Roles of Hydrogen Peroxide in Thyroid Physiology and Disease

Article *in* Journal of Clinical Endocrinology & Metabolism · November 2007

DOI: 10.1210/jc.2007-0660 · Source: PubMed

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The Journal of Clinical Endocrinology & Metabolism 92(10):3764–3773

doi: 10.1210/jc.2007-0660

REVIEW: Roles of Hydrogen Peroxide in Thyroid Physiology and Disease

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Context: The long-lived thyroid cell generates, for the synthesis of thyroid hormones, important amounts of H_2O_2 that are toxic in other cell types. This review analyzes the protection mechanisms of the cell and the pathological consequences of disorders of this system.

Evidence Acquisition: The literature on H_2O_2 generation and disposal, thyroid hormone synthesis, and their control in the human thyroid is analyzed.

Evidence Synthesis: In humans, H_2O_2 production by dual-oxidases and consequently thyroid hormone synthesis by thyroperoxidase are controlled by the phospholipase C-Ca^{2+}-diacylglycerol arm of TSH receptor action. H_2O_2 in various cell types, and presumably in thyroid cells, is a signal, a mitogen, a mutagen, a carcinogen, and a killer. The various protection mechanisms of the thyroid cell against H_2O_2 are analyzed. They include the separation of the generating enzymes (dual-oxidases), their coupling to thyroperoxidase in a proposed complex, the thyroxisome, and H_2O_2 degradation systems.

Conclusions: It is proposed that various pathologies can be explained, at least in part, by overproduction and lack of degradation of H_2O_2 (tumorigenesis, myxedematous cretinism, and thyroiditis) and by failure of the H_2O_2 generation or its positive control system (congenital hypothyroidism). **(*J Clin Endocrinol Metab* 92: 3764–3773, 2007)**

General Effects of H_2O_2

H_2O_2 in signal transduction

Since the pioneering work of de Haen and colleagues (1, 2) and Mukherjee *et al.* (3) on insulin-induced generation of H_2O_2, the role of this molecule as an intracellular signal, at first controversial, has become widely accepted (4). In vertebrates, H_2O_2 is generated in response to insulin and growth factors in many systems. Through inhibition of tyrosine phosphatases, by oxidation of a cysteine at the catalytic site (5), it enhances the protein tyrosine phosphorylations caused by the activated receptors of these hormones. It is a classical synergic double-action regulation: stimulation of the cascade and inhibition of the negative control. Some of the effects of growth factors, such as proliferation and/or survival, are therefore mimicked by low physiological levels of H_2O_2.

The cellular H_2O_2-generating systems belong to the family of reduced nicotinamide adenine dinucleotide phosphate oxidase (NOX) enzymes. These enzymes produce H_2O_2 or the O_2 superoxide O_2^-, which is rapidly converted to H_2O_2 by superoxide dismutases. The role of the various NOXs has been recently reviewed (6–8). The links between receptors and NOXs are still debated. Intracellular H_2O_2 is also generated by intracellular metabolism, for instance by mitochondria and peroxisomes presumably as a byproduct. The former process is enhanced by a blockade of the electron transport chain.

At physiological levels (1–10 μM extracellular), H_2O_2 enhances proliferation in a variety of vertebrate cells (9) as well as overexpression of NOXs. Conversely, intracellular and even extracellular catalase may inhibit proliferation. Several biochemical effects of H_2O_2 account for its activation of cell proliferation: activation of the growth receptor tyrosine kinase pathways by direct inhibition of protein tyrosine phosphatases (10–13) and by activation of kinases such as Src kinase (14), stimulation of the phosphoinositide-3-kinase pathway through activation of phosphoinositide-3-kinase (15) or inhibition of phosphatidylinositol-3,4,5-trisphosphate (PIP3) phosphatase and tensin homolog (PTEN) (16, 17), activation of cyclin-dependent kinases and of the degradation of inhibitors of these kinases. *etc.* In the case of platelet-derived growth factor and arterial cells, the *in vivo* role of H_2O_2 is supported by the fact that the signaling is suppressed by overexpression of peroxiredoxin and enhanced in peroxiredoxin knockout cells (18).

Through its stimulatory effect on various kinase pathways (*e.g.* c-Jun N-terminal kinase, p38, *etc.*) and through oxidized thioredoxin, H_2O_2 stimulates not only transcription factors such as nuclear factor-κB, activator protein-1, and p53 and induces specific protective genes (19–21) but also directly or indirectly many other genes (22). H_2O_2 acts as an intracellular and extracellular NO oxidizer and destroyer and as an extracellular paracrine vascular activator (23).

H_2O_2 has several roles other than signaling in normal cell biology. It is produced as a toxic metabolite in host defense

First Published Online July 31, 2007

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Abbreviations: DUOX, Dual-oxidases; EFP1, EF-hand fragment partner 1; GSH, glutathione; NOX, reduced nicotinamide adenine dinucleotide phosphate oxidase; PIP2, phosphatidylinositol-4,5-bisphosphate; TPO, thyroperoxidase.

by polymorphonuclear neutrophils, monocytes, and macrophages, and it may have a similar role in gastrointestinal mucosa and lung epithelium. It is used as a cofactor for iodide oxidation and thyroid hormone synthesis in thyroid and for protein cross-linking and cuticle formation in insects (24). It is therefore very probable that different NOX and peroxidase regulation and structural organization correspond to these different physiological functions. One example is the necessary role of Rac1 in the activation of most NOXs but not thyroid dual-oxidases (DUOXs) (25).

Toxic effects of H_2O_2

The levels of H_2O_2 reached physiologically in cells vary from a low 0.001 μM to a maximum of 0.7 μM. When H_2O_2 is applied to the exterior of cultured cells, the intracellular concentrations are approximately 10-fold lower than the extracellular concentrations (9, 26). Because there are great variations in the rate of H_2O_2 degradation in different cell types and models, it is difficult to compare concentration-effect relations. In most cell cultures, H_2O_2 in the medium disappears in less than 1 h.

At higher concentrations than those that have a signaling role, H_2O_2 induces oxidative stress, DNA oxidation and damage, and consequent mutagenesis and apoptosis (9). For the phagocytes, H_2O_2 has been designated as "the enemy within" (27). Oxidative stress involves the oxidation of various cellular components, proteins, lipids, nucleic acids, *etc.* The accumulation of oxidatively damaged proteins accelerates chaperone-mediated autophagy, which will degrade them (28).

Oxidative damage to DNA produces adducts (including 8-oxo-deoxyguanosine and thymine glycol), single-strand breaks, and at high levels double-strand breaks (29). Positive Comet assays demonstrate these breaks. The half-life of these damages varies for the various lesions (from 9–62 min for the adducts, more for the breaks) (30). The positive Comet assays for thyroid cells incubated with 50 μM H_2O_2 disappear by 80% in 2 h (31).

Mutagenesis, if it leads to constitutive activation of a protooncogene or to inactivation of tumor suppressor genes is carcinogenic, especially if it is combined to a proliferative effect. Thus, H_2O_2 is carcinogenic and has been found to play a role in several human cancers (7) even if it may not be sufficient (32).

Conversely, selenium, the essential constituent of protective enzymes, prevents tumor development in rats submitted to chemical carcinogenesis (33). Lack of protective systems in knockout mice such as lack of peroxiredoxin or glutathione (GSH) peroxidases indeed leads to malignant cancers (34, 35). Transfection of an H_2O_2-generating system transforms epithelial cells (36).

High-level acute H_2O_2 treatment of various cells *in vitro* leads to apoptosis (37). This effect has been linked to a loss of GSH and reduced glutaredoxin and consequent activation of apoptosis signal-regulating kinase (ASK) and of an apoptosis program (38). These effects are stronger in actively proliferating cells (39).

Chronic H_2O_2 administration at low levels induces senescence in cultured cells *in vitro* in human fibroblasts (40, 41). H_2O_2 favors inflammation (42), and its inhibitory effect on indoleamine dioxygenase, which by depriving lymphocytes of tryptophan is immunosuppressive, would enhance immune reactions.

It is therefore not astonishing that even in relatively short-lived (7 h) neutrophils (43) and macrophages, H_2O_2 generation is tightly regulated by a synergic two-pronged mechanism involving both intracellular calcium and diacylglycerol protein kinase C (41, 44).

H_2O_2 in the Thyroid

Physiological role

Until now, no signaling role of H_2O_2 has been demonstrated directly in the thyroid. Such a role can, however, be inferred from general work on other cell types.

To synthesize thyroid hormones, the thyrocyte takes up iodide from the blood and extracellular fluid and oxidizes it to bind it to selected tyrosines of thyroglobulin. Iodide is actively transported by the Na^+/I^- symporter in the cell at the basal membrane and leaked out along the electrical gradient (from the negative interior to the positive exterior) by an iodide channel at the apical membrane. Pendrin is a candidate for this role. Iodide in the follicular lumen is oxidized at the apical membrane by thyroperoxidase (TPO) using H_2O_2 as the other substrate. The latter originates from an H_2O_2-generating system whose main enzymes are the recently cloned thyroid DUOX1 and DUOX2 (45, 46). Oxidized iodide is linked covalently to tyrosines of thyroglobulin by TPO (47). The same system, by an oxidizing reaction, links covalently some iodotyrosines into iodothyronines within thyroglobulin. H_2O_2 is produced in large excess compared with the amounts of iodide incorporated into proteins. This may be necessary owing to the relatively high Michaelis Menten constant (K_m) of TPO for H_2O_2 (48, 49). It is interesting that iodide leakage, *i.e.* presumably the iodide channel that releases iodide at the apical membrane, is acutely regulated by the same cascades and with the same timing as H_2O_2 generation (50).

Although DUOX1 and/or DUOX2 are expressed in several organs (*e.g.* gastrointestinal mucosa, lung epithelium, and oocyte) (51–53), TPO is specific for the thyroid. The TPO-like N-terminal domain of DUOX lacks the histidines that link the heme group in peroxidases (54, 55) and therefore presumably does not have any peroxidase activity. The specificity of the thyrocyte thyroid hormone synthesis machinery therefore rests on TPO. The function of other NOXs, in particular NOX2, is tightly linked to H^+ and other ion transport (56, 57), but this has not yet been studied for the DUOXs.

Thus, the normal physiology of the thyroid cell requires the generation of H_2O_2 by DUOXs and not O_2^- as for other NOXs (46). DUOX1 and/or DUOX2 are responsible for the generation of H_2O_2, as demonstrated in transfected cells expressing both DUOX and DUOXA (58). Both DUOXs contain intracellular EF-hands and respond to increase in intracellular calcium by a marked activation. They are inactive in its absence. Both are stimulated by phorbol esters and thus presumably diacylglycerol through protein kinase C. The defect in iodide organification in congenital inactivation of

DUOX2 shows that this isozyme is fully necessary for H_2O_2 generation (59).

In thyrocytes of most species, including humans and pigs, TSH and its receptor activate both Gs and Gq, *i.e.* the cAMP and the phospholipase C-Ca^{2+} signaling cascades (Fig. 1). In such thyrocytes, the cAMP cascade inhibits, whereas the phospholipase C-Ca^{2+} cascade activates H_2O_2 generation, iodide binding to proteins, and thyroid hormone formation; the cAMP cascade activates secretion (8, 48, 60). In dog thyrocytes, in which the TSH receptor does not activate Gq, cAMP activates both H_2O_2 generation and thyroid hormone synthesis and the secretion of these hormones (8, 61). In all species studied, iodide at high concentrations presumably through an iodinated lipid, iodohexadecanal, inhibits H_2O_2 generation (the Wolff-Chaikoff effect) and adenylate cyclase (62–64).

The control of the thyroid hormone-synthesizing system is exerted at least at two levels: acute regulation of H_2O_2 generation by calcium diacylglycerol and iodide and delayed regulation of TPO expression by cAMP (8). Induction of TPO by TSH takes place at the transcription level, does not require intermediary protein synthesis, and is very rapid (already after 1 h) (65, 66).

The dynamics of TPO localization in the rat thyrocyte has been beautifully demonstrated by the groups of Wollman and Ekholm (67, 68). We presume DUOX behaves similarly (Fig. 2). TPO in the thyrocyte at rest is concentrated in secretory granules just inside of the apical membrane. The iodination system is inactive there. After TSH stimulation, the granules fuse to the membrane between microvilli, allowing TPO to migrate to the microvillous membrane. It never locates on the pseudopods engulfing thyroglobulin. The main location of TPO and iodination and therefore presumably DUOX is thus in the microvillous membranes (68–70). This is supported by the demonstration by histochemistry-electron microscopy of NOX activity in the microvilli (71). Such a localization already appears, early in evolution, in the endostyle of larval amphioxus (72). In lung epithelium

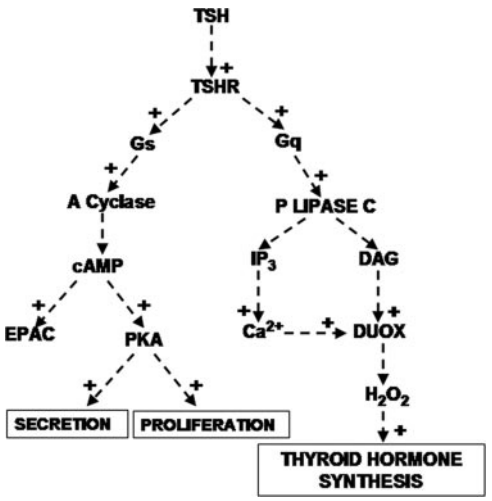

FIG. 1. Regulation of the human thyrocytes by TSH. The *dashed arrows* with the *plus sign* indicate positive direct control. DAG, Diacylglycerol; EPAC, exchange protein activated by cAMP; PKA, cAMP-dependent protein kinase; TSHR, TSH receptor.

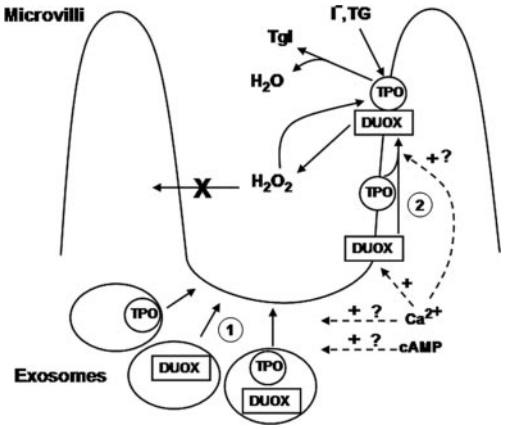

FIG. 2. Proposed steps in the activation of the postulated thyroxisome. 1) Translocation of inactive DUOX and/or TPO from near membrane intracellular granules (exosomes) to the membrane. 2) Association of DUOX and TPO. TG, Thyroglobulin; TgI, iodinate thyroglobulin.

and in the gastrointestinal mucosa, DUOX also preferentially localizes at the brush border (51, 52).

H_2O_2 toxicity on thyroid cells

H_2O_2 generation in the thyroid is quantitatively important, especially in stimulated cells. It is of the same order as the production of activated leukocytes. Stimulated dog thyroid slices and FRTL5 and PCCl3 rat thyroid cell lines produce around six, pig thyroid slices and thyroid cells in primary cultures around 10, and human leukocytes around 17 nmol H_2O_2/10min·10 μg DNA (60, 73, 74). However, although an activated leukocyte lives a few hours, the life of the thyrocyte in human adult is 7 yr (75, 76). Thus, the thyroid cells may be exposed to high doses of H_2O_2 and have to adapt to it (Fig. 3).

By a sort of leakage of the iodination system, some oxidized iodide is bound to phospholipids (77). Such iodination is presumed to be toxic.

H_2O_2 exerts on thyrocytes the same toxicity as on other cell types. In dog and human thyrocytes in primary culture, H_2O_2 at concentrations of less than 0.1 mM induces DNA single-strand breaks as demonstrated by the Comet assay at alkaline pH (31). Presumably, some of these correspond to the repair of 8-oxo-guanine bases. Such strand breaks are mostly repaired in 2 h.

At higher concentration (0.1 mM and above), H_2O_2 induces DNA double-strand breaks as demonstrated by the Comet assay at neutral pH and by the immunodetection of the phosphorylation of histone H2AX on serine 139 by Western blotting. In PCCl3 cell lines, as in other cells (78), the majority of the breaks are fully repaired after 18 h (Mondello, C., and N. Driessens, unpublished). Such effects are, when they are not or badly repaired, potentially mutagenic. Similarly, $Tg_{\alpha 1B}$ AR thyroid transgenic mice, with constitutive activation of a mutated α_{1B} adrenergic receptor and thus of both the cAMP and the PIP3 Ca^{2+} cascade, have increased H_2O_2 generation. They exhibit thyroid cell mortality and later tumorigenesis (79).

At high concentration (above 0.1 mM), H_2O_2 induces

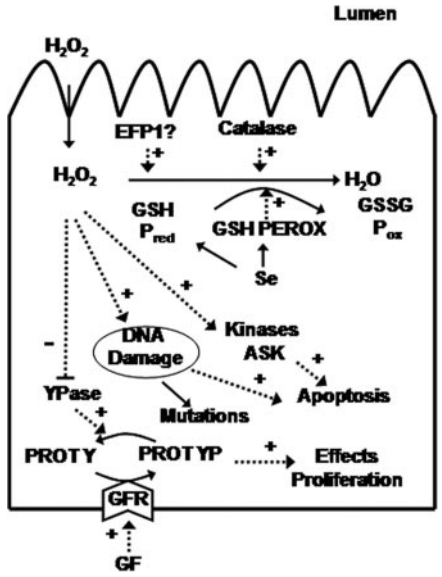

FIG. 3. Fate of H_2O_2 leaking back in the thyroid cell: catabolism by GSH peroxidase (GSH PEROX), peroxiredoxin reduced or oxidized (P_{red} or P_{ox}), and catalase; proposed effects on protein tyrosine phosphatase (YPase) and kinases such as apoptosis signal kinase (ASK); induction of DNA damage; and growth factor (GF) receptor (GFR). EFP1, EF-hand fragment partner 1 (thioredoxin-like DUOX binding protein); GSSG, oxidized glutathione; Se, selenium incorporated in peroxiredoxin and GSH PEROX. *Solid arrows* indicate chemical transformation; *dashed arrows* with a *plus sign* indicate positive direct control; and *dashed lines* with a *minus sign* indicate negative direct control.

apoptosis in thyroid cells, and at even higher levels (above 0.4 mM), necrosis (80–82), an effect that is potentiated by selenium deprivation and consequent GSH peroxidase depletion.

The human *in vivo* thyroid relevance of these *in vitro* observations on H_2O_2-induced mutagenesis is supported by several facts: 1) a greatly increased spontaneous mutation frequency in the thyroid compared with other organs of mice (83); 2) in human disease, a higher frequency of those somatic mutations of the TSH receptor that result from DNA oxidations; 3) a somewhat higher positivity of Comet assay of thyroid permeabilized tissue treated with the DNA excision repair enzyme; and 4) *in vivo*, a basal global DNA damage in normal thyroid comparable to the one of other tissues, as shown by Paschke's group by using the Comet assay at alkaline pH. When using lesion-specific enzymes during the assay, he has shown that the thyroid presented more oxidized pyrimidines and purine oxidation products such as 8-oxoguanine compared with lung, liver, and to a lesser extent spleen. He also reported a most prominent follicular cell immunohistochemical distribution of 8-hydroxydeoxyguanosine and 8-hydroxyguanosine near the lumen where H_2O_2 is produced (83).

Defense Mechanisms in the Thyroid

A proposed iodination complex: the thyroxisome

As suggested in 1971 (84) and experimentally demonstrated later (67, 84), a main protection of the thyroid cell against the H_2O_2 that it generates is the strict separation of the iodination system acting at the apical membrane of the cell in the follicular lumen from the interior of the cell (Table 1).

The iodination complex is composed at least of TPO and DUOXs. We have shown that these enzymes are associated in transfected cells expressing both enzymes intracellularly (85) and also in membranes of the human thyroid cell (Song, Y., unpublished). In the first case, the complexes as judged by H_2O_2 generation and iodination are inactive; in the second case, as judged by the same criteria, they are active. The association of DUOX and TPO might be correlated to the addition in the DUOX structure, to the usual NOX archetype, of an extracellular segment homologous to TPO. We propose to call the assembly of these proteins, and of putative others participating in their function, the thyroxisome (Fig. 4). This concept of association does not necessarily imply a stoichiometric one to one complex. The fact that TPO and DUOX coimmunoprecipitate in transfected cells, which do not express them at the membrane, suggests that they could associate in the cells somewhere after their biosynthesis. TPO, in the presence of I^-, oxidizes I^- and in doing so, as other peroxidases such as myeloperoxidase (86), reduces H_2O_2. Besides, such enzymes have a catalase-like effect: in the absence of iodide, they oxidize other potential available substrates, depending on their specific affinities, and thus catabolize H_2O_2 (87, 88). For instance, they all oxidize thiocyanide (89). In simple Krebs phosphate buffer, TPO has weak catalase-like activity that is enhanced by iodide (90). The relative importance of this effect *in vivo* is unknown. Indirect arguments for the *in vivo* validity of this concept are

TABLE 1. Levels of thyroid cell protection against H_2O_2

Thyroid cell protection
1 Association of DUOX and TPO in membrane: the thyroxisome; H_2O_2 produced is degraded *in loco* by TPO in the presence of iodide and perhaps in its absence (catalase-like effect of peroxidase); possible role of thioredoxin like EFP1; regulation of this association: role of raft-like membrane structure, caveolin?
2 Poor permeability of apical membrane to H_2O_2: raft-like composition
3 Localization of thyroxisomes mostly in microvillous membrane, *i.e.* at a distance from the body of the cell
4 Tight control of the activity of DUOX and H_2O_2 generation in the cell and perhaps in the membrane; role of calcium and DUOX EF hands and of protein kinase C; possible role of ionic composition and pH of the intraluminal colloid
5 Control of access of thyroxisome to apical membrane: regulated exocytosis
6 Tight control of export of DUOX from the reticulum to the membrane: role of DUOXA1 and DUOXA2 on delivering fully glycosylated and active DUOX at the cell membrane
7 Intracellular H_2O_2 detoxifying mechanisms: GSH peroxidase and GSH reductase, peroxiredoxin, thioredoxin and thioredoxin reductase, and catalase
8 Induction of the protective mechanisms by the same agents and cascades stimulating H_2O_2 generation?
9 Control of DUOX mRNA and protein expression: very loose in humans

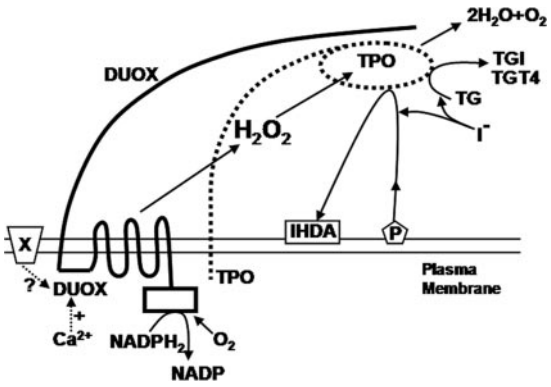

FIG. 4. The postulated thyroxisome. The producer-consumer unit is composed of the associated DUOX and TPO at the membrane. Generation of H_2O_2 is in a restricted space where it is consumed by the oxidation of iodide and its binding to thyroglobulin (TG) and plasmalogen (P), generating iodotyrosines and iodothyronines in thyroglobulin (TGI and TGT4) and iodohexadecanal (IHDA) in the membrane. A possible catalase effect of TPO is represented. The participation of other proteins X in H_2O_2 disposal, such as EFP1, is represented. $NADPH_2$, Nicotinamide adenine dinucleotide phosphate.

the finding of normal expression of DUOX, overexpression of TPO, and decreased H_2O_2 generation in autonomous adenomas in humans (91) and in chronically stimulated mice thyroids (92). It would explain why expression is far more regulated for TPO than for DUOX. Also, iodide, at high concentration through an iodinated lipid derivative, most probably iodohexadecanal, inhibits H_2O_2 generation by open follicles (73, 93). This, in a medium containing iodide and that traps very efficiently H_2O_2 (homovanillic acid and horseradish peroxidase), shows that the H_2O_2 necessary for TPO iodination of membrane lipids must be protected from horseradish peroxidase at the apical membrane, a hand to mouth effect presumably in the thyroxisome.

A dual role of peroxidase would also explain why, although DUOX activity, which generates H_2O_2, is tightly regulated, TPO activity, which consumes it, is apparently constitutive. TPO certainly does not contain in its intracellular part any phosphorylation consensus sequence.

On the other side, TPO, like other peroxidases, may be inactivated by excess H_2O_2 (87, 94, 95).

If some DUOX-produced H_2O_2 leaks back at the level of production, the newly discovered DUOX partner EF-hand fragment partner 1 (EFP1), a thioredoxin-related protein, could perhaps destroy it (85) on the spot.

The NOX2 complex in leukocytes and macrophages is tightly controlled by an on/off regulation. H_2O_2 generation by the thyroid cells in slices is also controlled, but less stringently, the basal level of production being less than 1/10 of the stimulated level.

Location

The low diffusion conditions of the colloid, between the microvilli and the preferential localization of TPO and presumably DUOX in microvilli separate the H_2O_2 produced from the body of the cell (96) in a restricted space. These characteristics ensure that only part of the H_2O_2 produced can diffuse back to the cell.

Moreover, H_2O_2 does not freely diffuse across biomembranes. This role of barrier, coupled to the H_2O_2 detoxifying mechanism explains the important gradient between extracellularly applied H_2O_2 and intracellular H_2O_2 for cells *in vitro* (97). The high level of gangliosides and cholesterol in apical membranes, *i.e.* its raft-like composition, would further impair H_2O_2 diffusion in the cell (98). Indeed yeast cells lacking ergosterol become much more sensitive to the toxic effects of H_2O_2 (97). The membrane of the enterocyte microvilli has a raft-like structure maintained by glycolipids but not cholesterol (99). Because some aquaporins are permeable to H_2O_2 (100), it would be interesting to know whether aquaporins are excluded from the microvillous membranes.

The complex DUOX-TPO is inactive inside the cell. There is little activation by calcium ionophore of H_2O_2 generation in transfected cells that fail to bring DUOX to the plasma membrane. Moreover, even though the majority of DUOX and TPO proteins are inside the thyroid cell, no intracellular iodination takes place in the presence of radioiodide, whereas the cells concentrate it. If exogenous H_2O_2 is provided, intracellular iodination takes place, which shows that H_2O_2 is limiting (101) and suggests that it is not generated endogenously in the cell. Several mechanisms account for this regulation:

1. DUOX is active only in its fully glycosylated form, which is present only at the plasma membrane and perhaps in juxtamembrane vesicles (45).

2. DUOX proteins require specific maturation proteins (chaperone), DUOXA1 and DUOXA2, to get to the membrane (58).

3. The bulk of DUOX proteins is not detected at the cell surface but is inactive in intracellular compartments, providing a stimulus-recruitable pool. TPO and presumably DUOX are stored in granules below the apical membrane. Because no iodination takes place, they are inactive (102). Access of the components of the thyroxisome or the thyroxisome itself to the apical membrane is tightly regulated by the controlled exocytosis of secretory granules (68). Stimulation of the cells induces granule fusion to the plasma membrane. This has been clearly shown for TPO (102).

4. DUOX is linked to caveolin in the membrane. DUOX contains the peptide sequence insuring such linkage. TPO is bound to DUOX. The possible regulatory role of caveolin and other proteins of the thyroxisome is unknown.

5. Disruption of follicles leads to a loss of polarity and to the generation of intracellular lumina in which iodination takes place (103). Thus, the follicular structure ensures the polarity necessary for the apical sequestration of H_2O_2 and of iodination. The strict segregation of H_2O_2 generation at the apex, plus the intracytoplasmic localization of H_2O_2-degrading enzymes ensures the absence of H_2O_2 in the cell that would immediately lead to intracellular iodination by the intracellular TPO. Such iodination takes place only with large toxic amounts of extracellular H_2O_2 and when the defense mechanisms are impaired (*e.g.* by selenium deficiency) (101), the H_2O_2 penetrating the cells presumably through their basal membrane.

Other defenses

The existence in some species of a positive control of H_2O_2 generation by low concentration of iodide, *i.e.* by the cosubstrate of TPO, is another way to restrict H_2O_2 synthesis to the appropriate time (73). This control is weak and inconsistent in humans.

The thyroid cell contains all the biochemical systems that detoxify H_2O_2 in other cells, notably the selenoproteins: GSH peroxidases (cytoplasmic, plasma, and phospholipid) and thioredoxin reductases (104, 105). The concept of the TSH role in the activation of H_2O_2 generation and of the protective role of GSH peroxidase coupled to the hexose monophosphate pathway had already been developed in 1971 (84).

In the cell, the GSH peroxidase system plays the major role, at physiological levels of H_2O_2, whereas catalase with its higher K_m and V_{max}, allows the cell to metabolize even very large, toxic levels of H_2O_2 (106). The first system follows Michaelis-Menten kinetics, whereas the second follows first-order kinetics (107). Peroxiredoxin directly and thioredoxin and metallothioneins indirectly inactivate H_2O_2. Peroxiredoxin also reduces peroxidized membrane phospholipids. Oxidized thioredoxin is reduced by thioredoxin reductase and peroxiredoxin by thioredoxin (108, 109). The role of catalase does not appear to be very important because there is no thyroid phenotype reported in acatalasemia. The cytosolic localization of the disposal enzymes in the cytosol ensures that, even if some leakage in the cell occurs, the H_2O_2 will be reduced at the very periphery. The importance of these systems in physiology is suggested by the fact that the thyroid is, with the brain, the privileged organ retaining Se in Se-deprived rats (105).

There is no general induction of antioxidant defenses by H_2O_2 in mammalian cells, at least in cancer cell lines (110), but this needs to be investigated in heavy H_2O_2-producing cells such as thyrocytes and macrophages. Indeed, some of these protective systems are induced in stimulated thyroids: GSH reductases 1 and 3 and thioredoxin reductase 1 (104, 111). Another little considered potential defense is ascorbate, which is highly concentrated in the thyroid (112).

Thyroid Diseases Related to H_2O_2

H_2O_2 and TPO are necessary for the oxidation of iodide and for the synthesis of thyroid hormones. In their absence, the thyroid still takes up iodide but does not metabolize it, leading to absence of thyroid hormone synthesis, hypothyroidism, and consequent high TSH secretion and goiter, a classical congenital hypothyroidism category. The stimulated thyroid highly concentrates iodide, which remains in equilibrium with serum iodide. Administration of perchlorate, inhibiting the sodium/iodide transporter, induces an immediate release of this iodide from the gland (the perchlorate discharge test). These schemes allow us to explain the consequences of known enzyme defects and to predict the consequences of still to be defined defects (Table 2).

Inactivation of DUOX and TPO would have the same consequences on iodide organification and retention in the thyroid: a high iodide uptake and a positive perchlorate discharge test. On the other hand, although the DUOX defect would suppress H_2O_2 generation, a TPO defect might increase H_2O_2 generation by decreasing its inactivation and allowing an increased generation in response to TSH. The first defect would decrease, whereas the second would not reduce but on the contrary might increase the effects of H_2O_2. Both defects would lead to congenital hypothyroidism and goiter, but the TPO defect would more likely lead to severe thyroid disease and cancer. Presumably, inactivating mutations of DUOXA1 and/or -A2 would have the same consequences as those of DUOX. The majority of these iodination defects is due to inactivating mutations of TPO, but a few cases of DUOX2 inactivation have now been found which suggests that DUOX1 gene is not sufficient (113, 114). Interestingly, in view of the possible catalase effect of peroxidase described above, the congenital goiters originating from TPO defects, compared with those of other defects in thyroid metabolism, are characterized by their severity and their frequent evolution to nodularity and tumorigenesis (115).

The DUOX defect entails the lack of thyroid hormone synthesis with positive perchlorate discharge but, because H_2O_2 is not produced, is not expected to lead to such extreme goitrogenesis and tumors. On the other hand, given the role

TABLE 2. Thyroid H_2O_2 diseases

Disease	Enzyme defect	Model	Phenotype
Congenital hypothyroidism	Thyroperoxidase defect, loss of H_2O_2 inactivation (?)	Human	Goiter, hypothyroidism, carcinomas, ClO_4 discharge
Congenital hypothyroidism	DUOX2 defect, DUOXA defect (?) loss of H_2O_2 generation	Human	Goiter, hypothyroidism, ClO_4 discharge
Congenital hypothyroidism	TSH receptor defect with lack of Gq activation (?)	?	Goiter, hypothyroidism, ClO_4 discharge (?)
Tumor	α1R constitutive activation leading to activation of cAMP, *i.e.* cell proliferation; PIP2 Ca^{2+} cascade, *i.e.* H_2O_2 generation, apoptosis	Tgα1R mice	Rapid cell turnover, carcinomas
Thyroid tumors?	TSH receptor overactivation of Gq phospholipase C cascade (?)	?	Frequent thyroid nodules
Sporadic papillary carcinoma	Abnormal DNA repair response to H_2O_2 (?)	Human	Papillary carcinoma
Thyroiditis	Se deficiency and impaired H_2O_2 catabolism	Human	Thyroid necrosis and inflammation
Myxedematous endemic cretinism	I^- and Se deficiency, SCN in food, lack of H_2O_2 catabolism	Human, rat	Atrophy, hypothyroidism

A *question mark in parentheses* indicates a hypothesis.

of DUOX in oocytes and reproduction, inactivating mutations of the isozyme expressed in oocyte DUOX1 might tend to eliminate themselves. This and the possible redundancy of the DUOXs could explain why among thyroid organification defects TPO mutations are the most prevalent. In view of their necessary role in the transport from reticulum to membranes of the DUOX, defects in the chaperones DUOXA1 and DUOXA2 would necessarily have at least the same effects as defects in DUOXs.

Inactivating mutations of the TSH receptor in human lead to thyroid atrophy and hypothyroidism when they affect the cAMP pathway but would, as in the case of DUOX defects, cause organification defects, loss of iodine, positive perchlorate discharge, hypothyroidism, and compensatory hypertrophy and goiter if they affected only the phosphatidylinositol-4,5-bisphosphonate (PIP2)-Ca^{2+} cascade.

Overactivation of the cAMP branch by a mutated TSH receptor or Gsα leads to cell proliferation and decreased H_2O_2 generation in autonomous adenomas that very rarely evolve into carcinomas. Similarly, the thyroid-stimulating antibodies do not stimulate the Gq phospholipase C in human thyroid (116) and Graves' disease and, despite a marked thyroid stimulation, only rarely leads to thyroid carcinomas.

Overactivation of the PIP2-Ca^{2+} cascade by a mutated TSH receptor or Gq would lead, on the contrary, to greater H_2O_2 generation and its mutagenic and carcinogenic consequences in the presence of a normal cAMP trophic stimulus and to necrosis in its absence. An experimental model of the constitutive activation of both Gs-adenylate cyclase and Gq-phospholipase C cascades is provided by the Tgα1 adrenergic receptor transgenic mice. Contrary to the Tg adenosine A2 receptor mice, with only constitutive activation of the cAMP cascade, the Tgα1 adrenergic receptor mice indeed develop malignant nodules (79). It might be interesting in this regard to investigate the few hyperthyroidism cases due to excess of TSH that does activate both cascades. A follicular carcinoma has been observed in one such case (117).

We have repeatedly suggested that the important generation of H_2O_2 in thyroid cells might account for mutagenesis and the important generation of nodules in the thyroid (79, 118). This would also explain in part why more nodules are found in iodine-deficient areas. In the same framework, although sporadic and post-Chernobyl radioinduced carcinomas represent the same disease (118, 119), they are distinguishable, with molecular signatures reflecting specific responses to γ-radiation and H_2O_2 and with a signature of genes involved in homologous recombination that repairs DNA double-strand breaks. This suggests that, in a population, thyroid carcinoma would preferentially affect those patients with less effective specific repair mechanisms (Detours, V., submitted for publication) against H_2O_2 for sporadic carcinomas and against x-rays for post-Chernobyl cancers.

Excess H_2O_2 in thyroid is neutralized in the thyrocytes by second-line mechanisms, the most efficient ones being GSH peroxidases, peroxiredoxin, and other Se-containing enzymes. Se deficiency should weaken such defenses. Indeed, low levels of serum Se have been associated with thyroid cancer (120–122). NOX and H_2O_2 through its mutagenic effect have been implicated in carcinogenesis in other tissues (7).

Myxedematous endemic cretinism, caused by thyroid destruction after birth, has been linked to low iodine supply in early life, leading to intense stimulation and presumably H_2O_2 generation, to passage from low O_2 to high O_2 at birth, to selenium deficiency, and thus to decreases in GSH peroxidase and thioredoxin reductase activity and to dietary thiocyanate. The experimental reproduction of this scenario in newborn rats confirms the validity of these conclusions (105, 123).

Interestingly, a similar scenario has been proposed for the physiopathology of thyroiditis (120, 124). Selenium dietary supplementation has therefore been proposed for prevention and treatment of thyroiditis and has indeed alleviated it (124, 125).

Questions Pending

Although we now know the general outline of the role and generation of H_2O_2 in the thyroid many questions remain, among which:

- The role of H_2O_2 as a signal for proliferation is assumed on the basis of experiments in other cells but has still to be proved in thyroid.
- The relative concentration-effect relationships for the various H_2O_2 effects on the thyrocytes.
- The relative roles of DUOX1 and DUOX2 in the thyroid.
- The relative roles of vesicle DUOX mobilization to the apical membrane and of the activation of already present membrane DUOXs in the stimulation of H_2O_2 generation.
- The role of TPO-DUOX caveolin assembly in the apical membrane.
- The role of ion transport and pH at the apical membrane in H_2O_2 generation.
- The importance of the catalase-like action of TPO in the absence of iodide.
- The proportion of the generated H_2O_2 that leaks back in the cell and is reduced there by GSH peroxidase.
- The role of thyroid ascorbate in the reduction of H_2O_2.
- The presence of thyroid carcinoma in cases of TSH-hypersecreting adenoma.
- Defects in DUOXA and in TSH receptor-Gq interactions in congenital iodination defects.

Acknowledgments

Received March 22, 2007. Accepted July 24, 2007.

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X.D.D. is chargé de recherche Fonds National de la Recherche Scientifique (FNRS). The work reported in this article has been supported by the Action Concertée de la Communauté Française, the Pôle d'attraction interuniversitaire of the Ministère de la Recherche Scientifique, and the Fonds National de la Recherche Scientifique (FNRS and Fonds de la Recherche Scientifique Médical).

Disclosure Summary: The authors have nothing to disclose.

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