

# 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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> generation and disposal, thyroid hormone synthesis, and their control in the human thyroid is analyzed.

**Evidence Synthesis:** In humans, H<sub>2</sub>O<sub>2</sub> production by dual-oxidases and consequently thyroid hormone synthesis by thyroperoxidase are controlled by the phospholipase C-Ca<sup>2+</sup>-diacylglycerol arm of TSH receptor action. H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> are analyzed. They include the separation of the generating enzymes (dual-oxidases), their coupling to thyroperoxidase in a proposed complex, the thyroxosome, and H<sub>2</sub>O<sub>2</sub> degradation systems.

**Conclusions:** It is proposed that various pathologies can be explained, at least in part, by overproduction and lack of degradation of H<sub>2</sub>O<sub>2</sub> (tumorigenesis, myxedematous cretinism, and thyroiditis) and by failure of the H<sub>2</sub>O<sub>2</sub> generation or its positive control system (congenital hypothyroidism). (*J Clin Endocrinol Metab* 92: 3764–3773, 2007)

## General Effects of H<sub>2</sub>O<sub>2</sub>

### H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub>, the role of this molecule as an intracellular signal, at first controversial, has become widely accepted (4). In vertebrates, H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub>.

The cellular H<sub>2</sub>O<sub>2</sub>-generating systems belong to the family of reduced nicotinamide adenine dinucleotide phosphate oxidase (NOX) enzymes. These enzymes produce H<sub>2</sub>O<sub>2</sub> or the O<sub>2</sub> superoxide O<sub>2</sub><sup>-</sup>, which is rapidly converted to H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> is also gen-

erated 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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> acts as an intracellular and extracellular NO oxidizer and destroyer and as an extracellular paracrine vascular activator (23).

H<sub>2</sub>O<sub>2</sub> has several roles other than signaling in normal cell biology. It is produced as a toxic metabolite in host defense

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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.

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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  $\mu M$  to a maximum of 0.7  $\mu M$ . 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  $\mu M$   $H_2O_2$  disappear by 80% in 2 h (31).

Mutagenesis, if it leads to constitutive activation of a proto-oncogene 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 DUOX2 (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, iodoheptadecanal, 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

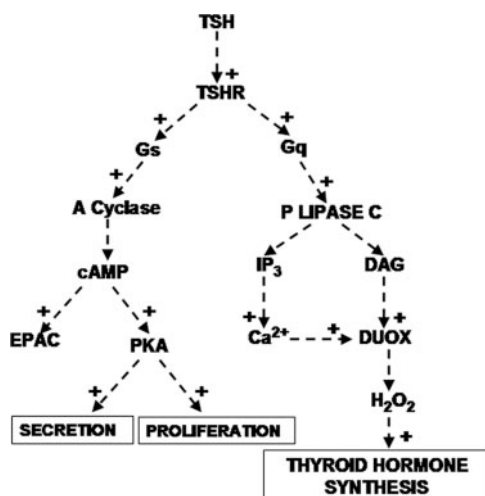


FIG. 1. Regulation of the human thyrocytes by TSH. The dashed arrows with the plus sign indicate positive direct control. DAG, Diacylglycerol; EPAC, exchange protein activated by cAMP; PKA, cAMP-dependent protein kinase; TSHR, TSH receptor.

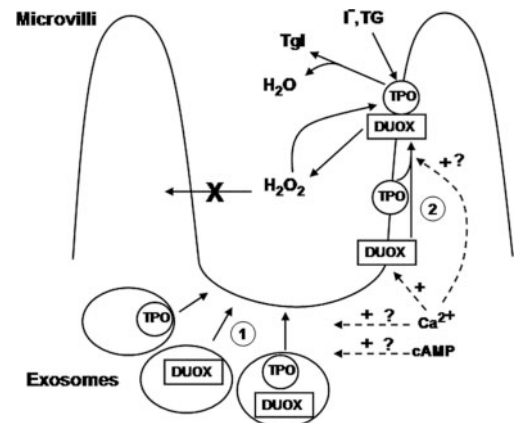


FIG. 2. Proposed steps in the activation of the postulated thyrocyte. 1) Translocation of inactive DUOX and/or TPO from near membrane intracellular granules (exosomes) to the membrane. 2) Association of DUOX and TPO. TG, Thyroglobulin; TgI, iodinate thyroglobulin.

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 PCCI3 rat thyroid cell lines produce around six, pig thyroid slices and thyroid cells in primary cultures around 10, and human leukocytes around 17 nmol  $H_2O_2/10\text{min}\cdot 10\ \mu\text{g DNA}$  (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 PCCI3 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  $\alpha_{1B}$  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

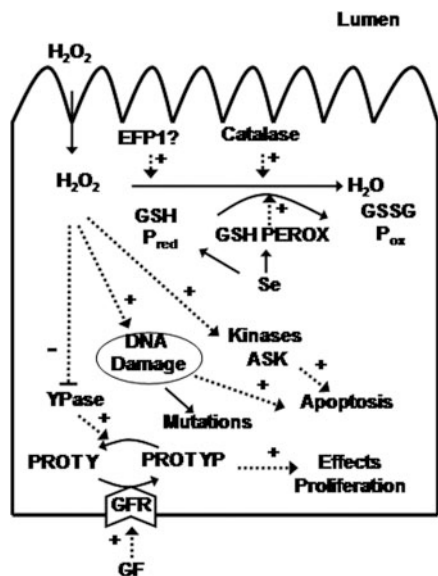


FIG. 3. Fate of H<sub>2</sub>O<sub>2</sub> leaking back in the thyroid cell: catabolism by GSH peroxidase (GSH PEROX), peroxiredoxin reduced or oxidized (P<sub>red</sub> or P<sub>ox</sub>), and catalase; proposed effects on protein tyrosine phosphatase (YPase) and kinases such as apoptosis signal kinase (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<sub>2</sub>O<sub>2</sub>-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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> 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<sup>-</sup>, oxidizes I<sup>-</sup> and in doing so, as other peroxidases such as myeloperoxidase (86), reduces H<sub>2</sub>O<sub>2</sub>. 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<sub>2</sub>O<sub>2</sub> (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<sub>2</sub>O<sub>2</sub>

Thyroid cell protection
1 Association of DUOX and TPO in membrane: the thyroxisome; H <sub>2</sub> O <sub>2</sub> produced is degraded <i>in loco</i> 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 <sub>2</sub> O <sub>2</sub> : raft-like composition
3 Localization of thyroxisomes mostly in microvillous membrane, <i>i.e.</i> at a distance from the body of the cell
4 Tight control of the activity of DUOX and H <sub>2</sub> O <sub>2</sub> 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 <sub>2</sub> O <sub>2</sub> 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 <sub>2</sub> O <sub>2</sub> generation?
9 Control of DUOX mRNA and protein expression: very loose in humans

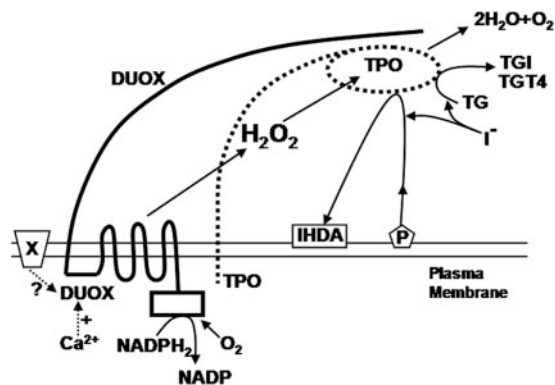


FIG. 4. The postulated thyroxisome. The producer-consumer unit is composed of the associated DUOX and TPO at the membrane. Generation of  $H_2O_2$  is in a restricted space where it is consumed by the oxidation of iodide and its binding to thyroglobulin (TG) and plasmalogen (P), generating iodotyrosines and iodothyronines in thyroglobulin (TGI and TGT4) and iodoheptadecanal (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<sub>2</sub>, 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 iodoheptadecanal, 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<sub>2</sub>O<sub>2</sub> generation by low concentration of iodide, *i.e.* by the cosubstrate of TPO, is another way to restrict H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub>, whereas catalase with its higher K<sub>m</sub> and V<sub>max</sub> allows the cell to metabolize even very large, toxic levels of H<sub>2</sub>O<sub>2</sub> (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<sub>2</sub>O<sub>2</sub>. 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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> in mammalian cells, at least in cancer cell lines (110), but this needs to be investigated in heavy H<sub>2</sub>O<sub>2</sub>-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<sub>2</sub>O<sub>2</sub>

H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> generation, a TPO defect might increase H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub>. 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 DUOX1 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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> diseases

Disease	Enzyme defect	Model	Phenotype
Congenital hypothyroidism	Thyropoxidase defect, loss of H <sub>2</sub> O <sub>2</sub> inactivation (?)	Human	Goiter, hypothyroidism, carcinomas, ClO <sub>4</sub> discharge
Congenital hypothyroidism	DUOX2 defect, DUOX1 defect (?) loss of H <sub>2</sub> O <sub>2</sub> generation	Human	Goiter, hypothyroidism, ClO <sub>4</sub> discharge
Congenital hypothyroidism	TSH receptor defect with lack of Gq activation (?)	?	Goiter, hypothyroidism, ClO <sub>4</sub> discharge (?)
Tumor	α1R constitutive activation leading to activation of cAMP, <i>i.e.</i> cell proliferation; PIP2 Ca <sup>2+</sup> cascade, <i>i.e.</i> H <sub>2</sub> O <sub>2</sub> 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 <sub>2</sub> O <sub>2</sub> (?)	Human	Papillary carcinoma
Thyroiditis	Se deficiency and impaired H <sub>2</sub> O <sub>2</sub> catabolism	Human	Thyroid necrosis and inflammation
Myxedematous endemic cretinism	I <sup>-</sup> and Se deficiency, SCN in food, lack of H <sub>2</sub> O <sub>2</sub> 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<sup>2+</sup> cascade.

Overactivation of the cAMP branch by a mutated TSH receptor or Gs $\alpha$  leads to cell proliferation and decreased H<sub>2</sub>O<sub>2</sub> 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<sup>2+</sup> cascade by a mutated TSH receptor or Gq would lead, on the contrary, to greater H<sub>2</sub>O<sub>2</sub> 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 Tg $\alpha$ 1 adrenergic receptor transgenic mice. Contrary to the Tg adenosine A2 receptor mice, with only constitutive activation of the cAMP cascade, the Tg $\alpha$ 1 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<sub>2</sub>O<sub>2</sub> 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  $\gamma$ -radiation and H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> for sporadic carcinomas and against x-rays for post-Chernobyl cancers.

Excess H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> through its mutagenic ef-

fect 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<sub>2</sub>O<sub>2</sub> generation, to passage from low O<sub>2</sub> to high O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> in the thyroid many questions remain, among which:

- The role of H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> generation.
- The importance of the catalase-like action of TPO in the absence of iodide.
- The proportion of the generated H<sub>2</sub>O<sub>2</sub> that leaks back in the cell and is reduced there by GSH peroxidase.
- The role of thyroid ascorbate in the reduction of H<sub>2</sub>O<sub>2</sub>.
- The presence of thyroid carcinoma in cases of TSH-hypersecreting adenoma.
- Defects in DUOXA and in TSH receptor-Gq interactions in congenital iodination defects.

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### References

1. May JM, de Haen C 1979 The insulin-like effect of hydrogen peroxide on pathways of lipid synthesis in rat adipocytes. *J Biol Chem* 254:9017–9021

2. Muchmore DB, Little SA, de Haen C 1982 Counterregulatory control of intracellular hydrogen peroxide production by insulin and lipolytic hormones in isolated rat epididymal fat cells: a role of free fatty acids. *Biochemistry* 21:3886–3892
3. Mukherjee SP, Lane RH, Lynn WS 1978 Endogenous hydrogen peroxide and peroxidative metabolism in adipocytes in response to insulin and sulfhydryl reagents. *Biochem Pharmacol* 27:2589–2594
4. Rhee SG, Kang SW, Jeong W, Chang TS, Yang KS, Woo HA 2005 Intracellular messenger function of hydrogen peroxide and its regulation by peroxiredoxins. *Curr Opin Cell Biol* 17:183–189
5. Bokoch GM, Knaus UG 2003. NADPH oxidases: not just for leukocytes anymore! *Trends Biochem Sci* 28:502–508
6. Geiszt M 2006 NADPH oxidases: new kids on the block. *Cardiovasc Res* 71:289–299
7. Quinn MT, Ammons MC, Deleo FR 2006 The expanding role of NADPH oxidases in health and disease: no longer just agents of death and destruction. *Clin Sci (Lond)* 111:1–20
8. Dumont JE, Christophe D, Vassart G, Roger P, Maenhaut C 2005 The phylogeny, ontogeny, anatomy and regulation of the iodine metabolizing thyroid. In: DeGroot LJ, ed. *Dartmouth, MA: Endocrine Education*
9. Stone JR 2004 An assessment of proposed mechanisms for sensing hydrogen peroxide in mammalian systems. *Arch Biochem Biophys* 422:119–124
10. Cho SH, Lee CH, Ahn Y, Kim H, Kim H, Ahn CY, Yang KS, Lee SR 2004 Redox regulation of PTEN and protein tyrosine phosphatases in H<sub>2</sub>O<sub>2</sub> mediated cell signaling. *FEBS Lett* 560:7–13
11. Kamata H, Honda S, Maeda S, Chang L, Hirata H, Karin M 2005 Reactive oxygen species promote TNF $\alpha$ -induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell* 120:649–661
12. Tonks NK 2003 PTP1B: from the sidelines to the front lines! *FEBS Lett* 546:140–148
13. Tonks NK 2005 Redox redux: revisiting PTPs and the control of cell signaling. *Cell* 121:667–670
14. Giannoni E, Buricchi F, Raugeri G, Ramponi G, Chiarugi P 2005 Intracellular reactive oxygen species activate Src tyrosine kinase during cell adhesion and anchorage-dependent cell growth. *Mol Cell Biol* 25:6391–6403
15. Markadieu N, Crutzen R, Blero D, Erneux C, Beauwens R 2005 Hydrogen peroxide and epidermal growth factor activate phosphatidylinositol 3-kinase and increase sodium transport in A6 cell monolayers. *Am J Physiol Renal Physiol* 288:F1201–F1212
16. Kwon J, Lee SR, Yang KS, Ahn Y, Kim YJ, Stadtman ER, Rhee SG 2004 Reversible oxidation and inactivation of the tumor suppressor PTEN in cells stimulated with peptide growth factors. *Proc Natl Acad Sci USA* 101:16419–16424
17. Leslie NR, Bennett D, Lindsay YE, Stewart H, Gray A, Downes CP 2003 Redox regulation of PI 3-kinase signalling via inactivation of PTEN. *EMBO J* 22:5501–5510
18. 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, Kang SW 2005 Regulation of PDGF signalling and vascular remodelling by peroxiredoxin II. *Nature* 435:347–353
19. Aggeli IK, Gaitanaki C, Beis I 2006 Involvement of JNKs and p38-MAPK/MSK1 pathways in H<sub>2</sub>O<sub>2</sub>-induced upregulation of heme oxygenase-1 mRNA in H9c2 cells. *Cell Signal* 18:1801–1812
20. Bubici C, Papa S, Dean K, Franzoso G 2006 Mutual cross-talk between reactive oxygen species and nuclear factor- $\kappa$ B: molecular basis and biological significance. *Oncogene* 25:6731–6748
21. Terada LS 2006 Specificity in reactive oxidant signaling: think globally, act locally. *J Cell Biol* 174:615–623
22. Fratelli M, Goodwin LO, Orom UA, Lombardi S, Tonelli R, Mengozzi M, Ghezzi P 2005 Gene expression profiling reveals a signaling role of glutathione in redox regulation. *Proc Natl Acad Sci USA* 102:13998–14003
23. Ardanaz N, Pagano PJ 2006 Hydrogen peroxide as a paracrine vascular mediator: regulation and signaling leading to dysfunction. *Exp Biol Med (Maywood)* 231:237–251
24. Bedard K, Krause KH 2007 The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev* 87:245–313
25. Fortemaion N, Miot F, Dumont JE, Dremier S 2005 Regulation of H<sub>2</sub>O<sub>2</sub> generation in thyroid cells does not involve Rac1 activation. *Eur J Endocrinol* 152:127–133
26. Stone JR, Yang S 2006 Hydrogen peroxide: a signaling messenger. *Antioxid Redox Signal* 8:243–270
27. Spletstoesser WD, Schuff-Werner P 2002 Oxidative stress in phagocytes: "the enemy within". *Microsc Res Tech* 57:441–455
28. Kiffin R, Christian C, Knecht E, Cuervo AM 2004 Activation of chaperone-mediated autophagy during oxidative stress. *Mol Biol Cell* 15:4829–4840
29. Cantoni O, Cattabeni F, Stocchi V, Meyn RE, Cerutti P, Murray D 1989 Hydrogen peroxide insult in cultured mammalian cells: relationships between DNA single-strand breakage, poly(ADP-ribose) metabolism and cell killing. *Biochim Biophys Acta* 1014:1–7
30. Beckman KB, Ames BN 1997 Oxidative decay of DNA. *J Biol Chem* 272:19633–19636
31. Chico G, V, Massart C, Jin L, Vanvooren V, Caillet-Fauquet P, Andry G, Lothaire P, Dequanter D, Friedman M, Van Sande J 2006 Acrylamide, an in vivo thyroid carcinogenic agent, induces DNA damage in rat thyroid cell lines and primary cultures. *Mol Cell Endocrinol* 257–258:6–14
32. Halliwell B 2007 Oxidative stress and cancer: have we moved forward? *Biochem J* 401:1–11
33. Bjorkhem-Bergman L, Torndal UB, Eken S, Nystrom C, Capitanio A, Larsen EH, Bjornstedt M, Eriksson LC 2005 Selenium prevents tumor development in a rat model for chemical carcinogenesis. *Carcinogenesis* 26:125–131
34. Lee DH, Esworthy RS, Chu C, Pfeifer GP, Chu FF 2006 Mutation accumulation in the intestine and colon of mice deficient in two intracellular glutathione peroxidases. *Cancer Res* 66:9845–9851
35. Neumann CA, Krause DS, Carman CV, Das S, Dubey DP, Abraham JL, Bronson RT, Fujiwara Y, Orkin SH, Van Etten RA 2003 Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression. *Nature* 424:561–565
36. Chu R, Lin Y, Reddy KC, Pan J, Rao MS, Reddy JK, Yeldandi AV 1996 Transformation of epithelial cells stably transfected with H<sub>2</sub>O<sub>2</sub>-generating peroxisomal urate oxidase. *Cancer Res* 56:4846–4852
37. Reinehr R, Becker S, Eberle A, Grether-Beck S, Haussinger D 2005 Involvement of NADPH oxidase isoforms and Src family kinases in CD95-dependent hepatocyte apoptosis. *J Biol Chem* 280:27179–27194
38. Song JJ, Rhee JG, Suntharalingam M, Walsh SA, Spitz DR, Lee YJ 2002 Role of glutaredoxin in metabolic oxidative stress. Glutaredoxin as a sensor of oxidative stress mediated by H<sub>2</sub>O<sub>2</sub>. *J Biol Chem* 277:46566–46575
39. Rancourt RC, Hayes DD, Chess PR, Keng PC, O'Reilly MA 2002 Growth arrest in G1 protects against oxygen-induced DNA damage and cell death. *J Cell Physiol* 193:26–36
40. Duan J, Duan J, Zhang Z, Tong T 2005 Irreversible cellular senescence induced by prolonged exposure to H<sub>2</sub>O<sub>2</sub> involves DNA-damage-and-repair genes and telomere shortening. *Int J Biochem Cell Biol* 37:1407–1420
41. Groemping Y, Rittinger K 2005 Activation and assembly of the NADPH oxidase: a structural perspective. *Biochem J* 386(Pt 3):401–416
42. Poljak A, Grant R, Austin CJ, Jamie JF, Willows RD, Takikawa O, Littlejohn TK, Truscott RJ, Walker MJ, Sachdev P, Smythe GA 2000 Inhibition of indoleamine 2,3 dioxygenase activity by H<sub>2</sub>O<sub>2</sub>. *Arch Biochem Biophys* 450:9–19
43. Zhang B, Hirahashi J, Cullere X, Mayadas TN 2003 Elucidation of molecular events leading to neutrophil apoptosis following phagocytosis: cross-talk between caspase 8, reactive oxygen species, and MAPK/ERK activation. *J Biol Chem* 278:28443–28454
44. Gorzalczyk Y, Sigal N, Itan M, Lotan O, Pick E 2000 Targeting of Rac1 to the phagocyte membrane is sufficient for the induction of NADPH oxidase assembly. *J Biol Chem* 275:40073–40081
45. De Deken X, Wang D, Dumont JE, Miot F 2002 Characterization of ThOX proteins as components of the thyroid H<sub>2</sub>O<sub>2</sub>-generating system. *Exp Cell Res* 273:187–196
46. Dupuy C, Virion A, Ohayon R, Kaniewski J, Deme D, Pommier J 1991 Mechanism of hydrogen peroxide formation catalyzed by NADPH oxidase in thyroid plasma membrane. *J Biol Chem* 266:3739–3743
47. Nunez J, Pommier J 1982 Formation of thyroid hormones. *Vitam Horm* 39:175–229
48. Corvilain B, Laurent E, Lecomte M, Van Sande J, Dumont JE 1994 Role of the cyclic adenosine 3',5'-monophosphate and the phosphatidylinositol-Ca<sup>2+</sup> cascades in mediating the effects of thyrotropin and iodide on hormone synthesis and secretion in human thyroid slices. *J Clin Endocrinol Metab* 79:152–159
49. Rousset B, Poncet C, Dumont JE, Mornex R 1980 Intracellular and extracellular sites of iodination in dispersed hog thyroid cells. *Biochem J* 192:801–812
50. Raspe E, Dumont JE 1994 Control of the dog thyrocyte plasma membrane iodide permeability by the Ca<sup>2+</sup>-phosphatidylinositol and adenosine 3',5'-monophosphate cascades. *Endocrinology* 135:986–995
51. El Hassani RA, Benfares N, Caillou B, Talbot M, Sabourin JC, Belotte V, Morand S, Gnidehou S, Agnandji D, Ohayon R, Kaniewski J, Noel-Hudson MS, Bidart JM, Schlumberger M, Virion A, Dupuy C 2005 Dual oxidase2 is expressed all along the digestive tract. *Am J Physiol Gastrointest Liver Physiol* 288:G933–G942
52. Schwarzer C, Machen TE, Illek B, Fischer H 2004 NADPH oxidase-dependent acid production in airway epithelial cells. *J Biol Chem* 279:36454–36461
53. Wong JL, Creton R, Wessel GM 2004 The oxidative burst at fertilization is dependent upon activation of the dual oxidase Udx1. *Dev Cell* 7:801–814
54. De Deken X, Wang D, Many MC, Costagliola S, Libert F, Vassart G, Dumont JE, Miot F 2000 Cloning of two human thyroid cDNAs encoding new members of the NADPH oxidase family. *J Biol Chem* 275:23227–23233
55. Dupuy C, Ohayon R, Valent A, Noel-Hudson MS, Deme D, Virion A 1999 Purification of a novel flavoprotein involved in the thyroid NADPH oxidase. Cloning of the porcine and human cDNAs. *J Biol Chem* 274:37265–37269
56. Cross AR, Segal AW 2004 The NADPH oxidase of professional phagocytes: prototype of the NOX electron transport chain systems. *Biochim Biophys Acta* 1657:1–22
57. Maturana A, Arnaudeau S, Ryser S, Banfi B, Hossle JP, Schlegel W, Krause KH, Demaurex N 2001 Heme histidine ligands within gp91(phox) modulate

- proton conduction by the phagocyte NADPH oxidase. *J Biol Chem* 276:30277–30284
58. **Grasberger H, Refetoff S** 2006 Identification of the maturation factor for dual oxidase. Evolution of an eukaryotic operon equivalent. *J Biol Chem* 281:18269–18272
  59. **Ris-Stalpers C** 2006 Physiology and pathophysiology of the DUOXes. *Antioxid Redox Signal* 8:1563–1572
  60. **Bjorkman U, Ekholm R** 1992 Hydrogen peroxide generation and its regulation in FRTL-5 and porcine thyroid cells. *Endocrinology* 130:393–399
  61. **Corvilain B, Van Sande J, Laurent E, Dumont JE** 1991 The H<sub>2</sub>O<sub>2</sub>-generating system modulates protein iodination and the activity of the pentose phosphate pathway in dog thyroid. *Endocrinology* 128:779–785
  62. **Panneels V, Van Sande J, Van den BH, Braekman JC, Dumont JE, Boeynaems JM** 1994 Inhibition of human thyroid adenylyl cyclase by 2-iodoaldehydes. *Mol Cell Endocrinol* 106:41–50
  63. **Panneels V, Van den BH, Jacoby C, Braekman JC, Van Sande J, Dumont JE, Boeynaems JM** 1994 Inhibition of H<sub>2</sub>O<sub>2</sub> production by iodoaldehydes in cultured dog thyroid cells. *Mol Cell Endocrinol* 102:167–176
  64. **Corvilain B, Van Sande J, Dumont JE** 1988 Inhibition by iodide of iodide binding to proteins: the “Wolff-Chaikoff” effect is caused by inhibition of H<sub>2</sub>O<sub>2</sub> generation. *Biochem Biophys Res Commun* 154:1287–1292
  65. **Gerard CM, Lefort A, Christophe D, Libert F, Van Sande J, Dumont JE, Vassart G** 1989 Control of thyroperoxidase and thyroglobulin transcription by cAMP: evidence for distinct regulatory mechanisms. *Mol Endocrinol* 3:2110–2118
  66. **Uyttersprot N, Pelgrims N, Carrasco N, Gervy C, Maenhaut C, Dumont JE, Miot F** 1997 Moderate doses of iodide in vivo inhibit cell proliferation and the expression of thyroperoxidase and Na<sup>+</sup>/I<sup>-</sup> symporter mRNAs in dog thyroid. *Mol Cell Endocrinol* 131:195–203
  67. **Ekholm R** 1981 Iodination of thyroglobulin. An intracellular or extracellular process? *Mol Cell Endocrinol* 24:141–163
  68. **Tice LW, Wollman SH** 1974 Ultrastructural localization of peroxidase on pseudopods and other structures of the typical thyroid epithelial cell. *Endocrinology* 94:1555–1567
  69. **Ofverholm T, Ericson LE** 1984 Intraluminal iodination of thyroglobulin. *Endocrinology* 114:827–835
  70. **Strum JM, Karnovsky MJ** 1970 Cytochemical localization of endogenous peroxidase in thyroid follicular cells. *J Cell Biol* 44:655–666
  71. **Mizukami Y, Matsubara F, Matsukawa S** 1985 Cytochemical localization of peroxidase and hydrogen-peroxide-producing NAD(P)H-oxidase in thyroid follicular cells of propylthiouracil-treated rats. *Histochemistry* 82:263–268
  72. **Fredriksson G, Ofverholm TELE** 1985 Electron-microscopic studies of iodine-binding and peroxidase activity in the endostyle of the larva amphioxus. *Cell Tissue Res* 241:257–266
  73. **Corvilain B, Collyn L, Van Sande J, Dumont JE** 2000 Stimulation by iodide of H<sub>2</sub>O<sub>2</sub> generation in thyroid slices from several species. *Am J Physiol Endocrinol Metab* 278:E692–E699
  74. **Ruch W, Cooper PH, Baggiolini M** 1983 Assay of H<sub>2</sub>O<sub>2</sub> production by macrophages and neutrophils with homovanillic acid and horse-radish peroxidase. *J Immunol Methods* 63:347–357
  75. **Coclet J, Foureau F, Ketelbant P, Galand P, Dumont JE** 1989 Cell population kinetics in dog and human adult thyroid. *Clin Endocrinol (Oxf)* 31:655–665
  76. **Saad AG, Kumar S, Ron E, Lubin JH, Stanek J, Bove KE, Nikiforov YE** 2006 Proliferative activity of human thyroid cells in various age groups and its correlation with the risk of thyroid cancer after radiation exposure. *J Clin Endocrinol Metab* 91:2672–2677
  77. **Rabinowitz JL, Tavares CJ** 1977 Iodinated phospholipids and the in vitro iodination of proteins of dog thyroid gland. *Biochem J* 168:155–160
  78. **Mondello C, Guasconi V, Giulotto E, Nuzzo F** 2002 Gamma-ray and hydrogen peroxide induction of gene amplification in hamster cells deficient in DNA double strand break repair. *DNA Repair (Amst)* 1:483–493
  79. **Ledent C, Denef JF, Cottecchia S, Lefkowitz R, Dumont J, Vassart G, Parmentier M** 1997 Costimulation of adenylyl cyclase and phospholipase C by a mutant  $\alpha$ 1B-adrenergic receptor transgene promotes malignant transformation of thyroid follicular cells. *Endocrinology* 138:369–378
  80. **Demelash A, Karlsson JO, Nilsson M, Bjorkman U** 2004 Selenium has a protective role in caspase-3-dependent apoptosis induced by H<sub>2</sub>O<sub>2</sub> in primary cultured pig thyrocytes. *Eur J Endocrinol* 150:841–849
  81. **Riou C, Remy C, Rabilloud R, Rousset B, Fonlupt P** 1998 H<sub>2</sub>O<sub>2</sub> induces apoptosis of pig thyrocytes in culture. *J Endocrinol* 156:315–322
  82. **Riou C, Tonoli H, Bernier-Valentin F, Rabilloud R, Fonlupt P, Rousset B** 1999 Susceptibility of differentiated thyrocytes in primary culture to undergo apoptosis after exposure to hydrogen peroxide: relation with the level of expression of apoptosis regulatory proteins, Bcl-2 and Bax. *Endocrinology* 140:1990–1997
  83. **Maier J, van Steeg H, van Oostrom C, Karger S, Paschke R, Krohn K** 2006 Deoxyribonucleic acid damage and spontaneous mutagenesis in the thyroid gland of rats and mice. *Endocrinology* 147:3391–3397
  84. **Dumont JE** 1971 The action of thyrotropin on thyroid metabolism. *Vitam Horm* 29:287–412
  85. **Wang D, De Deken X, Milenkovic M, Song Y, Pirson I, Dumont JE, Miot F** 2005 Identification of a novel partner of duox: EFP1, a thioredoxin-related protein. *J Biol Chem* 280:3096–3103
  86. **Arnhold J, Furtmuller PG, Regelsberger G, Obinger C** 2001 Redox properties of the couple compound I/native enzyme of myeloperoxidase and eosinophil peroxidase. *Eur J Biochem* 268:5142–5148
  87. **Ehrenshaft M, Mason RP** 2006 Protein radical formation on thyroid peroxidase during turnover as detected by immuno-spin trapping. *Free Radic Biol Med* 41:422–430
  88. **Mathy-Hartert M, Bourgeois E, Grulke S, Deby-Dupont G, Caudron I, Deby C, Lamy M, Serteyn D** 1998 Purification of myeloperoxidase from equine polymorphonuclear leucocytes. *Can J Vet Res* 62:127–132
  89. **Taugog A, Dorris ML** 1992 Myeloperoxidase-catalyzed iodination and coupling. *Arch Biochem Biophys* 296:239–246
  90. **Magnusson RP, Taugog A, Dorris ML** 1984 Mechanisms of thyroid peroxidase- and lactoperoxidase-catalyzed reactions involving iodide. *J Biol Chem* 259:13783–13790
  91. **Deleu S, Allory Y, Radulescu A, Pirson I, Carrasco N, Corvilain B, Salmon I, Franc B, Dumont JE, Van Sande J, Maenhaut C** 2000 Characterization of autonomous thyroid adenoma: metabolism, gene expression, and pathology. *Thyroid* 10:131–140
  92. **Milenkovic M, De Deken X, Jin L, De Felice M, Di Lauro R, Dumont JE, Corvilain B, Miot F** 2007 Duox expression and related H<sub>2</sub>O<sub>2</sub> measurement in mouse thyroid: onset in embryonic development and regulation by TSH in adult. *J Endocrinol* 192:615–626
  93. **Ohayon R, Boeynaems JM, Braekman JC, Van den BH, Gorin Y, Virion A** 1994 Inhibition of thyroid NADPH-oxidase by 2-iodohexadecanal in a cell-free system. *Mol Cell Endocrinol* 99:133–141
  94. **Ohtaki S, Nakagawa H, Nakamura S, Nakamura M, Yamazaki I** 1985 Characterization of hog thyroid peroxidase. *J Biol Chem* 260:441–448
  95. **Wildberger E, Kohler H, Jenzer H, Kampf J, Studer H** 1986 Inactivation of peroxidase and glucose oxidase by H<sub>2</sub>O<sub>2</sub> and iodide during in vitro thyroglobulin iodination. *Mol Cell Endocrinol* 46:149–154
  96. **Zimmer KP, Scheumann GF, Bramswig J, Bocker W, Harms E, Schmid KW** 1997 Ultrastructural localization of IgG and TPO in autoimmune thyrocytes referring to the transcytosis of IgG and the antigen presentation of TPO. *Histochem Cell Biol* 107:115–120
  97. **Branco MR, Marinho HS, Cyrne L, Antunes F** 2004 Decrease of H<sub>2</sub>O<sub>2</sub> plasma membrane permeability during adaptation to H<sub>2</sub>O<sub>2</sub> in *Saccharomyces cerevisiae*. *J Biol Chem* 279:6501–6506
  98. **Danielsen EM, Hansen GH** 2003 Lipid rafts in epithelial brush borders: atypical membrane microdomains with specialized functions. *Biochim Biophys Acta* 1617:1–9
  99. **Danielsen EM, Hansen GH** 2006 Lipid raft organization and function in brush borders of epithelial cells. *Mol Membr Biol* 23:71–79
  100. **Bienert GP, Moller AL, Kristiansen KA, Schulz A, Moller IM, Schjoerring JK, Jahn TP** 2007 Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. *J Biol Chem* 282:1183–1192
  101. **Ekholm R, Bjorkman U** 1997 Glutathione peroxidase degrades intracellular hydrogen peroxide and thereby inhibits intracellular protein iodination in thyroid epithelium. *Endocrinology* 138:2871–2878
  102. **Bjorkman U, Ekholm R, Ericson LE, Ofverholm T** 1976 Transport of thyroglobulin and peroxidase in the thyroid follicle cell. *Mol Cell Endocrinol* 5:3–17
  103. **Ekholm R, Bjorkman U** 1984 Localization of iodine binding in the thyroid gland *in vitro*. *Endocrinology* 115:1558–1567
  104. **Beckett GJ, Arthur JR** 2005 Selenium and endocrine systems. *J Endocrinol* 184:455–465
  105. **Kohrle J, Jakob F, Contempre B, Dumont JE** 2005 Selenium, the thyroid, and the endocrine system. *Endocr Rev* 26:944–984
  106. **Bjorkman U, Ekholm R** 1995 Hydrogen peroxide degradation and glutathione peroxidase activity in cultures of thyroid cells. *Mol Cell Endocrinol* 111:99–107
  107. **Hashida K, Sakakura Y, Makino N** 2002 Kinetic studies on the hydrogen peroxide elimination by cultured PC12 cells: rate limitation by glucose-6-phosphate dehydrogenase. *Biochim Biophys Acta* 1572:85–90
  108. **Bozonet SM, Findlay VJ, Day AM, Cameron J, Veal EA, Morgan BA** 2005 Oxidation of a eukaryotic 2-Cys peroxiredoxin is a molecular switch controlling the transcriptional response to increasing levels of hydrogen peroxide. *J Biol Chem* 280:23319–23327
  109. **Kim HS, Manevich Y, Feinstein SI, Pak JH, Ho YS, Fisher AB** 2003 Induction of 1-cys peroxiredoxin expression by oxidative stress in lung epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 285:L363–L369
  110. **Desaint S, Luriau S, Aude JC, Rousset G, Toledano MB** 2004 Mammalian antioxidant defenses are not inducible by H<sub>2</sub>O<sub>2</sub>. *J Biol Chem* 279:31157–31163
  111. **Howie AF, Arthur JR, Nicol F, Walker SW, Beech SG, Beckett GJ** 1998 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
  112. **Suzuki M, Nagashima M, Yamamoto K** 1961 Studies on the mechanism of iodination by the thyroid gland: iodide-activating enzyme and intracellular inhibitor of iodination. *Gen Comp Endocrinol* 1:103–116

113. **Moreno JC, Bikker H, Kempers MJ, van Trotsenburg AS, Baas F, de Vijlder JJ, Vulsma T, Ris-Stalpers C** 2002 Inactivating mutations in the gene for thyroid oxidase 2 (THOX2) and congenital hypothyroidism. *N Engl J Med* 347:95–102
114. **Vigone MC, Fugazzola L, Zamproni I, Passoni A, Di Candia S, Chiumello G, Persani L, Weber G** 2005 Persistent mild hypothyroidism associated with novel sequence variants of the DUOX2 gene in two siblings. *Hum Mutat* 26:395
115. **Knobel M, Medeiros-Neto G** 2003 An outline of inherited disorders of the thyroid hormone generating system. *Thyroid* 13:771–801
116. **Laurent E, Van Sande J, Ludgate M, Corvilain B, Rocmans P, Dumont JE, Mockel J** 1991 Unlike thyrotropin, thyroid-stimulating antibodies do not activate phospholipase C in human thyroid slices. *J Clin Invest* 87:1634–1642
117. **Calle-Pascual AL, Yuste E, Martin P, Aramendi T, Garcia-Maurino ML, Argente J, Catalan MJ, Uriá J, Cabranes JA, Charro AL** 1991 Association of a thyrotropin-secreting pituitary adenoma and a thyroid follicular carcinoma. *J Endocrinol Invest* 14:499–502
118. **Duprez L, Hermans J, Van Sande J, Dumont JE, Vassart G, Parma J** 1997 Two autonomous nodules of a patient with multinodular goiter harbor different activating mutations of the thyrotropin receptor gene. *J Clin Endocrinol Metab* 82:306–308
119. **Detours V, Wattel S, Venet D, Hutsebaut N, Bogdanova T, Tronko MD, Dumont JE, Franc B, Thomas G, Maenhaut C** 2005 Absence of a specific radiation signature in post-Chernobyl thyroid cancers. *Br J Cancer* 92:1545–1552
120. **Duntas LH** 2006 The role of selenium in thyroid autoimmunity and cancer. *Thyroid* 16:455–460
121. **Glattre E, Thomassen Y, Thoresen SO, Haldorsen T, Lund-Larsen PG, Theodorsen L, Aaseth J** 1989 Prediagnostic serum selenium in a case-control study of thyroid cancer. *Int J Epidemiol* 18:45–49
122. **Jellum E, Andersen A, Lund-Larsen P, Theodorsen L, Orjasaeter H** 1993 The JANUS serum bank. *Sci Total Environ* 139–140:527–535
123. **Contempre B, de Escobar GM, Deneff JF, Dumont JE, Many MC** 2004 Thiocyanate induces cell necrosis and fibrosis in selenium- and iodine-deficient rat thyroids: a potential experimental model for myxedematous endemic cretinism in central Africa. *Endocrinology* 145:994–1002
124. **Gartner R, Gasnier BC, Dietrich JW, Krebs B, Angstwurm MW** 2002 Selenium supplementation in patients with autoimmune thyroiditis decreases thyroid peroxidase antibodies concentrations. *J Clin Endocrinol Metab* 87:1687–1691
125. **Duntas LH, Mantzou E, Koutras DA** 2003 Effects of a six month treatment with selenomethionine in patients with autoimmune thyroiditis. *Eur J Endocrinol* 148:389–393

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