Molecular Analysis of the Sodium/Iodide Symporter: Impact on Thyroid and Extrathyroid Pathophysiology

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De La Vieja, Antonio, Orsolya Dohan, Orlie Levy, and Nancy Carrasco. Molecular Analysis of the Sodium/Iodide Symporter: Impact on Thyroid and Extrathyroid Pathophysiology. Physiol Rev 80: 1083–1105, 2000.—The Na⁺/I⁻ symporter (NIS) is an intrinsic membrane protein that mediates the active transport of iodide into the thyroid and other tissues, such as salivary glands, gastric mucosa, and lactating mammary gland. NIS plays key roles in thyroid pathophysiology as the route by which iodide reaches the gland for thyroid hormone biosynthesis and as a means for diagnostic scintigraphic imaging and for radioiodide therapy in hyperthyroidism and thyroid cancer. The molecular characterization of NIS started with the 1996 isolation of a cDNA encoding rat NIS and has since continued at a rapid pace. Anti-NIS antibodies have been prepared and used to study NIS topology and its secondary structure. The biogenesis and posttranslational modifications of NIS have been examined, a thorough electrophysiological analysis of NIS has been conducted, the cDNA encoding human NIS (hNIS) has been isolated, the genomic organization of hNIS has been elucidated, the regulation of NIS by thyrotropin and I⁻ has been analyzed, the regulation of NIS transcription has been studied, spontaneous NIS mutations have been identified as causes of congenital iodide transport defect resulting in hypothyroidism, the roles of NIS in thyroid cancer and thyroid autoimmune disease have been examined, and the expression and regulation of NIS in extrathyroidal tissues have been investigated. In gene therapy experiments, the rat NIS gene has been transduced into various types of human cells, which then exhibited active iodide transport and became susceptible to destruction with radioiodide. The continued molecular analysis of NIS clearly holds the potential of an even greater impact on a wide spectrum of fields, ranging from structure/function of transport proteins to the diagnosis and treatment of cancer, both in the thyroid and beyond.
I. INTRODUCTION

The thyroid is a master endocrine gland that plays a central role in the intermediary metabolism of virtually all tissues and is of fundamental importance for the development of the central nervous system in the fetus and the newborn. The widespread effects of the thyroid result from the biosynthesis and secretion of two rather unique hormones, triiodothyronine (T₃) and thyroxine (or tetraiodothyronine) (T₄), the only iodine-containing hormones in vertebrates. Iodide (I⁻) is an essential constituent of T₃ and T₄ so that both thyroid function as a whole and its systemic ramifications depend on an adequate supply of I⁻ to the gland. A remarkably efficient and specialized system has evolved in the thyroid that ensures that most of the ingested dietary I⁻ (the only source of I⁻) is accumulated in the gland and thus made available for T₃ and T₄ biosynthesis. The significance of this becomes more apparent when one considers that I⁻ is scarce in the environment. Endemic goiter and cretinism caused primarily by insufficient dietary supply of I⁻ remain a major health problem in many parts of the world, affecting millions of people (24). I⁻ deficiency still often leads to various degrees of impaired brain development, mostly in populations of children living in poor regions (114). These public health problems could conceivably be solved relatively easily by ensuring that all table salt consumed in the affected areas is iodized, as has been done in many countries. However, the sociopolitical realities of the affected regions have prevented solutions like this from being implemented, at great human cost. Still, this situation dramatizes not only the health value of I⁻ as a nutrient and the consequences to society of its environmental scarcity, but also underscores how difficult it would be for people to stay euthyroid and healthy in the absence of a specialized I⁻ transport mechanism in the thyroid.

The ability of thyroid follicular cells to concentrate I⁻ was first reported as early as 1915 (68). The thyroid gland was found to be capable of concentrating I⁻ by a factor of 20–40 with respect to its concentration in the plasma under physiological conditions. Hence, the existence of a thyroid I⁻ transporter was inferred, and some of its properties were elucidated over the years (see Refs. 16, 42, 43, 61, 63, 98, 107 and 125 for reviews). Briefly, I⁻ accumulation in the thyroid has long been shown to be an active transport process that occurs against the I⁻ electrochemical gradient, stimulated by thyrotropin (TSH), and blocked by the well-known “classic” competitive inhibitors, the anions thiocyanate and perchlorate. Eventually, it was determined that the thyroid I⁻ transporter is a Na⁺/I⁻ symporter (NIS) (6, 9, 45, 79, 122), i.e., an intrinsic plasma membrane transport protein that couples the inward “downhill” translocation of Na⁺ to the inward “uphill” translocation of I⁻ (Fig. 1). The driving force for the process is the inwardly directed Na⁺ gradient generated by the Na⁺-K⁺-ATPase. The ability of the thyroid to accumulate I⁻ via NIS has long provided the basis for diagnostic scintigraphic imaging of the thyroid with radioiodide and has served as an effective means for pharmacological doses of radioiodide to target and destroy hyperfunctioning thyroid tissue, such as in Graves’ disease or I⁻-transporting thyroid cancer and its metastases (23, 124). Therefore, the study of NIS is of great relevance to thyroid pathophysiology. Nevertheless, no molecular information on NIS was available until 1996, when after a decades-long search by numerous investigators, a cDNA encoding rat NIS was finally isolated by expression cloning in Xenopus laevis oocytes (21). This development, a major breakthrough in the study of I⁻ transport processes and thyroid physiology, marked the beginning of the molecular characterization of NIS.

On the basis of the cloned cDNA, rat NIS was determined to be a protein of 618 amino acids (relative molecular mass 65,196) (Fig. 2). The hydrophatic profile and initial secondary structure predictions (20, 21) of the protein suggested an intrinsic membrane protein with 12 putative transmembrane segments. The NH₂ terminus was originally placed on the cytoplasmic side, given the absence of a signal sequence. The COOH terminus, which was also predicted to be on the cytoplasmic side, was found to contain a large hydrophilic region of ~70 amino acids within which the only potential canonical cAMP-dependent protein kinase A (PKA) phosphorylation sequence of the molecule was located (positions 549–552). Three potential Asn-glycosylation sites were identified in the deduced amino acid sequence at positions 225, 485, and 497. The first was located in a predicted intracellular hydrophilic loop, while the last two were located in the last hydrophilic loop, a segment predicted to be on the extracellular face of the membrane. The length of the 12 transmembrane segments in this original model ranged from 20 to 28 amino acid residues, except transmembrane segment V, which contained 18 residues. Only three charged residues were predicted to lie within transmembrane segments, namely, Asp-16 in transmembrane segment I, Glu-79 in transmembrane segment II, and Arg-208 in transmembrane segment VI. Out of a total of eight Trp residues found in the membrane, six were located near the extremes of transmembrane segments. Four Leu residues (positions 199, 206, 213, and 220) appeared to comprise a putative leucine zipper motif in transmembrane segment VI. This motif, which has been proposed to play a role in the oligomerization of subunits in the membrane, has been conserved in all cloned neurotransmitter transporters (20, 21). NIS is often regarded to be a member of the Na⁺/glucose cotransporter (SGLT1) family of transporters. For a detailed review of this family of transporters, see Reference 113a.

The molecular characterization of NIS has proceeded at an astounding pace with a wide variety of
approaches and techniques, leading to numerous reports in just the last 3 years (61, 63, 98, 107). This review provides an overview of the most recent developments on the molecular analysis of NIS, among which are the following: anti-NIS antibodies have been prepared and used to study the topology of NIS and to experimentally test the proposed NIS secondary model so that some aspects of the above-described model have been experimentally confirmed and others revised. The most recent revised secondary structure model for NIS proposes 13 (Fig. 2B) rather than 12 transmembrane segments (Fig. 2A). The biogenesis and posttranslational modifications of NIS have been examined; a thorough electrophysiological analysis of NIS has been conducted, in which NIS specificity and stoichiometry were studied and a mechanistic model was proposed; the cDNA encoding human NIS (hNIS) has been isolated; the genomic organization of hNIS has been elucidated (Fig. 7) and the hNIS gene has been mapped to chromosome 19p; the regulation of NIS by TSH and I\(^2\) has been analyzed; the regulation of NIS transcription has been studied; spontaneous NIS mutations have been identified as causes of congenital hypothyroidism; and the roles of NIS in thyroid cancer and thyroid autoimmune disease have been examined.

In addition, it has been shown that I\(^-\) transport in at least some extrathyroidal tissues, such as breast and gastric mucosa, is also mediated by NIS expressed in these tissues, in which NIS is differently regulated and subjected to distinct posttranslational modifications. This notion effectively invalidates the previously held view that NIS was a major thyroid-specific protein, like thyroglobulin (Tg) and thyroid peroxidase (TPO), presumably not expressed in any other tissues. Gene therapy experiments have recently been reported in which the rat NIS gene was transduced into human melanoma, murine liver, murine colon, and human carcinoma cells, all of which then exhibited active I\(^-\) transport and became susceptible to destruction with radioiodide. Therefore, it is now clearer than ever before that the continued molecular analysis of NIS holds the potential of an even greater impact on a wide spectrum of fields,

FIG. 1. Schematic representation of the biosynthetic pathway of thyroid hormones triiodothyronine (T\(_3\)) and thyroxine (T\(_4\)) in the thyroid follicular cell. Thyroid follicles are comprised of a layer of epithelial cells surrounding the colloid. The basolateral surface of the cell is shown on the left, and the apical surface on the right. Circle, active accumulation of I\(^-\), mediated by the Na\(^+\)/I\(^-\) symporter (NIS); triangle, Na\(^+-\)K\(^+\)-ATPase; square, thyrotropin (TSH) receptor; diamond, adenylate cyclase; ellipse, G protein; cylinder, I\(^-\) efflux toward the colloid; TPO, thyroid peroxidase (TPO)-catalyzed organification of I\(^-\); arrows, endocytosis of iodinated thyroglobulin (Tg), followed by phagolysosomal hydrolysis of endocytosed iodinated Tg and secretion of both thyroid hormones.

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ranging from structure/function of transport proteins to the diagnosis and treatment of cancer, both in the thyroid and beyond.

II. CHARACTERIZATION OF THE SODIUM/IODIDE SYMPORTER PROTEIN

A. Generation of Anti-NIS Antibodies

A major development in the molecular characterization of NIS has been the generation of anti-NIS antibodies (Ab). Levy et al. (62) generated a high-affinity [dissociation constant (Kd) ~100 pM] site-directed polyclonal anti-NIS Ab against the last 16 amino acid residues of the COOH terminus of the protein. The Ab immunoreacts with a mature ~87-kDa polypeptide (i.e., NIS) and a partially glycosylated (~56 kDa) polypeptide in a line of highly functional thyroid rat cells (FRTL-5 cells). Immunoreactivity is also observed in *X. laevis* oocytes and COS cells expressing NIS, and it is competitively blocked by the presence of excess synthetic peptide. This anti-COOH-terminal NIS Ab was the first available tool to experimentally probe the NIS secondary structure model, and it was used to confirm the model-predicted cytosolic-side location of the COOH terminus by indirect immunofluorescence experiments in permeabilized FRTL-5 cells (62). Subsequently and independently, another site-directed Ab against the same COOH-terminal segment of NIS was generated by Paire et al. (81), which similarly immunore-
acts with an ~80- to 90-kDa glycosylated NIS protein from FRTL-5 cells.

**B. N-Linked Glycosylation of NIS: Implications for the NIS Secondary Structure Model**

Paire et al. (81) used their anti-NIS COOH Ab to explore the regulation of NIS by TSH in FRTL-5 cells. They observed that tunicamycin, an inhibitor of the synthesis of N-linked oligosaccharides, prevented both the synthesis of mature NIS and the TSH-dependent reinduction of NIS activity in FRTL-5 cells. On this basis, they suggested that N-linked glycosylation of NIS was essential for NIS biosynthesis, correct folding, and stability. However, because tunicamycin inhibits the synthesis of N-linked oligosaccharides and thus prevents N-linked glycosylation of all proteins in the cell, it is clear that the observed effect of tunicamycin on NIS activity is not necessarily due specifically to the lack of N-linked glycosylation of NIS. Levy et al. (64) have obtained conclusive evidence showing that, contrary to the conclusion of Paire et al., neither partial nor total lack of N-linked glycosylation impairs activity, stability, or targeting of NIS. Using site-directed mutagenesis, Levy et al. (64) substituted both separately and simultaneously the Asn residues (amino acids 225, 485, and 497, see Fig. 2) in all three putative N-linked glycosylation consensus sequences of NIS with Gln and assessed the effects of the mutations on function, targeting, and stability of NIS in COS cells. All mutants were active and displayed 50–100% of wild-type NIS activity, including the completely nonglycosylated triple mutant, which migrated as a ~50-kDa NIS polypeptide. They observed that the half-life of nonglycosylated NIS was similar to wild-type NIS and that the Michaelis constant ($K_m$) value for $\Gamma^- (\sim 30 \mu M)$ in nonglycosylated NIS was virtually identical to wild-type NIS. These findings demonstrate that, to a considerable extent, function, targeting, and stability of NIS are present even in the total absence of N-linked glycosylation (64). Therefore, a bacterial expression system, in which no N-linked glycosylation occurs, may be used to overproduce NIS for structural studies.

In their report of N-linked glycosylation of NIS, Levy et al. (64) demonstrated that the putative N-linked glycosylation site at N225, which had originally been predicted to face intracellularly (Fig. 2A), is indeed glycosylated. Therefore, it is now clear that the hydrophilic loop that contains this sequence faces the extracellular milieu rather than the cytosol. They have proposed a 13-transmembrane segment model to be the most likely secondary structure for NIS. In contrast to the original model, in which the NH$_2$ terminus was predicted to face the cytosol based on the lack of a signal sequence in NIS, in the current model both the NH$_2$ terminus and the hydrophilic loop containing N225 are predicted to be on the extracellular side, and the COOH terminus facing the cytosol, as confirmed previously (Fig. 2B).

**C. Extracellular Orientation of the NH$_2$ Terminus of NIS**

Levy et al. (64) have recently demonstrated unequivocally that the NH$_2$ terminus faces the external milieu, as proposed in the current model. This conclusion was reached using two independent experimental approaches. First, these authors introduced a FLAG (MDYKDDDDK) epitope into the NH$_2$ terminus. COS cells transfected with FLAG-containing NIS displayed undistinguishable $\Gamma^-$ uptake accumulation from COS cells transfected with wild-type NIS. Immunofluorescence experiments demonstrated positive immunoreactivity with anti-Flag Ab in nonpermeabilized COS cells transfected with FLAG-containing NIS. Positive immunoreactivity in nonpermeabilized cells indicates that the NH$_2$ terminus faces externally. In contrast, immunoreactivity using anti-COOH Ab requires permeabilization because the COOH terminus faces the cytosol. The second technique took advantage of a previous observation that unglycosylated NIS is active. The N-linked glycosylation amino acid sequence NNSS was introduced into the NH$_2$ terminus of unglycosylated NIS (25). They observed glycosylation of NIS at the NH$_2$ terminus upon transfection of NNSS-containing NIS into COS cells, thus proving that the NH$_2$ terminus faces the lumen of the endoplasmic reticulum during biosynthesis and therefore faces the external milieu upon reaching the plasma membrane (25).

In addition, utilizing the same strategy of N-linked glycosylation scanning mutagenesis, De la Vieja et al. (25) have demonstrated that the hydrophilic loop between putative transmembrane segments VIII and IX faces the external milieu (Fig. 2B). In a complementary approach to study the topology of NIS in the plasma membrane, a Cys residue was placed at position 160 (in the hydrophilic loop between putative transmembrane segments IV and V) in an outside Cys-less background, a mutant that retains total activity. NIS activity was modified by membrane-impermeable sulfhydryl reagents such as sodium (2-sulfonatoethyl) methanethiosulfonate (MTSES) and 2-(trimethylammonium)ethyl methanethiosulfonate (MTSET), indicating the external localization of this residue. In summary, 5 NIS loops (NH$_2$ terminus, loops between transmembrane segments IV and V, VI and VII, VIII and IX, and XII and XIII) out of a total of 7 have experimentally been confirmed to have the external disposition predicted in the current 13-transmembrane segment secondary structure model. Additional experiments are being carried out to complete the topological analysis of NIS.
D. Several Hydroxyl-Containing Amino Acid Residues in the Transmembrane Segment IX (Ser-353, Thr-354, Ser-356, and Thr-357) Are Important for NIS Function

Levy et al. (65) have demonstrated that a hydroxyl group at the β-carbon at position 354 (in the transmembrane segment IX) is essential for NIS function. Such a hydroxyl group is present in Thr-354. This discovery followed reports that a spontaneous mutation consisting of the single amino acid substitution of Pro instead of Thr at position 354 (T354P) is the cause of congenital lack of iodide transport in several patients (39, 40, 55, 70). Patients with this condition do not accumulate iodide in their thyroids, often resulting in severe hypothyroidism. Seeking to characterize this transport defect at the molecular level, Levy et al. (65) set out to determine whether T354P NIS is a nonfunctional but stable polypeptide properly targeted to the plasma membrane, or a fully or partially functional protein that is retained in intracellular organelles as a result of the mutation. For this purpose, Levy et al. (65) generated T354P NIS by site-directed mutagenesis and used their high-affinity anti-NIS Ab to monitor T354P expression in COS cells transfected with NIS cDNA. COS cells transfected with T354P NIS cDNA were assayed for iodide uptake activity and found to display no iodide accumulation. After demonstrating that T354P was properly targeted to the plasma membrane, these authors carried out several additional amino acid substitutions at position 354 and determined that the lack of iodide transport activity is not due to a structural change induced by proline, as had been previously proposed (39), but rather to the absence of a hydroxyl group at the β-carbon at position 354.

Significantly, the transmembrane segment IX, in which Thr-354 is located, is where the highest incidence of hydroxyl-containing amino acids is found in NIS (Fig. 2B). Hence, De La Vieja et al. (unpublished data) assessed the role played by these other hydroxyl groups in NIS function by replacing the corresponding amino acid residues with Ala and Pro. Substituting Ser-349, Thr-351, Ser-358, and Thr-366 (Fig. 3) for similar amino acid residues devoid of hydroxyl groups did not affect either NIS expression or activity. In contrast, the hydroxyl groups of Ser-353, Ser-356, and Thr-357 seem to be essential for NIS activity, given that NIS functioned to a significant extent only when Ser or Thr was present at these positions. The lack of function when Ala or Pro was present instead at these positions was not due to an effect on expression or trafficking, given that the mutant NIS proteins were shown by confocal immunofluorescence to reach the plasma membrane properly. Additional experiments are being carried out to determine the mechanistic role of these hydroxyl groups in NIS activity. Kinetic analyses are also being performed to differentiate between kinetically impaired and uncoupled NIS molecules.

E. Electrophysiological Analysis of NIS: Mechanism, Stoichiometry, and Specificity

Eskandari et al. (34) have examined the mechanism, stoichiometry, and specificity of NIS by means of electrophysiological, tracer uptake, and electron microscopic methods in X. laevis oocytes expressing NIS. These investigators obtained electrophysiological recordings using the two-microelectrode voltage-clamp technique. They showed that an inward steady-state current (i.e., a net influx of positive charge) is generated in NIS-expressing oocytes upon addition of iodide to the bathing medium, leading to depolarization of the membrane. Because the recorded current is attributable to NIS activity, this observation confirms that NIS activity is electrogenic. Simultaneous measurements of tracer fluxes and currents revealed that two Na+/I− stoichiometry. Therefore, the observed inward steady-state current is due to a net influx of Na+(Fig. 4).

Eskandari et al. (34) observed also that in response to step voltage changes, NIS exhibited current transients that relaxed with a time constant of 8–14 ms and that pre-steady-state charge movements (integral of the current transients) versus voltage relations obeyed a Boltzmann distribution. These charge movements are attrib-
The kinetic data suggest that Na

substrate is able to cross the membrane via NIS in a Na

2

formational change to expose the bound I

the cell (CNa

2

I

0

). Both Na

' and I

are released into the cytoplasmic compartment, and the empty carrier undergoes another conformational change to expose the binding sites to the external solution again. Charge movement data suggest that the Na

' binding dissociation does not contribute greatly to the total observed charge. Thus it is proposed that NIS charge movements arise primarily from conformational changes of the empty carrier (C

'= C'). For more details, refer to Reference 34.

FIG. 4. Schematic representation of a NIS mechanistic model. As shown in this scheme, first 1 Na

' binds to NIS, which in the absence of substrate is able to cross the membrane via NIS in a Na

' uniport mode (CNa

'= CNa

; C, carrier). Release of Na

' into the cytoplasm is followed by the return of the empty binding site to complete the pathway (C

= C'). The kinetic data suggest that Na

' binds to NIS before I

'. In the presence of I

, the complex CNa

I

is formed which undergoes a conformational change to expose the bound I

' and 2 Na

' to the interior of the cell (CNa

I

' = CNa

I

). Both Na

' and I

' are released into the cytoplasmic compartment, and the empty carrier undergoes another conformational change to expose the binding sites to the external solution again. Charge movement data suggest that the Na

' binding dissociation does not contribute greatly to the total observed charge. Thus it is proposed that NIS charge movements arise primarily from conformational changes of the empty carrier (C

'= C'). For more details, refer to Reference 34.

F. The NIS Inhibitor Perchlorate Is Not Translocated by NIS Into the Cell

Similar steady-state inward currents were generated by a wide variety of anions in addition to I

( including ClO

3

, SCN

, SeCN

, NO

3

, Br

, BF

, IO

, and BrO

), indicating that these anions are also transported by NIS. However, perchlorate (ClO

3

) (Fig. 5), the most widely characterized inhibitor of thyroidal I

uptake, was surprisingly found not to generate a current, strongly sug-

gesting that it is not transported (34). Yoshida et al. (130) have reported, similarly, that perchlorate did not induce an inward current in Chinese hamster ovary (CHO) cells stably expressing NIS, as measured using the whole cell patch-clamp technique. The most likely interpretation of these observations is that perchlorate is not transported by NIS, although the unlikely possibility that perchlorate is translocated by NIS on a 1:1 Na

'/ClO

3

 stoichiometry cannot be ruled out. Therefore, perchlorate is a potent inhibitor of NIS most probably acting as a blocker, not as a substrate.

These results raise the question of whether or not perchlorate is indeed translocated into thyroid cells expressing endogenous NIS or into cells other than oocytes expressing transfected NIS, and this question has been the subject of some controversy. In the acknowledgments section of his recent extensive review on perchlorate and the thyroid gland, Wolff (127) asserts that “perchlorate accumulation in thyroid tissue has been repeatedly demonstrated” and suggests that the absence of perchlorate transport by NIS in oocytes reported by Eskandari et al. (34) may be due to differences between the oocyte system and thyroid tissue. However, Yoshida et al. (131) thereafter showed that perchlorate elicits no change in the membrane current in the highly functional rat thyroid cell line FRTL-5, as revealed by the whole cell patch-clamp technique, thus strongly suggesting that perchlorate is not transported into FRTL-5 cells and supporting both their previous observations in CHO cells (130) and the results of Eskandari et al. (34) in oocytes.

With the consideration that the properties of NIS expressed in oocytes are virtually indistinguishable from those of endogenous NIS in thyroid cells, including FRTL-5 cells, it appears that earlier experiments (42, 43, 49, 50) were not done under conditions where a detectable inward current were as follows: NO

, HCO

, CO

2

, SO

2

, HPO

3

, PO

4

. Data are reported as means ± SE (n = 3). [Adapted from Eskandari et al. (34).]
125) ostensibly showing that $^{36}\text{Cl}$-labeled perchlorate enters the cell may have been misinterpreted. Because $[^{36}\text{Cl}]{\text{Cl}}$-chlorate ($\text{ClO}_4^-$) is a $^{36}\text{Cl}$-labeled by-product of the reaction employed to chemically synthesize $[^{36}\text{Cl}]{\text{Cl}}$- perchlorate ($\text{ClO}_4^-$) for these uptake studies, it seems likely that $[^{36}\text{Cl}]{\text{Cl}}$-chlorate, rather than perchlorate, accounts for the presence of label in the cytosol of thyrocytes, given that chlorate is readily translocated via NIS into the cell (34). Current electrophysiological data, in conclusion, strongly indicate that perchlorate is not translocated via NIS into the cell.

**III. REGULATION OF SODIUM/IODIDE SYMPORTER PROTEIN EXPRESSION**

TSH is the primary hormonal regulator of thyroid function overall and has long been known to stimulate thyroidal $\Gamma^-$ accumulation. TSH is a glycoprotein of $\sim 30,000$ Da, biosynthesized in the adenohypophysis by basophilic cells known as thyrotropes. The release of TSH from the pituitary is stimulated by thyrotropin-releasing hormone (TRH) from the hypothalamus and inhibited through a negative-feedback mechanism by the thyroid hormones $T_3$ and $T_4$. Most actions of TSH take place through activation of adenylate cyclase via the GTP binding protein $\Gamma\alpha$ (117). This cascade of events is initiated by the interaction of TSH with its receptor [i.e., TSH receptor (TSHr)] on the basolateral membrane of the follicular cells (Fig. 1). Early observations made before the isolation of the NIS cDNA suggested that TSH stimulation of $\Gamma^-$ accumulation results, at least in part, from the cAMP-mediated increased biosynthesis of NIS (51, 122). Using high affinity anti-NIS Ab, Levy et al. (62) demonstrated in rats that NIS protein expression is upregulated by TSH in vivo. They prepared thyroid membrane fractions from control, propylthiouracil (PTU)-treated, iodine-deficient, and hypophysectomized rats, the latter with or without subsequent injection of TSH. Membrane fractions were then subjected to immunoblot analysis with anti-NIS Ab. They observed NIS upregulation in rats with increased TSH circulating levels caused either by PTU treatment (which inhibits $\Gamma^-$ organification) or an $\Gamma^-$-deficient diet. Conversely, NIS protein expression was decreased in hypophysectomized rats, which exhibit markedly lower TSH levels. A single injection of TSH to hypophysectomized rats reinduced the expression of NIS protein back to basal levels. Consistent with these findings is a later observation by Uyttersprot et al. (115) that the expression of NIS mRNA in dog thyroid ($\sim 3.9$ kb) is dramatically upregulated by goitrogenic treatment (i.e., PTU treatment, which leads to elevated TSH circulating levels in vivo).

The main factor regulating the accumulation of $\Gamma^-$ in the thyroid (i.e., NIS activity) other than TSH has long been considered to be $\Gamma^-$ itself. As early as 1944, Morton et al. (75) reported that the biosynthesis of thyroid hormones by sheep thyroid slices was inhibited by high doses of $\Gamma^-$. Wolff and Chaikoff reported in 1948 (128) that organic binding of iodide in the rat thyroid was blocked when $\Gamma^-$ thyroid levels reached a critical high threshold, a phenomenon known as the acute Wolff-Chaikoff effect. These researchers observed further that $\sim 2$ days later, in the presence of continued high plasma $\Gamma^-$ concentrations, an “escape” or adaptation from the acute effect is observed so that the level of organification of $\Gamma^-$ is restored and normal hormone biosynthesis resumes (129). Whereas the mechanism responsible for the acute Wolff-Chaikoff effect has yet to be fully elucidated, it has been proposed to be the result of organic iodocompounds acting as mediators. The iodolipid $\alpha$-iodohexadecanal has been suggested to be one such mediator, on account of its ability to inhibit NADPH oxidase, TPO, and TSH-induced cAMP formation in the thyroid (28). The less studied mechanism for the escape from the acute Wolff-Chaikoff effect has been proposed by Braverman and Ingbar (12) to be due to a decrease in $\Gamma^-$ transport, which would presumably lead to sufficiently low intracellular $\Gamma^-$ concentrations to remove inhibition of $\Gamma^-$ organification.

As in the case of NIS regulation by TSH, the regulatory role played by $\Gamma^-$ on NIS function was explored at the molecular level only after the cDNA that encodes NIS was isolated. In vivo studies have shown that $\Gamma^-$ inhibits the expression of both TPO and NIS mRNA in dog thyroid (115), a finding consistent with the Wolff-Chaikoff effect. More recently, Eng et al. (33) measured the levels of NIS mRNA and NIS protein in response to both chronic and acute $\Gamma^-$ excess in rats in vivo, thus showing, specifically, that the decrease of $\Gamma^-$ transport observed during the escape from the Wolff-Chaikoff effect is due to a decrease in NIS expression by a mechanism that is at least in part transcriptional.

**IV. TRANSCRIPTIONAL REGULATION OF THE SODIUM/IODIDE SYMPORTER**

**A. Thyroid-Specific Transcription Factors**

The transcriptional regulation of three other genes of major significance in thyroid physiology had been analyzed, namely, the Tg, TPO, and TSHr genes. Initially, all three gene products were proposed to be thyroid specific, i.e., to be expressed exclusively in the thyroid. However, TSHr expression and activity have recently been demonstrated in adipocytes (100), leaving Tg and TPO as the only thyroid-specific proteins.

Three different transcription factors have been implicated in thyroid-specific gene transcription (22, 73): I) thyroid transcription factor (TTF)-1, a homeodomain (HD)-containing protein present in the developing thy-
B. Transcriptional Regulation of Tg and TPO

The Tg and TPO minimal promoter structures are very similar to each other but different from the TSHr promoter (22) (Fig. 6). The Tg and TPO promoters from different species exhibit three binding sites for TTF-1 (A, B, and C), one binding site for TTF-2, and one for Pax8. All of these sites are localized 5′-upstream between −170 and +1 bp (A in the ATG initiation codon) and both contain a TATA box. The TTF-1 site C and Pax8 site overlap, suggesting that this overlapping site area may play an important role in thyroid-specific transcription. TTF-2 plays an important role in the modulation of the Tg and TPO genes by insulin and insulin-like growth factor I, but this action is more evident for the TPO than for the Tg promoter (8, 80, 94). Both promoters have been shown to have binding sites for different ubiquitous transcription factors, namely, ubiquitous factor A (UFA) in the Tg promoter and ubiquitous factor B (UFB) in the TPO promoter. Sato et al. (95) have recently shown that both TTF-2 and HNF-3β, another forkhead transcription factor also present in the thyroid, bind to the same site and thus participate in the regulation of the TPO but not the Tg promoter. In addition, upstream enhancers have also been identified in both promoters. Three TTF-1 binding sites for the bovine Tg promoter are present 2 kb upstream. Two TTF-1 and one Pax8 binding site, recently reported (35), are found in a 230-bp enhancer located 5.5 kb upstream of the TPO initiation codon (22).

C. Transcriptional Regulation of the TSHr

The TSHr promoter is very different from the Tg and TPO promoters (Fig. 6). The TSHr minimal promoter region is localized in the 5′-upstream region between −220 and −39 bp and shows thyroid-specific expression and TSH/cAMP regulation. Several binding regulatory elements have been proposed to play a role in the regulation of TSHr transcription. A canonical cAMP responsive element (CRE) (−139 to −131 bp) site that is autoregulated by TSH via cAMP has been shown to first increase and then decrease the expression of TSHr as a function of time (93). One TTF-1 binding site is present at −189 to −175 bp; binding of phosphorylated TTF-1 to this site produces a modest effect on the activation of transcription (77, 101). Single-strand DNA-binding proteins (SSBP), whose binding sites overlap with the 5′-end of the TTF-1 site, have been implicated in the regulation of the TSHr (102). In contrast to the Tg and TPO promoters,
neither TTF-2 nor Pax8 binding sites have been identified in the TSHr promoter. The TSHr transcript has also been found in retro-orbital fibroblasts and in adipose tissue (32, 60). Rat adipose tissue expresses TSHr mRNA levels similar to those found in the thyroid (100). The promoter regulation is different in adipocytes from thyroid cells, but both display multiple start sites and important regulation by CREB (99).

TPO and Tg expression are both upregulated by TSH (41, 116) but by different regulatory mechanisms. TPO and TSHr regulation takes place faster than Tg regulation. Although Tg transcriptional activation kinetics depend on the experimental model used, i.e., it is rapid (~1 h) in thyroid slides and slow (~8 h) in primary cultures, TPO induction is rapid in both experimental models (41). All three genes are controlled in the thyroid at the transcriptional level mainly by a cAMP-dependent mechanism, whose intracellular level is elevated by TSH/forskolin. However, only the TSHr gene has been shown to be regulated by cAMP through a CRE sequence. The exact mechanism by which cAMP regulates Tg and TPO is still an open question.

D. Analysis of the Human NIS Promoter

It has long been established that TSH stimulates NIS activity via the cAMP pathway (62, 81, 122) and, more recently, that it upregulates NIS mRNA levels (53). However, to fully understand these mechanisms, it is necessary to study the transcriptional regulation of NIS. The human NIS promoter has been sequenced by three different groups (10, 90, 118). Venkataraman et al. (118) isolated a 1.2-kb fragment of the 5′-flanking region of the hNIS gene. Significantly, they characterized the promoter in a human thyroid cell line, KAT-50, and found thyroid-specific expression between ~1,044 and ~336 bp. Some putative transcription factor binding sites, both thyroid and nonthyroid, are localized in this region (1.2 kb) but were not analyzed. It is noteworthy that this list includes the putative TTF-2 and Pax8 binding sites, both of which are found within the 2-kb portion of the 5′-flanking region in the rat promoter (see below). Ryu et al. (90) isolated 2 kb of the 5′-flanking region from the hNIS promoter. They mapped the start site to ~375 bp and delimited the minimal promoter between ~478 and ~389 bp. A 90-bp fragment exhibited a high identity (73%) with the minimal rNIS promoter. These investigators reported some mismatches with the previously reported sequence. However, this group did not find thyroid-specific regulatory elements. Finally, Behr et al. (10) isolated 1.6 kb of the 5′-flanking region of the hNIS gene. Like Ryu et al. (90), Behr et al. (10) also found strong promoter activity in thyroid and nonthyroid cells within the minimal promoter. Behr et al. (10) suggested that the nonspecificity could result from the presence of an upstream tissue-specific enhancer or from the influence of chromatin structure and/or methylation, as there are numerous CpG dinucleotides.

E. Analysis of the Rat NIS Promoter

Tong et al. (112) isolated a 16.4-kb fragment of genomic rNIS DNA. They localized the start site at ~98 bp and one TATA box between ~124 and ~118 (AATAAAAT), with a minimal promoter localized between ~199 and ~110 bp. Surprisingly, however, they concluded that 8 kb of the 5′-flanking region of the NIS gene is not sufficient to confer thyroid-specific transcription. Neither TTF-2 nor Pax8 putative binding sites were localized within 2 kb upstream of the initiation codon. However, the conclusion of Tong et al. (112) proved incorrect, because Endo et al. (30) localized a TTF-1 binding site between ~245 and ~230 bp, in the proximal rNIS promoter (2 kb), that confers thyroid-specific transcription but only exerts a modest effect.

The same group (76) subsequently identified, in the 5′-flanking region between ~1,968 and ~1 bp, a novel TSH-responsive element (TRE) between ~420 and ~385 bp upstream of the TTF-1 site in the rNIS promoter, that upregulated two- to threefold NIS expression. The TSH effect is cAMP mediated and thyroid specific. They showed that the protein that binds this site is different from TTF-1, TTF-2, Pax8, or other known transcription factors, and the researchers named the putative binding protein in the TRE site NIS TSH-responsive factor 1 (NTF-1). However, because the TSH upregulation of NIS via this TRE site is lower than the regulation that the same group reported previously (~6-fold) (53), they have suggested that other transcription binding sequences may be present upstream from the 1,968-bp region that they studied.

A thorough characterization of the upstream enhancer of the rNIS gene has recently been reported by Ohno et al. (78). These investigators showed that the rNIS regulatory region contains a nonthyroid-specific promoter between ~564 and ~2 bp and an enhancer located between ~2,264 and ~2,495 bp that recapitulates the most relevant aspects of NIS regulation. This rNIS enhancer mediates thyroid-specific gene expression by the interaction of Pax8 with a novel cAMP-dependent pathway. The NIS upstream enhancer (NUE) stimulates transcription in a thyroid-specific and cAMP-dependent manner. NUE contains the following: two Pax8 binding sites (PA and PB); two TTF-1 binding sites (TA and TB) that have no effect on rNIS transcription; and a degenerate CRE sequence (5′-TGACGCA-3′), which is important for NUE transcriptional activity. Interestingly, the same degenerate CRE sequence has been implicated in tissue-specific cAMP response of other promoters, such as the dopamine
The main vertebrate nonthyroid tissues reported to actively accumulate I\(^{-}\) are salivary glands, gastric mucosa, lactating mammary gland, choroid plexus, and the ciliary body of the eye. Many of these transport systems exhibit functional similarities with their thyroid counterpart, notably a susceptibility to inhibition by thiocyanate and perchlorate, but they also display important differences: 1) nonthyroid I\(^{-}\) transporting tissues do not have the ability to organify accumulated I\(^{-}\); therefore, they behave like PTU-treated thyroid tissue; 2) TSH exerts no regulatory influence on nonthyroid I\(^{-}\) accumulation; and 3) at least salivary glands and gastric mucosa concentrate thio-cyanate, unlike the thyroid where it is metabolized (oxidized). Despite these differences, several reports of patients suffering the simultaneous genetic absence of I\(^{-}\) transport in the thyroid, the salivary glands, and the gastric mucosa strongly hinted at a "genetic link" among these I\(^{-}\) transport systems, suggesting that extrathyroidal I\(^{-}\) transport is catalyzed by plasma membrane proteins that are very similar, if not identical, to thyroid NIS (13, 16, 42, 125). Moreover, thyroidal and extrathyroidal I\(^{-}\) accumulation generate I\(^{-}\) concentration gradients of similar magnitude (~20- to 40-fold under steady-state conditions). Hence, the isolation and characterization of the NIS cDNA from rat thyroid (21) made it possible to examine NIS expression in nonthyroid tissues, leading to the conclusion that I\(^{-}\) transport in most (and probably all) extrathyroidal tissues in which it is present is also mediated by NIS itself, like in the thyroid. However, NIS is clearly regulated and processed differently in each tissue.

A. NIS in Salivary Glands, Stomach, Mammary Gland, and Other Tissues

Spitzweg et al. (108) subjected several extrathyroidal human tissues to Northern blot and RT-PCR analysis with human thyroid NIS cDNA and detected the NIS transcript by Northern blot predominantly in the parotid salivary gland but not in the other tissues. With the use of RT-PCR, hNIS gene expression was detectable in salivary glands, pituitary gland, pancreas, testis, mammary gland, gastric mucosa, prostate, and ovary but not in orbital fibroblasts, colon, and nasopharyngeal mucosa. Kotani et al. (56), who carried out Northern analyses with rat thyroid NIS cDNA in extrathyroidal rat tissues, detected the NIS transcript primarily in the stomach. Subsequently, Spitzweg et al. (108) reported the cloning of human NIS cDNA from human gastric mucosa, parotid, and mammary glands, all of which exhibited full identity to thyroid hNIS cDNA. Consistent with these findings, immunohistochemical studies by Jhiang et al. (48) and by Kotani et al. (56) detected NIS protein in the acinar cells of human salivary glands and in surface epithelial and parietal cells of the rat stomach, respectively. Ajan et al. (4) have reported variable amounts of hNIS amplified product, as detected by RT-PCR, in human stomach, salivary gland, and mammary tissue, but not in the ovary, esophagus, colon, extraocular fat, or skin. Surprisingly, they failed to detect NIS expression using Northern blot analysis in stomach, salivary gland, intestinal fat, and multinodular goiter tissue samples.

It must be pointed out that the RT-PCR technique
yields a large number of false positives due to the high sensitivity of the RT-PCR technique (38). Therefore, the detection of the NIS amplified product by RT-PCR in a given tissue cannot be regarded as sufficient evidence that NIS is functionally expressed in that tissue. A thorough characterization of NIS protein expression is necessary to properly evaluate the significance or results obtained by RT-PCR and even Northern analyses, and once NIS protein expression has been demonstrated, a correlation with Na\(^+\)-dependent, perchlorate-sensitive, active I\(^-\) accumulation in that tissue must be established. By these criteria, and with the consideration of the above results, NIS appears to be expressed and active in extrathyroidal tissues previously known to exhibit NIS activity, such as salivary glands and gastric mucosa. The significance of the detection of the NIS-amplified product by RT-PCR in other human and rat tissues still awaits further characterization.

B. Identification of Mammary Gland NIS and Gastric NIS

Levy et al. (62) performed immunoblot analyses to assess whether a high-affinity antithyroid NIS Ab would react with a mammary gland membrane protein (111a). They observed immunoreactivity against a single broad ~75-kDa polypeptide in rat lactating mammary gland membranes, but not in membranes from nonlactating mammary gland or from lung, muscle, or heart, all tissues that do not transport I\(^-\). This immunoreactive polypeptide is mammary gland NIS (mg-NIS). These authors then investigated the difference in electrophoretic mobilities between mg-NIS (~75 kDa) and thyroid NIS (~90 kDa) and found that it is due to differences in their posttranslational modifications. They treated membrane proteins from thyroid and lactating mammary gland with N-glycosidase F, an enzyme that removes N-linked carbohydrates, and probed membranes with anti-NIS Ab. Under these conditions, anti-NIS Ab recognized a ~50-kDa polypeptide in membranes from both thyroid and lactating mammary gland. Significantly, both nonglycosylated NIS in FRTL-5 cells and NIS expressed in Escherichia coli exhibit an identical electrophoretic mobility (i.e., ~50 kDa). These results demonstrate that the ~75- and ~50-kDa immunoreactive polypeptides detected in lactating mammary gland correspond to glycosylated and nonglycosylated mg-NIS, respectively. Moreover, Tazebay et al. (111a) also observed immunoreactivity of anti-NIS Ab with a ~100-kDa gastric polypeptide, which upon deglycosylation migrated, too, at ~50 kDa. In all likelihood, these polypeptides correspond, respectively, to glycosylated and nonglycosylated gastric NIS (g-NIS). CNBr treatment of rat thyroid NIS, mg-NIS, and g-NIS proteins yielded the same peptide map in each case (Levy et al., Ref. 62), a finding consistent with the identity among human thyroid NIS, mg-NIS, and g-NIS predicted by the cloning of human NIS cDNA from gastric mucosa and mammary glands by Spitzweg et al. (108).

C. Regulation of Mammary Gland NIS

Subsequently, Tazebay et al. (111a) investigated whether a correlation existed between I\(^-\) accumulation activity and mg-NIS expression in the various physiological stages of the mammary gland. They found that mg-NIS was absent in nubile and pregnant mammary gland, but clearly present in lactating mammary gland. To ascertain whether weaning had an effect on mg-NIS expression, mother rats were removed from their litters. Twenty-four hours after weaning, mg-NIS expression was significantly decreased, and after 48 h, it was barely detectable. Furthermore, mg-NIS expression was reversible upon reestablishment of nursing. These results indicate that mg-NIS expression is clearly upregulated during active nursing, rapidly downregulated upon cessation of it, and exquisitely regulated in a reversible manner by suckling.

To assess the issue of tissue distribution of I\(^-\) transport activity, Tazebay et al. (111a) carried out in vivo scintigraphic imaging of \(^{131}\text{I}^-\) (or the substitute isotope \(^{99m}\text{TcO}_4^-\)) tissue distribution in rats. In nonlactating rats, the isotope was concentrated in the stomach and later in the thyroid, whereas in lactating rats it was evident in the stomach and soon thereafter in all pairs of mammary glands. Concentration of the isotope in all these tissues was inhibited by perchlorate.

VI. THE SODIUM/IODIDE SYMPORTER IN AUTOIMMUNE THYROID DISEASE

An important aspect of the wide pathophysiological impact of Tg, TPO, and TSHr is the fact that autoAb against all three proteins have been demonstrated in patients suffering from autoimmune thyroid disease (AITD). AITD is significantly more prevalent in women; it affects ~2% of the female population but only ~0.2% of males (121). The pathogenesis of AITD across the spectrum from Graves’ disease to Hashimoto’s thyroiditis has been suggested to involve a deficiency in the number and/or function of antigen-specific and nonspecific population of T lymphocytes (37). As is the case in all autoimmune disorders, multiple factors are believed to play a role in the development of AITD, including genetic predisposition, environmental factors, and a state of dysregulation of the immune system (37). In the presence of AITD, the thyroid is infiltrated with mononuclear cells, including activated T and B plasma cells and macrophages, thus creating a local cytokine milieu (120). A distinctive fea-
ture of AITD is the expression of HLA class II molecules, induced by the T cell secreted interferon (IFN)-γ, on the follicular epithelial cells, which are normally class II negative. Another characteristic feature of AITD is the presence in patients’ sera of autoAb that are directed against specific thyroid antigens (Tg, TPO, and TSHr) (121). Iodine is an important susceptibility and modulating factor in the development of thyroid autoimmunity. High levels of iodine can cause thyroid damage and thyroiditis, particularly against the background of a previously low intake of iodine. Significantly, the incidence of AITD is higher in areas of iodine sufficiency than in areas of iodine deficiency (37).

Given that thyroid-associated proteins such as Tg, TPO, and TSHr are known targets for autoAb in AITD, the molecular identification of NIS has made it possible for the first time to experimentally assess whether NIS is also a target autoantigen, and whether any anti-NIS autoAb (if present) have an effect on NIS function. In addition, it is also possible that NIS is one of the modulators of the local autoimmune processes, conceivably regulating the individual Γ− supply to the follicular cell.

Even before the isolation of the NIS cDNA, Raspe et al. (88) screened sera from 147 patients with AITD by measuring the effect of the sera on Γ− transport activity in primary cultures of dog thyrocytes. They observed that the serum from a 58-yr-old female patient suffering from Hashimoto’s thyroiditis, autoimmune gastritis, and rheumatoid arthritis, with high autoAb titers against Tg, TPO, and gastric parietal cells, completely blocked TSH-induced Γ− uptake. Inhibition was specific, given that the serum was still active at 1:1,000 dilution and did not inhibit the activity of the Na+−K+−ATPase. Unfortunately, no further experiments were performed because only a limited amount of serum was available. Nevertheless, the investigators still concluded that the serum contained NIS-inhibiting autoAb. The low prevalence of the autoAb positivity (1 of 147 sera) may be due to the purely functional nature of the assay, as only Ab against external epitopes and with an Γ− transport inhibitory effect would be detected, as well as to the use of dog thyrocytes to screen human sera.

Endo et al. (31) screened sera from patients withAITD by recombinant NIS protein slot blotted onto nitrocellulose sheets. Twenty-two of 88 Graves’ disease sera and 3 of 20 Hashimoto’s thyroiditis sera seemed to detect the recombinant NIS protein, but the effect of these positive sera on Γ− uptake was not tested. In a subsequent study, the same group (29) concentrated exclusively on Hashimoto’s thyroiditis samples. Four of 34 sera from patients with Hashimoto’s thyroiditis immunoreacted with a ~80-kDa polypeptide, as seen upon immunoblot analysis of FRTL-5 cell membranes (which contain not only NIS but also other thyroid antigens), and these same four sera caused 14−62% inhibition of Γ− accumulation in CHO cells stably expressing recombinant rat NIS. The investigators observed also that some normal sera and patients’ sera that did not immunoreact on immunoblots nevertheless caused ~90% inhibition of NIS activity. Because this inhibitory effect was lost after dialyzing these sera, the authors concluded that some sera contained a dialyzable inhibitor, independent of the presence or absence of anti-NIS autoAb, and thus suggested that purified IgG be used to accurately evaluate the putative autoAb activity. The investigators also concluded that the presence of anti-NIS autoAb may play a role in the pathogenesis of Hashimoto’s thyroiditis and thus may modulate thyroid function. All of these preliminary observations call for further study and confirmation.

Morris et al. (74) synthesized 21 peptides corresponding to putative extracellular segments of rat NIS, based on the first 12 transmembrane segment secondary structure model proposed for rat NIS (21). Samples were analyzed by ELISA using the synthetically made peptides. Because of the high variability of background binding, the normal range of binding was determined in the case of each peptide by a pool of normal IgG, and thereafter the number of standard deviation units from the mean of the control was calculated. Eighty-eight percent of Graves’ (n = 27) and 63% of Hashimoto (n = 27) serum samples recognized at least one synthetic peptide. The most highly recognized eight peptides were those corresponding to the fourth, fifth, and sixth extracellular loops and the intracellular COOH-terminal tail of the first secondary structure model (Fig. 2A), which correspond, respectively, to the fourth and sixth intracellular and sixth extracellular loops, and the intracellular COOH-terminal tail of the current 13 transmembrane segments model (Fig. 2B). In contrast, none of the control sera displayed any immunoreactivity. The occurrence of Ab that recognized intracellular epitopes was explained by the investigators as a result of exposure of these internal sequences to the thyroiditis-induced follicular cell damage. Unfortunately, there were no data regarding recognition of the entire NIS molecule by these antisera.

Ajjan et al. (3) established a CHO cell line stably expressing human NIS devoid of the last 31 amino acids, thus generating a valuable system (CHO-NIS9 cells) for the evaluation of anti-NIS Abs, on account of the absence of other thyroid-specific antigens. Eighty-eight sera from patients with Graves’ disease were tested for their effect on Γ− uptake. Twenty-seven of 88 (30.7%) of the Graves’ disease sera (and also their corresponding purified IgG) be used to accurately evaluate the putative autoAb activity. The investigators also concluded that the presence of anti-NIS autoAb may play a role in the pathogenesis of Hashimoto’s thyroiditis and thus may modulate thyroid function. All of these preliminary observations call for further study and confirmation.

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underlying pathogenesis of the autoimmune process.

Considering the limitations of RT-PCR as a quantitative method, Arturi et al. (7) were able to evaluate only the presence or absence of the NIS transcript in a series of malignant and benign thyroid tumors. They first analyzed 26 primary thyroid carcinomas (19 papillary, 5 follicular, and 2 anaplastic), and subsequently a new series of 15 follicular adenomas (11 cold and 4 hot as classified by thyroid scintigraphy), and found loss of expression of NIS in 5 of 19 papillary cancers, 1 of 5 follicular cancers, and none detected in anaplastic tissues. All the (benign) follicular adenomas except one expressed hNIS, whereas one papillary and two follicular cancers expressed hNIS at variable levels.

The treatment of differentiated thyroid carcinoma is total or near-total thyroidectomy followed by 131I ablation. Radioiodide ablation destroys occult microscopic carcinomas and also any remaining normal thyroid tissue, permitting postablative 131I total body scanning search for possible persistent carcinoma (119). The tools that allow the sensitive and specific detection and subsequent treatment of differentiated thyroid carcinoma are the uptake (mediated by NIS) and organification (mediated by TPO) of $\Gamma^-$, both TSH-regulated processes. The tumorigenic role of radioiodine in the thyroid, transported also by NIS into the follicular cell, is debated, but the increased incidence of papillary thyroid carcinomas in children (100 times higher than in nonexposed children) after nuclear testing and nuclear accidents suggests that radioactive isotopes of iodine have a direct tumorigenic effect on the thyroid (97).

To understand the involvement of NIS in the observed patterns of $\Gamma^-$ transport in thyroid carcinomas with respect to healthy thyroid tissue, several groups have looked for a possible correlation between the lower or absent radioiodine uptake activity in thyroid carcinomas and the expression of NIS mRNA or protein, using Northern blot analysis, RT-PCR, or immunological analyses. Smanik et al. (106) observed, by Northern blot analysis, lower expression of hNIS in two papillary, one follicular, and one anaplastic carcinoma. Upon further analysis of additional papillary carcinomas by RT-PCR, a more sensitive technique, they found that different papillary cancers expressed hNIS at variable levels.

VII. THE SODIUM/IODIDE SYMPORTER AND CANCER

Although cancer of the thyroid is a relatively infrequent condition in the United States (0.6% of all cancers in men and 1.6% in women, Ref. 104), it has a considerably higher impact on endocrinological practice than would be expected solely on the basis of its incidence. This is so because the possible existence of thyroid cancer must be ruled out whenever a thyroid nodule is detected. There is a high estimated incidence of solitary palpable nodules in United States adults (2–4%) (89). The degree of accumulation of $\Gamma^-$, as revealed by scans of the gland, is used as an aid in the differential diagnosis of thyroid nodules. Thyroid nodules that accumulate $\Gamma^-$ equally or more efficiently than the normal surrounding tissue are generally benign, whereas most thyroid cancers display markedly reduced $\Gamma^-$ accumulation relative to healthy tissue. On the other hand, carcinomas behaving as hyperfunctioning hot nodules are very rare. Thus radioactive $\Gamma^-$ plays a major diagnostic and therapeutic role in the management of differentiated thyroid carcinoma. The main morphological types of differentiated thyroid cancer are the papillary carcinoma (75–85% of all thyroid cancers) and the follicular carcinoma (10–20%). In most instances, both types are well-differentiated tumors that originate in the thyroid follicular cells (89). Well-differentiated thyroid carcinoma, either follicular or papillary, is one of the most curable cancers; the overall survival rate at 10 years for middle-aged adults with thyroid carcinoma is $\sim$80–90% (97). The reduced $\Gamma^-$ accumulation detected in the majority of thyroid cancers suggests that malignant transformation of these cells may have an effect on the NIS molecule. Thus the continued characterization of NIS may shed light on $\Gamma^-$ transport changes observed in thyroid cancer.

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To understand the involvement of NIS in the observed patterns of $\Gamma^-$ transport in thyroid carcinomas with respect to healthy thyroid tissue, several groups have looked for a possible correlation between the lower or absent radioiodine uptake activity in thyroid carcinomas and the expression of NIS mRNA or protein, using Northern blot analysis, RT-PCR, or immunological analyses. Smanik et al. (106) observed, by Northern blot analysis, lower expression of hNIS in two papillary, one follicular, and one anaplastic carcinoma. Upon further analysis of additional papillary carcinomas by RT-PCR, a more sensitive technique, they found that different papillary cancers expressed hNIS at variable levels.

Considering the limitations of RT-PCR as a quantitative method, Arturi et al. (7) were able to evaluate only the presence or absence of the NIS transcript in a series of malignant and benign thyroid tumors. They first analyzed by RT-PCR 26 primary thyroid carcinomas (19 papillary, 5 follicular, and 2 anaplastic), and subsequently a new series of 15 follicular adenomas (11 cold and 4 hot as classified by thyroid scintigraphy), and found loss of expression of NIS in 5 of 19 papillary cancers, 1 of 5 follicular cancers, and none detected in anaplastic tissues. All the (benign) follicular adenomas except one expressed NIS. According to these authors, the fact that even one benign follicular adenoma failed to express NIS means that the absence of the NIS mRNA signal may not be restricted to the malignant phenotype. In all the studied tumors, except anaplastic, both Tg and TPO transcripts were expressed.
Caillou et al. (14) conducted an immunohistochemical analysis of NIS protein expression in thyroid pathological tissues, including six benign adenomas, five follicular, and nine papillary carcinomas. No immunostaining for hNIS was detected in any of the six benign adenomas that appeared to be hypofunctioning on thyroid scintigraphy. hNIS expression was lower or absent in the papillary and follicular well-differentiated carcinomas, but more NIS was present in these than in the poorly differentiated follicular carcinomas. Consistent with these results, Jhiang et al. (48) detected no hNIS by immunohistochemical staining in any of the three papillary or the single follicular carcinoma that they studied. In contrast, Saito et al. (92) found increased NIS expression in papillary carcinomas. They investigated 31 papillary carcinomas by either Northern blot or immunological analysis. Based on their observations of higher NIS expression in ~50% of the analyzed carcinomas compared with nonmalignant thyroid tissue, the investigators concluded that despite the high amounts of NIS protein present, well-differentiated thyroid carcinomas do not accumulate radioiodide, suggesting that the activity of NIS is impaired or absent as a result of different posttranslational modifications, trafficking alterations, or other unknown modulatory factors. In cancerous follicular cells positive for hNIS, the localization of the protein was described as basolateral and intracellular (92).

In light of the data summarized above, one may conclude that hNIS expression may be either increased, decreased, or absent in well-differentiated thyroid cancer, even though I– transport activity is consistently decreased in the majority of tumors. Therefore, the explanation for the decreased I– uptake in well-differentiated thyroid carcinomas lies not simply in lower NIS expression, but more likely involves a more complex combination of regulatory changes ultimately affecting NIS expression, targeting, and/or activation. To date, there are still no data concerning the regulation of expression, posttranslational modifications, targeting to the plasma membrane, or other factors regulating NIS in cancerous follicular cells. Thus there clearly is a pressing need to investigate a much larger number of thyroid cancer specimens by more quantitative techniques to better understand the expression of hNIS both at the transcript and protein levels, to elucidate the cellular localization of NIS, and to be able to compare such findings to the clinical behavior of the carcinomas.

Some in vitro experiments concerning NIS-based gene therapy for both diagnostic and therapeutic purposes have been reported, in which NIS-mediated radioiodide uptake was used to visualize and destroy malignant tumor cells. Schimura et al. (96) transfected the hNIS gene into malignant rat thyroid cells that did not concentrate I–, resulting in increased I– accumulation in these cells in vitro. Using a retroviral vector, Mandell et al. (67) have recently introduced rNIS into melanoma, ovarian, liver, and colon carcinoma cells. The resulting rNIS transduced tumor cells exhibited I– uptake activity. In vitro experiments showed that these transduced cells could be destroyed by accumulation of 131I. If positive results were obtained in subsequent in vivo experiments, this gene therapy approach would undoubtedly be one of the most promising developments concerning the possible uses of the molecular characterization of NIS in the diagnosis and treatment of cancer.

VIII. CONGENITAL IODIDE TRANSPORT DEFECT DUE TO SODIUM/IODIDE SYMPORTER MUTATIONS

Congenital lack of I– transport is a rather uncommon thyroid condition that has been defined as an I– transport defect (ITD). The general clinical picture consists of hypothyroidism (which can be normalized in some cases with high I– supplementation or L-T4 substitutive therapy), goiter, low thyroid I– uptake (as determined by scintigraphy), and low saliva-to-plasma I– ratio (109, 126). Even though congenital hypothyroidism is an infrequent disease (incidence 1:3,000–1:4,000 in neonates) (113), it has an irreversible deleterious effect on the development of the newborn, finally resulting in cretinism if untreated. Mutations in thyroid-specific molecules, such as TPO (2, 11), Tg (60, 72), and TSHr (1, 110), have been identified among causes of congenital hypothyroidism. Most recently, NIS mutations have also been demonstrated to cause this disease. In the absence of a functional NIS molecule, I– has no access to the thyroid epithelial cells, resulting in decreased thyroid hormone biosynthesis (see sect. 1) and higher circulating levels of TSH, which in turn stimulate the morphological and biochemical changes in the thyroid that lead to the development of goiter.

Ever since the first case of congenital hypothyroidism due to an I– transport defect was described by Federmann et al. (36), several explanations have been proposed to better define the nature of the defect. However, the molecular basis of this condition has only begun to be examined after the cloning of NIS cDNA (21, 105) and the elucidation of the exon-intron organization of the NIS gene (106) (Fig. 7). About 48 cases of ITD, belonging to 33 families, have been reported worldwide to date. Seventeen cases from 13 families have been studied at the molecular level and shown to have a mutation in NIS (see Table 1).

Shortly after isolation of the cDNA that encodes NIS, two groups reported almost simultaneously a homozygous missense mutation in patients that had previously been diagnosed with congenital hypothyroidism caused by ITD (39, 70) (Table 1, cases 1 and 6, respectively). Both groups found a DNA substitution of adenine with cytosine
in nucleotide 1060 in exon 9, resulting in a Pro instead of Thr at position 354 (T354P) in NIS. The T354P mutation is located in the putative transmembrane segment IX of the NIS protein (Figs. 2B, 3, and 7).

The patient in the report of Fujiwara et al. (39) was homozygous for the T354P mutation and had consanguineous parents; her two siblings were heterozygous for T354P and were unaffected, suggesting the recessive nature of the disease. Extremely high doses of potassium iodide treatment maintained this patient euthyroid. HEK-293 cells (human embryonic kidney cells) transfected with the mutant T354P NIS cDNA did not exhibit any I\(^2\) uptake activity. Based on these results, the authors proposed that T354P probably disrupts (due to the presence of proline), through a structural change, the putative transmembrane segment IX of NIS, where the mutation is located.

The second case (70) was diagnosed 23 years ago with an I\(^-\) transport defect (44) (Table 1, case 6). The patient was euthyroid apparently on account of a very high iodide dietary intake. However, diffuse goiter with slightly elevated TSH was noted at 18 yr of age. The patient exhibited greatly increased NIS mRNA levels (>100-fold) in his thyroid. Hence, these investigators proposed that the observed increase in NIS transcription may reflect a compensating mechanism for low NIS activity, possibly related to transcriptional regulation of NIS by I\(^-\) itself. This patient was born from a consanguineous marriage, and his daughter was heterozygous for the mutation but had no abnormal phenotype, as would be expected in a recessive condition.

Subsequently, Levy et al. (65) studied the T354P mutation by site-directed mutagenesis and transfection of COS cells. They determined that the T354P NIS was not active, but they observed by immunofluorescence analysis that it was properly targeted to the plasma membrane. Then various other amino acids substitutions (Ala, Pro, Cys, Tyr, Ser) at position 354 were explored to determine the structural change in putative transmembrane segment IX, leading to the conclusion that the hydroxyl group at the \(\beta\)-carbon of the amino acid residue at position 354 is essential for NIS function (see sect. ii).

Fujiwara et al. (40) have reported four new cases of patients with the T354P mutation from two families (Table 1, cases 2–5). Case 2 was diagnosed at age 5, treated with thyroid powder, and thus maintained euthyroid; however, this patient's thyroid gland enlarged gradually and developed multinodular goiter at the age of 14. The patient's parents were heterozygous for the T354P mutation without thyroid disorders. The other three patients reported by Fujiwara et al. (40) are siblings (Table 1, cases 3–5). These patients and their mother were heterozygous for T354P, but their father was negative for this mutation. The patients presented a small goiter that developed late in life with only slight symptoms of hypothyroidism. Even though the patients were heterozygous for the T354 mutation, they were previously diagnosed as having ITD but were not screened for other NIS mutations.

Kosugi et al. (55) have reported more cases of T354P mutations, including three new and four reevaluated cases with known ITD from five families (Table 1, cases 6–12). All these cases were homozygous for the T354P
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SP, salivary to plasma ratio of I; FA, follicular adenoma; empty box, test not done or unknown; Cons, parental consanguinity; TRIU, thyroid radioiodide uptake (%); T<sub>4</sub>, thyroxine; T<sub>3</sub>, triiodothyronine; TSH, thyrotropin; TM, transmembrane segment. Patients 3-5 only have been screened for the T354P mutation.
mutation. Whenever the patients’ parents were analyzed, they were heterozygous for the T354P mutation and had no obvious thyroid disorders. The first case (case 6) was previously described by the same group (see above) (70). Cases 6 and 7, siblings, were euthyroid due to an extremely high I\(^{-}\) diet. They presented large diffuse goiters at ages 18 and 20, respectively. In cases 9 and 12, hypothyroidism was diagnosed early so that L-T\(_4\) treatment was started and goiter development was prevented. The rest of the cases had different degrees of diffuse goiter and hypothyroidism. NIS protein determinations were carried out in cases 6 and 10 and were found to be increased ~10-fold with respect to normal tissue. Immunohistochemical staining of NIS in these two patients indicated that NIS was overexpressed and properly localized in the basolateral plasma membrane of the thyroid cells (55).

The same group has subsequently reported three patients with ITD from two families, presenting new mutations of the NIS gene (54) (Table 1, cases 13–15). Case 13 had already been reported (91) and reviewed earlier by Wolff (126). This patient carried compound heterozygous mutations. From the paternal allele he presented a DNA substitution of guanidine with cytosine in the 277 nucleotide in exon 1. From the maternal allele he presented a substitution of adenine with cytosine in nucleotide 1060 in exon 9. These resulted in the compound G93R/T354P mutation, affecting the putative transmembrane segments III and IX of NIS (Fig. 7). The patient’s father was heterozygous for the G93R mutation and his mother for the T354P mutation, both without thyroid disorders. They were not consanguineous. The patient had developed diffuse goiter at age 15, and he had I\(^{-}\) responsive hypothyroidism.

Cases 14 and 15 are siblings. They carried the homozygous substitution of guanidine for adenine in the 1,628 nucleotide in exon 13. The resulting G543E mutation is located in the putative transmembrane segment XIII of NIS (Fig. 7). The patients’ parents, who were not consanguineous, were heterozygous for G543E, without thyroid disorders. The patients’ younger sister did not carry the mutation. Neither G93R, T354P, G93R/T354P, nor G543E mutant cDNA transiently transfected into COS-7 displayed any I\(^{-}\) transport activity (54). Nevertheless, these mutations were not characterized at the protein level so that the possibility that these mutations resulted in a nonfunctional protein or one not properly targeted to the plasma membrane remains open.

Only two ITD cases characterized at the molecular level are not from Japan (85, 86) (Table 1, cases 16 and 17). The first one (Table 1, case 16), from Brazil, was also reviewed by Camargo et al. (15). The patient presented a substitution of cytosine 1163 with adenine in exon 6, resulting in a premature stop codon at position 272 (C272X). The mutant NIS protein is truncated in the hydrophilic segment between the putative transmembrane segments VII and VIII (Fig. 6), and no I\(^{-}\) uptake activity was detected after transfecting mutant NIS cDNA into COS-7 cells. The patient was homozygous for the mutation. His parents were not consanguineous; his mother, son, and a paternal aunt were heterozygous for C272X, and his father was not investigated. All of the carriers were euthyroid with normal or slightly enlarged thyroid glands. The patient’s goiter was noted at 3 mo of age and treated with L-T\(_4\), but examination after several years revealed low T\(_4\) and high TSH levels. A nodule developed in each thyroid lobe. Subsequent treatment with high I\(^{-}\) restored the patient to the euthyroid state.

Case 17 in Table 1 was reported by Pohlenz et al. (86). This was a patient of Hispanic ancestry who carried a compound heterozygous mutation. From the paternal allele she presented a substitution of cytosine for guanidine in nucleotide 1146 in exon 6 that resulted in a Gln for Gln amino acid substitution (Q267E) in NIS. This mutation is located between the putative transmembrane segments VII and VIII of NIS (Fig. 7). COS cells transfected with the mutated cDNA did not exhibit I\(^{-}\) transport activity. The patient’s brother, father, and a paternal uncle and aunt were heterozygous for the mutation, and they were clinically unaffected. From the maternal allele she presented a substitution of cytosine with guanidine in nucleotide 1940 in exon 13. This point mutation has a double effect. First, it creates a stop codon at amino acid 531 (Y531X) with the consequent deletion of the entire hydrophilic COOH terminus (Fig. 7). Second, it generates a new 3’-acceptor splice site for intron 12 that produces a 67-nucleotide deletion in exon 13, rendering a protein with only 515 amino acids that has a deleted part of the putative transmembrane segment XIII and II of the COOH terminus of NIS. The in vitro activity of these truncated proteins was not evaluated. None of the heterozygous maternal members was affected. This patient was diagnosed with hypothyroidism during neonatal screening and treated with L-T\(_4\). At 12 yr of age she was diagnosed as having ITD. She presented a nodule that had not been observed 1 yr before. Histological investigation revealed a follicular adenoma.

In vitro experiments in cells transfected with the various NIS mutants found in ITD patients have shown that the mutant NIS proteins do not elicit I\(^{-}\) transport. However, the precise structural change brought about by a NIS mutation has only been determined for T354P. Therefore, it remains to be determined whether the other NIS mutations give rise to proteins that are nonfunctional but properly targeted to the plasma membrane, proteins that are rapidly degraded, or retained in intracellular organelles. It is unknown how I\(^{-}\) enters the thyroid follicular cells in these patients. However, a high I\(^{-}\) diet has been shown to be able to maintain some of these patients in a euthyroid state. It is possible that thyroidal I\(^{-}\) transport in these patients occurs by “simple diffusion” or by
nonspecific channels, as suggested by Wolff (126). Moreover, Cl\(^-\) channels have been shown to be able to transport other halides with different affinities, and in some cases, these channels display a considerable affinity for I\(^-\) (26, 27, 52). Administration of high doses of potassium iodide (KI) (1–200 mg/day) has been reported to maintain these patients euthyroid, without apparent long-term side effects.

The inheritance pattern of ITD is autosomal recessive in all cases. In the investigated families, the heterozygous carriers were always clinically unaffected, and only a few of them presented slight goiter. Nevertheless, cases 3–5 in Table 1, reported by Fujiwara et al. (40), were heterozygous for T354P. However, other mutations could have been present in these patients and missed in the study because only T354P was screened.

Most of the patients suffering from this condition have been from Japan. The high incidence of ITD in the Japanese population could be related to the high I\(^-\) diet prevalent in Japan, which provides an adequate I\(^-\) supply even for ITD patients, and allows them to maintain acceptable thyroid hormone synthesis. In a few ITD cases, expression and activity of the other TSH-regulated thyroid-specific proteins (TPO and Tg) were also investigated. Low or normal levels of Tg protein were observed in the cases that were studied (Table 1, cases 13 and 16; and 5 more cases reported by Wolff (126)). In case 16, Tg was poorly iodinated, but the patient was euthyroid as a result of a high I\(^-\) diet. This indicates that under certain circumstances “non-NIS mediated transport” is able to supply enough I\(^-\) into the follicle to ensure hormonogenesis. Furthermore, Lamas et al. (58) have shown that the efficiency of hormonogenesis is determined by the available amount of I\(^-\). Lamas et al. (57) also reported that under conditions of low I\(^-\) intake the synthesis of thyroid hormones on the Tg molecule occurred at the main hormonogenic site, and Passler et al. (83) showed that this utilization was regulated by TSH.

In two of the patients reported by Wolff (126), TPO activity was normal or slightly increased. In case 16 (15), the activity and immunostaining of TPO were threefold with respect to normal thyroid tissue, probably due to the high TSH levels. These findings suggest that only NIS is affected in ITD patients, whereas Tg and TPO expression and activity remain intact despite the upregulated TSH levels. Anti-Tg, anti-TPO, and anti-TSHr autoantibodies were negative when tested in all these patients.

Thyroid morphological findings are fairly heterogeneous, even in patients carrying the same NIS mutation. Among the patients in which NIS mutations have been characterized at the molecular levels (as summarized in Table 1), we find 2 cases without goiter (cases 9 and 12), 11 cases with small or large diffuse goiter (cases 3–8, 10, 11, and 13–15), 1 case with nodular goiter (case 16), and 3 cases with follicular adenoma (cases 1, 2, and 17). Two cases (cases 6 and 7) remained euthyroid. Most probably, this variability reflects the roles played by multiple factors in the onset and evolution of ITD due to NIS mutations, including the type of mutation, time of detection of the condition, time and type of administered therapy, and I\(^-\) content in the diet. In any case, the considerable strides made in this area of research in recent years illustrate the powerful impact that detailed function/structure studies of this key molecule can have on health and disease.

**IX. CONCLUDING REMARKS**

The landscape of NIS research has been dramatically transformed in a very short time. Just six years ago, in 1993, the following was written in a review on “Iodide Transport in the Thyroid Gland” (16): “The chemical nature of the putative I\(^-\) carrier has been the subject of some debate in the past. At one time, phospholipids were considered potential I\(^-\) carriers as much as proteins, but it has long been clear that transport of ions and small molecules (such as carbohydrates and amino acids) across cell membranes is mediated by intrinsic membrane proteins, not phospholipids.” Later in the same article, it was stated that “the Na\(^+/\)I\(^-\) symporter molecule has not been fully characterized yet, as its size, number of subunits, and amino acid sequence remain unknown,” and with respect to NIS the article concludes by saying that “there is no question that our understanding of I\(^-\) transport processes, both in the thyroid and in other tissues, will be greatly enhanced when this molecule is fully identified and characterized. Once this is achieved, insight may subsequently be gained into various important related research fields, given the relevance of I\(^-\) transport to Na\(^+/\)I\(^-\)-dependent symport mechanisms, thyroid physiology and pathophysiology, hormone action mechanisms, and cell differentiation.” The present review, in fact, focuses on reports that started to appear shortly after publication of the quoted article, covering the isolation of a cDNA encoding rat NIS, the preparation of anti-NIS antibodies to study NIS topology and its secondary structure, the examination of the biogenesis and posttranslational modifications of NIS, its electrophysiological analysis, the isolation of the cDNA encoding hNIS, the elucidation of the genomic organization of hNIS, the analysis of NIS regulation by TSH and I\(^-\), and the regulation of NIS transcription, among several other topics. It also discusses spontaneous NIS mutations that have been identified as causes of congenital ITD resulting in hypothyroidism, the roles of NIS in thyroid cancer and thyroid autoimmune disease, and the expression and regulation of NIS in extrathyroidal tissues. Perhaps most significantly, the above accomplishments have made it possible to carry out gene therapy experiments in which the rat NIS gene has been transduced into various types of hu-
man cells, which then exhibited active iodide transport and became susceptible to destruction with radioiodide. Hence, the impact of the continued molecular analysis of NIS has already reached farther than predicted a few years ago, given that it extends not only to structure/ function of transport proteins, thyroid pathophysiology, hormone action mechanisms, and cell differentiation, but also, quite possibly, to the diagnosis and treatment of cancer, both in the thyroid and beyond.

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REFERENCES


MARINE D AND FEISS HO. The absorption of potassium iodide by perfused thyroid glands and some of the factors modifying it. J Pharmacol Exp Ther 7: 557–567, 1915.


