The iodide-containing thyroid hormones T₃ and its precursor T₄ are crucial for normal development, growth, and regulation of numerous metabolic pathways. The main function of the thyroid gland is to concentrate iodide and to make it available for biosynthesis of thyroid hormones. The significance of this mechanism is evident in light of the scarcity of iodine in most of the environment and the fact that insufficient dietary supply of iodide remains a major public health issue in many parts of the world (1).

The synthesis of thyroid hormones requires a normally developed thyroid gland, an adequate nutritional intake of iodide, and a series of sequential biochemical steps. Thyroid hormone synthesis takes place in the follicles, the functional units of the gland (2). Each follicle consists of a single layer of thyroid epithelial cells surrounding the follicular lumen. The follicular lumen is filled with colloid, which is predominantly composed of thyroglobulin, a large glycoprotein that serves as the scaffold for thyroid hormone synthesis (3).

The synthesis of thyroid hormones requires uptake of iodide across the basolateral membrane into the thyrocytes, transport across the cell, and efflux through the apical membrane into the follicular lumen. Uptake of iodide is mediated by the sodium-iodide symporter (NIS), which cotransports two sodium ions along with one iodide ion, with the sodium gradient serving as the driving force (Fig. 1) (1). The energy required to produce the sodium gradient is provided by the ouabain-sensitive Na⁺/K⁺-ATPase (4). The efflux of iodide across the apical membrane is mediated, at least in part, by pendrin (5). Once iodide reaches the cell-colloid interface, it is oxidized and rapidly organified by incorporation into selected tyrosyl residues of thyroglobulin. This reaction, referred to as organification, is catalyzed by thyroid peroxidase (TPO) in the presence of hydrogen peroxide and results in the formation of mono- and diiodotyrosines (MIT and DIT). The generation of hydrogen peroxide is mediated by the calcium-dependent reduced nicotinamide adenine dinucleotide phosphate (NADPH) dual oxidase type 2 (DUOX2), which oxidizes hydrogen peroxide to form diiodotyrosines (MIT and DIT) (6). The generation of hydrogen peroxide is mediated by the calcium-dependent reduced nicotinamide adenine dinucleotide phosphate (NADPH) dual oxidase type 2 (DUOX2), which oxidizes hydrogen peroxide to form diiodotyrosines (MIT and DIT). The generation of hydrogen peroxide is mediated by the calcium-dependent reduced nicotinamide adenine dinucleotide phosphate (NADPH) dual oxidase type 2 (DUOX2), which oxidizes hydrogen peroxide to form diiodotyrosines (MIT and DIT). The generation of hydrogen peroxide is mediated by the calcium-dependent reduced nicotinamide adenine dinucleotide phosphate (NADPH) dual oxidase type 2 (DUOX2), which oxidizes hydrogen peroxide to form diiodotyrosines (MIT and DIT). The generation of hydrogen peroxide is mediated by the calcium-dependent reduced nicotinamide adenine dinucleotide phosphate (NADPH) dual oxidase type 2 (DUOX2), which oxidizes hydrogen peroxide to form diiodotyrosines (MIT and DIT). The generation of hydrogen peroxide is mediated by the calcium-dependent reduced nicotinamide adenine dinucleotide phosphate (NADPH) dual oxidase type 2 (DUOX2), which oxidizes hydrogen peroxide to form diiodotyrosines (MIT and DIT).
The molecular characterization of NIS began in 1996 when the cDNA encoding rat NIS was isolated by expression-cloning in Xenopus laevis oocytes (6). Subsequently, the human cDNA has been cloned by a RT-PCR approach taking advantage of the homology to rat NIS (8). Rat NIS is predicted to have 618 amino acids with a relative molecular mass of 65,196 Daltons (6), and human NIS contains 643 amino acids and exhibits an 84% amino acid identity and 93% similarity to rat NIS (9). The human NIS gene is located on chromosome 19p12-13.2 and contains 14 introns and 15 exons (9).

NIS (SLC5A5) belongs to the solute carrier family 5A (SLC5A). All members of this protein family depend on an electrochemical sodium gradient as the driving force for transport of anions across the plasma membrane (10). The current secondary structure model predicts that NIS contains 13 transmembrane domains with the amino terminus located extracellularly and the carboxy terminus facing the cytosol (1). The mature NIS protein is approximately 87 kDa in size and has three asparagine-linked glycosylation sites (1). Glycosylation does not seem to be required for stability, activity, or targeting of the NIS molecule to the plasma membrane (11). The expression of NIS is differentially regulated and is subjected to various posttranslational modifications in each tissue in which it is expressed (1, 12).

In addition to thyroid follicular cells, NIS is expressed in several other tissues, including the salivary glands, gastric mucosa, and the lactating mammary gland, where it mediates active transport of iodide (1). In the lactating mammary gland, NIS plays an important role by concentrating iodide in the milk, thereby supplying newborns with iodide for thyroid hormone synthesis.

Although NIS has a high affinity for iodide, it is able to mediate transport of other ions (13). Large anions such as thiocyanate and perchlorate can inhibit accumulation of iodide in the thyroid by competing with iodide (14, 15). Perchlorate is 100 times more potent than thiocyanate in inhibiting iodide accumulation in the thyroid (1). The ability of perchlorate to block iodide transport has been used in the therapy of hyperthyroidism, and it is used in the perchlorate discharge test, which serves to detect defects in iodide organification (16). In normal individuals, administration of perchlorate blocks subsequent accumulation of iodide in the thyrocytes but does not cause any discharge of previously accumulated radioactive iodine because of its rapid organification. In contrast, in individuals with a total or partial iodide organification defect, administration of perchlorate results in the rapid release of the unorganified fraction of the tracer from the thyrocytes (16). Until recently, it has been controversial whether perchlorate acts as a blocker or as a substrate that is transported by NIS (13, 17, 18). Two recent studies provide evidence that perchlorate is actively transported by NIS (19, 20). Perchlorate transport could be demonstrated in a polarized cell system (19) as well as by direct measurement with mass spectrometry (20). Moreover, it has been shown that perchlorate, a widely found pollutant, is transported into the milk (19). Remarkably, the stoichiometry of the NIS-mediated Na⁺/Cl⁻ transport is electroneutral, which contrasts with the electrogenic transport of iodide (two Na⁺ and one I⁻) (19). These findings indicate that NIS is able to transport different substrates with distinct stoichiometries (19).
Regulation of NIS protein expression

TSH is the major regulator of thyroid cell proliferation, differentiation, and function, including iodide uptake (21). The effects of TSH are primarily mediated through the activation of the cAMP cascade via the GTP-binding protein Gsα (22). TSH stimulates iodide accumulation by positively regulating NIS expression at the protein and mRNA level via the cAMP pathway (23). Hypophysectomized rats with low circulating levels of TSH have a decreased protein expression of NIS, whereas a single injection of TSH leads to a prompt increase in NIS expression (24). Rats maintained on an iodide-deficient diet or treated with propylthiouracil, an agent blocking iodide organification, have high concentrations of TSH, which correlates with an increase in NIS protein expression. These findings are in agreement with the results obtained in human thyroid primary cultures (25, 26) and the rat thyroid FRTL-5 cell line (23). In FRTL-5 cells, withdrawal of TSH results in a decrease in intracellular cAMP concentration and iodide uptake activity (23). Re-addition of TSH increases NIS mRNA and protein expression and subsequently restores iodide uptake activity.

Recent studies have shown that TSH not only regulates NIS transcription and biosynthesis but also mediates NIS activity by posttranscriptional mechanisms (27). In the presence of TSH, NIS is active and inserted in the basolateral membrane of thyrocytes (27). Upon TSH withdrawal, NIS protein half-life decreases from 5 to 3 d, and it translocates from the plasma membrane to intracellular compartments (27). Of yet, the mechanisms regulating the subcellular distribution of NIS are only partially elucidated. It is known that NIS has several consensus sites for kinases, including protein kinase A (PKA) and protein kinase C (1). Although it has been shown that NIS is phosphorylated in vivo (27), the functional significance of this modification needs further characterization. A more recent study identified five amino acid residues in NIS that are phosphorylated in vivo, but the phosphorylation status of these amino acid residues does not affect targeting of NIS to the plasma membrane (28). NIS contains several sorting sequences that are known to play a role in targeting, retention, and endocytosis of other membrane proteins (29). For example, the PDZ motif (T/S-X-V/L) located at the carboxy terminus of NIS is one of the sequences involved in protein-protein interactions (1). The PDZ motif is recognized by PDZ-binding proteins that have been implicated in internalization of other transporters (29). NIS also has a dileucine motif, L(37)L(538), which is known to interact with the clathrin-coated system (30). This interaction leads to incorporation of integral membrane proteins into coated vesicles that are then carried to different destinations within the cell (31).

Iodide is another factor that can regulate iodide accumulation in the thyroid. In 1948, Wolff and Chaihoff (32) reported that high doses of iodide block iodide organification in the rat thyroid in vivo. This phenomenon, known as the acute Wolff-Chaikoff effect, is a reversible process, because iodide organification resumes when the iodide concentration in the serum decreases. The mechanisms underlying the Wolff-Chaikoff effect are complex and involve acute regulation of several key genes and proteins within the thyrocytes. Several studies have examined the effect of iodide on NIS mRNA and protein expression in vivo and in vitro (33–35). In vivo data suggest that high concentrations of iodide lead to reduction in both NIS mRNA and protein levels, partially by a transcriptional mechanism. In vitro results suggest that exposure to high doses of iodide results in a decrease in NIS protein levels that is, at least in part, due to an increase in NIS protein turnover (33–35).

Congenital iodide transport defect (ITD)

Biallelic mutations in the NIS gene cause a congenital ITD. ITD is an autosomal recessive condition characterized by hypothyroidism, goiter, reduced or absent thyroid uptake of radioiodide, and a low saliva/plasma iodide ratio (1, 36). Currently, at least 12 ITD-causing mutations of NIS have been identified (37). Six of these mutations, namely 226delH, T354P, G395R, Q267E, G543E, and V59E have been characterized more thoroughly (38–41). The G543E substitution leads to retention of NIS in intracellular compartments as a result of improper maturation and trafficking of the protein, the other mutants are still targeted to the membrane but result in a loss of function (38–41). Structural and functional analysis of the T354P mutant protein demonstrated that a hydroxyl group at the β-carbon of the residue at position 354 is crucial for proper NIS function (38). Substitutions of the glycine residue at position 395 residue with several amino acids indicated that the presence of a small and an uncharged amino acid residue at this position is required for NIS function (39). Lastly, a recent study revealed that the histidine residue at position 226 is important for the iodide transport activity of NIS (37).

Pendrin

Pendrin is a highly hydrophobic membrane protein located at the apical membrane of thyrocytes (2, 4). In addition to the thyroid, pendrin is also expressed in the kidney and in the inner ear (42, 43). In the kidney, pendrin plays an important role in acid-base metabolism as an exchanger of chloride and bicarbonate in β-intercalated cells (44). In the inner ear, pendrin is important for generation of the endocochlear potential (45).

Pendrin belongs to the SLC26A family, which includes several anion transporters, as well as the motor protein prestin that is expressed in outer hair cells (46, 47). Pendrin is encoded by the SLC26A4 gene, which was cloned in 1997 (48). The SLC26A4 gene is located on chromosome 7q21-31 and contains 21 exons with an open reading frame of 2343 bp (48). Pendrin is a glycoprotein composed of 780 amino acids (2). It contains three putative extracellular asparagine-glycosylation sites (4, 49). Pendrin usually appears as a single protein band with a molecular mass of 110–115 kDa when isolated from human thyroid membranes (49). Pendrin is proposed to have 12 transmembrane domains with both amino and carboxy termini located inside the cytosol (4, 50). Like other members of the SLC26A family, pendrin contains a so-called STAS (sulfate transporter and antisigma factor antagonist) domain (51). The
exact function of this domain has not been elucidated. Recent studies, however, suggest that the STAS domain can interact with the regulatory domain of CFTR (cystic fibrosis transmembrane conductance regulator) in certain epithelial cells (52–54).

**Pendred syndrome**

Mutations in the *SLC26A4* gene lead to Pendred syndrome (2). Pendred syndrome is an autosomal recessive disorder characterized by sensorineural deafness, goiter, and a partial defect in iodide organification (55, 56). Deafness or hearing impairment is the leading clinical sign of Pendred syndrome (56). In many patients, hearing loss is prelingual; in some individuals, however, the hearing loss develops later in childhood (57). Patients with Pendred syndrome display an enlarged endolymphatic sac and duct (58–60). A subset of patients presents with a so-called Mondini defect, which is characterized by replacement of the cochlear turns by a single cavity or a rudimentary cochlea (57, 58). Variability in the hearing loss observed in patients with *SLC26A4* mutations suggests that the phenotype is influenced by environmental factors and/or genetic modifiers (61).

Goiter usually develops during childhood. There is, however, a substantial variation within and between families and different geographic regions (62–64). Nutritional iodide intake appears to play an important role as a modifier of the thyroidal phenotype (65, 66). Under conditions of high iodide intake, most individuals have no or only a mild enlargement of the thyroid (60, 67). If the nutritional iodide is scarce, patients with Pendred syndrome not only develop goiter but may also present with mild or overt hypothyroidism (68, 69).

Mutations in the *SLC26A4* gene, found in patients with Pendred syndrome, are highly heterogeneous (56). Currently, more than 150 mutations of the *SLC26A4* gene have been reported (56). Most of the mutations are missense mutations, and a smaller number of mutations consists mainly of nonsense and intronic mutations (56). Loss of function of some of the mutants results from the retention of the mutated and misfolded protein in intracellular compartments, most likely the endoplasmic reticulum (70, 71).

**Role of pendrin in the thyroid**

Initial functional studies of pendrin in *Xenopus* oocytes have demonstrated that pendrin is able to mediate transport of chloride and iodide (5) and that it can act as a chloride/formate exchanger (72). The ability of pendrin to mediate iodide efflux (5), the localization of pendrin at the apical membrane of thyrocytes (4, 73), as well as the defect in iodide organification observed in patients with Pendred syndrome (62, 74), suggested that pendrin could function as an apical iodide transporter in thyroid cells (46). The results obtained from a number of independent studies performed in heterologous systems support the role of pendrin in mediating, at least in part, apical iodide efflux. Yoshida *et al.* (75) have demonstrated that iodide efflux is much higher in nonpolarized Chinese hamster ovary cells expressing NIS and pendrin than in cells expressing NIS alone. Electrophysiological studies with transfected COS-7 cells also indicate that pendrin mediates iodide transport and that it is more efficient at high extracellular concentrations of chloride (76). In addition, iodide efflux/chloride influx appears to be more efficient than chloride efflux/iodide influx (76). These findings are consistent with results obtained in polarized Madin-Darby canine kidney (MDCK) cells (50). MDCK cells expressing NIS and pendrin independently or simultaneously were cultured in a bicameral system, which allowed measuring iodide uptake at the basolateral membrane and iodide efflux at the apical membrane. Cells transfected with NIS alone have a significant increase in intracellular iodide uptake compared with untransfected control cells. In contrast, cells expressing NIS and pendrin show a significant increase in iodide transport into the apical chamber and consequently a significant decrease in the intracellular iodide content. These findings support the notion that pendrin may have a role in facilitating vectorial iodide transport at the apical membrane (50). The partial organization defect found in patients with Pendred syndrome suggests, however, that iodide can reach the follicular lumen independently of the presence of pendrin.

**Questions concerning the role of pendrin as an apical iodide transporter**

The traditional concept held that iodide simply crosses the apical membrane due to the electrochemical gradient that is present between the cytosol and the follicular lumen (77). However, autoradiography studies revealed that iodide first accumulates in the cytosol and subsequently moves to the follicular lumen (78). This transport of iodide across the apical membrane is rapidly stimulated by TSH (64, 79). Hence, it has been proposed that apical iodide efflux is mediated through a specific transporter or channel. This concept has risen based on several findings. Electrophysiological studies performed with thyroid membrane vesicles suggested the presence of two apical iodide transporters (80). Functional studies performed in heterologous cells, including polarized cells (50, 75, 76, 81), along with the iodide organization defects found in patients with Pendred syndrome (62, 74), suggested that pendrin mediates apical iodide efflux in thyrocytes. The physiological role of pendrin has, however, been questioned for several reasons (77). Patients with biallelic mutations in the *SLC26A4* gene display a mild or no thyroidal phenotype under conditions of sufficient iodide intake (65). The pendrin knockout mice, studied under normal iodide intake conditions, do not develop a goiter or abnormal thyroid hormone levels (45). In addition, it is intriguing that pendrin may have distinct roles in the thyroid, the inner ear, and the kidney (77). This led to the proposal that pendrin may be a part of multiprotein complex, the composition of which may vary among different cell types, thereby potentially explaining a variability in anion selectivity of pendrin (77). Other proteins (SLC5A8 and chloride channel 5 CICN5) have been proposed to mediate apical iodide efflux (82, 83). Functional studies performed in *Xenopus* oocytes and polarized MDCK cells clearly demonstrate that
SLC5A8, originally designated as human apical iodide transporter (hAIT) (82), does not mediate iodide uptake or efflux (84). Localization of the ClCn5 protein at the apical membrane of thyrocytes and a thyroidal phenotype of the ClCn5-deficient mice that is reminiscent of Pendred syndrome suggest that ClCn5 could be, possibly in conjunction with other chloride channels, involved in mediating apical iodide efflux or iodide/chloride exchange (83). This possibility has, as of yet, not been corroborated by further experimental data.

**Regulation of pendrin expression**

TSH stimulates iodide efflux across the apical membrane of thyrocytes (79, 85, 86). After exposure to TSH, iodide efflux is rapidly stimulated in FRTL-5 cells (85) and in polarized porcine thyrocytes (79, 86). In polarized porcine thyrocytes grown in a bicameral system, measurement of iodide transport in both directions demonstrates that TSH up-regulates iodide efflux at the apical membrane, whereas efflux across the basolateral membrane does not change (79). Treatment of rat thyroid PCC13 cells with TSH for a short time results in the rapid translocation of pendrin from intracellular compartments, specifically endosomes, to the plasma membrane, thus suggesting a role of pendrin in rapid regulation of apical iodide efflux (87). Insertion of pendrin in the apical membrane correlates with the phosphorylation of pendrin, but it remains unknown whether phosphorylation is necessary or sufficient for this translocation. These events occur through the PKA pathway and can be inhibited by H89, a specific PKA inhibitor (87). Interestingly, translocation of pendrin from the cytosol to the plasma membrane through a PKC-dependent pathway has been demonstrated after exposure of cultured rat thyroid cells to insulin for 10, 20, and 40 min (88).

At least in rat FRTL-5 cells, TSH does not significantly modify SLC26A4 gene expression (4). Interestingly, thyroglobulin has been shown to up-regulate SLC26A4 mRNA levels in FRTL-5 cells while suppressing expression of several thyroid-specific genes, including the TSH receptor, NIS, TPO, and TG genes (4). Treatment with iodide does not affect expression of the SLC26A4 gene (89). Exposure to thyroglobulin leads to a decreased NIS gene and protein expression and subsequently results in an reduced iodide uptake in vitro (90). In contrast, accumulation of thyroglobulin in the follicular lumen suppresses iodide uptake in vitro (90). It has been suggested that the inverse relationship between the concentration of thyroglobulin in the follicular lumen and iodide uptake in vivo may be important in the regulation of thyroid function under constant TSH levels (91) and promote iodide efflux into the follicular lumen (89).

**Conclusions**

- NIS mediates the active transport of iodide at the basolateral membrane of thyrocytes.
- Biallelic mutations in NIS cause a congenital iodide transport defect, an autosomal recessive condition, characterized by hypothyroidism, goiter, low thyroid iodide uptake, and low saliva/plasma iodide ratio.
- Pendrin is involved in the apical iodide efflux in thyroid cells. It can also exchange chloride and bicarbonate.
- Pendrin is encoded by the SLC26A4 gene. Biallelic mutations in the SLC26A4 gene cause Pendred syndrome, an autosomal recessive disorder, characterized by deafness, goiter, and impaired iodide organization.
- In the inner ear, pendrin plays a role in acid-base metabolism as a chloride/bicarbonate exchanger.
- In addition to pendrin, other apical iodide channels or transporters may be involved in regulation of apical iodide efflux in thyrocytes.

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