Hormones and Transport Systems

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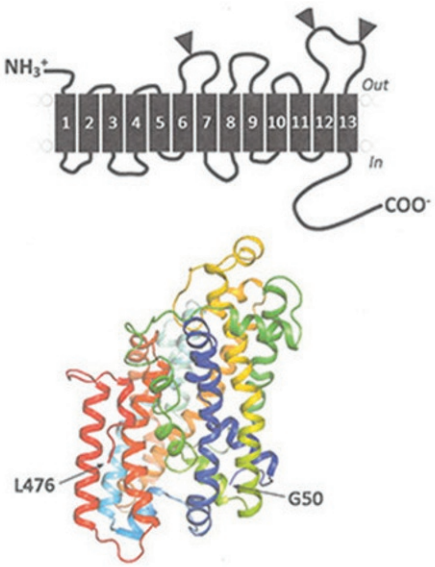

VITAMINS AND HORMONES, VOLUME 98

CHAPTER ONE

Dietary I^- Absorption: Expression and Regulation of the Na^+/I^- Symporter in the Intestine

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Abstract

Thyroid hormones are critical for the normal development, growth, and functional maturation of several tissues, including the central nervous system. Iodine is an essential constituent of the thyroid hormones, the only iodine-containing molecules in vertebrates. Dietary iodide (I^-) absorption in the gastrointestinal tract is the first step in I^- metabolism, as the diet is the only source of I^- for land-dwelling vertebrates. The Na^+/I^- symporter (NIS), an integral plasma membrane glycoprotein located in the brush border of enterocytes, constitutes a central component of the I^- absorption system in the small intestine. In this chapter, we review the most recent research on structure/function relations in NIS and the protein's I^- transport mechanism and stoichiometry, with a special focus on the tissue distribution and hormonal regulation of NIS, as well as the role of NIS in mediating I^- homeostasis. We further discuss recent findings concerning the autoregulatory effect of I^- on I^- metabolism in enterocytes: high

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intracellular I^- concentrations in enterocytes decrease NIS-mediated uptake of I^- through a complex array of posttranscriptional mechanisms, e.g., downregulation of NIS expression at the plasma membrane, increased NIS protein degradation, and reduction of NIS mRNA stability leading to decreased NIS mRNA levels. Since the molecular identification of NIS, great progress has been made not only in understanding the role of NIS in I^- homeostasis but also in developing protocols for NIS-mediated imaging and treatment of various diseases.

1. THE IMPORTANCE OF IODIDE IN HUMAN HEALTH

Iodide (I^-) uptake in the thyroid gland is the first step in the biosynthesis of thyroid hormones—triiodothyronine (T_3) and thyroxine (T_4) (Portulano, Paroder-Belenitsky, & Carrasco, 2014). Thyroid hormones are the only iodine-containing hormones in vertebrates and are required for the development and maturation of the central nervous system, skeletal muscle, and lungs in the fetus and the newborn. They are also primary regulators of intermediate metabolism and effect pleiotropic modulation in virtually all organs and tissues throughout life (Yen, 2001).

Iodine is an extremely scarce element in the environment and is supplied to the body exclusively through the diet. Insufficient dietary I^- intake may cause mild to severe hypothyroidism and subsequently goiter, stunted growth, retarded psychomotor development, and even cretinism (impairment of physical growth and irreversible mental retardation due to severe thyroid hormone deficiency during childhood) (Zimmermann, 2009). I^- deficiency-associated diseases are the most common preventable cause of mental retardation in the world and were slated for global eradication by iodination of table salt by the year 1990 by the World Health Organization. Although significant progress has been made, there were still an estimated 1.88 billion people suffering from insufficient I^- intake in 2011 (Andersson, Karumbunathan, & Zimmermann, 2012).

As iodine is an irreplaceable component of thyroid hormones, normal thyroid physiology relies on adequate dietary I^- intake, gastrointestinal I^- absorption, and proper I^- accumulation in thyrocytes. Therefore, the evolution of a highly efficient system to avidly accumulate I^- appears to be a physiological adaptation to compensate for the environmental scarcity of iodine.

2. THE Na^+/I^- SYMPORTER

The thyroid gland has developed a remarkably efficient system to ensure an adequate supply of I^- for thyroid hormone biosynthesis. Under

physiological conditions, the thyroid concentrates I^- approximately 40-fold with respect to the plasma concentration (Wolff & Maurey, 1961). Moreover, the ability of the thyroid to concentrate I^- has provided the molecular basis for the use of radioiodide in the diagnosis, treatment, and follow-up of thyroid pathology (Bonnema & Hegedus, 2012; Reiners, Hanscheid, Luster, Lassmann, & Verburg, 2011). A major breakthrough in the field—as important as the introduction of radioactive I^- isotopes into the study of thyroid physiology near the middle of the twentieth century (Hertz, Roberts, Means, & Evans, 1940)—was the identification of the complementary DNA (cDNA) encoding the Na^+/I^- symporter (NIS), the protein that mediates I^- transport in the thyroid (Dai, Levy, & Carrasco, 1996). The identification of NIS started a new era of intensive I^- research.

2.1 Molecular identification of NIS

The journey toward the identification of NIS began with the isolation of poly(A^+) RNA from FRTL-5 cells, a line of highly differentiated rat thyroid-derived cells which, microinjected into *Xenopus laevis* oocytes, produced Na^+-dependent I^- transport (Vilijn & Carrasco, 1989). Thereafter, the cDNA encoding NIS was isolated by expression cloning in *X. laevis* oocytes using cDNA libraries generated from FRTL-5 cells (Dai et al., 1996). The full nucleotide sequence revealed an open reading frame of 1,854 nucleotides encoding a protein of 618 amino acids. Shortly thereafter, the screening of a human thyroid cDNA library with rat NIS probes enabled the identification of human NIS (Smanik et al., 1996), which exhibits 84% identity and 93% similarity to rat NIS. The human NIS gene was mapped to chromosome 19p13.11 and comprises 15 exons with an open reading frame of 1,929 nucleotides, giving rise to a protein of 643 amino acids (Smanik, Ryu, Theil, Mazzaferri, & Jhiang, 1997).

NIS is an intrinsic plasma membrane glycoprotein. The current, experimentally tested NIS secondary structure model shows a hydrophobic protein with 13 transmembrane segments (TMSs), an extracellular amino terminus and an intracellular carboxy terminus (Levy et al., 1997, 1998; Fig. 1A). Moreover, NIS is a highly *N*-glycosylated protein, although *N*-glycosylation is not essential for I^- transport or NIS trafficking to the plasma membrane (Levy et al., 1998).

NIS-driven active transport of I^- into the thyroid is electrogenic and relies on the driving force of the Na^+ gradient generated by the Na^+/K^+ ATPase and the electrical potential across the plasma membrane. By

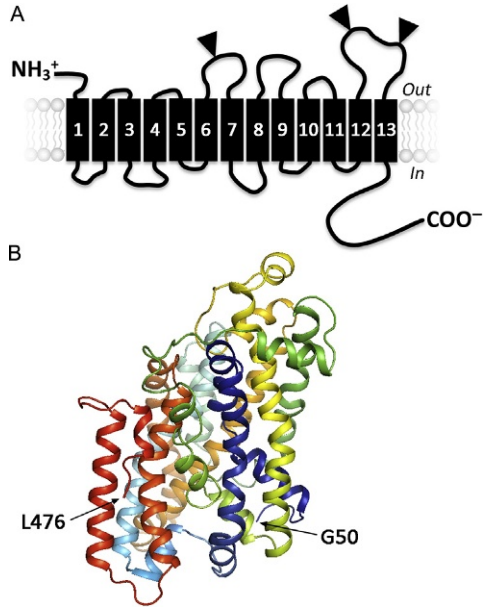

Figure 1 NIS secondary and tertiary structure. (A) Secondary structure. NIS secondary structure model showing the 13 transmembrane segments from the extracellular amino terminus to the intracellular carboxy terminus. Black triangles mark N-linked glycosylation sites at N225, N485, and N497. (B) Tertiary structure. Membrane plane of the NIS homology model built using the rat NIS sequence including residues G50 through L476 (Paroder-Belenitsky et al., 2011), based on the X-ray structure of vSGLT. The NIS homology model is shown as a ribbon representation and rainbow colored by sequence, from the amino terminus (blue) to the carboxy terminus (red).

coupling the inward transport of Na^+ down its electrochemical gradient to the translocation of I^- against its electrochemical gradient across the plasma membrane, NIS avidly concentrates I^- into the cells (Dai et al., 1996; Eskandari et al., 1997).

Like all membrane transporters, NIS belongs to the solute-carrier gene (SLC) superfamily. In particular, NIS is a member of solute-carrier family 5A (SLC5A) and has been designated SLC5A5 according to the Human Genome Organization (HUGO) Gene Nomenclature Committee. To date, the only crystal structure of a member of SLC5A is that of the *Vibrio parahaemolyticus* Na^+/galactose transporter (vSGLT), a bacterial homologue of the human SGLT1 (SLC5A1) (Faham et al., 2008). Despite the lack of sequence homology, as predicted by De la Vieja, Reed, Ginter, and Carrasco (2007), the structure of vSGLT revealed the same fold—an

inverted topology repeat and unwound helices in regions critical for substrate binding—and a Na^+ coordination similar to that observed in the high-resolution (1.65 Å) crystal structure of the leucine transporter (LeuT) from *Aquifex aeolicus* (LeuT) (Yamashita, Singh, Kawate, Jin, & Gouaux, 2005). Remarkably, NIS shares significant identity (27%) and homology (58%) with vSGLT—almost as much as SGLT1 does (31% identity, 62% homology). Therefore, Paroder-Belenitsky et al. (2011) generated a structural homology model for rat NIS, comprising residues 50–476, using as template the crystal structure of vSGLT (Fig. 1B). Importantly, the development of the 3D homology model helped bridge the gap between the secondary and tertiary structures and further contributed to our understanding of the relation between NIS structure and function. Using our NIS homology model, we uncovered the interaction between the δ-amino group of Arg-124 with the thiol group of Cys-440, concluding that the interaction between intracellular loop (IL)-2 and IL-6 is critical for the local folding required for NIS maturation and targeting to the plasma membrane (Paroder, Nicola, Ginter, & Carrasco, 2013). Moreover, we proposed that the side chain of Asn-441 interacts with the main chain amino group of Gly-444, capping the α-helix of TMS XII and thus stabilizing NIS structure (Li, Nicola, Amzel, & Carrasco, 2013).

2.2 NIS-mediated transport: Substrates and stoichiometry

Using electrophysiological techniques, Eskandari et al. (1997) demonstrated NIS-elicited inward currents when Na^+-dependent I^- accumulation occurs in NIS-expressing *X. laevis* oocytes. Simultaneous flux experiments with radioactive tracers and electrophysiological data established that NIS-mediated I^- transport is electrogenic, with a 2 Na^+/1 I^- stoichiometry (Eskandari et al., 1997). Similar inward currents were observed with different NIS-transported anions. However, surprisingly, the environmental pollutant and well-known inhibitor of thyroidal I^- uptake perchlorate (ClO_4^-) did not elicit currents and, further, abolished I^--induced inward currents (Eskandari et al., 1997). The blockage of I^- transport by ClO_4^- has been used in the treatment of hyperthyroidism and is currently used in the detection of I^- organification defects (ClO_4^- discharge test) (Hilditch, Horton, McCruden, Young, & Alexander, 1982). As radioactive $^{36}ClO_4^-$ was not available for flux experiments, the most likely interpretation was that ClO_4^- blocked NIS activity. A decade later, Dohan et al. (2007) conclusively demonstrated that ClO_4^- is actively transported by NIS. The kinetic

parameters of NIS-mediated ClO_4^- transport were determined using the structurally related anion perrhenate (ReO_4^-). Flux experiments using ^{186}Re revealed active accumulation of $^{186}ReO_4^-$, and Na^+-dependent initial rates of ReO_4^- transport indicated an electroneutral stoichiometry (1 Na^+/1 ReO_4^- or ClO_4^-). Therefore, these results demonstrated that NIS translocates different substrates with different stoichiometries (Dohan et al., 2007). NIS-mediated ClO_4^- accumulation has been reported using chromatography-electrospray ionization-tandem mass spectrometry (Tran et al., 2008) and yellow fluorescent protein-based genetic biosensors (Cianchetta, di Bernardo, Romeo, & Rhoden, 2010).

2.3 The role of physiological Na^+ concentrations in NIS affinity for I^-

Na^+-driven symporters such as NIS are expected to exist in at least two conformations, an open-out conformation in which they are open to the extracellular milieu and bind the substrates to be transported, and an open-in state where they are open to the cytoplasm and release the substrates (Krishnamurthy & Gouaux, 2012; Yamashita et al., 2005). The current model of coupled transport is the alternating access model, according to which structural changes occur between the two conformations, allowing the transport of substrates across biological membranes. During the transition, uncoupled flux is prevented by intermediate states that close off access to the binding sites, and after the substrates are released into cytoplasm, the transporter reverts to the open-out state with the binding sites empty.

In Na^+-driven symporters, the coupling mechanism requires that the conformational changes occur when the transporter has bound both Na^+ and substrate. To fulfill this requirement, the transporter must use binding site occupancy to control conformational transitions. Experimental evidence suggests that Na^+ triggers a conformational change, as Na^+ stabilizes the open-out state until the substrate binds (Zhao et al., 2010). Moreover, substrate binding to the open-out conformation was proposed to initiate the conformational change by overcoming the stabilizing effect of Na^+ binding (Zhao et al., 2010). Nevertheless, the coupling mechanism remains poorly understood at the molecular level.

Very recently, Nicola, Carrasco, and Amzel (2014) addressed a fundamental mechanistic question: how NIS binds and releases its substrates. Taking advantage of the fact that NIS translocates I^- and ReO_4^- with different stoichiometries, the authors analyzed initial rates of transport

measured at different concentrations of substrates using statistical thermodynamics and determined the affinity of NIS for the transported ions as well as the relative populations of the different NIS species present during the transport cycle. They showed that empty NIS has a very low intrinsic affinity for I^- ($K_d = 224$ μM), but it increases 10 times ($K_d = 22.4$ μM) when two Na^+ ions are bound to the transporter. Moreover, at physiological Na^+ concentrations, approximately 79% of NIS molecules are occupied by two Na^+ ions, and hence poised to bind and transport I^-, even though the physiological concentration of I^- in the blood is in the submicromolar range, well below the affinity of NIS for I^- (Nicola et al., 2014). Ultimately, understanding the conformational changes that NIS undergoes during the transport cycle and the changes in Na^+/anion stoichiometry will require us to obtain structural information on NIS with different substrates bound and in different conformations.

3. NIS EXPRESSION BEYOND THE THYROID

In addition to the thyroid, I^- uptake has been demonstrated in other tissues, including the lacrimal drainage system, choroid plexus, salivary glands, stomach, and lactating breast. Indeed, radioiodide accumulation outside the thyroid is routinely observed in whole-body radioiodide scintiscans (Bruno et al., 2004). Interestingly, patients with congenital hypothyroidism due to NIS mutations display no I^- transport in the thyroid or any extrathyroidal tissue, highlighting the role of NIS in mediating I^- transport in all these tissues (Spitzweg & Morris, 2010). NIS was initially thought to be a thyroid-specific protein, but since NIS was cloned and NIS-specific antibodies generated, various groups have detected NIS protein expression in extrathyroidal locations previously known to actively accumulate I^-, such as salivary glands, stomach, and lactating breast (Altorjay et al., 2007; La Perle et al., 2013; Spitzweg, Joba, Schriever, et al., 1999; Tazebay et al., 2000; Vayre et al., 1999; Wapnir et al., 2003). In addition, NIS expression was demonstrated in the lacrimal sac and nasolacrimal duct, kidney, placenta, and ovary (Di Cosmo et al., 2006; Donowitz et al., 2007; Mitchell et al., 2001; Morgenstern et al., 2005; Riesco-Eizaguirre et al., 2014; Spitzweg et al., 2001).

The functional significance of NIS expression is clear in some extrathyroidal tissues but in others remains largely unknown. The placenta allows I^- to pass from the maternal to the fetal circulation for normal fetal thyroid function. The observation that NIS is mainly expressed at the apical

membrane of cytotrophoblasts is consistent with this (Di Cosmo et al., 2006; Mitchell et al., 2001). In the lactating breast, NIS is expressed at the basolateral membrane of ductal epithelial cells (Tazebay et al., 2000). NIS translocates I^- from the bloodstream to the maternal milk, where it reaches a concentration of approximately 150 μg/L, thus providing the nursing newborn with a supply of I^- adequate for thyroid hormone biosynthesis. Although basolateral NIS expression has been demonstrated in the mucus-secreting and parietal cells of the stomach and ductal epithelial cells in the salivary glands (Altorjay et al., 2007; La Perle et al., 2013; Spitzweg, Joba, Schriever, et al., 1999; Vayre et al., 1999; Wapnir et al., 2003), the physiological role of I^- accumulation in the saliva and gastric juice is a matter of debate. Given the scarcity of I^-, some authors have speculated that the secretion of I^- into the gastrointestinal tract may serve as an I^- recycling mechanism (Venturi & Venturi, 2009), as I^- that is not accumulated in the thyroid or released by the action of iodothyronine deiodinases in peripheral tissues is secreted into the saliva and gastric juice and likely reabsorbed further down the gastrointestinal tract along with newly ingested I^-, thus preventing excessive renal excretion. Moreover, I^- has been proposed to serve antioxidant and antimicrobial functions in these tissues (El Hassani et al., 2005; Geiszt, Witta, Baffi, Lekstrom, & Leto, 2003). It is worth emphasizing that NIS in the stomach is not involved absorbing dietary I^- from the stomach lumen into the bloodstream, as previously suggested (Kotani et al., 1998). Importantly, I^- accumulation in the saliva has long served as a key diagnostic tool in the detection of genetic defects in I^- transport (patients with NIS-inactivating mutations do not accumulate I^- in the saliva; Portulano et al., 2014).

The excretion of I^- occurs primarily through glomerular filtration in the kidney. Measurement of urinary I^- is the simplest method to assess I^- intake, as under I^- sufficiency almost all ingested I^- is excreted in the urine (Vejbjerg et al., 2009). I^- clearance involves glomerular filtration and partial tubular reabsorption as well as secretion from the plasma. However, the events that regulate tubular I^- handling remain poorly understood. Immunohistochemical analysis has revealed NIS expression in the tubular system of the human kidney. Using a monoclonal anti-human NIS antibody, Spitzweg et al. (2001) observed predominant intracellular immunostaining throughout the entire tubular system, without evidence of plasma membrane localization. Later, Wapnir et al. (2003) showed NIS expression in six out of six tissue microarray cores derived from normal human kidney samples. The protein was localized at the apical surface of principal and

intercalated cells of renal distal and collecting tubules, suggesting a potential role for NIS in mediating I^- reabsorption. Other immunohistochemical studies did not reveal NIS staining in kidney tissue (Lacroix et al., 2001; Vayre et al., 1999). However, none of these studies measured NIS-mediated renal I^- transport, either absorption or secretion. So, it is still an open question whether NIS is functionally expressed and regulated in the kidney.

Recently, Riesco-Eizaguirre et al. (2014) reported NIS expression at the basolateral membrane of ovarian surface epithelial cells and in secretory cells of the epithelium of the fallopian fimbriae, but not in ovarian stromal cells, in 14 out of 14 healthy women. NIS expression in the ovary was functionally evaluated using a gamma camera; 49 out of 345 women (15%) accumulated $^{99m}TcO_4^-$ in the ovary region, suggesting that NIS mediates physiological I^- accumulation in the reproductive tract.

Elucidating the mechanisms of NIS expression and regulation in extrathyroidal tissues may help us not only to understand I^- metabolism and prevent or minimize side effects of radioiodide therapy but also to better handle patients under treatment (Bonnema & Hegedus, 2012; Reiners & Luster, 2012). The most common side effects are swelling; nausea and vomiting; gastritis; dry mouth, taste changes, and sialadenitis; dry eyes and conjunctivitis; disturbances of female reproductive function; and decreased testicular function. Unsurprisingly, these side effects may be related to NIS-mediated radioiodide accumulation in the relevant tissues. Therefore, understanding tissue-specific NIS regulation may help us selectively downregulate NIS expression to minimize side effects as well as enhance NIS expression in particular tissues to increase the efficiency of radioiodide therapy.

Immunohistochemical analysis of frozen or paraffin-embedded tissue sections offers the advantage of revealing not only the expression of NIS but also its subcellular localization. However, as mentioned, conflicting results have been reported regarding NIS expression in several extrathyroidal tissues. This may be related to the quality of the different anti-NIS antibodies used, in terms of specificity and affinity for the relevant epitope and the procedures used to obtain and preserve the tissue. Moreover, studies in tissue microarrays are optimal for high-throughput screening but intrinsically limited because of the size of the samples and uncertainty about tissue preservation conditions. Detecting NIS mRNA or protein expression by real-time PCR or immunoblot analysis, respectively, may not be trivial. The samples would have to be highly enriched for a specific cell type before preparing

tissue lysates to ensure that NIS expression is not diluted out to undetectable levels (see NIS expression in the small intestine).

4. TARGETING OF NIS TO THE PLASMA MEMBRANE

Epithelial tissues are composed of polarized cells with an apical membrane facing the external or internal surface of the body (as in the skin or small intestine), or the lumen of a gland (i.e., as in thyroid), and a basolateral membrane facing the connective tissue. Apical-to-basolateral polarity defines different domains in terms of membrane protein expression and determines normal cell function. Therefore, differential sorting of membrane proteins to specific membrane domains is necessary for the generation and maintenance of biochemical polarity.

As previously mentioned, NIS displays different polarized localizations in different tissues. NIS is expressed at the basolateral membrane in the thyroid, stomach, salivary gland, and lactating breast. In contrast, NIS is targeted to the apical surface of placental cytotrophoblasts and the collecting tubules of the kidney. Although one may interpret this as indicating that different polarized NIS targetings arise from different tissue-specific I^--handling requirements, the mechanisms responsible for this behavior remain largely uncharacterized. Thus, these findings have raised new and intriguing biological questions about the posttranslational regulation of NIS in different tissues and about how different epithelia selectively interpret NIS sorting signals.

Little has been reported on the signals and molecular regions involved in the polarized targeting of NIS in the thyroid or other tissues. NIS sequencing in different tissues yielded the same protein identity (Spitzweg, Joba, Eisenmenger, & Heufelder, 1998), suggesting that factors other than the NIS sequence may regulate the polarized targeting of NIS. Analysis of the NIS intracellular carboxy terminus revealed the presence of conserved sorting sequences known to participate in retention, endocytosis, and targeting to the plasma membrane of proteins. In particular, the last four amino acids of the carboxy terminus of NIS constitute a putative class I PDZ-binding motif potentially involved in basolateral targeting. In addition, L556 and L557 constitute a potential di-leucine motif which may interact with the clathrin-coated system involved in protein endocytosis.

A major limitation in the study of NIS polarized targeting has been the nonexistence of highly functional polarized thyroid cell lines for *in vitro* studies. However, the Madin–Darby canine kidney cell line has been shown to

recapitulate the native polarity of several thyroid proteins (Paroder et al., 2006; Zhang, Riedel, Carrasco, & Arvan, 2002) and is therefore an interesting cell system in which to study NIS polarization signals.

NIS-mediated radioiodide therapy used to ablate thyroid cancer metastases and remnants after thyroidectomy has been the most successful targeted internal radiation anticancer therapy ever designed (Bonnema & Hegedus, 2012; Reiners et al., 2011). Radioiodide therapy depends on the ability of thyroid tumors to accumulate radioiodide, which is ultimately dependent on functional NIS expression at the plasma membrane (Schlumberger, Lacroix, Russo, Filetti, & Bidart, 2007). However, thyroid tumors often exhibit less I^- transport than normal thyroid tissue (or even no detectable transport) and are diagnosed as cold nodules on thyroid scintigraphy. Several reports have demonstrated that 70–80% of thyroid tumors in fact overexpress NIS when compared to surrounding normal tissue, suggesting the presence of trafficking abnormalities (Dohan, Baloch, Banrevi, Livolsi, & Carrasco, 2001; Kollecker et al., 2012; Tonacchera et al., 2002; Wapnir et al., 2003). No NIS mutations have been identified in thyroid tumors (Neumann et al., 2004; Russo et al., 2001), so it cannot be structural defects that impair targeting of NIS in these tumors; this stands in contrast to the situation in some patients with congenital I^- transport deficiency (Li et al., 2013; Paroder et al., 2013). Therefore, it is crucial that we understand the mechanisms that regulate the trafficking of NIS to the cell surface in normal and diseased tissue.

To date, only one NIS-interacting protein has been reported that may be involved in NIS plasma membrane targeting: the pituitary tumor-transforming gene binding factor (PBF). PBF expression is frequently upregulated in thyroid tumors. Smith et al. (2009) reported that ectopic PBF overexpression resulted in the redistribution of NIS from the plasma membrane into CD63-positive intracellular vesicles associated with clathrin-dependent endocytosis. Therefore, improving NIS-mediated radioiodide therapy for thyroid cancer may require that greater priority be given to developing strategies aimed at enhancing NIS plasma membrane expression, as opposed to just stimulating NIS transcription.

5. HORMONAL REGULATION OF NIS EXPRESSION

Hormonal regulation of NIS expression seems to be tissue specific. Thyrotropin (TSH) has long been known to be a key regulator of NIS expression and activity in the thyroid. Transgenic mice that do not express

the TSH receptor do not show detectable thyroidal NIS expression (Marians et al., 2002). Similarly, hypophysectomized rats show the same phenotype, but NIS expression can be restored in these animals by TSH administration (Levy et al., 1997). TSH regulates several steps in the biogenesis of NIS, including NIS expression at both the transcriptional and the posttranscriptional level (Kogai et al., 1997; Ohno, Zannini, Levy, Carrasco, & di Lauro, 1999; Riedel, Levy, & Carrasco, 2001). Detailed functional analysis of the NIS promoter has revealed that the transcription factor Pax8 plays a critical role in NIS transcription (Ohno et al., 1999).

The role of TSH in regulating NIS expression in the thyroid has been well established, but TSH does not regulate NIS expression in any extrathyroidal tissue. Importantly, withdrawal of thyroid hormone to increase endogenous TSH concentrations and administration of recombinant TSH are routinely used to stimulate I^- uptake in differentiated thyroid cancer to prepare patients receiving radioiodide for diagnostic scintigraphy and radioiodide therapy (Schlumberger et al., 2007). Tissue-specific NIS regulation makes it possible to improve the therapeutic outcome of stimulating radioiodide accumulation in the tumor cells and to simultaneously reduce the therapeutic dose of radioiodide, thereby decreasing its side effects.

NIS expression seems to be constitutive in the stomach and salivary glands and no hormonal regulation has yet been reported in these tissues. The mechanisms behind the differential regulation of NIS in different tissues remain largely unknown; clearly, the elucidation of these mechanisms will be a valuable contribution to basic science and likely to clinical medicine as well. For example, the development of novel strategies for allowing selective inhibition of NIS expression in salivary glands and stomach, thereby reducing tissue damage in thyroid cancer patients undergoing radiotherapy and decreasing radioiodide clearance, may permit a reduction of the therapeutic dose of radioiodide.

Although NIS is not expressed in healthy nonlactating breast tissue, NIS expression becomes evident toward the end of gestation and persists throughout lactation (Cho et al., 2000; Tazebay et al., 2000). In the lactating breast, NIS expression is stimulated by a combination of various hormones, including estrogen, prolactin, and oxytocin (Cho et al., 2000; Tazebay et al., 2000), and suckling is essential for maintaining NIS expression in the lactating breast after delivery (Tazebay et al., 2000). The combined administration of 17-β-estradiol and oxytocin in ovariectomized mice resulted in NIS expression, indicating that the effect of oxytocin on NIS expression in the mammary gland requires the presence of estrogen (Tazebay et al., 2000).

Placental NIS expression is regulated by pregnancy-related hormones such as human chorionic gonadotropin (hCG), prolactin, and oxytocin. These hormones increase I^- uptake in primary cultures of human placental cytotrophoblasts and human placental choriocarcinoma cell lines (Arturi et al., 2002; Burns, O'Herlihy, & Smyth, 2013). However, although neither 17-β-estradiol nor progesterone itself had any significant effect on NIS expression levels, the two hormones appear to work synergistically by increasing the effect of prolactin and oxytocin on NIS expression in the placenta (Burns et al., 2013). Pax8 expression has been described in placental tissue and placental cell lines. hCG increased cAMP-dependent Pax8 expression and DNA-binding activity. However, placental cells transfected with a Pax8-specific small interfering RNA did not show changes in NIS mRNA expression in response to hCG stimulation (Ferretti et al., 2005). These findings indicate that NIS expression in trophoblasts is modulated by transcription factors other than Pax8.

Physiological I^- accumulation in the rat female reproductive tract correlates with the reproductive cycle: NIS-mediated I^- accumulation coincides with the rise of estrogens during the follicular phase (Riesco-Eizaguirre et al., 2014). Interestingly, unligated estrogen receptor α cooperates with Pax8 to upregulate NIS transcriptional expression in transiently transfected HeLa cells. On the basis of these findings, Riesco-Eizaguirre et al. (2014) suggested that attention should be paid to when in their menstrual cycle women are given radioiodide.

6. DIETARY I^- ABSORPTION

I^- is supplied to the body exclusively through the diet; therefore, I^- absorption in the gastrointestinal tract constitutes the first step in I^- metabolism. Given the physiological importance of I^-, it has long been of major interest where and how dietary I^- is absorbed in the gastrointestinal tract.

To our knowledge, I^- absorption in the gastrointestinal tract was first reported by Hanzlik in 1912 (Hanzlik, 1912). This author showed that the most I^- absorption took place between the pylorus and the colon, and that the duodenum, jejunum, and ileum maintain this absorption rate. Later, Cohn (1932) measured I^- absorption in isolated canine small intestine, reporting that ingested inorganic iodine and iodate may be reduced to I^- in the gastrointestinal tract before being absorbed in the small intestine. Ingested I^- appears to be absorbed almost entirely in the gastrointestinal

tract. When euthyroid human patients were given a single oral dose of radioiodide, less than 1% of it was found in their feces, suggesting that ingested radioiodide is absorbed remarkably efficiently (Fisher, Oddie, & Epperson, 1965).

An important step toward the characterization of I^- absorption in the small intestine was the study by Josefsson, Grunditz, Ohlsson, and Ekblad (2002) involving ligation of the gastrointestinal tract. The authors demonstrated that pyloric ligation virtually abolished I^- accumulation in the thyroid after oral administration of radioiodide, but did not modify thyroid I^- accumulation after parenteral administration (Fig. 2). The reduction in I^- accumulation in the thyroid after oral administration in pylorus-ligated animals was accompanied by lower levels of I^- in the blood, indicating deficient I^- absorption (Fig. 2). Furthermore, animals receiving I^- intravenously showed substantial accumulation of I^- in the stomach, confirming that I^- is secreted into the lumen of the stomach rather than absorbed from it (Josefsson et al., 2002).

Although I^- absorption was restricted to the small intestine, it was initially not known whether there was a dedicated intestinal I^- transporter. Shortly after the cloning of NIS, several studies investigated NIS expression in the small intestine to test the hypothesis that NIS participates in dietary I^- accumulation; conflicting results were obtained. Using semiquantitative RT-PCR, Perron, Rodriguez, Leblanc, and Pourcher (2001) detected low

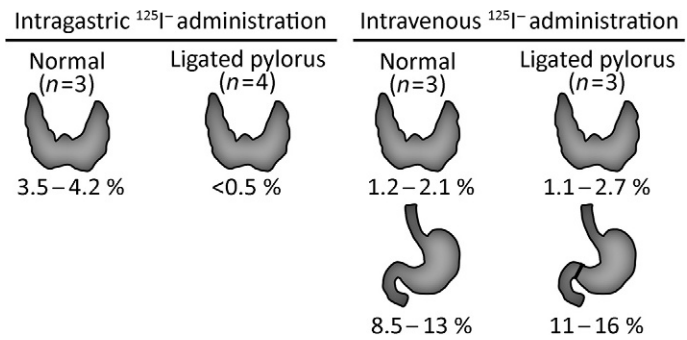

Figure 2 Gastrointestinal absorption and secretion of I^-. Untreated or pylorus-ligated rats received a bolus dose of radioiodide by intragastric or intravenous administration. After 60 min, radioactivity of thyroid glands and gastric washouts were determined and expressed as percentage of the total administered radioiodide dose. Values are indicated as ranges for each group and *n* indicates the number of animals per group (Josefsson et al., 2002). *Adapted from Josefsson (2009). Reproduced with permission.*

levels of NIS mRNA in the mouse small intestine. In contrast, previous reports did not detect NIS mRNA expression in whole human small intestine extracts by Northern blot analysis using a radiolabeled human NIS-specific probe (Spitzweg et al., 1998) or real-time PCR analysis (Lacroix et al., 2001).

The presence in a cell or tissue of NIS mRNA does not in itself show that the NIS protein is biosynthesized, targeted to the plasma membrane, or functional. Indeed, several independent studies using immunohistochemical procedures with unrelated anti-NIS antibodies failed to detect NIS protein expression in frozen and paraffin-embedded normal or pathological human small intestine specimens (Altorjay et al., 2007; Lacroix et al., 2001; Vayre et al., 1999). In contrast, Wapnir et al. (2003) investigated NIS protein expression in tissue microarrays containing cores from normal human small intestine by immunohistochemical analysis and found weak expression in two out of three samples. In agreement with this result, Donowitz et al. (2007) performed a detailed proteomic analysis of mouse jejunal brush-border enterocytes and demonstrated NIS protein expression by immunoblot and immunofluorescence staining, which validated NIS as a brush-border protein. These reports furnished more persuasive evidence that NIS may be involved in I^- absorption in the small intestine, but this evidence was not quite conclusive, as no functional data were provided.

Consistent with these findings, we have characterized I^- absorption in the small intestine and concluded that NIS may play a key role in dietary I^- absorption, since we demonstrated that NIS is functionally expressed on the apical surface of the absorptive epithelium (Nicola et al., 2009). We analyzed paraffin-embedded sections of small intestine from rats and mice by immunohistochemistry using an affinity-purified polyclonal anti-NIS antibody (Levy et al., 1997). In an immunohistochemistry study in which tissue samples were collected with special care, we consistently observed NIS protein expression along all three sections of the small intestine (duodenum, jejunum, and ileum), but exclusively on the brush border or microvilli, the finger-like projections that protrude from the apical membrane of absorptive enterocytes into the intestinal lumen (Fig. 3A–C; Nicola et al., 2009). This observation is compatible with the notion that NIS may translocate I^- from the intestinal lumen into absorptive enterocytes.

To demonstrate NIS protein expression by immunoblot, we followed a protocol described by Weiser (1973) to isolate villus-tip epithelial cells and further purify brush-border apical membranes because NIS expression is restricted to the most villus-tip-differentiated enterocytes. As expected, the procedure resulted in a pronounced enrichment (~20-fold) of the

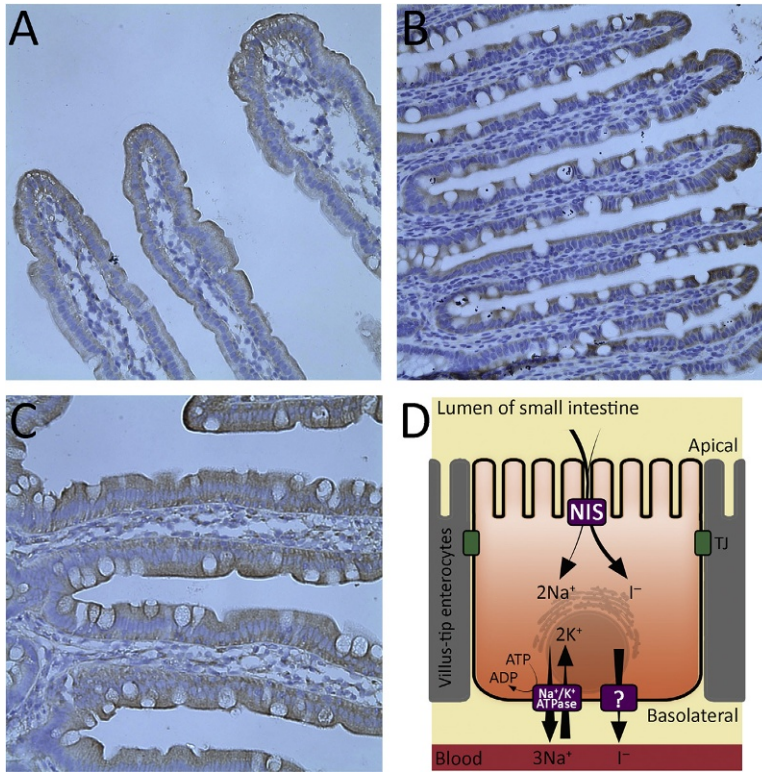

Figure 3 NIS expression in rat small intestine. Immunohistochemistry analysis demonstrating NIS expression in the three sections of the rat small intestine. NIS-specific immunostaining is evident in the apical membrane of the absorptive enterocytes (Nicola et al., 2009). (A) Duodenum, (B) jejunum, and (C) ileum. All pictures are presented at 40 × magnification. (D) Schematic representation of NIS-mediated I^- absorption in villus-tip small intestine enterocytes. NIS mediates transcellular apical transport of dietary I^- against its concentration gradient by coupling it to Na^+ transport. The Na^+ gradient is generated by the Na^+/K^+ ATPase in the basolateral membrane, maintaining the Na^+ electrochemical gradient that favors the Na^+-dependent accumulation of substrates. Transport of I^- into the blood may be facilitated by still uncharacterized channels or transporters. It is not known whether I^- is translocated across the intestinal epithelium through the intercellular space between the enterocytes (paracellular transport). TJ, tight junctions.

activity of alkaline phosphatase, a villus-tip marker, in membranes over cell homogenates. Immunoblot analysis of enriched brush-border membranes from villus-tip enterocytes revealed a 90-kDa polypeptide corresponding to intestinal NIS, whose electrophoretic mobility was identical to that of NIS from the thyroid cell line FRTL-5 (Nicola et al., 2009). It is worth

noting that NIS expression was undetectable in intestinal cell homogenates, but became evident upon enrichment.

To determine more precisely the functional significance of NIS expression in the enterocyte, we prepared sealed brush-border membrane vesicles (BBMVs) from isolated villus-tip enterocytes and performed steady-state I^- transport assays. I^- uptake in BBMVs was both Na^+-dependent and ClO_4^- – sensitive, two hallmarks of NIS activity. Moreover, kinetic analysis of I^- transport in BBMVs showed an affinity for I^- of 13.4 ± 2.0 μM, a value comparable to those reported for NIS-mediated I^- transport in thyroid membrane vesicles (O'Neill, Magnolato, & Semenza, 1987). We also investigated whether NIS mediates I^- absorption *in vivo*. We administered pertechnetate ($^{99m}TcO_4^-$), a widely used radioactive NIS substrate with a short half-life, alone or together with the NIS inhibitor ClO_4^- via duodenal catheterization, and collected blood samples through a jugular catheter placed in the right atrium. Interestingly, rats simultaneously treated with ClO_4^- absorbed 27–48% less $^{99m}TcO_4^-$ than rats treated with $^{99m}TcO_4^-$ alone (Nicola et al., 2009). These data provide strong evidence that NIS is a significant and possibly central component of the I^- absorption system in the small intestine (Fig. 3D).

However, our data do not rule out the possibility that channels or transporters other than NIS, such as chloride channels or anion exchangers participate, or the possibility that passive paracellular transport is involved in the absorption of I^- from the intestinal lumen—both of which are consistent with the partial inhibition of $^{99m}TcO_4^-$ absorption by ClO_4^-. de Carvalho and Quick (2011) have reported that the Na^+/multivitamin transporter (SLC5A6), the protein with the highest sequence homology with NIS, actively mediates Na^+-dependent but ClO_4^- – insensitive I^- transport, albeit with a lower affinity than NIS. On the basis of the intestinal expression of the Na^+/multivitamin transporter, this protein has been proposed to provide a complementary pathway for I^- absorption in the small intestine. This hypothesis can now be fruitfully tested in enterocyte-specific Na^+/multivitamin transporter knockout mice, a recently developed system (Ghosal, Lambrecht, Subramanya, Kapadia, & Said, 2013).

One important finding in the study of intestinal NIS has been the detection of functional NIS expression in late-passage IEC-6 cells (IEC-6 cells are a line of rat small intestine-derived cells). Performing flux experiments under steady-state conditions, we showed Na^+-dependent, ClO_4^- – sensitive I^- accumulation in IEC-6 cells (Nicola et al., 2009). Active I^- accumulation levels were higher in IEC-6 cells than in FRTL-5 cells, a result consistent

with the higher NIS protein expression levels observed by immunoblot in IEC-6 than in FRTL-5 cells. We analyzed the kinetic properties of NIS in IEC-6 cells and FRTL-5 thyroid cells. Intestinal NIS exhibited an affinity for I^- (K_m $I^- = 20.3 \pm 3.9$ μM) similar to that of thyroid NIS (K_m $I^- = 23.2 \pm 3.7$ μM) (Nicola et al., 2009). Therefore, IEC-6 cells may constitute a good *in vitro* model in which to study NIS regulation in intestinal cells.

Crohn's disease is an inflammatory bowel disease that may affect any part of the gastrointestinal tract from mouth to anus, most commonly the terminal ileum of the small intestine. The disease causes a wide variety of symptoms including diarrhea and malabsorption syndrome. Jarnerot (1975) investigated I^- metabolism in patients with chronic inflammatory bowel disease, including Crohn's disease. His results demonstrated that 10 out of 50 patients with chronic inflammatory bowel disease excreted less than 40 μg I^- in the urine over a 24-h period, compared with 5 out of 102 healthy controls. Moreover, 16 out of 38 patients showed a 24-h thyroid radioiodide uptake higher than 50% of the administered dose, compared with 4 out of 36 controls. Although these results suggested an increased occurrence of I^- deficiency in patients with chronic inflammatory bowel diseases, no evidence was found of impaired absorption of inorganic iodide from the gut as the amount of orally administered radioiodide they absorbed was not significantly different from the corresponding amount for control patients (Jarnerot, 1975). However, an accurate classification of patients with Crohn's disease according to the location of the inflammation (ileum, colon, or both) will shed light on the role of the small intestine in dietary I^- absorption.

Navarro, Suen, Souza, De Oliveira, and Marchini (2005) investigated the possible influence of intestinal malabsorption on I^- status in patients with severe bowel malabsorption due to chronic pancreatitis or short bowel syndrome who were fed exclusively parenterally and in control subjects. The study demonstrated that severe bowel malabsorption does not significantly affect I^- status, as patients and control subjects receiving equal dietary intakes over a period of 24 h did not show significant changes in daily urinary I^- excretion. However, a major limitation of the study was the small size of the population analyzed—only nine patients per group. Follow-up studies with more patients are needed to obtain conclusive data.

Recently, Michalaki et al. (2014) reported that dietary I^- absorption is not influenced by malabsorptive bariatric surgery. Urinary excretion of I^- was not reduced in obese patients following malabsorptive bariatric

surgery, although the stomach, the duodenum, and a substantial part of the jejunum were bypassed. This indicates that sufficient I^- is absorbed along the remainder of the gastrointestinal tract, as expected, given that NIS is expressed all along the small intestine (Nicola et al., 2009).

7. REGULATION OF INTESTINAL NIS EXPRESSION

I^- plays a key role in thyroid physiology, not only as an irreplaceable constituent of the thyroid hormones but also as a regulator of thyroid physiology and NIS expression and function (Portulano et al., 2014). Saturating concentrations of I^- downregulate thyroid function by inhibiting thyroid hormone biosynthesis, a phenomenon, ill understood at the molecular level, known as the Wolff–Chaikoff effect (Wolff & Chaikoff, 1948). This effect is followed by downregulation of I^- uptake leading to an "escape" from the effect, which restores thyroid hormone biosynthesis (Braverman & Ingbar, 1963). High I^- concentration-reduced I^- transport has been associated with a decrease in NIS expression. In thyroid cells, the regulation of NIS mRNA levels by I^- excess has mainly been attributed to a transcriptional effect (Eng et al., 1999; Spitzweg, Joba, Morris, & Heufelder, 1999; Uyttersprot et al., 1997). However, more recent data suggest that NIS regulation by I^- takes place at the posttranscriptional and posttranslational levels (Dohan, De la Vieja, & Carrasco, 2006; Leoni, Kimura, Santisteban, & De la Vieja, 2011; Serrano-Nascimento, Calil-Silveira, & Nunes, 2010). The "escape" from the Wolff–Chaikoff effect seems to be an adaptive response that serves to reduce intracellular I^- levels, thus protecting thyrocytes from the oxidative effects of I^- excess. Very recent evidence suggests a link between thyroid oxidative state and the Wolff–Chaikoff effect. Serrano-Nascimento et al. (2014) reported the involvement of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) signaling activation in I^- excess-downregulated NIS function in thyroid cells. Interestingly, I^- excess led to increased generation of mitochondrial reactive oxygen species that trigger activation of PI3K signaling (Serrano-Nascimento et al., 2014).

We investigated the regulatory effect of dietary I^- intake on NIS expression and function in rat small intestine. Animals were divided into four groups, three of which received a high concentration of I^- (0.05%) in their drinking water for 12–48 h. The effect of I^- administration was compared with that in a fourth group of animals, which received regular water. After treatment, villus-tip enterocytes were isolated and sealed BBMVs prepared from treated and nontreated rats to perform steady-state I^- transport studies.

High concentrations of I^- significantly reduced NIS-mediated I^- uptake by 55% at 24 h, and the decrease in I^- transport became more pronounced with longer exposure times. Moreover, BBMVs were subjected to immunoblot analysis to assess NIS protein expression. Consistent with the reduced I^- uptake, high dietary I^- levels decreased NIS protein levels by 49% at 12 h, relative to the levels of the control group. The same treatment given for 48 h decreased NIS protein expression by as much as 83% (Nicola et al., 2009). Importantly, no significant changes were observed in the expression of the differentiation marker alkaline phosphatase. Thus, our data demonstrate that high I^- concentrations inhibit NIS-mediated I^- uptake *in vivo* in the small intestine, just as in the thyroid.

To further our understanding of the molecular mechanism involved in the high I^- concentration-regulated expression of intestinal NIS, we investigated the effect of I^- *in vitro* using IEC-6 cells (Nicola, Reyna-Neyra, Carrasco, & Masini-Repiso, 2012). When these cells were incubated with a high concentration of I^- (100 μM), there was a significant reduction in I^- transport 3 h after I^- treatment, which became more pronounced with longer incubation times. This is due to a decrease in I^- influx rather than an increase in I^- efflux. Analysis of the kinetic parameters of I^- transport demonstrated that I^- excess did not affect the apparent affinity of NIS for its substrates. Together, these findings suggested that high concentrations of I^- may decrease the number of functional NIS molecules at the plasma membrane of enterocytes.

Indeed, surface biotinylation experiments revealed a significant time-dependent reduction in NIS expression at the plasma membrane by 6 h after I^- treatment. However, total NIS protein levels were only reduced 24 h after I^- treatment. Moreover, immunofluorescence studies showed time-dependent decreased colocalization of NIS and the Na^+/K^+ ATPase (a plasma membrane marker) and increased NIS intracellular staining in response to I^- excess by 6 h. Complementarily, we determined intracellular NIS protein levels by assaying the supernatant remaining after streptavidin-purified biotin-labeled surface proteins. Immunoblot analysis showed that NIS intracellular expression increased from 6 to 12 h after I^- treatment, but the amount of intracellular NIS decreased after 24 h, consistent with the observed reduction in total lysates (Nicola et al., 2012).

Given that only NIS molecules at the plasma membrane take up I^-, we established a correlation between the reduction in I^- accumulation and the corresponding lowering of NIS expression at the cell surface induced by I^- excess (Nicola et al., 2012). The prompt recruitment of NIS from the

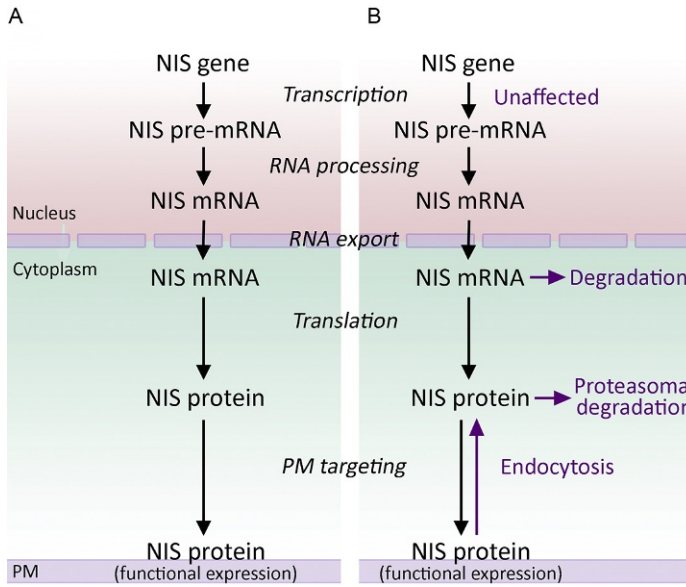

Figure 4 Different levels of intestinal NIS regulation induced by high concentrations of I^-. (A) Under normal conditions, the NIS gene is transcribed into NIS pre-mRNA. Then introns are removed and exons reconnected to generate mature NIS mRNA, which is exported to the cytoplasm and translated into protein. NIS protein is glycosylated and targeted to the plasma membrane of small intestine absorptive enterocytes where it mediates I^- transport. (B) Under I^- excess, although the NIS gene is normally transcribed, mature NIS mRNA levels are decreased due to a reduction in its stability. NIS protein expression levels are diminished in response to increased proteasomal degradation, and NIS expression at the plasma membrane is downregulated due to increased amiloride-sensitive internalization. As a result, I^- excess decreased NIS-mediated accumulation of I^- in enterocytes (Nicola et al., 2012).

plasma membrane to intracellular compartments upon I^- treatment suggests the existence of posttranslational mechanisms for reducing the number of NIS molecules at the plasma membrane. Interestingly, we observed that the physiological control of NIS expression at the cell surface of enterocytes seems to involve constitutive macropinocytosis-dependent endocytosis, as amiloride treatment increased I^- transport in IEC-6 cells and abolished I^- excess-induced NIS internalization (Nicola et al., 2012) (Fig. 4).

In addition to NIS endocytosis, we observed a significant reduction in NIS protein expression in I^--treated IEC-6 cell lysates after 24 h. Therefore, we investigated the effect of I^- on NIS levels after cycloheximide treatment in IEC-6 cells to study any changes there might be in NIS protein stability (Nicola et al., 2012). We found that the half-life of NIS in cells treated with

excess I^- was 36% lower than that of NIS in control cells, suggesting that increased NIS protein degradation is partially responsible for the lower levels of I^--induced NIS protein expression in intestinal cells. To determine the proteolytic pathways involved in I^--induced NIS protein degradation, we incubated IEC-6 cells with lysosomal or proteasome inhibitors (Nicola et al., 2012). The lysosomal inhibitor cocktail chloroquine plus ammonium chloride did not have an effect on NIS levels, independently of the presence of I^-. In contrast, the proteasome inhibitor MG132 markedly increased NIS protein expression levels and prevented excess I^--induced reduction of NIS expression, indicating that NIS protein turnover in enterocytes is regulated via the ubiquitin–proteasome system (Fig. 4).

Although increased NIS protein degradation could by itself account for the observed reduction in NIS expression in response to excess I^-, a reduction of NIS mRNA levels is also compatible with a decreased protein translation, which in turn may lead to reduced protein biosynthesis. Indeed, real-time PCR analysis demonstrated an I^--induced time-dependent reduction in NIS mRNA levels in IEC-6 cells, without a significant change in the mRNA levels of alkaline phosphatase (Nicola et al., 2012) (Fig. 4). We further determined NIS mRNA expression *in vivo* in response to an I^--rich diet. Rats received 0.05% I^--supplemented drinking water for different periods of time, whereas control rats received regular water. Villus-tip small intestine epithelial cells were isolated and further processed for total RNA extraction. Quantification of NIS mRNA levels showed substantial reduction in enterocytes subjected to the I^--supplemented diet after 24 h, while I^- had no effect on alkaline phosphatase mRNA expression. In a complementary experiment, we observed a significant increase in intestinal NIS mRNA levels in enterocytes of animals fed with an I^--deficient diet for 2 and 4 weeks, but no effect on alkaline phosphatase mRNA expression (Nicola et al., 2012).

Messenger RNA expression levels are the result of gene transcription and mRNA stability and degradation. Therefore, we investigated a potential negative transcriptional effect exerted by excess I^- on NIS transcriptional activity (Nicola et al., 2012). We transiently transfected IEC-6 cells with a luciferase reporter construct containing a 2867-bp DNA fragment from the rat NIS promoter (−2854 to +13, +1 being the adenosine of the start codon) and determined whether excess I^- had an effect on transcriptional promoter activity. Although the NIS regulatory region showed strong transcriptional activity in intestinal cells, this activity was not modified by I^- excess; however, the possibility cannot be entirely ruled out that

transcriptional regulation occurs outside the region tested (Fig. 4). In agreement with our data, Leoni et al. (2011) reported that high concentrations of I^- do not modulate NIS promoter activity in thyroid cells.

As excess I^- reduced NIS mRNA levels in the absence of a transcriptional effect, we investigated whether excess I^- regulates NIS mRNA stability in IEC-6 cells (Nicola et al., 2012). We evaluated the half-life of NIS mRNA in cells treated (or not) with I^- in the presence of the mRNA synthesis inhibitor actinomycin D for several periods of time. Whereas I^- significantly shortened the half-life of NIS mRNA by almost 75%, I^- did not have an effect on the half-life of alkaline phosphatase mRNA. Our data taken together are consistent with the notion that there is in enterocytes a previously unknown mode of posttranscriptional regulation of NIS by its own substrate. Consistent with this, several findings have suggested that thyroid NIS mRNA stability or translation efficiency may decrease owing to shortening of the molecule's poly-A tail in response to I^- administration (Serrano-Nascimento et al., 2010).

Untranslated regions (UTRs) of mRNAs play crucial roles in the posttranscriptional regulation of gene expression. In particular, 3′-UTRs harbor determinants that control mRNA stability and translation efficiency (Jackson, Hellen, & Pestova, 2010; Sonenberg & Hinnebusch, 2009). Therefore, to study the involvement of NIS UTRs in the I^--triggered regulation of NIS mRNA, we generated heterologous green fluorescent protein (GFP) reporters containing both the NIS mRNA 5′-UTR sequence (−92 to −1) upstream of the GFP open reading frame (5′-UTR-GFP) and the 3′-UTR sequence (+1858 to +2761) downstream of the GFP coding sequence (GFP-3′-UTR) (Nicola et al., 2012). IEC-6 cells were transiently transfected with the aforementioned reporters or a control vector expressing GFP and treated with excess I^-. Interestingly, I^- excess had no effect on GFP mRNA expression in GFP- and 5′-UTR-GFP-transfected cells but markedly reduced GFP mRNA expression in GFP-3′-UTR-transfected cells. Given that all the GFP-based reporters are controlled by the cytomegalovirus promoter, these results suggest that the NIS 3′-UTR sequence regulated GFP mRNA stability, rather than its transcription in the presence of I^-.

Interestingly, other trace elements also regulate at the posttranscriptional level the expression of genes coding for proteins involved in their own transport or metabolism. For example, iron, selenium, zinc, and calcium regulate the mRNA abundance of transferrin receptor, glutathione peroxidase, the zinc transporter ZnT5, and parathyroid hormone, respectively. The

mechanisms that underlie this regulation involve mRNA UTRs, particularly 3′-UTRs (Bermano, Arthur, & Hesketh, 1996; Erlitzki, Long, & Theil, 2002; Jackson et al., 2007; Moallem, Kilav, Silver, & Naveh-Many, 1998; Nechama, Uchida, Mor Yosef-Levi, Silver, & Naveh-Many, 2009; Owen & Kuhn, 1987). Although the mechanism related to I^--induced NIS mRNA decay remains unknown, our findings suggest the presence of functional *cis*-acting elements in the NIS mRNA 3′-UTR sequence related to the I^- regulatory effect. We identified a homozygous mutation -54C>T in the NIS 5′-UTR as responsible for dyshormonogenic congenital hypothyroidism due to reduced NIS mRNA translation efficiency (Nicola et al., 2011), highlighting the importance of UTR sequences in gene expression regulation. These results taken together demonstrate that dietary I^- plays an essential role in enterocyte physiology by controlling its own NIS-mediated absorption and thus regulating the supply of I^- to the body (Nicola et al., 2009, 2012).

There are currently no data regarding hormonal regulation of NIS expression in the small intestine. However, given the great importance of I^- in thyroid hormone biosynthesis, the thyroid hormones themselves might regulate intestinal I^- absorption and NIS expression. Thyroid hormones have been shown to modulate the developmental processes responsible for intestinal maturation, such as the onset of digestive enzyme expression and the regulation of intestinal homeostasis. Thyroid hormone receptor (TR)-α is mainly involved in postnatal small intestine development, as TR-α-deficient mice display alterations in bone and small intestine development, in contrast to TR-β-deficient mice, which do not display retarded intestinal development (Plateroti et al., 1999). Importantly, strong nuclear expression of TR-α was observed in the differentiated epithelial cells of the intestinal villi, indicating that thyroid hormones may regulate gene expression in enterocytes (Gauthier et al., 2001). Therefore, we hypothesize that thyroid hormone levels decrease under chronic I^- deficiency, increasing intestinal NIS expression to maximize dietary I^- absorption.

8. CONCLUSIONS AND FUTURE DIRECTIONS

In light of the findings here reviewed, we propose that NIS is a key molecule in I^- metabolism: it mediates dietary I^- absorption in the small intestine; I^- uptake in the thyroid; and I^- accumulation in breast milk, fetal blood, saliva, and gastric juice. In these last two secretions, I^- accumulation

causes the anion to return to the gastrointestinal lumen, where it is again reabsorbed by NIS in the small intestine as part of the I^- conservation system.

NIS expression at the apical surface of the absorptive epithelium of the small intestine is crucial for I^- absorption, the first step in I^- metabolism. These findings underscore the physiological and regulatory significance of the apical localization of intestinal NIS, which contrasts with the basolateral localization observed in virtually all other tissues that express NIS, including the thyroid.

Regulation of intestinal NIS may be elucidated by experiments on the NIS promoter aimed at understanding the molecular mechanisms involved in the expression and regulation of intestinal NIS, as well as the identification of transcription factors required for intestinal NIS expression.

A topic of great significance closely related to that of NIS expression in the small intestine is the fact that NIS mediates active transport of the environmental pollutant ClO_4^-. As ClO_4^- is a frequent contaminant in drinking water sources, intestinal NIS may be a conduit through which ClO_4^- enters the bloodstream. Because NIS actively transports ClO_4^-, the effects of ClO_4^- exposure on public health are more detrimental than previously supposed, particularly for pregnant and nursing women with partial I^- deficiency and, even more worryingly, for their children, the exposure of whom to high ClO_4^- levels puts them at risk of impaired development, not only physically but also intellectually.

There is no pathological condition currently known to result in gastrointestinal I^- malabsorption. Although we have mentioned a few studies investigating I^- absorption in bowel malabsorption syndromes, most of them suffer from several limitations, mainly having to do with sample size. In our view, studies involving more patients may shed light on whether or not patients suffering malabsorption syndromes need I^- supplementation. It will be of great interest to investigate frequent small intestine-restricted malabsorption syndromes such as celiac disease.

We expect that generating small intestine-specific NIS knockout mice will unequivocally establish the role of NIS expression in dietary I^- absorption. This aim will also be well served by a systematic study of patients with NIS mutations that cause congenital I^- transport defect.

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