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Forum Review

Physiology and Pathophysiology of the DUOXes

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ABSTRACT

The dual oxidases (DUOXes) 1 and 2 are named based on their having both a domain homologous to the NADPH-oxidase of the phagocyte NADPH-oxidase gp91*phox*/NOX2 and a domain homologous to thyroid peroxidase. The DUOX1 and DUOX2 mRNAs were originally cloned from thyroid tissue, and the corresponding proteins were recognized as intricate components of the thyroid hormone synthesis process, providing hydrogen peroxide essential for the organification of iodide. The function of DUOX2 in thyroid hormonogenesis has been firmly established by linking the congenital hypothyroid phenotype "total iodide organification defect" to biallelic inactivating DUOX2 mutations. Based on the expression of both DUOXes in combination with a peroxidase in a range of different tissues and functional studies; the concept evolves that DUOX is important not only for thyroid hormonogenesis but also as an integral part of the host defense system of mucosal surfaces, participates in the control of epithelial infection, augments surface B-cell receptor signaling in lymphocytes, and is involved in generating a respiratory burst at fertilization. *Antioxid. Redox Signal.* 8, 1563–1572.

THE SYNTHESIS OF HYDROGEN PEROXIDE as a prerequisite for thyroid hormone synthesis on the apical membrane of thyroid follicular cells has been well documented for decades (7, 54).

After reports of partial purification and characterization of the thyroid hydrogen peroxide generation system (5, 13, 40, 64), the cloning of the full-length thyroidal oxidases (11) allowed their recognition as novel homologs of the phagocyte NADPH-oxidase gp91*phox*/NOX2, with an additional N-terminal peroxidase-homology domain. Because of their dual nature, having both an NADPH-oxidase and a peroxidase domain, they are referred to as dual oxidases (DUOXes) (33).

Of the two DUOXes currently cloned from thyroid tissue (11), only the functionality of DUOX2 in thyroid hormonogenesis has been established without doubt (38).

In addition to thyroid, DUOX expression is reported for a variety of other tissues. In general, the expression of DUOX in mucosal surfaces is seen as an integral part of the host defense system (23). DUOX1 is constitutively expressed in airway epithelia and the respiratory tract (52), whereas DUOX2 expression can be induced in response to pathologic changes (21, 26, 51). DUOX2 is expressed in bowel (2) and salivary gland (23).

THYROID PHYSIOLOGY

Thyroid hormone (thyroxine, tetraiodothyronine, T_4) in general stimulates growth, metabolism, and differentiation but is particularly important for optimal prenatal and early postnatal development of the central nervous system. T_4 is considered a prohormone and requires metabolization by iodothyronine deiodinases (3) to the active thyroid hormone metabolite T_3, which exerts its biologic function through the nuclear thyroid hormone receptors (35, 42). Thyroid hormone is exclusively synthesized in the thyroid gland (Fig. 1). Biochemically thyroid hormone consists of two iodinated tyrosine amino acid residues, and its synthesis requires a series of tuned reactions in which a large number of proteins and factors are involved (Fig. 2). In general, thyroid hormone synthesis is stimulated by the pituitary-derived hormone thyrotropin (TSH). TSH stimulates the thyrocyte through the G-protein–coupled TSH receptor (TSH-R) (61) (Fig. 2[1]).

The rare element iodine is ingested via the food, absorbed through the small intestine, transported in plasma to the thyroid, where it is imported across the basal membrane of the thyrocyte against an electrochemical gradient by the sodium–iodide symporter (NIS) (9) (Fig. 2[2]). The efflux of iodide over the

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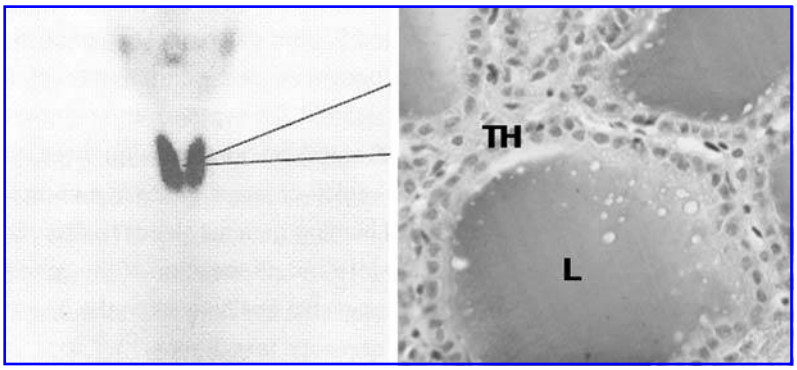


FIG. 1. The thyroid gland visualized by technetium uptake (left) and a microscopic section showing thyroid follicles with thyrocytes (TH) encircling the lumen (L) (right).

apical membrane is currently attributed to pendrin, which transports iodide and chloride in a Na^+-independent fashion (16, 69) (Fig. 2[3]).

Thyroid hormone is formed in an intricate process involving thyroidperoxidase (TPO) (28), a hydrogen peroxide (H_2O_2)-generating system and a polypeptide backbone for thyroid hormone synthesis and storage: thyroglobulin (TG). H_2O_2 is the final electron acceptor essential for the oxidization of iodide, and oxidized iodide is rapidly bound to tyrosyl residues TG (57) (Fig. 2[4]). This process takes place in the follicular lumen at the apical border of the thyrocyte. Subsequently, the iodotyrosine residues couple to form iodothyronines, mainly T_4 (Fig. 2[5]), and thyroid hormones can be liberated from thyroglobulin and released to the circulation, where they are transported to their target tissues.

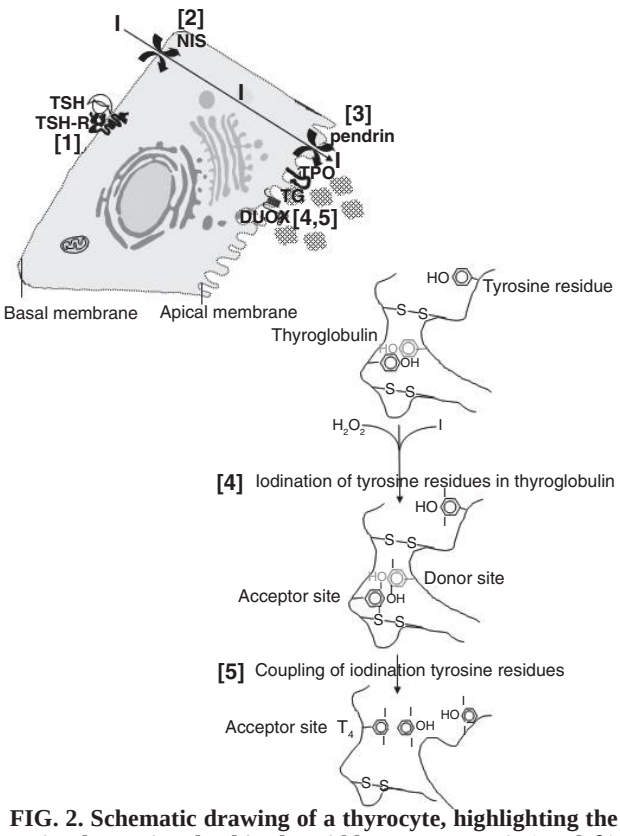


FIG. 2. Schematic drawing of a thyrocyte, highlighting the main players involved in thyroid hormonogenesis (top left) and schematic representation of iodination and coupling to T_4 within thyroglobulin that takes place at the apical border in the thyroid follicle (bottom right). Numbers in brackets refer to the text. (Adapted from van de Graaf SAR, Ris-Stalpers C, Pauws E, Mendive FM, Targovnik HM, de Vijlder JJM. Up to date with human thyroglobulin. *J Endocrinol* 170: 307–321, 2001, with permission.)

Both iodination and coupling are attributed to TPO and require H_2O_2 at the apical border of the thyrocyte. H_2O_2 is the rate-limiting step in this iodide oxidation essential to thyroid hormone synthesis (7). Although the presence of a H_2O_2 generation system in thyroid has been generally accepted for decades, the molecular cloning of the enzyme is of recent date.

THE CLONING OF DUOX1 AND DUOX2

Microsequencing of a flavoprotein isolated from pig thyroid plasma membrane resulted in the synthesis of primers suitable for 5′ and 3′ rapid amplification of cDNA ends and the cloning of pig and human $p138^{tox}$ (13). The human variant encoded a protein of 1,210 amino acids, which later proved to be a partial DUOX2 clone. Seven months later, De Deken *et al*. (11) reported the full-length DUOX2/THOX2 sequence, encoding a 1,548-amino acid protein, after low-stringency screening of a thyroid cDNA library with a probe specific for $gp91^{phox}$. $Gp91^{phox}$ equals NOX2 and is the catalytic core of the phagocytic oxidase that is activated when phagocytes ingest bacteria. Additionally they identified a second transcript encoding a 1,551-amino acid protein (THOX1 or DUOX1). Both glycoproteins are highly homologous, include seven transmembrane α-helices, have a 500-amino acid amino terminus that shows 43% homology with thyroid peroxidase, and contain two EF-hand motifs, an FAD-binding domain, and four NADPH-binding sites. Immunostaining localizes DUOX in the apical poles of thyroid cells, where it colocalizes with TPO. Western blot analysis of normal human thyroid membranes reveals DUOX proteins with molecular masses of 180 and 190 kDa that correspond to two different *N*-glycosylation states (10). In total, at least 10 to 20 kDa of the protein species observed by Western blot analysis is due to *N*-glycosylation (6).

The DUOX1 and DUOX2 genes are closely linked on chromosome 15q15.3. Initially the genes were reported to be ap-

proximately 3 kb apart (39). More recently, the distance has been mapped to 16 kb (43). Current sequences available through NCBIs Entrez Nucleotide NT_010194 show that both genes are indeed 16 kb apart, separated by the gene encoding the homolog of *Drosophila* Numb-interacting protein (NIP). The DUOX1 and 2 genes have opposite transcriptional orientations. The DUOX1 gene is more telomeric, spans 36 kb, and is composed of 35 exons, of which the first two are noncoding. TheDUOX2 gene spans 21.5 kb and contains 34 exons (of which the first is noncoding) (39). Exons 4, 5, and 6 of DUOX2 as well as the intervening sequences are more than 99% similar to DUOX1. Although initially cloned from thyroid tissue, cDNA DUOX clones from 20 human tissues are present in the publicly available databases (Table 1).

TISSUE-SPECIFIC DUOX EXPRESSION

A combination of Northern blot analysis, *in situ* hybridization, reverse transcription–polymerase chain reaction (RT-PCR), Western blot, and immunohistochemical analysis showed that DUOX expression and H_2O_2 generation are not restricted to thyroid.

The choice of the tissues studied is largely dependent on the specific interest of the research group in question. To objectively evaluate DUOX1 and DUOX2 tissue-specific gene expression, SAGE and EST libraries were used (data have been extracted from http://cgap.nci.nih.gov/SAGE) (Table 1, Fig. 3). In these cases, the reported tissue-specific expression is not steered by specific research interests but rather by overall interest in the organ, reflected by the size of the EST and SAGE libraries. SAGE (62) provides a complete and quantitative transcription profile of the tissue studied. Because SAGE libraries from many tissues are publicly available from the Internet, these data can be used to compare tissue-specific gene expression, just like the comparison of tissue-specific EST expression levels. Overall, the expression of both DUOXes observed in EST and SAGE libraries largely overlaps with the expression documented by cDNA libraries, apart from the presence of DUOX2 ESTs in stem cell and DUOX1 SAGE tags in vascular tissue.

TABLE 1. DUOX EXPRESSION DOCUMENTED BY PRESENCE IN TISSUE-SPECIFIC cDNA, EST, OR SAGE LIBRARIES

	DUOX1			*DUOX2*		
	cDNA	*EST*	*SAGE*	*cDNA*	*EST*	*SAGE*
Brain	+	+	+	+	+	
Cerebellum	+	+				
Cervix				+		
Colon			+	+	+	+
Gastrointestinal tract	+			+		+
Genitourinary	+			+		
Head and neck	+	+		+		
Lung	+	+	+	+	+	
Mammary gland	+					+
Muscle	+	+		+	+	+
Nervous	+			+		
Pancreas				+		+
Pancreatic islet				+	+	
Placenta	+	+				
Prostate	+	+	+	+	+	+
Skin	+	+	+			
Stem cell					+	
Stomach	+					+
Testis	+	+				
Thyroid	+	+	+	+		+
Uterus	+			+		
Vascular			+			

Data extracted from NCBIs CGAP (http://cgap.nci.nih.gov/SAGE).

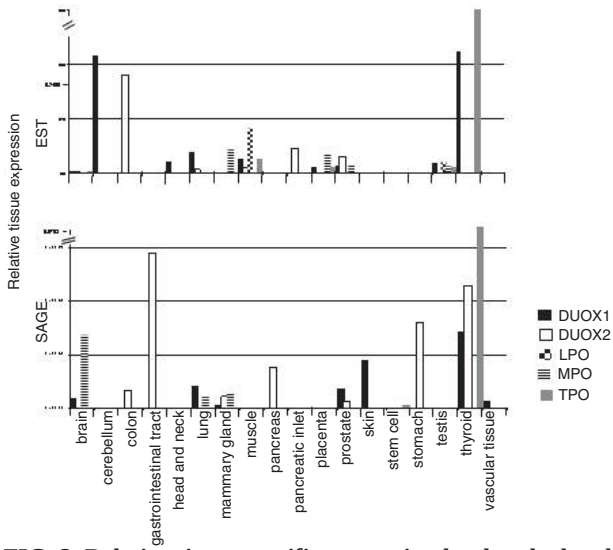


FIG. 3. Relative tissue-specific expression levels calculated from EST libraries (top) and SAGE libraries (bottom), available through NCBIs GCAP, of DUOX1, DUOX2, LPO, MPO, and TPO.

In *thyroid tissue*, DUOX2 mRNA is a factor 1.5 to 5 times more abundant than DUOX1 mRNA (43). Although this also is visible when comparing SAGE expression levels, it is not supported by the respective expression levels in EST libraries, probably because of the relative small available thyroid EST library. Compared with older patients, younger children in general have higher DUOX expression contained in smaller follicles (18). Apart from being expressed in normal thyroid tissue, both DUOXes also are expressed in Graves thyroid tissue, toxic adenoma, multinodular goiter, and hypofunctioning adenoma in the same order of magnitude (6). Although at variable levels, DUOX is also expressed in thyroid carcinomas (31) where, in contrast to normal thyroid, it shows a cytoplasmic localization. In papillary thyroid carcinoma, expression is increased (24). The expression of DUOX proteins does not appear to correlate with progression of the disease (31).

Both DUOXes are expressed in *airway epithelial cells* (21, 51), although the mucosal surface of the trachea and the bronchi, large airways, preferentially express DUOX1 (23, 52). This is reflected by the higher expression of DUOX1 ESTs and SAGE tags compared with those of DUOX2.

DUOX2 is widely expressed in the *intestinal tract*, where it is mainly located at the apical membrane of enterocytes and in the brush border of the cecum and sigmoidal colon (2). DUOX2 is also abundantly expressed in colon, stomach, and the gastrointestinal tract EST and SAGE libraries, whereas DUOX1 is currently not present. *Salivary gland* and *rectum* also express DUOX2 (23).

Additionally, DUOX2 SAGE tags have been reported for *pancreas*, and substantial DUOX1 expression is present in SAGE/EST libraries from *cerebellum* and *skin*.

THE REGULATION OF DUOX EXPRESSION

Thyroid metabolism is under close surveillance of the hypothalamus and pituitary gland, and expression of most genes involved in thyroid hormonogenesis is controlled by cyclic adenosine monophosphate (cAMP) and phospholipase C cascades through TSH-receptor signaling.

The promoters of both DUOX genes are dissimilar and differ from the promoters of other known thyroid-specific genes. The human DUOX1 promoter is GC-rich and has three putative SP1-binding elements. The human DUOX2 promoter lacks a TATA box and does not contain any SP1-binding elements (43). Although the structural differences suggest that the DUOXes are differentially regulated, this has not been observed for thyroid. Normal thyroid tissue, as well as thyroid adenoma and thyroid carcinoma, express DUOX1 and DUOX2 genes in parallel, indicating that in thyroid, both are controlled by similar mechanisms (6, 31).

In FRTL-5 cells, insulin increases DUOX2 mRNA, an effect antagonized by cAMP at low serum concentrations (36). Furthermore, in hyperstimulated rat thyroid gland, the DUOX2 mRNA expression was lower than in normal tissue, whereas the expression of other thyroid cAMP-regulated genes was markedly increased (12).

Although in dog thyroid, expression of the DUOXes is activated through the cAMP pathway, this is less pronounced in human thyroid (11), and quite unlike most thyroid-specific genes, the activity of the DUOX promoters is not stimulated by cAMP. Although rather unexpected, the lack of cAMP-induced DUOX mRNA increase actually prevents thyroid damage. If DUOX expression were under positive cAMP control, H_2O_2 generation would be increased in all cases of primary hypothyroidism in which TSH is increased. In these cases, the cytotoxic and mutagenic effects of H_2O_2 would evoke damage in critical biomolecules such as DNA and lipids within the thyroid gland, in effect worsening integrity of the gland are reducing the chances of ever achieving euthyroidism. In contrast to neutrophils, in which intracellular H_2O_2 levels reach toxic levels aimed at cell destruction that eradicates the pathogens, which is followed by replenishing of neutrophils by the hematopoietic stem cell, the whole system in the thyroid should be tuned to reaching strictly localized adequate levels favoring thyroid hormone synthesis while preventing damage through excessive and uncontrolled H_2O_2 levels.

The retaining of immature DUOX protein in the Golgi and endoplasmic reticulum (ER) (10), as a stock of nearly completely processed proteins that can be rapidly delivered to the membrane when its full activity is required for thyroid hormone synthesis, might be another measure to limit expression of the active protein at the cell surface, regulating the generation of deleterious oxidative agents essential for thyroid hormone synthesis (10).

Both DUOX1 and DUOX2 promoter constructs display marked transcriptional activity in nonthyroidal cell lines in accordance with their documented expression in more than 20 human tissues (43).

In the respiratory tract, concomitant with H_2O_2 production, DUOX1 gene expression is modestly induced by the Th2-specific cytokines interleukin (IL)-4 and IL-13, whereas DUOX2 is markedly induced by the Th-1–specific cytokine interferon (IFN)-ɣ (26).

THE PHYSIOLOGICAL ASPECTS OF DUOX

Classically reactive oxygen species (ROS; superoxide, hydrogen peroxide, and the hydroxyl radical) have been considered accidental by-products of respiration with cytotoxic and deleterious effects on biologic systems. They are held responsible for aging, somatic mutations associated with cancer, and neurodegenerative diseases (32), with peroxidases as one of the cellular strategies to eliminate ROS.

Currently it is accepted that ROS generation is regulated and has a broad range of physiologic effects ranging from host defense, to hormonogenesis, to apoptosis, to fertilization.

The physiologic effects of ROS are a result from the concerted action between heme peroxidases such as lactoperoxidase (LPO), myeloperoxidase (MPO) and thyroidperoxidase (TPO), and H_2O_2 (17). For several tissues, EST and SAGE libraries show tissue-specific coexpression of DUOX with one or more of these peroxidases (Fig. 3). Coexpression of DUOX with LPO is documented for muscle and testis, and with MPO for brain, lung, mammary gland, placenta, prostate, and testis. Coexpression with TPO occurs in thyroid, but also in brain, muscle, placenta, stem cell, and testis. Although cerebellum, colon, gastrointestinal tract, head and neck, pancreatic islets, skin, stomach, and vascular EST and/or SAGE libraries contain DUOX, they do not report expression on LPO, MPO, or TPO.

The protein characteristics of the DUOX proteins fit with the biochemistry and physiology of the *thyroid*. Together with their localization at the apical membrane of the thyrocyte and their colocalization with TPO, this provides convincing arguments that the DUOXes constitute the thyroidal H_2O_2-generating system. Several groups, however, have encountered major difficulties in reconstituting the functional H_2O_2-generating properties of DUOX in transfected cells. Presumably this is caused by two main factors. First, in transfected cells, the DUOX protein is not properly expressed on the plasma membrane but is retained in the ER. Residues 596–685 of a truncated form of the human DUOX2 protein are responsible for this ER retention (37). The second factor is the absence of cofactors.

Chronic granulomatous disease is characterized by severe and recurrent infection due to the inability of neutrophils and macrophages to mount a respiratory burst and kill invading

bacteria. The disease is caused by a defect in any of the genes encoding gp91^{phox}, p67^{phox}, p47^{phox}, and p22^{phox}, that together form the phagocyte oxidase system (50, 56). Defects in none of these genes have been associated with any form of thyroid dysfunction, making it unlikely that any of these components contribute to an active and functional NADPH oxidase system in thyroid, especially taking into account the fact that p47^{phox} and p67^{phox} are not present in thyroid. Nevertheless, the role of individual components of the phagocyte oxidase system in generating a functional DUOX system in transfected cells has been investigated. DUOX expression in nonthyroid cell lines results in an 180,000 intracellular protein that does not have any enzymatic activity, and despite the high homology with gp91^{phox}/NOX2, they cannot fulfil a similar function in leukocytes already expressing the cofactors indicating that additional thyroid-specific cofactors are required to accomplish thyroidal DUOX activity. Simultaneous expression of DUOXes with TPO and p22^{phox} in transfected cells also does not have any positive effect on DUOX activity (10).

Recently, yeast-two-hybrid screening identified EF-hand binding protein (EFP1) as a DUOX1-binding protein that also interacts with TPO. EFP1 is also known as thioredoxin domain–containing protein 11 (TXNDC11), which contains two thioredoxin domains. Thioredoxins are redox-active proteins with antioxidant properties. Although EFP1 clearly is part of the DUOX protein assembly, it is not sufficient to get DUOX at the membrane or to induce H_2O_2 generation (67). Rac, a cytosolic GTP-binding protein and a member of the Rho family, is another cofactor controlling O_2^{2} production by NOX2. Rac is expressed in thyroid but is not involved in thyroidal H_2O_2 generation (20).

Recently, measurements using spin-trapping combined with electron paramagnetic resonance spectroscopy in particulate fractions of cells transfected with DUOX demonstrated calcium-dependent H_2O_2 generation via dismutation of the superoxide anion. Activity was generated by partially glycosylated DUOX2, located in the ER, and DUOX1 showed less activity (1).

Contrary to some reports (30), currently no evidence supports the role of DUOX1 in thyroid hormone synthesis.

The role of the peroxidase domain, characteristic of the dual oxidases, is currently not clear. Because T_4 can be formed nonenzymatically, it has been speculated that although TPO is essential for the organification process, it might not be required for the coupling of iodotyrosines to T_4 (8).

In studies with purified TPO, H_2O_2 is usually generated by the system glucose-glucoseoxidase, a flavoprotein enzyme that, in the reduced form, directly reacts with O_2 with the formation of H_2O_2. Within this experimental system, only the iodination potential of TPO is measured (4). Based on the work of Edens *et al.* (15), it is tempting to speculate that the peroxidase domain of DUOX is required for cross-linking of iodinated tyrosines in thyroglobulin, reducing the contribution of TPO in the process of thyroid hormonogenesis to the organification of iodide. They showed that a portion of DUOX, corresponding to the TPO-like domain expressed in bacteria, has peroxidase activity, and conclude, based on the phenotypes induced by RNA interference, that DUOX in *Caenorhabditis elegans* catalyzes the cross-linking of tyrosine ethyl ester residues through di- and tri-tyrosine formation involved in the stabilization of cuticular extracellular matrix (55).

Low levels of DUOX2 expression have been documented for the *intestinal tract* (2, 23), and based on the difference between fasting patients and normally fed animals, it is postulated that the H_2O_2 production resulting from DUOX2 expression in human colon, small intestine, and duodenum is associated with digestive function, although it cannot be excluded that these differences are species related. In intestine, DUOX2 constitutes another source of ROS in addition to NOX1, and the regulation and respective roles of both NADPH oxidases are currently not clear. Overall the substantial DUOX2 expression in digestive tract compared with the absent or weak expression of DUOX1 demonstrates that DUOX2 is functionally independent of DUOX1 (2).

DUOX2 expression in bowel is implicated in the elevation of ROS levels and the pathogenesis of inflammatory bowel disease (27) where oxidative damage is a key contributor to a loss of barrier integrity.

In *Drosophila*, DUOX silencing in gut is accompanied by an increased mortality rate after infection induced by the ingestion of contaminated food. This demonstrates that the oxidant-mediated defense system is not restricted to phagocytes but is also present in barrier epithelia and that ROS generated by DUOX are important for controlling epithelial infection (25).

Lactoperoxidase (LPO) is an integral part of the anti-infection system of *mucosal surfaces* and has potent antimicrobial properties in airway epithelia, saliva, milk, and tears, oxidizing thiocyanate and iodide into reactive compounds. Analogous to the thyroidal thyroidperoxidase, LPO requires an H_2O_2 source and, for several tissues, coexpression of LPO, the sodium iodide symporter, and DUOX has been documented, suggesting that they act in concert. DUOX2 mRNA expression has been demonstrated in the human *salivary glands*, whereas DUOX1 mRNA is not expressed. More specifically in rat, DUOX2 is expressed within epithelial cells of intralobal, interlobal, and main secretory ducts, suggesting that H_2O_2 production occurs in the final steps of saliva formation, preventing oxidant-induced glandular tissue damage. DUOXes are capable of supporting the LPO system on mucosal surfaces and can be considered a component of the host defense supporting the LPO system on mucosal surfaces (23).

In *airway epithelia*, DUOX is the enzymatic source able to provide H_2O_2 essential for the airway LPO anti-infection system (21). DUOX1 in respiratory tract epithelium is an essential component of oxidant-regulated signaling pathways and plays a critical role in mucin expression by airway epithelial cells. The ROS production stimulated by DUOX1 activates TNF-a–converting enzyme, the subsequent TGF-a release, and mucin production. The proposed mechanism includes joining of DUOX1 with p47^{phox} and p67^{phox} at the plasma membrane to constitute an active enzyme system (51).

Mucus hypersecretion is associated with chronic inflammatory airway diseases, and the discovery of DUOX1 in mucin induction suggests new therapies for hypersecretory airway diseases.

Human tracheal epithelial cells express DUOX1, DUOX2, p22^{phox}, p40^{phox}, p47^{phox}, p67^{phox}, and the apically DUOX-

based NADPH oxidase is involved in intracellular H^+ production and secretion. The acidic environment of the airway-surface liquid is critical for normal function of airway epithelium, including bacterial killing by potentiating bactericidal activity of H_2O_2 and HOCl and mucociliary clearance (51). Although DUOX2 is considered to be the inducible airway isoform important for monitoring pathologic changes in the respiratory tract that is probably not required under basal conditions, DUOX1 is the constitutive isoform modulating long-term changes (26).

Furthermore, DUOX1 is implicated in the NADPH oxidase–induced expression of matrix-metalloproteinase-12 by cigarette smoke in airway epithelia (34).

Rat *hepatocytes* express DUOX1 and DUOX2, and their activation by CD95L may induce oxidative stress that ultimately induces CD95 activation and apoptosis (47).

Activation of the cell-surface B-cell receptor (BCR) in *lymphocytes* results in the production of reactive oxygen intermediates by DUOX1, which act in a feedback manner to amplify the original signal (53).

The sea urchin egg shows a calcium-dependent respiratory burst at fertilization that has recently been attributed to urchin dual oxidase1, the sea urchin homolog of NADPH oxidases (Udx1, duox1.urchin). Udx1 expression expressed in the *egg* is responsible for the generation of hydrogen peroxide that, in concert with ovoperoxidase, blocks polyspermy by cross-linking of the fertilization envelope (68). The calcium-dependent, pH-sensitive activation of the reductase domain converts extracellular O_2 to H_2O_2 (14), whereas the catalase-like peroxidase domain is able to reverse this process, thereby neutralizing the toxic reactive oxygen ROS before they can penetrate the irreplaceable and nonrenewable cell. It is tempting to speculate that a similar dual oxidase–dependent mechanism is functional throughout fertilization in other species (68).

The initial simultaneous cloning of the highly homologous DUOX1 and DUOX2 from thyroid tissue suggested that both were components of the thyroid H_2O_2-generating system. Currently more evidence exists that both proteins are functionally independent and have separate physiologic functions.

DUOX PATHOPHYSIOLOGY

Congenital hypothyroidism (CH) is the most frequent occurring congenital endocrine disorder, affecting about 1 in every 3,000 newborns. The major problems, both in CH and iodine deficiency, are life-long cognitive and motor problems as a consequence of impaired brain development. The extent of these features depends on the severity and duration of the hypothyroid state.

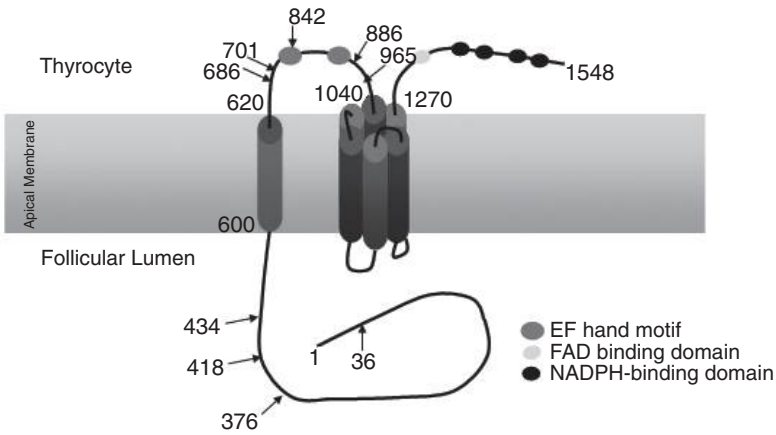
Low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy, demonstrating that maternal plasma thyroxine concentrations are important for optimal development of the fetal central nervous system (45). Because of the protective effects of a substantial maternal–fetal transfer of T_4 (66), the delay in cerebral development is, for the greater part, caused by lack of thyroid hormone after birth. The largest beneficial effect of T_4 supplementation is obtained when diagnosis is made and treatment is initiated immediately after birth. When T_4 supplementation starts in the first weeks of life, and plasma TSH concentrations are kept within the lower half of the normal range, goiter development can be suppressed permanently (65).

Genetic abnormalities in any of the key proteins involved in thyroid hormonogenesis (44) can result in hypothyroidism that, if untreated, will cause goiter as a result of persistent stimulation of the TSH receptor through increased plasma TSH levels. This has been well documented for the genes encoding NIS (SLC5A5) (22, 29), TPO (4, 49), pendrin (SLC26A4) (46, 59) and TG (48, 58). Defects in these genes leading to thyroid dyshormonogenesis are transmitted as classic autosomal mendelian traits.

The molecular action of DUOX, the generation of thyroidal H_2O_2 as a key step in iodide organification, makes the DUOXes prime candidates to explain the genetic basis of partial or total organification defects. Low thyroid NADPH oxidase activity has been reported for thyroid tissue of patients with hypothyroidism and goiter, indicating that lack of this enzymatic activity *in vivo* results in hypothyroidism (19, 41).

The DUOX1 and DUOX2 genes of eight patients with mild congenital hypothyroidism (due to a partial iodide-organification defect and one patient with a total iodide-organification defect that could not be attributed to inactivating TPO mutations) were screened for mutations. The permanently and severely hypothyroid TIOD patient is homozygous for a premature stop codon in DUOX2 that results in a protein lacking all functional domains essential for H_2O_2 generation (Fig. 4). This case constitutes the first *in vivo* evidence that DUOX2 is crucial for thyroid hormonogenesis and that DUOX1 is not able to compensate when DUOX2 is lacking. In three of the eight PIOD patients who were transiently and mildly hypothyroid at birth, monoallelic inactivating mutations in the THOX2 gene were detected (Fig. 4). These mutations prevent the synthesis of sufficient amounts of thyroid hormones to meet the relatively large requirements in the first few years of life (38). Compound heterozygosity for a nonsense and a missense substitution has been reported for two siblings with mild but permanent hypothyroidism due to a partial iodide-organification defect (Fig. 4). For one of the affected sibs with a perinatal iodine overload, TSH elevation was not detected at birth but became apparent when the urinary iodine excretion declined. Although both parents were carriers for a mutant allele, their thyroid function in adulthood was unaffected, apart from a slight partial iodide-organification defect (63). It cannot be excluded that they exhibited the mild transient form of congenital hypothyroidism previously associated with monoallelic DUOX2 mutations (38).

Additionally, two families have been reported with index patients who had hypothyroidism and an iodide-organification defect. Both patients are compound heterozygous for at least one clearly inactivating mutation, inducing a premature stop-codon combined with either a mutation inducing an amino-acid substitution or a mutation likely affecting splicing. Most heterozygous family members were euthyroid, with the exception of a carrier for a premature stop codon who was mildly hypothyroid (60). Additionally, in one family, several members were affected by autoimmune thyroid disease, a phenotype that does not correlate with the DUOX2 genotype in this family.



Genotype		Phenotype	Reference
Allele 1	Allele 2		
c.1300 C>T p.Arg434X	c. 1300 C>T p. Arg434X	TIOD permanent CH	Moreno et al.
c.2056 C>T p.Gin686X	wt	PIOD transient CH	Moreno et al.
c.2101 C>T p.Arg701X	wt	PIOD transient CH	Moreno et al.
c.1126 C>T p.Arg376Trp	c.2524 C>T p.Arg842X	PIOD permanent	Vigore et al.
c.2895-2898 delGTTC p.Ser965fsX994	wt	PIOD transient CH	Moreno et al.
c.108G>C p.Q36His	c.2895-2898 delGTTC p.Ser965fsX994	IOD	Varela et al.
c.1253delG p.G418fsX482	g.IVS19-2A>C*	Major IOD	Varela et al.
c.1253delG p.G418fsX482	wt	PIOD	Varela et al.

•Aberrant splicing at this position affects the DUOX2 protein from amino acid residue 886 onwards

FIG. 4. Schematic DUOX2 representation. *Arrows*, Mutations causing hypothyroidism identified to date. *Numbers* refer to amino acid residues. Wt, wild type; IOD, iodide organification defect; T, total; P, partial; CH, congenital hypothyroidism.

Overproduction of ROS has a role in the pathogenesis of pulmonary fibrosis, asthma, and other respiratory distress syndromes. The discovery of DUOX1 as a novel source of ROS can aid in unravelling the underlying mechanisms of these diseases and provide a new candidate putatively to mediate the process of these diseases. The compromised airway defenses inherent in cystic fibrosis might in part be related to alterations in the effectiveness of the LPO-DUOX host-defense system resulting from changes in the surface liquid, in the case of cystic fibrosis (23). Animal knockout models for DUOX1 are urgently needed to unravel further the functional properties of this protein.

The homozygous mutation encoding a premature DUOX2 termination signal, eliminating all functional domains of the protein (38), is currently the only *in vivo* evidence in humans establishing the role of DUOX2 in thyroid hormonogenesis. The isolated thyroidal phenotype of this patient does not support the concept that DUOX2 expression in human intestine is associated with digestive function or that, analogous to DUOX in *Drosophila* (25), ROS generated by DUOX2 are important for controlling gut epithelial infection.

ABBREVIATIONS

CH, congenital hypothyroidism; DUOX1, dual oxidase 1; DUOX2, dual oxidase 2; EST, expressed sequence tag; LPO, lactoperoxidase; MPO, myeloperoxidase; NIS, sodium iodide symporter; NOX2, gp91^{phox}; ROS, reactive oxygen species; RT-PCR, reverse transcriptase–polymerase chain reaction; SAGE, serial analysis of gene expression; T_4, thyroxine, tetraiodothyronine; TG, thyroglobulin; TPO, thyroidperoxidase; TSH, thyrotropin; TSH-R, TSH receptor.

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