, and Laloraya M. Regulation of superoxide anion radical-superoxide dismutase system in the avian thyroid by TSH with reference to thyroid hormonogenesis. *Biochem Biophys Res Commun* 239: 212–216, 1997.
81. Song Y, Driessens N, Costa M, De Deken X, Detours V, Corvilain B, Maenhaut C, Miot F, Van Sande J, Many MC, and Dumont JE. Roles of hydrogen peroxide in thyroid physiology and disease. *J Clin Endocrinol Metab* 92: 3764–3773, 2007.

82. Sun Y, Ha PC, Butler JA, Ou BR, Yeh JY, and Whanger P. Effect of dietary selenium on selenoprotein W and glutathione peroxidase in 28 tissues of the rat. *J Nutr Biochem* 9: 23–27, 1997.
83. Takano T, Hasegawa Y, Matsuzuka F, Miyauchi A, Yoshida H, Higashiyama T, Kuma K, and Amino N. Gene expression profiles in thyroid carcinomas. *Br J Cancer* 83: 1495–1502, 2002.
84. Taurog A. *The Thyroid*. Philadelphia: Lippincott Williams & Wilkins, 2000.
85. Turker O, Kumanlioglu K, Karapolat I, and Dogan I. Selenium treatment in autoimmune thyroiditis: 9-month follow-up with variable doses. *J Endocrinol* 190: 151–156, 2006.
86. Verma S, Kumar P, Laloraya M, Nivsarkar M, and Singh A. Superoxide dismutase activation in thyroid and suppression in adrenal. Novel pituitary regulatory routes. *FEBS Lett* 282: 310–312, 1991.
87. Wang D, De Deken X, Milenkovic M, Song Y, Pirson I, Dumont JE, and Miot F. Identification of a novel partner of duox: EFP1, a thioredoxin-related protein. *J Biol Chem* 280: 3096–3103, 2005.
88. Yanagawa T, Ishikawa T, Ishii T, Tabuchi K, Iwasa S, Bannai S, Omura K, Suzuki H, and Yoshida H. Peroxiredoxin I expression in human thyroid tumors. *Cancer Lett* 145: 127–132, 1999.
89. Yant LJ, Ran Q, Rao L, Van Remmen H, Shibatani T, Belter JG, Motta L, Richardson A, and Prolla TA. The selenoprotein GPX4 is essential for mouse development and protects from radiation and oxidative damage insults. *Free Radic Biol Med* 34: 496–502, 2003.

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3. Moses O Akiibinu, Omobola A Ogundahunsi, Ebenezer O Ogunyemi. 2012. Inter-relationship of plasma markers of oxidative stress and thyroid hormones in schizophrenics. *BMC Research Notes* **5**:1, 169. [CrossRef]
4. Lutz Schomburg. 2011. Selenium, selenoproteins and the thyroid gland: interactions in health and disease. *Nature Reviews Endocrinology* . [CrossRef]
5. Ulrich Schweizer, Nora Dehina, Lutz Schomburg. 2011. Disorders of selenium metabolism and selenoprotein function. *Current Opinion in Pediatrics* **23**:4, 429-435. [CrossRef]
6. J. Köhrle, G. Brabant. 2010. Synthese, Stoffwechsel und Diagnostik der Schilddrüsenhormone. *Der Internist* **51**:5, 559-567. [CrossRef]
7. Marcus Conrad , Ulrich Schweizer . 2010. Unveiling the Molecular Mechanisms Behind Selenium-Related Diseases Through Knockout Mouse Studies. *Antioxidants & Redox Signaling* **12**:7, 851-865. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
8. Josef Köhrle, Roland Gärtner. 2009. Selenium and thyroid. *Best Practice & Research Clinical Endocrinology & Metabolism* **23**:6, 815-827. [CrossRef]
9. Leopold Flohé , Fulvio Ursini . 2008. Peroxidase: A Term of Many Meanings. *Antioxidants & Redox Signaling* **10**:9, 1485-1490. [Abstract] [Full Text PDF] [Full Text PDF with Links]