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Review

Defects of thyroidal hydrogen peroxide generation in congenital hypothyroidism

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ABSTRACT

Thyroperoxidase-catalyzed iodination of thyroglobulin and subsequent oxidative coupling of iodinated tyrosyl residues to protein-bound iodothyronines are the key reactions in thyroid hormone biosynthesis. Under sufficient iodine supply, both synthesis steps are rate-limited by the availability of hydrogen peroxide (H_2O_2), which is required as final electron acceptor. The primary enzyme feeding H_2O_2 to thyroid peroxidase is a heterodimeric NADPH oxidase complex of dual oxidase 2 (DUOX2) and DUOX maturation factor 2 (DUOXA2) at the apical plasma membrane. While the thyrotropin receptor mediates most biological effects through the Gs/adenyl cyclase/cAMP pathway, the Gq/phospholipase C-β cascade induces H_2O_2 generation via synergistic effects of increased intracellular calcium and protein kinase C activation on DUOX2/DUOXA2. Defects in thyroidal H_2O_2 generation have been identified in a subset of patients with congenital hypothyroidism. These include loss-of-function mutations in *DUOX2* and *DUOXA2*. Thyrotropin receptor mutations with preferential loss of Gq-coupling may indirectly affect H_2O_2 production. Expressivity of the defects can be highly variable owning to the presence of genetic modifiers (e.g., the paralogs DUOX1 and DUOXA1), and environmental factors particularly nutritional iodide intake.

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1. The thyroidal H_2O_2-generating system

With a prevalence of 1 in 3000–4000 newborns, congenital hypothyroidism (CH) is the most common inborn endocrine disorder and one of the most common preventable causes of mental retardation. While frequently sporadic and due to developmental abnormalities (thyroid dysgenesis), approximately 15–20% are caused by defects in thyroid hormone synthesis (dyshormonogenesis).

The central steps in thyroid hormone synthesis take place at the apical membrane of polarized follicular thyroid cells (Fig. 1). First, iodide is oxidized and covalently linked to tyrosines of thyroglobulin (TG). Second, iodinated tyrosyl residues (mono- and di-iodinated tyrosines) are linked via phenoxy-ether bond formation to iodothyronines (thyroxine [T_4] and, to lesser degree, triiodothyronine [T_3] and reverse T_3). Both synthesis steps are catalyzed by thyroid peroxidase (TPO), which is anchored via a C-terminal transmembrane domain at the apical membrane of thyroid epithelial cells. As a heme peroxidase, TPO requires by definition hydrogen peroxide (H_2O_2) as the final electron acceptor.

In human thyroid *in vitro*, the iodination process is stimulated by intracellular calcium as well as phorbol esters and diacylglycerol, known activators of protein kinase C (PKC) (Corvilain et al., 1991). The effect of these agents on iodination is exclusively due to an increase in H_2O_2 generation (Bjorkman and Ekholm, 1988; Corvilain et al., 1991) and not phosphorylation of TPO, which lacks obvious phosphorylation sites in its intracellular tail. Thus, H_2O_2

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control has significance for not only the coupling of iodinated tyrosine but also iodination.

Early biochemical studies showed that the enzyme feeding H_2O_2 to TPO is a membrane-bound NADPH dependent flavoprotein (Bjorkman and Ekholm, 1984; Virion et al., 1984; Deme et al., 1985; Nakamura et al., 1987; Dupuy et al., 1989; Gorin et al., 1996; Leseney et al., 1999). The prototype of this class of enzymes is the phagosomal NADPH oxidase NOX2 (gp91phox) of leukocytes, which, during engulfment of invading microbes, transfers electrons from NADPH across the membrane to molecular oxygen thereby generating superoxide anions (O_2^-). Even though thyroidal H_2O_2 could result from spontaneous O_2^- dismutation, no detectable O_2^- intermediate is released from thyroid cells under physiological conditions (Dupuy et al., 1989).

It took 15 years from the initial biochemical characterization to the cloning of the dual oxidases (*DUOX*; originally called thyroid oxidases or THOX), which constitute the catalytic core of the thyroidal H_2O_2 generator. They were identified by purification and partial sequencing of a thyroidal NADPH oxidase flavoprotein (Dupuy et al., 1999) and via screening of a thyroid cDNA library for homologs of *NOX2* (De Deken et al., 2000). The predicted amino acid sequences of the two *DUOX* paralogs (DUOX1: 1551 amino acids; DUOX2: 1548 amino acids) share 83% sequence similarity. They are expressed as fully glycosylated forms (~190 kDa) found at the apical plasma membrane and as high mannose glycosylated forms (180 kDa) in the endoplasmic reticulum (ER) (De Deken et al., 2002; Morand et al., 2003).

The C-terminal region (~50% similarity with NOX2 over 569 amino acids) comprises six α-helical transmembrane segments harboring four invariant histidines, which are considered coordination sites for two nonidentical heme prosthetic groups, and a C-terminal cytosolic domain containing flavin adenine dinucleotide (FAD) and NADPH-binding sites. This arrangement of prosthetic groups provides an electron transport chain for transferring electrons from NADPH across the membrane (Fig. 1). In addition to the NOX2 homolog region, DUOX proteins feature an ecto-facing N-terminal peroxidase-*like* domain (hence named *dual* oxidases). It precedes a membrane-spanning segment and an additional cytosolic domain containing two EF-hand calcium-binding motifs. Whereas calcium reversibly activates the thyroidal H_2O_2 generator, limited proteolysis with α-chymotrypsin renders the enzyme fully active independently of calcium (Dupuy et al., 1992). It is thus possible that the EF-hands provide autoinhibition of DUOX by interfering with the function of the C-terminal NOX2 homolog domain. Binding of calcium (reversibly) and proteolytic removal of the autoinhibitory domain (irreversibly) would remove the constraint by inducing a conformational change.

DUOX differ from other essential factors in the conserved thyroid hormone biosynthesis pathway (e.g., TG and TPO) in two important ways. First, although both *DUOX* genes are highly expressed in follicular thyroid cells, their expression is not restricted to thyroid but rather is epithelial cell specific. For instance, in humans and other mammals, high level of *DUOX2* expression are found in polarized epithelial cells of the gastrointestinal mucosa (El Hassani et al., 2005; Geiszt et al., 2003) and both *DUOX* are expressed in ciliated airway epithelium (Forteza et al., 2005; Geiszt et al., 2003; Schwarzer et al., 2004). Secondly, DUOX homologs are evolutionary old molecules found in invertebrates lacking a follicular thyroid or an endostyle thyroid equivalent (e.g., in *Drosophila* (Ha et al., 2005) and *C. elegans* (Edens et al., 2001)). Exposure to various pathogens clearly indicates an important function of Drosophila DUOX in barrier defense of the gastrointestinal mucosa. Whether vertebrate DUOXs play a similar role in host defense has not been investigated in any *in vivo* model system. Such a role would, however, be consistent with the induction of DUOX in response to inflammatory mediators, e.g., the induction of *DUOX1* by interleukins 4 and 13, and of *DUOX2* by interferon-γ in respiratory tract epithelium (Harper et al., 2005).

Although DUOX sequences contain all features compatible with calcium-stimulated NADPH oxidases, initial molecular studies of

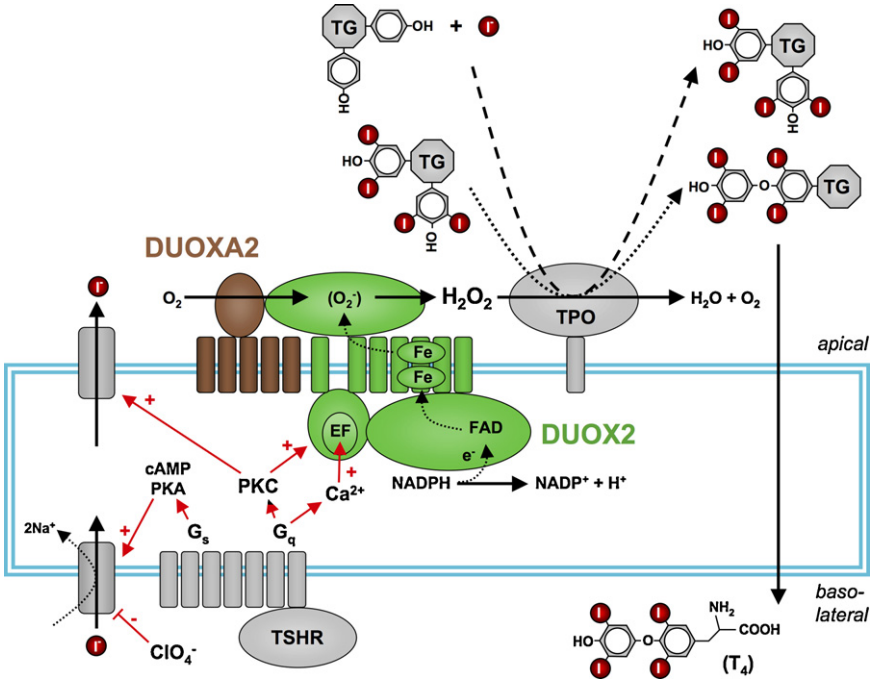

Fig. 1. The thyroidal H_2O_2-generating system. A heterodimeric DUOX2/DUOXA2 complex provides H_2O_2 for all TPO-catalyzed thyroid hormone synthesis steps. The latter include iodide oxidation and organification (binding to tyrosine residues of TG), and the subsequent coupling of mono- and di-iodinated tyrosines to iodothyronines (thyroid hormones). Stimulation of organification by TSH depends on activation of G_q linked signalling cascades leading to increased intracellular calcium (via PLC-β/IP_3) and PKC activation (via PLC-β/diacylglycerol). Calcium and PKC act synergistically in the activation of the DUOX2/DUOXA2 enzyme complex. Note that for clarity, the less expressed DUOX1 and DUOXA1 paralogs are not shown.