Recent Insights into the Cell Biology of Thyroid Angiofollicular Units

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Abstract

In thyrocytes, cell polarity is of crucial importance for proper thyroid function. Many intrinsic mechanisms of self-regulation control how the key players involved in thyroid hormone (TH) biosynthesis interact in apical microvilli, so that hazardous biochemical processes may occur without detriment to the cell. In some pathological conditions, this enzymatic complex is disrupted, with some components abnormally activated into the cytoplasm, which can lead to further morphological and functional breakdown. When iodine intake is altered, autoregulatory mechanisms outside the thyrocytes are activated. They involve adjacent capillaries that, together with thyrocytes, form the angiofollicular units (AFUs) that can be considered as the functional and morphological units of the thyroid. In response to iodine shortage, a rapid expansion of the microvasculature occurs, which, in addition to nutrients and oxygen, optimizes iodide supply. These changes are triggered by angiogenic signals released from thyrocytes via a reactive oxygen species/hypoxia-inducible factor/vascular endothelial growth factor pathway. When intra- and extrathyrocyte autoregulation fails, other forms of adaptation arise, such as euthyroid goiters. From onset, goiters are morphologically and functionally heterogeneous due to the polyclonal nature of the cells, with nodules distributed around areas of quiescent AFUs containing globules of compact thyroglobulin (Tg) and surrounded by a hypotrophic microvasculature. Upon TSH stimulation, quiescent AFUs are activated with Tg globules undergoing fragmentation into soluble Tg, proteins involved in TH biosynthesis being expressed and the local microvascular network extending. Over time and depending on physiological needs, AFUs may undergo repetitive phases of high, moderate, or low cell and tissue activity, which may ultimately culminate in multinodular goiters.

I. Introduction

The thyroid hormones (THs) T_3 and T_4 are essential for energy metabolism and embryonic development, especially for brain maturation. THs influence all anabolic and catabolic pathways involved in intermediary and structural metabolism ([1]–[3]). Claiming that the main role of the thyroid gland is the synthesis and secretion of THs seems, at first sight, a truism. Nevertheless, this task represents a continuous challenge, because the gland must not only deal with the scarcity of a trace element, iodine, but also perform within a potentially highly toxic biochemical environment. The goal of this review is to present the most recent and innovative data about TH synthesis and to explain how thyrocytes, within three-dimensional structures known as angiofollicular units (AFUs), adapt to iodine deficiency (ID). The existence of corrective mechanisms, including cross talk between epithelial and endothelial compartments, and the role of reactive oxygen species (ROS) under physiological and pathological conditions will be described to illustrate the continuous adaptation of thyroid cells to their ever-changing environment.

II. The Thyroid Gland: The Result of a Long Evolutionary Journey

Iodine (the 47th most abundant element in the earth's crust) is a nonmetallic element of the halogen family, which also includes bromine, fluorine, and chlorine. It was accidentally discovered by Bernard Courtois in 1811, when he was searching for new raw materials for explosives during the Napoleonic wars ([4], [5]). Over a century later, the biological relevance of iodine was recognized when E. C. Kendall isolated and crystallized T_4 in 1914 ([6]). The name *iode*, given by Gay-Lussac (thereafter anglicized to iodine), is derived from the Greek word *iodes*, which means violet-colored ([7]). The total mass of stable iodine on Earth is estimated to be 8.6×10^{15} kg ([8]). Although it is still expelled from hot water sources and rift faults, iodine was mainly generated early on in the earth's formation during the degasification of the asthenosphere. Since then, leaching from glaciation, flooding, and erosion have depleted surface soils of iodine, which explains why it is mostly found in oceanic sediments, the largest reservoir of iodine on Earth ([8], [9]).

From an evolutionary point of view, the thyroid gland in mammals is the most advanced system for storing and producing iodocompounds from an iodine-deficient milieu ([5], [10]). Mother Nature first used iodine for its potent antioxidant properties, for instance in algae, the first living cells that produced oxygen in the terrestrial milieu. Some marine seaweeds and algae can accumulate iodine to 4,000–30,000 times its seawater concentration, which results in levels of 0.8–4.5 mg of iodine per gram of dried material ([7], [9], [11], [25]). The human thyroid gland is the result of an endless adaptation that started millions of years ago when primitive marine animals left the sea, an iodine-enriched environment, to colonize the iodine-deficient terrestrial mainland. At that time, these organisms had to solve many problems, including the sequestration of iodine from an iodine-deficient ecosystem. Animal species have used different strategies to cope with this problem, from jellyfishes lacking a thyroid gland to chordates developing an organ called the endostyle on the floor of the pharynx, which concentrates iodine and produces THs ([13], [14]). In early vertebrates, such as lampreys, nonencapsulated follicles evolve from the larval endostyle during metamorphosis ([15]). It is worth noting that some endostyle cells produce thyroglobulin (Tg), display peroxidase activity, and incorporate iodine, which are all features of the thyroid gland in vertebrates ([15]–[22]). The homology between the endostyle and the thyroid gland of higher vertebrates is supported by the results of recent comparative analyses, which demonstrated

that genes (*Pax8* and *Ttf1*) involved in the expression of thyroid-specific transcription factors have similar expression patterns during the morphogenesis of the mouse thyroid and in the chordate endostyle ([13](), [22]()–[24]()). In higher vertebrates, the organ kept on evolving, following a process that culminated in the thyroid gland ([10](), [25]()–[28]()).

Over millions of years, T_4 had no hormonal actions *per se* in invertebrates, but instead served to transport the ancient antioxidant iodide (I^-), the reduced form of iodine (I_2), into peripheral cells, which, in more recent vertebrates, started using the remaining T_3 as an active hormone to control processes such as metamorphosis in amphibians, spawning changes in fishes, and thermogenesis. In the organs (stomach, salivary glands, and mammary tissue) of more recent mammals, I^- still acts as an antioxidant in the presence of hydrogen peroxide (H_2O_2). In the thyroid gland, after disposing of its electrons, I^- is instead transformed into oxidized forms that immediately iodinate tyrosyl residues of Tg, thereby neutralizing its own oxidant properties, while generating T_4 ([10](), [25](), [29]()–[31]()).

Thus, from this endless adaptation of iodine-concentrating cells, a highly sophisticated system emerged in mammals, known as the follicle. Each follicle is composed of polarized cells, called thyrocytes or follicular cells, which are perfectly adapted to achieve a task that is not only demanding, due to the scarcity of iodine, but also somewhat perilous because it requires the production of potentially cytotoxic ROS.

III. Thyroid Hormone Synthesis: A Dangerous Process

A. Iodine: intracellular journey and transformation

The intrathyroidal journey of iodide is shown in [Fig. 1](). TH synthesis requires the incorporation of iodine into Tg (iodine organification). The main sources of iodine and products containing high amounts of iodine are fish, shellfish, kelp and seaweed, milk, iodinated salt, bakery products where iodates are used as conditioners for dough, preservatives, therapeutics (amiodarone, vitamins, and Lugol's solution), topical antiseptics, and contrast dyes ([9](), [32]()). Under normal conditions, dietary iodine, which is found in nature in a reduced state (I^-), is nearly completely absorbed from the gastrointestinal tract to join the inorganic I^- pool in the extracellular fluid ([9](), [33]()). As for the thyroid, the intestinal transport of I^- occurs via the sodium-iodide symporter (NIS) [a 643-amino-acid, 13-transmembrane-domain glycoprotein encoded by the soluble carrier 5A5 *(SLC5A5)* gene on chromosome 19] ([34]()). Under conditions of sufficient iodine intake, the body of a healthy adult contains 15–20 mg of iodine, of which 70–80% is found in the thyroid. This content may fall to less than 20 µg under conditions of chronic ID ([33]()). In the thyroid, the local clearance of I^- varies with iodine intake, ranging from 10% uptake in situations of adequate iodine supply to 80% uptake under conditions of chronic ID ([33]()). To balance losses and preserve TH synthesis, an adult thyroid must trap at least 60 µg of I^- per day ([33]()). Under conditions of sufficient iodine nutrition, more than 90% of the ingested I^- is excreted in the urine ([9](), [33](), [35]()). Urinary iodine can therefore be used to assess iodine intake in human populations ([33](), [35]()).

After entering the thyroid capillaries, I^- is actively taken up by the thyrocytes. The active transport of I^- into thyrocytes is mediated by NIS that is localized at the basolateral membrane and the energy required for transport is provided by a ouabain-sensitive Na^+/K^+-ATPase ([33](), [36]()–[38]()). NIS couples the simultaneous transport of two Na^+ ions with one I^- ion by taking advantage of the favorable gradient concentration of Na^+. This pushes I^- into the cell against an electrochemical gradient, which results in intracellular concentrations that are 20–50 times higher than that of blood plasma. Thus, this transport is electrogenic, because two Na^+ cations are transported for each I^- anion. This is true for all NIS substrate anions (ClO_3^-, SCN^-, $SeCN^-$, NO_3^-, Br^-, BF_4^-, IO_4^-, and BrO_3^-), with the exception of perchlorate (ClO_4^-, a pollutant occurring widely in food and water in the United States), whose transport is electroneutral ([39](), [40]()). This indicates that NIS translocates different substrates with different stoichiometries ([39](), [40]()). The side chain of the amino acid Gly at NIS position 93 is crucial in this matter, because it is used as a pivot during the change from an outward to an inward open conformation during the transport cycle. This controls the size and chemical characteristics of the ion cavities as well as the kinetics and the stoichiometry of transport ([41]()). The basal expression of NIS is up-regulated by TSH ([42]()) and down-regulated by I^- ([43]()–[45]()), which is most likely caused by posttranslational modifications, including polyadenylation and mRNA destabilization ([46]()). The significantly decreased levels of NIS mRNA and protein have been implicated in escape from the Wolff-Chaikoff effect ([44]()), which corresponds to a blockade of I^- organification and of TH biosynthesis and release in response to elevated I^- plasma levels ([47]()). Of note, TSH fully induces NIS expression at the basolateral membrane only when thyrocytes are organized as follicles, suggesting that a signal (of unknown nature) resulting from specific interactions between

thyrocytes in follicles modulates TSH-induced NIS expression ([48](#)). This again emphasizes the importance of the follicle three-dimensional organization and of the cell polarization for a full expression and regulation of some thyroid-specific proteins. In models of cultured thyroid cells where TSH levels are kept constant, chronic ID does not influence NIS expression. This is in contrast to acute ID, which induces a clear up-regulation of NIS expression via a currently unknown mechanism ([49](#), [50](#)). Recently, other ionic channels were reported to play an important role in NIS-mediated I^- transport. Thus, the potassium voltage-gated channel, KQT-like subfamily, member 1-potassium voltage-gated channel, Isk-related family, member 2 (KCNQ1-KCNE2) complex, which is probably localized to the basolateral membrane, was identified as a TSH-stimulated thyrocyte K^+ channel that is critical for normal I^- accumulation ([51](#)). KCNQ1 and KCNE2 are two potassium channel subunits that function in repolarizing cardiac myocytes. They have been suggested to form a constitutively active K^+ channel in thyrocytes, which is required for normal TH biosynthesis ([51](#), [52](#)).

Because NIS mediates I^- uptake in organs other than the thyroid, it is considered to be the master regulator of iodine metabolism. Besides the intestine ([34](#)), NIS has also been identified in lactating mammary glands, gastric mucosa, and salivary glands (reviewed in Refs. [53](#) and [54](#)). In contrast to enterocytes where it is localized apically, NIS is found exclusively in the basolateral region of cells in other organs. In the breast, it is involved in I^- transport from the bloodstream to the milk, thereby fulfilling the iodine requirement of newborn babies. In gastric mucosa and salivary glands, NIS transports I^- in the gastric juice and in saliva as an antimicrobial and antioxidant substance before being reabsorbed by NIS located lower in the digestive tract at the apical surface of enterocytes. This reduces the loss of I^- ([34](#), [53](#), [54](#)).

After entering the cell, I^- crosses the apical membrane via a transporter [a candidate being the Cl^-/I^- exchanger, pendrin/Pendred syndrome (PDS); a 780-amino-acid, 12-transmembrane-domain, hydrophobic membrane protein encoded by the *SLC26A4* (also called the *PDS*) gene on chromosome 7] ([55](#)–[57](#)). Pendrin translocates to the membrane in response to TSH and forskolin through the protein kinase A pathway in PCCL-3 rat thyroid cells ([58](#)). However, the transport mechanism of I^- through the apical membrane remains a matter of debate. Other apical transporters, such as chloride channel protein 5 (ClC-5), a chloride/proton antiporter, and the chloride channel cystic fibrosis transmembrane conductance regulator (CFTR), have been proposed to mediate or comediate apical I^- efflux ([38](#), [56](#), [57](#), [59](#)–[63](#)). Studies using heterologous systems have indicated that the product of the *SLC5A8* gene, also known as sodium monocarboxylic acid transporter (SMCT1), human apical iodide transporter (AIT), or SLC5A8, is not involved in I^- efflux ([63](#)–[66](#)), as previously held ([67](#)). After transport through the apical membrane, I^- is covalently bound to the tyrosyl residues of Tg by thyroid peroxidase (TPO).

Tg has a relative molecular mass of 660,000 Da (660 kDa) and is a homodimeric glycoprotein protein. It is the product of a 270-kb gene with an 8.5-kb coding sequence divided into 48 exons on chromosome 8q24 (reviewed in Ref. [68](#)). Each chain consists of 2748 amino acids, 67 of which are tyrosines ([69](#)). Eighty percent of the molecule has three regions (called regions I-II-III) with cysteine-rich repeat domains covalently bound by disulfide bonds. These are followed by the cholinesterase-like carboxyl-terminal domain (~570 residues), which has two functions: 1) homodimerization and 2) binding to the I–II–III region to facilitate oxidative maturation required for intracellular protein transport ([70](#)). Correctly folded Tg homodimers are glycosylated in the Golgi and are secreted into the follicular lumen ([71](#)). It is worth noting that Tg is not only a scaffold protein for TH synthesis but also possibly a regulator of thyroid function. One research team repeatedly reported that the accumulation of Tg acts as a feedback suppressor of thyroid function, including the expression of important thyroid-specific transcription factors (*Titf1*/*Nkx2-1*, *Foxe1*, and *Pax8*), thereby decreasing the expression of Tg, TPO, and NIS (reviewed in Ref. [72](#)). However, many questions concerning the mechanisms controlling Tg-induced negative regulation remain unanswered.

TPO is a 933-amino-acid, transmembrane-bound glycoprotein protein of the mammalian peroxidase superfamily (which includes lactoperoxidase and myeloperoxidase) with a short intracellular C-terminal and a large N-terminal extracellular region. It is encoded by a 150-kb gene with 17 exons and 16 introns on chromosome 2 ([73](#)). The protein possesses a heme-containing, extracellular catalytic domain covalently linked to the protein, which uses H_2O_2 as an oxidizing agent for I^- ([74](#), [75](#)). TPO exists in two isoforms, TPO1 (110 kDa), which is active, and its alternatively spliced form, TPO2 (100 kDa). TPO2 is found only in intracellular compartments, has no prosthetic heme group, and

is inactive ([76](#), [77](#)). Until recently, it was thought that only a small fraction (~15%) of TPO was expressed in the microvilli of the apical plasmalemma; however, this notion has been called into question, at least in FRTL-5 cells (a rat thyroid cell line) where a large fraction of TPO is delivered to the plasma membrane ([78](#)).

The ability of the thyroid gland to produce H_2O_2 was first reported in 1971 ([79](#), [80](#)). Ten years later, it was demonstrated that H_2O_2 is generated by reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidases located in the apical plasma membrane ([81](#), [82](#)). Another 10 yr passed before the cloning of the two human cDNAs encoding these NADPH oxidases [now called dual oxidase 1 (DUOX1) and DUOX2], which share 83% sequence similarity ([83](#), [84](#)). DUOX proteins belong to a family of adenine dinucleotide phosphate oxidase (NOX) enzymes and exhibit the characteristics of heme-bound NADPH oxidases, like NOX1–NOX5 ([83](#)–[87](#)). Both enzymes have a seventh transmembrane domain and possess an extracellular NH_2 terminus peroxidase-like domain, also called the TPO-like N-terminal domain (43% similarity to TPO, even though no peroxidase activity is associated with human DUOX) ([88](#), [89](#)), which is able to associate with TPO ([83](#), [84](#), [90](#)). The C-terminal region contains the catalytic NADPH oxidase core, which is common to all NOX proteins ([83](#), [84](#)). DUOX proteins exist in two forms depending on their *N*-glycosylation states; only the fully *N*-glycosylated form (190 kDa) is expressed in the plasma membrane, whereas the high-mannose glycosylated immature form (180 kDa) is expressed in the endoplasmic reticulum (ER) in a nonfunctional state ([91](#), [92](#)). DUOX proteins need calcium to become fully activated ([91](#), [93](#)–[95](#)). Both proteins have EF-hand calcium-binding motifs in their first intracellular loop. EF-hand involvement in H_2O_2 production was demonstrated recently when mutations in this site were shown to block ionomycin-induced H_2O_2 production ([96](#)). In contrast to other genes involved in TH synthesis, DUOX expression is not influenced by TSH in human and mouse thyrocytes ([84](#), [97](#)). DUOX1 is responsive to cAMP and protein kinase A-mediated phosphorylation, whereas DUOX2 is stimulated by phospholipase cascade and protein kinase C-dependent phosphorylation ([96](#)). In the thyroid, the main source of H_2O_2 is DUOX2 ([98](#)), which, unlike other NOXs, does not produce the intermediate superoxide ($O_2^{\cdot-}$), although the immature partially glycosylated form of DUOX2 is thought to generate $O_2^{\cdot-}$ ([94](#)). The ability of DUOX2 to produce H_2O_2 instead of $O_2^{\cdot-}$ depends also upon associated maturation proteins as recently suggested by a study that showed that the NH_2-terminal tail of DUOX maturation factor 2 (DUOXA2) (one of the two maturation proteins as described below) favors the dismutation of $O_2^{\cdot-}$ to generate H_2O_2 ([99](#)). Congenital hypothyroidism due to an I^- organification defect can be associated with inactivating mutations in the *DUOX2* gene ([100](#)), even though rescue mechanisms by DUOX1 remain possible ([101](#), [102](#)). I^- plays an important role in the control of H_2O_2 production, regulating DUOX activity in a dual fashion: at low concentrations, I^- is stimulatory, thereby favoring the efficient iodination of Tg tyrosyl residues ([103](#)); however, when it is present in excess, H_2O_2 production is transiently blocked to protect the organism from overproduced TH (Wolff-Chaikoff effect) ([47](#)). The block may depend (at least partly) on the synthesis of iodolipids (6-iodo-δ-lactone and 2-iodo-hexadecanal) ([104](#)–[107](#)). I^- must anyway be oxidized to inhibit H_2O_2 production. This oxidized form acts at a posttranscriptional level by reducing the availability of the mature DUOX2 protein ([92](#)). Blocking H_2O_2 production when iodine is present in excess is one of the many iodine-induced autoregulatory mechanisms that protect the body against excessive TH release. Other mechanisms include the inhibition of TH secretion, cAMP generation, Tg proteolysis, glucose and amino acid transport, and protein and mRNA synthesis ([108](#)) and (reviewed in Refs. [109](#) and [110](#)). In the presence of sufficient amounts of I^-, H_2O_2 generation becomes the limiting step for TH synthesis ([111](#), [112](#)).

In addition to I^- organification, TPO also catalyzes H_2O_2-dependent head-to-tail coupling reactions of mono-iodotyrosine (MIT) and di-iodotyrosine (DIT) to form T_4 (80% of TH) and T_3 ([111](#), [113](#)) (reviewed in Ref. [5](#)). Forty of the 140 TPO tyrosyl residues are iodinated *in vivo*, but only a few are involved in coupling reactions ([111](#)). Four hormonogenic acceptor tyrosines were identified at positions 5 (the most favored one), 1291, 2554, and 2747 in human Tg, whereas tyrosines at positions 130, 847, and 1448 were identified as potential outer ring donor sites ([114](#)). The hormonogenic residues are located close to the extreme ends of the molecule to facilitate proteolytic cleavage and the release of T_4 and T_3. The iodinated residues that are not involved in hormonogenesis are readily deiodinated to a NADPH-dependent specific iodotyrosine dehalogenase 1 (DEHAL1) for recycling. DEHAL1 is a 33-kDa, transmembrane, nitroreductase-related enzyme present in intracellular vesicles and in the apical plasma membrane, which is located close to the organification site ([115](#)–[118](#)). Two other variant gene products (DEHAL1B and DEHAL1C) that are probably functionally inactive were also cloned ([119](#)).

The way in which I^- oxidation, iodination and coupling reactions occur in the thyroid gland remains controversial ([5], [120]). Concerning iodination, three mechanisms have been proposed: one includes a free radical scheme based on a one-electron transfer process ([111], [121]); the two others imply a two-electron transfer process with either iodinium cation (I^+) ([122]–[125]), or hypoiodite anion (IO^-) ([126]–[128]) as the iodinating species. Under conditions of normal iodine supply, H_2O_2 oxidizes the native ferric form (FeIII) of TPO to form compound I, a Fe(IV) porphyrin π-cation radical species that contains two oxidation equivalents above the ferric state of the free enzyme ([129]). According to the free radical model (which appears the less likely), I^- and tyrosine undergo one-electron oxidation to produce the radicals $I^{\cdot}$ and $Tyr^{\cdot}$, which readily combine to form MIT or DIT. In the I^+ hypothesis, or the IO^- hypothesis (the most widely accepted), I^- is oxidized in a two-electron transfer process in which $E\text{-}I^+$ or $E\text{-}OI^-$ acts as an intermediate. After this step, compound I reverts to its native state. As mentioned above, coupling reactions, which refer to the reaction between two DIT residues or between one MIT residue and one DIT residue to form T_4 or T_3, respectively, are also catalyzed by TPO ([113], [130]) (reviewed in Ref. [5]). This reaction occurs within the Tg molecule matrix and comprises three steps. The first involves the oxidation of iodotyrosyl residues by TPO. According to the most accepted univalent scheme, the second step involves the oxidation of two iodotyrosine residues to free iodotyrosyl radicals that then couple to form a quinol ether intermediate. In the third step, this unstable intermediate undergoes fission, leading to the formation of T_4 or T_3 in the hormonogenic site, whereas the alanine side chain of the donor tyrosyl remains in the Tg polypeptide chain as dehydroalanine.

After endocytosis, iodinated Tg is hydrolyzed by proteases in the lysosomes, which contributes to release TH from the Tg backbone. The last enigma related to TH secretion from thyrocytes is perhaps being clarified, as this process is mediated in the basolateral membrane by TH transporters of which the monocarboxylate transporter 8 [MCT8 (*SLC16A2*)], a protein with 12 predicted transmembrane domains, is an important contributor ([131], [132]) (reviewed in Ref. [133]). This does not exclude the possibility that other TH transporters will be discovered, as was suggested recently ([132], [133]). *MCT8* mutations are associated with the Allan-Herndon-Dudley syndrome (AHDS), which has been identified in more than 45 families worldwide. The symptoms of AHDS include severe X-linked mental retardation, disrupted locomotor development due to the hindered access of T_3 to target cells during development (because MCT8 is also involved in the transport of T_3 in peripheral cells, as described below), and abnormal TH levels, in particular, high serum T_3 levels ([133]–[137]). These anomalies are explained by the relative insensitivity of the pituitary to T_3 and the associated alteration in peripheral deiodination ([138]–[140]). MCT8 is thought to play a role in the maintenance of thyroid integrity, as recently reported in a paper describing severe thyroid morphological alterations (hyperplastic nodules, microfollicular areas with stromal fibrosis, and nuclear features close to papillary thyroid carcinoma) in an AHDS patient and in *Mct8*-deficient mice ([138]).

T_4 and T_3 are transported to their target tissues after binding plasma proteins (T_4-binding globulin, transthyretin, and albumin) ([141]). It was thought initially that THs passed through the plasma membrane of their target cells by passive transport. This simple view was challenged when it was discovered that MCT8 selectively transports T_3 across the membranes of target cells ([142]), indicating that, in contrast with previous thoughts, THs do not cross cell membranes passively. Other TH transporters have been discovered, and more are likely to be discovered in the near future, which will generate new knowledge on TH peripheral actions (reviewed in Ref. [133]). It is usually admitted that after T_4 deiodination, T_3, the active hormone, exerts genomic effects after binding TH receptors (TRs). In addition to this classic nuclear TH action, THs could also influence nongenomic pathways. TH interacts with αvβ3 integrins to induce ERK1/2 signaling-associated angiogenesis ([143]) and DNA synthesis ([144]). After binding TR, T_3 may engage in nongenomic activation of the phosphatidylinositol-3 kinase/AKT/mammalian target of rapamycin pathway in different cell types ([145]–[150]). Furthermore, T_4 and rT_3 were reported to regulate actin polymerization and microfilament organization independently of T_3 ([151]). Although it remains meaningful to evaluate the pathophysiological relevance of these TR-induced non-TH response element-dependent effects, the finding of nonclassical TH actions opens up new perspectives on TH biological actions in targets cells, as reviewed recently ([152]).

B. Colloid Tg: an easily accessible reserve

The colloid is not a homogeneous form of Tg storage ([Fig. 2]). It is rather heterogeneous with a more rapid Tg turnover at the periphery than in the center. This is because the iodination process first affects newly synthesized, soluble molecules at the periphery before affecting older molecules stored as compact Tg globules, as formulated in

the last-come-first-served principle ([153], [154]). Thus, when Tg molecules escape rapid uptake, they proceed to the colloid where they are stored as globules of highly concentrated, insoluble, covalently cross-linked, multimerized 19 Svedberg Tg, which is not immediately available for TH synthesis but is made available upon follicle stimulation ([155]–[157]). Therefore, Tg globules store high amounts of Tg in follicles, while preventing osmotic injury ([155], [156]). The iodine content of isolated globules is high, although hormones are not detected ([155], [158]). A mouse monoclonal antibody against iodinated Tg containing T_4 at the N-terminal hormonogenic site (TgI) ([159]) failed to detect globules ([157]), which could be due to the absence of TH ([158]) or to the limited access of the antibodies to epitopes buried in aggregates or covered by carbohydrate chains. However, the latter possibility seems unlikely, because the treatment of paraffin sections with neuraminidase, which breaks down the sialic acid-induced rigidity of the polysaccharide cover, did not restore the antigenicity ([157]). These globules are often observed in hypofunctioning follicles, but only very rarely during hyperactive endocytosis, as observed in Graves' disease and in autonomous adenomas ([157]).

TSH-stimulated thyrocytes metabolize globules in successive fragmentation steps from compact, insoluble Tg into soluble molecules ([157]). The fragments are then used for TH liberation. Several mechanisms may account for globule dissolution. Besides a first mechanism that involves protein disulfide isomerase (PDI)-induced reduction of intermolecular disulfide bridges ([156]) (very unlikely because PDI requires reducing and acidic conditions) and another involving H_2O_2-associated oxidative attacks of globule surface ([158]), a third mechanism involving lysosomal enzymes has received great attention over the last 15 yr ([160]–[164]). Tg proteolysis is a highly specific process, because Tg is resistant to proteolytic cleavage because of its size, its level of glycosylation, and the presence of many intra- and intermolecular bonds, which probably protects the protein from early degradation. The process starts in the colloid before endocytosis and involves the action of cysteine cathepsins in the pericellular space. These proteases are present in the follicle lumen after being released from the endolysosomal compartment. Before endocytosis, Tg undergoes a limited proteolysis in the follicular lumen by cathepsins B and L, which results in small fragments that are processed further by cathepsins K, L, and S ([163], [164]). T_4, but not T_3, is released to some extent by the extracellular proteolysis of Tg within the colloid, at the apical surface of thyrocytes ([160]). This form of proteolysis is not an easy process compared with bone resorption by osteoclasts. Although bone resorption occurs under acidic and reducing conditions, Tg proteolysis occurs at neutral pH and under oxidizing conditions, which are not ideal for maximum protease activity. However, evidence suggests that cathepsins conserve their proteolytic properties for awhile, even under oxidizing conditions ([165]). Upon TSH stimulation, the proteolytic process is activated as cathepsins are rapidly transported extracellularly through retrograde trafficking vesicles. TSH-induced cytosolic calcium release activates the fusion of apical vesicles with the plasma membrane within minutes. Thereafter, intracellular reserves of cathepsins are reconstituted, either from the extracellular colloid lumen or by *de novo* biosynthesis ([165]). A recent paper ([166]) reported that altered trafficking routes of cathepsins, for instance toward the basal membrane instead of the apical membrane, could have devastating consequences for malignant cell invasion because they degrade components of the extracellular matrix. This observation is in accord with another study published in the early 1990s ([167]).

In resting or moderately activated states, highly iodinated Tg is internalized via fluid-phase pinocytosis and receptor-mediated endocytosis (reviewed in Ref. [168]). Under TSH stimulation, pseudopods form at the apical membrane of thyrocytes and incorporate Tg as colloid droplets ([169]); however, this process has not been observed in species (including humans) other than rodents ([170]). The multiligand endocytic receptor megalin, a member of the low-density lipoprotein receptor family, acts as a high-affinity receptor for Tg ([171]). Megalin mediates Tg transcytosis from the apical to the basolateral surface, especially under intense TSH stimulation, which prevents excessive hormone release ([168]). Megalin also transports Tg molecules with a low hormone content away from lysosomes, which promotes the lysosomal degradation of hormone-rich Tg molecules ([172]). Evidence suggests that immature Tg molecules are internalized and recycled through the Golgi compartment ([173], [174]), and although still controversial, it is thought that this may occur via PDI ([175]) or via the vacuolar protein sorting 10 protein family member sortilin, which is expressed in thyroid epithelial cells in a TSH-dependent manner ([176]).

C. The TPO-DUOX couple: the heart of the synthesis complex

The multiprotein complex involved in TH biosynthesis is shown in Fig. 3. TH synthesis depends on biochemical reactions that would be highly toxic if not perfectly controlled. H_2O_2, a key element in this biosynthesis, would be lethal if not used immediately and/or properly detoxified ([177]–[182]).

For cells to be protected from life-long, cytotoxic free radical attacks, all oxidizing and coupling reactions must occur outside the cell, at the interface with the colloid, in a special biochemical environment that was called the thyroxisome by Song *et al.* ([178](#)). The thyroxisome cannot be considered as a true morphological entity or a cellular organelle because it has never been visualized as such. Rather, it should be viewed as a biochemical concept (that still needs to be further worked out) that helps to understand how TH biosynthesis occurs safely despite an *a priori* toxic environment. A harmless biochemical milieu is created in microvilli when the TPO-like extracellular N-terminal domain of DUOX interacts with TPO ([178](#), [181](#), [182](#)). How this specialized environment remains harmless needs to be further clarified, even though recent data, such as those reported below, brought new knowledge in this matter. Thus, both proteins are thought to be already closely related during their posttranslational maturation and transport across the Golgi apparatus, but in the absence of TSH, they are kept in an inactive state just below the apical membrane. One may admit that this assumption is still rather weak because it is based only on indirect arguments about location and activation of TPO and DUOX ([84](#), [181](#)–[184](#)). Under TSH-induced calcium signaling, DUOX is fully activated to form a competent H_2O_2-generating system after its integration into the apical membrane ([178](#), [180](#)–[182](#)).

In addition to the TPO-DUOX couple, other forms of autoregulation are required to make TH biosynthesis operate as a safe process. For instance, H_2O_2 favors the association between TPO and DUOX in human thyrocytes ([181](#)); however, when produced in the absence of I^-, H_2O_2 instead decreases the activity of both TPO and DUOX ([182](#)). TPO protects DUOX activity against the inhibitory effects of H_2O_2. This can be ascribed to its catalase-like activity, which requires a close interaction with the N-terminal domain of DUOX2 ([182](#)). DUOXs also need partners to work properly. One such partner is the thioredoxin-related protein EF-hand binding protein 1 (EFP1), which interacts with the intracellular region of DUOX1 and DUOX2 that contains two EF-hand domains. EFP1 may also be involved in the destruction of H_2O_2 when H_2O_2 is not used as an oxidizing substance in TH synthesis and leaks away from the site of its production ([177](#)). Two other partners are the DUOX-specific quality controlling maturation proteins DUOXA1 and DUOXA2, which are required in overcoming the ER retention of DUOX enzymes ([98](#), [185](#)). The critical role of the DUOX2/DUOXA2 complex in hormonogenesis was confirmed recently by results showing that loss-of-function mutations in DUOX2 and DUOXA2 cause congenital hypothyroidism ([100](#), [186](#)–[188](#)). Further support for this role comes from a mouse model of inactivated *Duoxa1* and *Duoxa2,* where DUOXA deficiency led to intracellular retention of DUOX subunits and the loss of calcium-inducible H_2O_2 release ([189](#)). Stable DUOX/DUOXA complexes at the apical membrane are necessary for proper H_2O_2 generation. Less stable combinations (such as DUOX2/DUOXA1) do not form complexes at the plasma membrane and produce $O_2^{\cdot-}$ instead of H_2O_2, which indicates that DUOX activators not only promote DUOX maturation but also take part directly in H_2O_2 production ([185](#)). Caveolin-1 (Cav-1) is another important member of the multiprotein complex involved in TH biosynthesis ([183](#)). Cav-1 is one of three caveolar proteins that are important components of caveolae ([190](#)). Caveolae are invaginated microdomains of the plasma membrane that influence endocytosis, transcytosis, lipid metabolism, signal transduction pathways, and cell proliferation in many eukaryotic cells (adipocytes, endothelial and epithelial cells, myocytes, and fibroblasts) (reviewed in Ref. [191](#)). In the thyroid, caveolae are required for TH synthesis and homeostasis, as recently demonstrated ([183](#), [192](#)). For instance, an increased apoptotic rate was reported in the thyroid of Cav-1-knockout mice ([183](#)). In these mice, the organification process is abnormal because the synthesis enzymatic complex is mislocalized to, and active in, the cytoplasm instead of the apical pole of the cell ([183](#)). There are reasons to believe that this mislocalization is responsible for the increased apoptotic rate observed in this knockout model, because apoptosis is associated with high oxidative stress (OS). High OS is a consequence of the increased expression and activity of H_2O_2-generating DUOX, which occurs when Cav-1-induced DUOX inhibition is insufficient. Thus, in the absence of Cav-1, DUOXs are localized to the cytosol, where they are abnormally active. It has been proposed, but not yet formally proven, that the Cav-1-induced inhibition of DUOX is caused by the presence of three amino acid sequences that may act as putative binding sites for the Cav-1 scaffolding domain ([183](#), [193](#)). This negative regulation is reminiscent of the role played by caveolar proteins in the negative regulation of signal transduction pathways and cell proliferation ([191](#)). Accordingly, as recently demonstrated in autonomous adenomas, in a model of adenosine A2-receptor transgenic mice, and in Cav-1-knockout mice, reciprocal negative regulation occurs between TSH/cAMP-mediated proliferation and Cav-1 expression via mRNA destabilization ([183](#), [192](#)). The increased proliferation rate of thyrocytes in Cav-1-knockout mice provides further evidence for the antimitogenic role of Cav-1 in the thyroid. This would explain why the overall size and function of the Cav-1-knockout thyroid remain unchanged, because the OS-mediated increase in the apoptotic rate is likely compensated by the increase in the proliferation rate in the Cav-1-knockout mice. A pathological mechanism similar to that observed

in Cav-1-knockout mice was described in a hypothyroid PDS patient with a large goiter ([184]). PDS is an autosomal recessive disorder caused by mutations in the *SLC26A4* gene. The disease is characterized by sensorineural deafness, diffuse goiter, and a positive perchlorate discharge test (reviewed in Ref. [59]). In this case, iodination processes were observed to also occur in the cytosol rather than in the apical membrane, suggesting that the disturbance of the TPO-DUOX molecular complex and its shift into the cytoplasm were, at least partly, responsible for the thyroid tissue destruction that is sometimes observed in PDS patients ([184]). This pathological mechanism cannot be generalized to all PDS patients because the phenotypes of PDS are highly variable and depend on nutritional iodine intake. Thus, in countries with a high iodine intake, PDS patients are usually euthyroid. This is in contrast to patients from iodine-deficient regions who may present with overt congenital hypothyroidism ([59]).

Thus, TPO and DUOX, the two main proteins involved in TH biosynthesis, interact with each other and are activated in microvilli under TSH stimulation. For TH biosynthesis occurring in safe conditions, additional proteins are required. How fine-tuned interactions physiologically occur between the different actors still requires further investigations, which opens new perspectives in this field of research. This sophisticated biochemical setting is responsible for the fact that H_2O_2 production is tightly controlled, because H_2O_2 is readily consumed during TPO-induced I^- oxidation, tyrosyl iodination, and iodophenoxy-ether coupling, whereas any H_2O_2 intracellular leakage is strictly contained ([178]). Consequently, H_2O_2 can be produced in the micromolar range similar to that in stimulated leukocytes ([103], [194]) without being lethal to cells. The local H_2O_2 production in a restricted area of the cell (*i.e.* the apical membrane) is reminiscent of the recently proposed model of compartmentalized redox signaling, which infers that, in response to the activation of receptor tyrosine kinases, H_2O_2 does not diffuse freely across the cytoplasm, but instead localizes to microdomains that are located close to sites of production (plasma membrane, endosomal compartment, and ER membranes) ([195]).

D. ROS: key factors in thyroid function and growth

Increasing evidence indicates that ROS other than H_2O_2, such as $OH^{\cdot}$ and $O_2^{\cdot-}$, are produced during TH synthesis, but in very small amounts ([130], [179], [196], [197]). These ROS do not harm thyroid cells, but they do increase the level of basal DNA damage in thyrocytes, which may increase the spontaneous mutation rate with functional and/or tumoral consequences ([198]–[203]), as explained later in this review. In fact, beyond the protection provided by the aforementioned regulatory mechanisms operating during TH biosynthesis, toxicity due to ROS leakage is kept under tight control by many potent other intracellular antioxidant defense systems, including catalases, superoxide dismutase, peroxiredoxins, and glutathione peroxidases ([178], [179], [203]–[207]). Peroxiredoxins degrade H_2O_2 and reduce peroxidized membrane phospholipids. They are associated with enzymes of the thioredoxin reductase family, which are selenoenzymes as is glutathione peroxidase. This emphasizes the role of selenium in the redox protection of the thyroid, its beneficial action during the course of thyroid autoimmune diseases, and its involvement in myxoedematous cretinism in conditions of combined iodine and selenium deficiency ([208]) (reviewed in Refs. [179], [209], and [210]). It was reported recently that the inactivation of mouse thyroid selenoproteins using a genetic loss-of-function approach was not associated with severe morphological or functional alterations. This suggests that selenoproteins are not essential for thyroid cell integrity, perhaps because other antioxidant enzymes can compensate for their activity when they are absent or inactivated ([207]).

ROS mediate a variety of signal transduction pathways (reviewed in Ref. [211]). In accordance with this, a recent report demonstrated that ROS levels corresponding to the physiological oxidative load of the cell are required to safeguard the function and integrity of the thyroid ([198]). In goitrous thyrocytes, the oxidative load is higher than under basal conditions, resulting in OS, likely because of increased H_2O_2 production or impaired consumption when iodide is lacking and/or when TPO is blocked ([212]). However, OS under these conditions is probably neutralized by antioxidant defenses and is thus harmless. Likewise, OS is required for cell division because goiter formation is significantly hampered when OS is blocked by *N*-acetylcysteine, a potent antioxidant ([212]). These results are not entirely surprising, because they are in accordance with the growth-promoting effects of ROS ([213]). For instance, ROS, including H_2O_2, stimulate the proliferation of many cell types through various mechanisms that affect growth receptor tyrosine kinase pathways, the MAPK pathway, the phosphoinositide-3-kinase pathway (PI3K/Akt), several cyclin-dependent kinases, and the nuclear factor κB pathway, making them important mediators of thyroid carcinogenesis ([213]–[221]). These observations support a role for H_2O_2 in the high frequency of thyroid tumors and microcarcinomas, particularly under conditions of antioxidant deficiency ([201]–[203]). They are also in agreement with a study that showed that the high rate of mutations in the thyroid is due to constitutive elevated H_2O_2 levels ([202]) and

with other studies showing that large amounts of H_2O_2 cause RET/PTC1 rearrangement and DNA double-strand and single-strand breaks in thyroid cells ([199], [200]). In addition, imbalances in intracellular redox systems have been linked to the molecular mechanisms of tumorigenesis in patients with thyroid cancers ([222]). Although the link between OS and thyroid carcinogenesis still needs to be worked out, some explanations are now put forward to explain why H_2O_2 becomes a carcinogen when not adequately contained. Thus, DUOX protein expression is generally normal or slightly increased in thyroid cancers, whereas NIS, TPO, and pendrin protein expression is decreased, mislocalized, or even sometimes absent, which implies less H_2O_2 utilization and therefore longer exposure to H_2O_2 ([54], [223]–[225]). This longer exposure, even though mild, may have long-term consequences considering the mean 8.5-yr life span of thyroid cells ([226]). In addition, it is not unusual to observe aberrant localization of the H_2O_2-DUOX-generating system, for instance in the cytoplasm, where antioxidant defenses are again inadequate to deal with increased OS ([223], [225]). Under such uncontrolled conditions, moderately high levels of H_2O_2 that are not enough to kill the cell (as seen in the aforementioned model of Cav-1-knockout mice and in PDS patients) might, however, diffuse freely into the nucleus and provoke DNA damage. Thyroid cancer tissues are also infiltrated by inflammatory cells that are sources of ROS, which may promote tumor growth by stimulating the major oncogene-initiated signaling pathways ([227]–[229]). TSH-stimulated H_2O_2 production could be an additional explanation for the carcinogenic properties of H_2O_2, because cancerous thyroid cells still express the TSH receptor ([224]). This may explain why high TSH plasma levels are associated with an increased risk of malignancy in thyroid nodules ([230], [231]).

OS is also highly toxic in experimental models of iodine-induced thyroid involution, Th1 cytokine-induced cytotoxicity, and autoimmune thyroiditis ([232]–[236]). Iodine treatment of hyperplastic thyrocytes results in their impairment, as shown by the increase in necrosis/apoptosis, the presence of many cellular fragments, and the massive interstitial inflammatory reaction ([234], [237]–[242]). In these cases, iodine-induced cytotoxicity is due to a more aggressive attack associated with a massive release of very toxic free radicals ($OH^{\cdot}$, $O_2^{\cdot-}$, $ONOO^-$, and iodocompounds) in addition to H_2O_2, most of which are also produced by infiltrating inflammatory cells ([196], [234]). Nevertheless, cell destruction is transient, and the thyroid always returns to its original aspect. This is not the case in individuals genetically susceptible to developing autoimmune diseases such as Hashimoto's thyroiditis. In experimental models of nonobese diabetic mice for instance, the acute inflammation that occurs after iodine administration nearly always evolves toward long-term destructive autoimmune thyroiditis ([236], [240], [243]–[246]). A clinical connection can be easily made with these experimental data, because human thyroid autoimmune diseases have been shown to be associated with increased OS ([247]–[250]). Taken together, the data indicate that, although thyroid antioxidant systems are ideally adapted for the detoxification of H_2O_2 and other ROS produced under normal conditions or even in excess in goiters, it is likely that they are overwhelmed under conditions of high free radical release, which would lead to neoplastic and autoimmune diseases.

IV. The Angiofollicular Unit: From Concept to Demonstration

A. The role of the local microvasculature in the maintenance of endocrine function

The complex functional and morphological multistep changes that occur during goitrogenesis have been described in great detail in the past ([196], [201], [240], [243], [251]–[257]). It is, of course, widely accepted that TSH stimulates thyrocytes, as shown by the increases in hypertrophy, hyperplasia, and expression of NIS, TPO, and Tg ([251]–[253], [255], [258]–[260]). It has also been known for decades that epithelial changes accompany increased thyroid blood flow and vascular expansion ([253]–[255], [261]–[267]). The microvascular expansion is even essential for goitrogenesis, as evidenced by the inhibition of goiter formation when three important angiogenic growth factor signaling pathways [vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and angiopoietins] are blocked ([268]).

Each follicle is enclosed by an independent basket-like plexus of anastomotic fenestrated capillaries that covers 20–50% of its surface ([269]). After ID or goitrogen treatment, each vascular nest increases in size as a result of cell proliferation and capillary fusion to cover up to 70–80% of the follicle surface area ([269]). This vascular expansion can be rather impressive; for example, a 250% increase in thyroid blood flow was observed in rats rendered iodine deficient for 2 wk ([270]), whereas in humans, a 44% decrease in iodine intake resulted in a 49% increase in thyroid blood flow ([271]). By contrast, thyroid blood flow falls promptly in cases of massive iodine supply (potentially triggered by endothelins), which is necessary to prevent excessive TH synthesis and to protect the gland against free radicals ([196], [239], [253], [272], [273]). Considering the emerging role of the microvasculature in thyroid homeostasis, it is

becoming clear that the morphological and functional unit of the thyroid is not restricted solely to the follicular cells of the follicle but involve also the surrounding capillaries that, along with thyrocytes, form the AFU ([258](#)–[260](#), [274](#)) ([Fig. 4](#)).

Such interconnected changes between epithelial and endothelial compartments are not unique to the thyroid. In the pancreatic islets of Langerhans, for example, dynamic interactions occur between blood vessels and ß-cells to improve insulin secretion and ß-cell proliferation ([275](#), [276](#)). Islet blood flow is for instance regulated by specific metabolic factors such as glucose that almost doubles it ([277](#), [278](#)), but also by less specific factors such as ATP signals through A1 adenosine receptors as well as neuronal and vasoactive factors ([279](#), [280](#)). In all cases, these changes occur independently of blood flow in the rest of the pancreas. As conceptualized in the case of the thyroid where capillaries close to thyrocytes behave differently from one AFU to another and from those irrigating the adjacent parathyroids, pancreatic islets are also arranged so that regions containing highly vascularized ß-cells are distinct from those containing other cell types ([281](#)). The pituitary gland is another interesting example where the microvasculature is involved in growth, development, and function ([282](#)), and there is evidence that abnormal microvascular development or altered angiogenesis is associated with some forms of central diabetes insipidus and pituitary adenomas ([283](#), [284](#)). Also bearing in mind the peculiar vascular architecture in the ovaries ([285](#)–[287](#)) and in the adrenals ([288](#), [289](#)), it appears that the involvement of the microvasculature is part of a more global system in endocrine glands where the local vasculature cooperates with endocrine cells to preserve their function and maintain homeostasis.

B. ID: the trigger of an immediate TSH-independent microvascular response

The question of how epithelial and endothelial growth is coordinated in the thyroid has been addressed extensively in the past. The involvement of TSH, although obvious at first sight ([290](#), [291](#)), was challenged quickly ([270](#), [292](#), [293](#)), and additional hypotheses, including the neuronal hypothesis and the growth and vasoactive factor hypothesis were proposed ([274](#), [294](#)). According to the neuronal hypothesis, thyroid blood flow and TH secretion are influenced by adrenergic, cholinergic, and peptidergic stimuli ([270](#), [292](#), [293](#), [295](#)–[301](#)), whereas the growth and vasoactive factor hypothesis implies the involvement of paracrine/autocrine factors of epithelial, neuronal, interstitial, and endothelial origin ([258](#)–[260](#), [268](#), [272](#)–[274](#), [302](#)–[306](#)). Additionally, the vascular status of the thyroid gland may also be influenced by estrogens ([307](#)). Again, this kind of complex regulation is not restricted solely to the thyroid because similar complex interactions between vasodilators and vasoconstrictors, gastrointestinal peptides, and the autonomic nervous system were reported to control islet blood flow in the pancreas ([308](#)).

In addition to not being the sole participant in this complex interplay, it is known that TSH acts somewhat late in response to ID, once intrathyroidal reserves of iodine become insufficient to maintain steady TH production (more than a week in animal models of ID-induced goiter) ([251](#)–[255](#), [258](#), [267](#), [273](#), [302](#)). Thus, to cope with daily variations in iodine supply, thyrocytes use various TSH-independent intra- and extrathyroidal self-regulatory mechanisms including the stimulation of I^- trapping by NIS, the preferential synthesis of T_3 over T_4, the recycling of intracellular I^- by DEHAL1, the peripheral conversion of T_4 into T_3 ([309](#)–[311](#)), and the rapid inflation of its own microcirculation ([251](#)–[255](#), [258](#)–[260](#), [267](#), [269](#)–[271](#), [273](#), [292](#), [293](#), [299](#), [302](#)).

These changes in the endothelial compartment are the earliest morphological events observed in response to ID and occur a few days before changes are visible in epithelial cells ([252](#), [254](#), [267](#)). The vascular reaction occurs in two waves. The delayed phase is TSH dependent, starts at least a week after goiter onset, and is accompanied by the hypertrophy and hyperplasia of follicular cells ([253](#)–[255](#), [261](#)–[267](#)). By contrast, the early phase is TSH independent and, besides endothelial cells, also involves pericytes, as evidenced by the increased expression of the proteoglycan neuron-glial antigen 2, a marker of pericyte activation ([263](#), [267](#)). Because this early phase takes place as an immediate response to the drop in intrathyroid I^- content, the intracellular I^- shortage has been suggested to initiate the changes that affect microvessels, even though a role for the organified form of I^- or for other iodinated molecules cannot be ruled out ([267](#)). A link between I^- shortage and the quick release of VEGF from thyroid epithelial cells was established recently *in vitro* ([49](#)) and in experiments where bevacizumab (an antibody against VEGF) was administered to iodine-deficient animals ([267](#)). In contrast to animals receiving control IgGs, this antibody prevented an increase in thyroid blood flow and blocked an increase in the relative volumes of vessels.

C. ID-induced microvascular response: which molecular mechanism?

The molecular links between ID and TSH-independent VEGF-driven vascular reactions were dissected recently in an *in vitro* model of thyrocytes acutely deprived of iodine (49). When the intracellular ^{127}I content drops sharply in thyrocytes incubated without I⁻ for 4 h or treated with perchlorate, an immediate increase in hypoxia-inducible factor-1α (HIF-1α) and VEGF expression occurs along with a concomitant surge in intracytoplasmic ROS. Thus, when I⁻ is no longer available or when its transport is blocked, its intracellular stocks drop rapidly, which causes an increase in ROS and the stabilization of HIF-1α. In turn, HIF-1α heterodimerizes with HIF-1ß, which is constitutively present in the cell. After binding to the promoter region of the VEGF gene, the HIF-1α/HIF-1ß heterodimer initiates transcription. As a result, VEGF mRNA and protein expression increases. VEGF is then secreted and activates adjacent pericytes and endothelial cells, which leads to microvascular expansion and increased blood flow (Fig. 5).

This pathway is transitory and is not associated with abnormal tissue growth, which implies tight control. By contrast, in malignant cells, the pattern of intracellular events in response to ID is somewhat different. ID-induced VEGF expression is stronger and is not controlled over time, which implies that the intracellular pathways are different from those activated in nonmalignant cells (50). Thus, although the angiogenic stimulus should be viewed as a physiological adaptation of the gland to optimize its functional yield in normal cells (49, 267), ID is associated with a less controlled angiogenic switch in malignant cells (50). This notion of microvascular control in normal tissues *vs.* malignant cells was also reinforced by the recent discovery of some components of the highly conserved cell signaling system Delta-Notch pathway in the thyroid (312). Beyond VEGF, angiogenic stimuli are monitored by many other controlling pathways, including the Delta-Notch pathway (313–316). This pathway is activated by VEGF to prevent excessive vessel proliferation. In humans, four Notch receptors (1–4), and five ligands [Delta-like ligand (DLL)-1, -3, and -4 and Jagged-1 and -2] were identified (316, 317). Notch1, -2, and -3 were reported in a study that suggested a role for the Notch pathway in thyroid cell differentiation (318), whereas Notch4 and DLL4 expression was reported to be lower in normal thyroids than in nonmalignant hyperplastic and malignant tissues (312). Only capillaries of Graves' disease samples were positive for DLL4, suggesting that DLL4 plays a role as a downstream controller of VEGF in hyperplastic nonmalignant tissues to keep the expanding microcirculation under control. Thus, in contrast to carcinomas where coherent and coordinated cross talk between cells of different tissue compartments is altered and leads to anarchic vascular growth, such cross talk is kept intact under nonmalignant conditions.

D. AFUs: a heterogeneous population of three-dimensional structures that protects the thyroid gland against functional failure

Although AFUs are perfectly adapted to respond to goitrogen stimuli in a synchronized manner, they do not work as a uniform population, but instead as heterogeneous clusters of distinct units characterized by particular growth and functional properties. This functional and morphological heterogeneity is a hallmark of the thyroid that helps the gland to adapt to changing physiological conditions and explains its propensity to form nodules.

In a model of elderly mice, characterized by marked morphological heterogeneity, thyroid capillaries are closely related to the functional status of the follicle upon which they depend. In fact, two types of follicles coexist in the thyroid of aged animals. Based on morphological and autoradiographic criteria, they are either active (hot) or inactive (cold) (258, 319–321). A correlation was drawn between the degree of activity of each follicle and the expansion of its respective microcirculation, which was significantly larger around active (hot) follicles than around inactive (cold) ones (258). When applied to humans, using tissue sections of multinodular goiters, the follicles were also sorted out into active or inactive, based on morphological criteria. In contrast to richly vascularized active follicles, inactive follicles, such as those in old mice, were surrounded by a hypotrophic microcirculation (259). Beyond epithelial function, vascular changes are also more marked around proliferative epithelial cells, as demonstrated in a model of transgenic hyperthyroid mice expressing the A2a adenosine receptor (Tg-A2aR) and in human Graves' disease (260) where the number of proliferating cell nuclear antigen-positive cells was highest in the most vascularized areas.

V. Angiofollicular Heterogeneity, Nodulogenesis, and Multinodular Goiter: Why and How?

The limitations of adaptive autoregulatory mechanisms in the thyroid gland in the face of a widely fluctuating iodine supply may lead to the development of goiters. Goiter formation should be considered as an adaptive process rather than pathological, as long as thyroid function is preserved and even though they may sometimes occur along with risks of local compression (35, 255). Throughout this process, not only TSH, but also many locally produced growth, vasoactive, and angiogenic factors [IGF-I, epidermal growth factor (EGF), FGF, endothelin-1, nitric oxide, VEGF,

and others] are involved in the growth and replication of epithelial and endothelial cells ([Fig. 2](#)) (reviewed in Refs. [274](#), [294](#), [303](#), and [306](#)). Of course, ID is not the unique etiological factor of goiters, because many individual genetic determinants and environmental factors (cigarette smoking, nutritional goitrogens, radiations, gender, age, and body mass index) are other well-known causative factors ([256](#), [257](#), [322](#)).

Starting from diffuse hyperplasia, nontoxic goiters always tend to become heterogeneous over time and are gradually transformed into multinodular goiters (MNGs) ([201](#), [256](#), [257](#), [322](#)). The tissue heterogeneity may be explained by two theories (perhaps complementary): the most studied and accepted polyclonal and mutation theory and the more recently proposed and less studied stem cell theory.

A. The polyclonal and mutation theory

The heterogeneity of MNG is evidenced in humans in ^{123}I and ^{99}technetium scans, which show a complex mixture of normal, hyperfunctioning (hot), and hypofunctioning (cold) nodules in variable proportions, the functional status (eu-, hyper-, or hypothyroidism) of a given individual being determined by the overall functional balance of these nodules ([257](#)). At the microscopic level, epithelial cells of each AFU also show heterogeneity in growth, endocytic responses, and Tg synthesis ([319](#), [323](#)–[329](#)). For instance, only a small fraction of thyrocytes reacts with TSH via the formation of endocytic colloid droplets ([324](#), [325](#)). In addition, the production of Tg varies considerably between cells; the colloid content of each follicle depends on the cells producing the largest amounts of Tg ([72](#), [319](#), [323](#)). The considerable tissue heterogeneity is therefore the result of the phenotypic diversity of thyroid cells. This phenotypic diversity may be explained by the polyclonality theory, which postulates that for a given stimulus, cells react differently from their neighbors depending on their intrinsic properties, which reflect their polyclonal origin ([330](#)–[336](#)). The principle of polyclonality can be understood at the cellular level. Thus, some cells, even within the same AFU, could for instance enter the cell cycle more quickly than others in response to growth stimuli. But the same principle can also be understood at the level of the organ as a whole. Accordingly, a rising nodule may result from the coordinated replication of a cluster of follicles characterized by the same growth properties, which may differ from those of their neighbors. When this trait is amplified, the process may eventually give rise to nodules having their own specific morphological and functional properties ([201](#), [255](#)–[257](#), [260](#), [322](#), [337](#)). This is supported by a study that reported that the thyroid epithelium exhibits large embryonal patch size. According to this paper, cellular monoclonal zones that react to growth-regulating signals would be relatively large instead of being restricted as initially thought ([338](#)). Thus, when it is applied to an organ made of many large monoclonal cell patches, but with different somatic genetic properties, the principle of polyclonality could account for organ heterogeneity and explain the development of macroscopic nodules distributed among otherwise morphologically and functionally quiescent areas. These resting zones are composed of large AFUs lined with flat epithelium and filled with dense colloid-containing globules of compact Tg (see *Section III.B* and [Fig. 2](#)) ([157](#), [258](#), [259](#), [274](#)). Their adjacent vasculature is hypotrophic. In experimental models, they are considered to act as a functional reserve that works as a rescue system when needed. Of note, proteins of the apical pole (TPO, pendrin, and DUOX) are absent in these hypofunctioning follicles, as is TgI. Upon TSH stimulation, the progressive dilution of dense Tg globules into soluble Tg is accompanied by the reexpression of proteins involved in TH synthesis (TPO, DUOX, and pendrin) and the proliferation of endothelial cells, as demonstrated by the increased number of [^{3}H]thymidine-labeled endothelial nuclei ([157](#), [258](#), [259](#)). This morphological and functional awakening is necessarily followed by a stabilization phase mediated by locally generated regulatory factors such as TGF-ß that, on the one hand, limit cell growth and function but, on the other hand, facilitate the deposition of connective tissue, which may aggravate tissue heterogeneity ([208](#), [303](#), [333](#), [339](#), [340](#)). Over time, nodules of different size and aspect develop. Sometimes they degenerate and a cyst is formed with traces of hemorrhage and calcium deposits, and it is not unusual to also observe areas of focal lymphocytic infiltration. As in cold hypofunctioning nontransformed nodules, there is also a loss of TgI in thyroid cancers, but the causative defects differ. In cold hypofunctioning nontransformed nodules, NIS and TSH receptor expression persists, whereas the apical markers (pendrin, DUOX, and TPO) vanish ([224](#)). In cancers, NIS expression either disappears or increases in association with a mislocalization of the protein that is no longer retained in the basolateral membrane, which suggests that the decrease in I$^-$ uptake in thyroid carcinomas is due instead to alterations in NIS trafficking (reviewed in Ref. [54](#)). The presence of apical markers depends on the type of tumor. For instance, pendrin and TPO are still observed in follicular carcinomas, in contrast to papillary carcinomas where they are undetectable. This suggests that the defect in Tg iodination has different causes in nontransformed and

transformed tissues and in different types of carcinomas. Accordingly, the anti-TgI antibody ([159](#)) was shown to help the diagnosis of thyroid carcinomas, especially when the differential diagnosis with nontransformed follicular adenomas is difficult ([224](#)).

During tissue transformation, some nodules may acquire growth and functional advantages, as in autonomously functioning thyroid nodules. This is likely to be associated with an increased number of mutation events resulting from increased cell proliferation and functional activity (reviewed in Refs. [201](#), [256](#), [257](#), and [322](#)). As already mentioned, during thyroid hyperplasia, DNA damage occurs because of increased H_2O_2 production, which results in a higher mutation load, and H_2O_2-associated mutagenesis is now a well-established fact ([178](#), [199](#), [200](#), [202](#), [203](#), [213](#)). Some of these mutations (*ras* mutations or those in the RAS/RAF/MEK/ERK/MAP cascade) confer a growth advantage, as is sometimes seen in cold thyroid nodules, whereas others (*TSHr* and *Gsα* mutations) rather favor both cell growth and function, as seen in autonomously functioning thyroid nodules, because they result in constitutive activation of the cAMP cascade ([201](#), [256](#), [257](#), [341](#)). These small clones with activating mutations will proliferate further irrespective of intra- and extrathyroid mechanisms (*e.g.* TSH) and even after cessation of the initial stimulatory stimulus (for example, ID). In addition, some defects in genes directly involved in thyroid physiology and TH synthesis (Tg, TPO, NIS, TSH receptor, PDS, and DEHAL1) will also predispose the thyroid to the development of goiter, especially in areas of borderline or insufficient iodine supply (reviewed in Ref. [201](#)).

B. The stem cell theory

Currently, the most widely accepted theory of thyroid tissue heterogeneity, ranging from adenomas to MNGs and finally carcinomas, is based on the notion that this is caused by multiple molecular aberrations in well-differentiated thyrocytes, including epigenetic changes, profound alterations in chromosomes, and dysregulated signaling and regulatory pathways. An alternative, and perhaps complementary, explanation for thyroid heterogeneity was presented recently using the concept of adult stem cells ([342](#)–[348](#)). Adult stem cells are undifferentiated, quiescent, or slow cycling cells found in differentiated tissues ([349](#)). In addition to colon, skin, pancreas, liver, and brain, they have been reported in the human thyroid gland ([342](#)–[345](#)). Through asymmetric cell division, adult stem cells are capable of making identical copies of themselves, resulting in one self-copy and one precursor or progenitor cell that divides further, eventually giving rise to differentiated cells. Adult stem cells are characterized by their resistance to growth stimulation, which is explained by their close interactions with niche cells (reviewed in Refs. [350](#) and [351](#)). These cells protect stem cells from uncontrolled differentiation stimuli, apoptotic stimuli, and excessive proliferation. Under conditions where strict niche cell-induced control is reduced, stem cells gradually escape growth control and start dividing. Some progenitor cells, under the influence of locally produced growth factors, may then proliferate faster than the surrounding differentiated thyrocytes to form nodules or hyperplastic lesions. If progenitor cells are not fully differentiated, they could serve as the starting point of cold nonfunctioning nodules ([347](#)). A paper by Lan *et al.* ([344](#)) in 2007 indicated that thyroid adult stem cells can be isolated directly from primary thyroid cultures as three-dimensional spheres (called thyrospheres) in a medium enriched with EGF and FGF, but only under serum-free medium conditions and in the absence of TSH (*i.e.* in conditions that reflect differentiated cell apoptosis). When isolated from these nonadherent, three-dimensional spheres, these stem cells can then differentiate into thyrocytes (with the ability to express *PAX8*, *Tg*, *NIS*, *TSHr*, and *TPO* mRNA and to show TSH-dependent ^{125}I uptake) when they are incubated into serum-enriched medium containing TSH, even though no TH secretion was found in this model ([344](#)) or in models of mouse embryonic stem cells ([352](#), [353](#)). Whether these stem cell-derived thyrocytes can reconstitute functional AFUs is currently unknown. Recently, thyroid cancerous stem cells derived from mutated adult stem cells or their progenitor cells were reported in papillary, follicular, and undifferentiated thyroid cancers ([12](#), [347](#), [348](#), [354](#)–[357](#)). Their identification is important because they could account for the aggressiveness and recurrence of undifferentiated carcinomas and for their resistance to radioiodine treatment and conventional chemo and radiotherapy ([12](#), [347](#), [348](#)). Further investigations are necessary to assemble into one coherent model current knowledge about the events affecting differentiated thyroid cells and stem cells.

VI. Concluding Remarks

The thyrocyte is a specialized cell that adapts itself continuously to fulfill its main function, that is, TH synthesis, a potentially hazardous biochemical reaction, while optimizing the uptake of an essential trace element, iodine. Oxidizing and coupling reactions occur at the interface with the colloid and are regulated in such a way that H_2O_2 and ROS production are tightly controlled. ROS are more than by-products of aerobic respiration; they are key players in thyroid function, and thyrocytes are well equipped to deal with ROS by virtue of their potent detoxifying

systems. When the synthesis complex is altered, oxidation reactions start to occur in the cytoplasm with devastating consequences, such as morphological and functional breakdown, which engender disease processes, including those of an autoimmune or neoplastic nature.

A crucial function that thyrocytes must perform on a daily basis is the constant incorporation of iodine into Tg. To face daily variations in iodine supply, each thyrocyte uses various intra- and extrathyroidal TSH-independent self-regulatory mechanisms, including the regulation of its own microvasculature. In this context, the structural and functional unit of the thyroid includes the adjacent microvasculature in the three-dimensional structure called AFU. According to this concept, the vascular plasticity acts in conjunction with the multiple homeostatic processes that control TH synthesis. ID-associated vascular responses occur in two successive phases: the first early phase involves a TSH-independent ROS/HIF/VEGF pathway, whereas the second delayed phase starts when TSH plasma levels rise; however, the nature of the intracellular sensor that triggers the quick vascular remodeling in reaction to ID is still unknown. It is worth investigating the identity of this trigger, because AFU must tightly control the adjacent vascular response at all times. It is easy to understand how strategic this control must be for the thyroid to avoid anarchic and uncontrolled growth of the vascular compartment. Such strict control of the endothelial compartment by epithelial cells, irrespective of TSH, is an inherent property of healthy AFU and could explain why so many *in situ* microcancers are quiescent in the thyroid gland. Recent evidence suggests that ID regulates vascular changes in thyroid carcinomas through VEGF, but in a way different from that in nonmalignant tissues or at least using an alternative sequence of events. This suggests that altered autocrine-paracrine signals in unstructured AFUs facing ID may induce an angiogenic switch that leads to uncontrolled cellular proliferation.

When protective measures become insufficient for thyrocytes to sustain TH synthesis, TSH leaps into action along with many other locally generated growth and vascular regulatory factors in very dense and complex paracrine networks, resulting in the emergence of a goiter. Although goiter growth is controlled in time and space, it is highly heterogeneous from inception because of the polyclonal origin of the cells. Evidence suggests that the concept of polyclonality accounts for organ heterogeneity and the growth of nodules distributed among morphologically and functionally quiescent areas. These zones correspond to tissue reserves that become easily accessible upon TSH stimulation. This morphological and functional awakening is always followed by a stabilization phase attributable to locally generated regulatory factors, such as TGF-ß that, on one hand, limit cell growth and function but, on the other hand, facilitate the deposition of connective tissue, which may aggravate the heterogeneous character of the thyroid tissue.

To conclude, the thyroid gland has somehow succeeded during its phylogenic maturation in squaring the circle between iodine uptake, TH synthesis, and protection against ROS by setting up highly efficient structures, termed AFUs, which enable it to face its own functional exigencies. This morphological and functional integration warrants an adequate TH supply, at any time and whenever necessary.

Acknowledgments

Footnotes

Abbreviations:

AFU Angiofollicular unit
AHDS Allan-Herndon-Dudley syndrome
Cav-1 caveolin-1
DEHAL1 dehalogenase 1
DIT di-iodotyrosine
DLL Delta-like ligand
DUOX1 dual oxidase 1
DUOXA2 DUOX maturation factor 2
EFP1 EF-hand binding protein 1
EGF epidermal growth factor
ER endoplasmic reticulum
FGF fibroblast growth factor

HIF-1α hypoxia-inducible factor-1α

ID iodine deficiency

MCT8 monocarboxylate transporter 8

MIT mono-iodotyrosine

MNG multinodular goiter

NADPH reduced nicotinamide adenine dinucleotide phosphate

NIS sodium-iodide symporter

NOX adenine dinucleotide phosphate oxidase

OS oxidative stress

PDI protein disulfide isomerase

PDS Pendred syndrome

ROS reactive oxygen species

SLC5A5 soluble carrier 5A5

Tg thyroglobulin

TgI antibody against iodinated Tg containing T$_4$ at the N-terminal hormonogenic site

TH thyroid hormone

TPO thyroid peroxidase

TR TH receptor

VEGF vascular endothelial growth factor.

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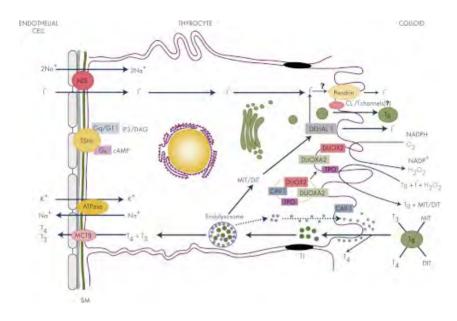
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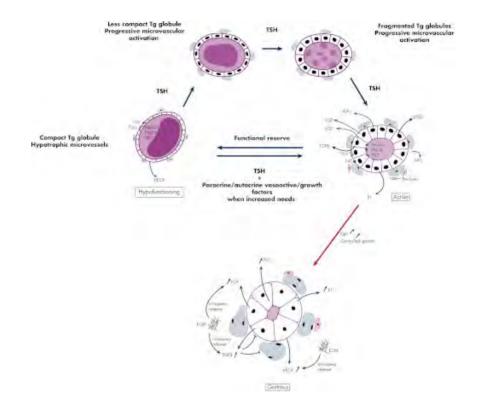
Figures and Tables

Figure 1.

The intrathyroidal journey of iodide. Iodide (I^-) entering the thyroid gland through fenestrated microvessels must sequentially cross the two basal laminae of endothelial and epithelial cells to be transported into the thyrocyte by NIS, which uses the energy provided by a Na^+-K^+-ATPase. Once inside the cell, I^- rapidly goes to the apical surface (according to a process that still remains elusive) where it is transported across the apical membrane by pendrin, even though other apical transporters have been proposed to be part of this process. I^- is readily oxidized by TPO in the presence of H_2O_2 and incorporated into tyrosine residues of Tg to form MIT and DIT, which are coupled, again by TPO, to form T_3 and T_4. H_2O_2 is produced by DUOX2 in coordination with DUOXA2. TH biosynthesis occurs at the interface with the colloid in a harmless environment for the cell. Iodinated Tg is stored in the colloid until use (see Fig. 2). T_4 can be partly released extracellularly by cathepsins K and L. In the human, the uptake of Tg by thyrocytes occurs by micropinocytosis, which can be either nonspecific (fluid phase) or receptor mediated. After endocytosis, colloid droplets containing partly digested Tg are transported to the endolysosomal compartment for complete hydrolysis by lysosomal enzymes including cathepsins. THs are released into the circulation via basal TH transporters (including MCT8) and transported to their target tissues via binding transport proteins. Unused MIT and DIT are dehalogenated by DEHAL1, and most of the I^- is recycled into TH synthesis. The binding of TSH to its receptor activates both Gs and Gq proteins. *Green dots* represent colloid droplets with partially degraded Tg, and *blue dots* represent cathepsins. BM, Basal membranes; DAG, diacylglycerol; Gq/G11, guanine nucleotide-binding protein αq and α11 subunits; Gs, guanine nucleotide-binding protein α-subunit; IP3, inositol triphosphate; TJ, tight junctions; TSHr, TSH receptor.
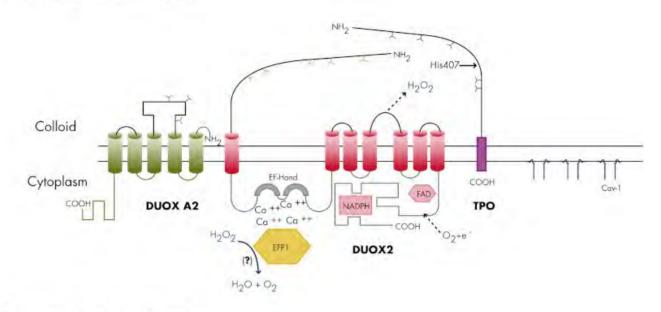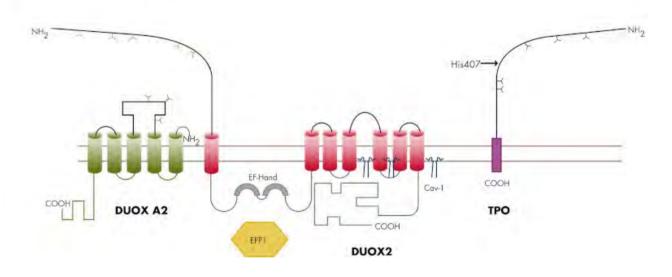
Figure 2.

Proposed mechanism of hypofunctioning follicle reactivation and processing of Tg globules upon TSH stimulation. In the normal human thyroid, active follicles coexist with many hypofunctioning follicles that are surrounded by hypotrophic capillaries and that contain compact Tg globules. These globules correspond to condensed Tg in covalently cross-linked form, not immediately available for TH synthesis, but eventually usable in case of increased hormonal needs. When this occurs, hypofunctioning follicles serve as a functional reserve that can be reactivated upon TSH stimulation. TPO, DUOX, and pendrin expression then reemerges along with progressive dilution of Tg globules into soluble Tg, under the action of secreted cathepsins. This occurs along with the expansion of adjacent microvessels, which, besides TSH, depends also upon many locally secreted growth and vasoactive factors. Persistent TSH stimulation induces thyrocyte hypertrophy and proliferation resulting in goitrous follicles with no more colloid Tg reserves. The microvascular reaction keeps evolving with the proliferation of endothelial cells, the fusion of adjacent microvessels, and the formation of larger microvessels. At this stage, in addition to being secreted by thyrocytes, VEGF, as well as FGF at least, could also function as crinopexins that are locally synthesized molecules sequestered in pericellular structures (extracellular matrix, cell surface, and proteoglycans) under a latent form (305). According to this theory, early alterations in local blood flow and in microvessel shape may alter the extracellular matrix, thereby provoking the release and the activation of crinopexins that could, in turn, stimulate endothelial cell proliferation. This would amplify local changes in the microvasculature initially originated from thyrocytes. Of note, VEGF expression is observed even in thyrocytes of hypofunctioning follicles. ECM, Extracellular matrix; ET, endothelins; TSHr, TSH receptor.

Figure 3.

A Active Synthesis Complex
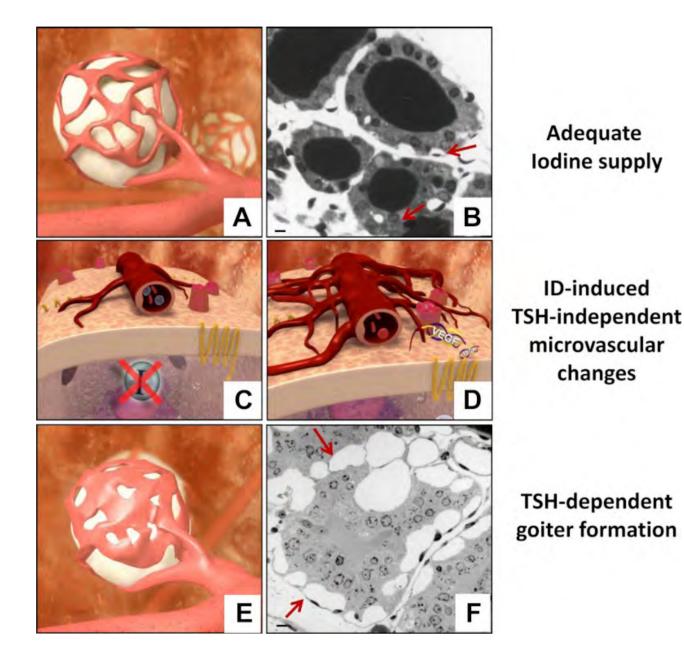

B Inactive Synthesis Complex

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The multiprotein complex involved in TH biosynthesis. A, Upon TSH stimulation, DUOX2 and TPO migrate into the plasma membrane where the complex is activated. TPO and DUOX2 are viewed as a producer-consumer unit that allows TPO to use H_2O_2 instantly to oxidize iodide (I^-) into iodine, to iodinate tyrosyl residues of Tg, and to couple iodotyrosyl residues to form T_3 and T_4, while protecting the cell against H_2O_2 possible oxidative damage. DUOX2 is represented here as a seven-transmembrane-domain glycoprotein with an extracellular NH_2-terminal peroxidase-like domain that appears essential for interaction with TPO, a long COOH-terminal region that contains the catalytic NADPH oxidase core along with the flavin adenine dinucleotide (FAD) and NADPH binding cavities, and two EF-hands in the first intracellular loop that are essential calcium binding sites. TPO is represented with a short intracellular COOH terminus and a long extracellular NH_2 terminus (90% of its amino acids) that exhibits a catalase-like activity in certain circumstances. His407 is involved in the covalent binding of the heme prosthetic group that is essential for enzyme activity. The manner in which TPO and DUOX2 interact makes the apical multiprotein complex perfectly suited to detoxify ROS and avoid H_2O_2 spillages. The local H_2O_2 concentration is kept under control by the TPO catalase-like action (which protects DUOX2, as long as both proteins are closely associated). In addition, the complex contains the thioredoxin-related protein EFP1, which interacts with the two EF-hands of DUOX2 and detoxifies H_2O_2 that is not readily used in the biosynthesis process. EFP1 may also be involved in the maturation process of DUOX2. For this biochemical unit to be activated, DUOX2 must be fully glycosylated (Y, *N*-glycosylation sites) following successive maturation steps in the ER and the Golgi apparatus. DUOX2 must also be associated with the

maturation factor DUOXA2, which is represented here as a five-transmembrane-domain protein with an extracellular loop between the second and the third transmembrane domains along with three glycosylation sites (Y). I^- and H_2O_2 regulate the activity of the enzymatic complex, depending on their local relative concentration. When I^- is present in adequate amounts, H_2O_2 is the limiting factor of the reaction and mediates the association between TPO and DUOX2. In thyroids with low intracellular I^- content, I^- instead exerts a stimulatory effect on H_2O_2 production. When I^- is present in excess, H_2O_2 synthesis is blocked. Because I^--induced effects are inhibited by methimazole or propylthiouracil, this indicates that I^- acts through oxidized species. B, In resting conditions, the complex TPO-DUOX2-DUOXA2 is kept inactive underneath the apical membrane. Cav-1 is required to keep DUOX2 quiescent, because it may interact with domains in the cytoplasmic region of the molecule that are putative sites for Cav-1 scaffolding domains (following a hypothesis that still needs to be proved). According to the most plausible explanation, Cav-1 would come off the enzyme complex after its incorporation into the apical membrane. The absence of Cav-1 is responsible for mislocalization and premature activation of the complex in the cytoplasm, creating potentially devastating consequences due to increased OS and/or lack of efficient antioxidant mechanisms naturally activated when I^- organification and coupling reactions normally occur in apical microvilli.
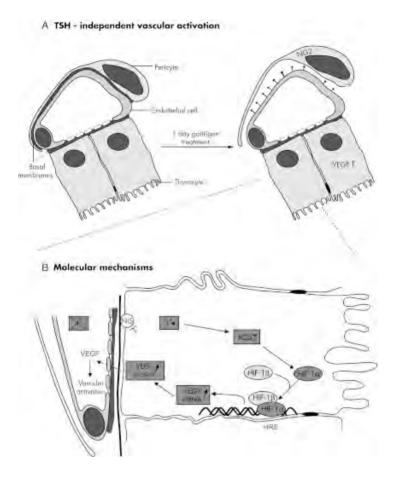
Figure 4.

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The concept of AFUs in the thyroid gland. The thyroid gland is composed of many AFUs. They are individualized entities with their own genotypic and phenotypic assets under the local control of a host of autocrine and paracrine factors, as well as exerted by TSH. A and B, Each unit is composed of thyrocytes gathered in a follicle that is surrounded by capillaries made of endothelial cells and pericytes independent from their neighbors. In unstimulated conditions (*i.e.* in conditions of adequate iodine supply), the vascular bed covers about 20–50% of the follicle surface (this AFU corresponds to the so-called active follicle in Fig. 2). C and D, As soon as the intracellular iodide (I^-) content drops, the thyrocytes react immediately by secreting VEGF, which triggers an early and modest TSH-independent microvascular reaction. If the increased local clearance of I^- associated with this compensatory mechanism is not enough to preserve TH synthesis, TSH plasma levels start rising. Upon TSH stimulation, a more robust but always tightly controlled microvascular reaction occurs. E and F, By the end of the process, the area occupied by the expanded vascular bed may cover up to 80% of the follicle surface made of hyperplastic and hypertrophic thyrocytes. *Scale bar*, 10 μm. *Arrows* indicate microvessels.

Figure 5.

Proposed mechanism of early ID-induced TSH-independent microvascular reaction in thyroid AFUs. A, TSH-independent microvascular activation. In unstimulated AFUs (*left*), capillaries close to thyrocytes are fenestrated and surrounded by pericytes. Endothelial cells and pericytes are separated only by fused basal membranes. If iodide (I⁻) supply drops, independently from any stimulation by TSH (*right*), VEGF expression increases in thyrocytes, which induces a microvascular activation, implying, as initial step, the detachment of chondroitin sulfate proteoglycan 4 (NG2)-expressing pericytes from endothelial cells. B, Molecular mechanisms. When I⁻ supply is altered or when its transport is blocked, the thyrocyte intracellular content drops rapidly, thereby increasing ROS, which leads to the stabilization of HIF-1α. The exact nature of ROS and where they are produced still deserve further investigation. HIF-1α heterodimerizes with HIF-1β, which is constitutively present in the cell. The HIF-1α/HIF-1β heterodimer, after binding to the hypoxia response element (HRE) site of the promoter region of the VEGF gene, turns on its transcription. As a result, VEGF mRNA and protein expression increases. VEGF is then secreted and activates adjacent endothelial cells and pericytes, thereby leading to microvascular activation, increased blood flow, and proliferation of endothelial cells and, in turn, to increased local clearance of I⁻. When this and other protective measures become insufficient to safeguard TH synthesis, TSH enters into the action, in coordination with many other locally generated growth and vascular regulatory factors.