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Ion channel-transporter interactions

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Abstract

All living cells require membrane proteins that act as conduits for the regulated transport of ions, solutes and other small molecules across the cell membrane. Ion channels provide a pore that permits often rapid, highly selective, and tightly regulated movement of ions down their electrochemical gradient. In contrast, active transporters can move moieties up their electrochemical gradient. The secondary active transporters (such as SLC superfamily solute transporters) achieve this by coupling uphill movement of the substrate to downhill movement of another ion, such as sodium. The primary active transporters (including H^+/K^+-ATPases and Na^+/K^+-ATPases) utilize ATP hydrolysis as an energy source to power uphill transport. It is well known that proteins in each of these classes work in concert with members of the other classes to ensure, for example, ion homeostasis, ion secretion, and restoration of ion balance following action potentials. More recently, evidence is emerging of direct physical interaction between true ion channels, and some primary or secondary active transporters. Here, we review the first known members of this new class of macromolecular complexes that we term "chansporters", explore their biological roles, and discuss the pathophysiological consequences of their disruption. We compare functional and/or physical interactions between the ubiquitous KCNQ1 potassium channel and various active transporters, and examine other newly discovered chansporter complexes that suggest we may be seeing the tip of the iceberg in a newly emerging signaling modality.

Keywords

active transport; ATPase; KCNQ1; NIS; SMIT1; voltage-gated potassium channel

INTRODUCTION

Ion channels constitute a numerous and eclectic class of membrane proteins that are essential for the function of most if not all cell types from the archaebacteria through to the plethora of specialized cells in higher mammals. Ion channels facilitate passive yet often rapid (e.g., tens of thousands of ions per second per channel pore) movement of aqueous ions across the otherwise forbidding barrier of the hydrophobic plasma membrane that evolved at least partly to prevent such leakage. Thus, ion channels rely on an electrochemical gradient for their activity. This property, together with their often

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remarkable ion selectivity (K^+ channels can readily permit K^+ movement but are proficient at limiting Na^+ leak, which bears similar charge to K^+ but is slightly smaller), has endowed cells with the ability to communicate on the millisecond timescale using action potentials. Conversely, some ion channels exist in non-excitable cells where their role is to ensure ion and fluid homeostasis or supply the necessary ingredients or conditions for biological functions such as hormone synthesis or mucous secretion. Another class of channels that primarily provides a conduit for water across the plasma membrane are the water channels, or aquaporins (Morelle et al. 2015).

Other types of membrane proteins can also mediate movement of ions or other moieties across cell membranes but do not fall into the category of ion channels, and are referred to as "transporters". The transporters that are perhaps furthest removed conceptually from the channels are the primary active transporters, some of which are also referred to as "pumps". Rather than relying on passive diffusion, the pumps can move molecules such as ions up an electrochemical gradient because they can utilize energy generated, for example, from hydrolysis of ATP (Pedersen & Carafoli 1987a; Pedersen & Carafoli 1987b).

Lying somewhere in the middle of the channels and the pumps are members of the Solute Carrier (SLC) superfamily that comprises facilitative transporters, which as the name suggests facilitate movement of solutes across the cell membrane down an electrochemical gradient ("downhill"), and the secondary active transporters, which can move their cargo uphill by coupling this transport to downhill movement of another solute.

Ion channels, solute carriers and pumps move ions and other solutes in and out of cells, covering a wide range of specificities, transport rates, and biological processes. Many of these functions are crucial production and secretion processes, others are events essential to intercellular communication. One would therefore expect a high degree of crosstalk between the different proteins in these classes. Recently, we and others have discovered a higher degree of crosstalk than expected, with some involving physical interaction between ion channels and transporters, with the formation of what we term "chansporter" complexes. Here, we review the current state of knowledge surrounding this newly-discovered biophysical phenomenon and distill what is known about the regulatory crosstalk between these two very different types of plasma membrane proteins. The focus is largely on a voltage-gated potassium (Kv) channel pore-forming (α) subunit, KCNQ1, that has been found to be particularly active in crosstalk with various transporters, but we discuss other examples that were also recently uncovered.

K+ CHANNELS AND SECONDARY ACTIVE TRANSPORTERS

The majority of the channel-transporter interactions identified to date are between K^+ channels and secondary active transporters that rely on coupling uphill movement of the desired solute to downhill movement of sodium ions (sodium-coupled solute transporters). In this category of interactions, KCNQ1 is the most often identified K^+ channel. Here, we first introduce the proteins involved before discussing their interaction.

Kv channels and Ca^{2+}-activated K^{+} channels

Voltage-gated potassium channel (Kv) pore-forming (α) subunits are encoded by genes of a 40-member family in humans. Conserved features among Kv α include a six transmembrane domains (S1-S6), a K^{+}-selective pore region formed by residues between S5 and S6, and a voltage sensor domain (VSD) comprising the S1-S4 segments, which moves in response to changes in membrane voltage (V$_m$) (Figure 1 A). The movement of the VSD controls channel opening by tugging on the intracellular S4-S5 linker region and inducing a conformational change to open the pore and permit K^{+} passage (Labro et al. 2011). Kv channels are especially well known for their essential functions in numerous excitable cell types including neurons, cardiac myocytes and skeletal muscle (Abbott et al. 2001; Jentsch 2000; Bellocq et al. 2004). KCNQ1, the primary Kv channel discussed in this review, is notable for its diverse functions in tissues including the heart, inner ear, thyroid, stomach, pancreas, lower gastrointestinal tract and choroid plexus (Lee et al. 2000; Harchi et al. 2010; Purtell et al. 2012; Warth et al. 2002; Abbott et al. 2014).

The *KCNQ1* gene encodes a protein spanning six transmembrane segments, and a tetramer of four such proteins forms a functional K^{+}-selective pore. KCNQ1 is best known for its crucial requirement in maintaining normal cardiac rhythm, arising from its role in repolarizing ventricular and atrial myocytes, responding to cellular depolarization by opening relatively slowly to permit K+ efflux and cellular repolarization to help end each heartbeat. Human KCNQ1 perturbation by, e.g., gene mutation, is associated with cardiac arrhythmias including Long QT syndrome (LQTS) (Tester et al. 2005) and atrial fibrillation (AF) (Chen et al. 2003; Bellocq et al. 2004), and has also been associated with extracardiac effects such as increased susceptibility to type II diabetes mellitus (T2DM) (Unoki et al. 2008; Yasuda et al. 2008; Zhou et al. 2010) and gastric cancer (Roepke et al. 2010; Than et al. 2014). In mice, *Kcnq1* gene deletion causes deafness, loss of gastric acid secretion, altered insulin sensitivity, and hypothyroidism (Lee et al. 2000; Frohlich et al. 2011; Grahammer et al. 2001; Boini et al. 2009). These extracardiac defects arise from perturbation of the role of KCNQ1 in non-excitable cells such as epithelial cells of the inner ear, stomach and thyroid (Lee et al. 2000; Frohlich et al. 2011)– highlighting the versatility of KCNQ1 and its necessity for many diverse biological processes.

Kv α subunits, including KCNQ1, can interact with smaller β subunits which drastically alter their function. KCNQ1 is most well-known for its interactions with β subunits of the KCNE family (Figure 1 A) (Abbott 2014). Each of the KCNE β-subunits alters KCNQ1 function differently. KCNE1 slows and right-shifts activation, increases conductance and removes inactivation (Tristani-Firouzi & Sanguinetti 1998; Sesti & Goldstein 1998; Sanguinetti et al. 1996; Barhanin et al. 1996); KCNE2 reduces peak outward current but makes KCNQ1 constitutively active (Tinel 2000); KCNE3 maintains peak outward current and makes KCNQ1 constitutively active (Schroeder et al. 2000); KCNE4 inhibits KCNQ1 (Grunnet et al. 2002); KCNE5 slows KCNQ1 activation and right-shifts the channel's voltage-dependence of activation so strongly as to render the channel inactive at physiological membrane potentials (Angelo et al. 2002).

Studies utilizing *Kcne2* knockout mice in particular have revealed the role of heteromeric KCNQ1-KCNE2 channels in supporting function of the thyroid epithelial cell sodium-iodide

symporter (NIS) and, in the choroid plexus epithelium, the sodium-dependent *myo*-inositol transporter (SMIT1)(Roepke et al. 2009; Roepke, Kanda, et al. 2011; Abbott et al. 2014). Investigation into the novel concept of KCNQ1-transporter complexes therefore has the potential to greatly improve our mechanistic understanding of KCNQ1- and KCNE2-linked human diseases as well as the generalizable mechanisms by which ion channels and transporters operate, signal to one another, and are regulated.

Calcium-activated K^+ (K_{Ca}) channels also play a critical role in many realms of physiology, including smooth muscle tone, action potential regulation, and cell metabolism (Rundén-Pran et al. 2002; Marrion & Tavalin 1998). K_{Ca} channels are generally grouped into three categories: large conductance (BK), small conductance (SK), and intermediate conductance (IK) (Vergara et al. 1998). MaxiK, a member of the BK subfamily of K_{Ca} channels and the primary K_{Ca} discussed in this review, is a seven transmembrane domain (S0-S6) protein with an extracellular N-terminus and large intracellular C-terminus, and must tetramerize to form a functional channel (Toro et al. 2014). MaxiK is involved in diverse protein-protein interactions, with proteins including tubulin (Ou et al. 2009), and voltage-gated calcium channel Cav2.1 (Indriati et al. 2013).

SLC-superfamily secondary active transporters

Sodium-dependent myo-inositol transporters—Both SMIT1 (*SLC5A3*) and SMIT2 (*SLC5A11*) are large transmembrane proteins of the Solute Carrier Family 5 (*SLC5A*) gene family, and they exhibit similar tissue expression patterns. These proteins, each spanning 14 transmembrane segments (Figure 1 B), function as sodium-coupled symporters and actively transport *myo*-inositol uphill into the cell by coupling to downhill transport of Na^+ ions. SMIT1 is additionally capable of transporting *scyllo*-inositol, L-fucose, L-xylose, L-glucose, D-glucose, α-methyl-D-glucopyranoside, and L-fucosea. SMIT2 transports *myo*-inositol, D-*chiro*-inositol, and D-xylose (Schneider 2015). The *myo*-inositol transported by each of these proteins functions as a crucial cellular osmolyte, is also a substrate of the phosphatidylinositol 4,5-bisphosphate (PIP$_2$) signaling pathway (Buccafusca et al. 2008; Holub 1986), and may also be converted to phosphatidylinositol (PI) – a crucial membrane component (Schneider 2015) (Figure 2). These functions are particularly pertinent to ion channel function, and especially for KCNQ1, because it (and to a greater or lesser degree many other channels) is highly sensitive to changes in both cellular volume and PIP$_2$ (Hammami et al. 2009; G Loussouarn 2003). Perturbations to the transporters' functions are therefore predicted to alter PIP$_2$-dependent processes such as KCNQ1 channel function as well as cellular responsiveness to osmotic stress, and have been implicated in pathologies including Down syndrome (Berry et al. 1995; Berry et al. 1999) and bipolar disorder (Davanzo et al. 2001; Harwood 2005; Willmroth et al. 2007).

The *myo*-inositol transported by the SMIT proteins serves many important functions within the cell, perhaps the most notable of which occurs after the conversion of *myo*-inositol to PI and PIP$_2$ by phosphatidylinositol 4-kinase (PI4K), followed by phosphatidylinositol 5-kinase (PI5K). Without PIP$_2$, many channels and cellular processes effectively shut off, including KCNQ channels, transient receptor potential (TRP) channels, inwardly-rectifying potassium (Kir) channels, plasmalemmal calcium pumps (PMCA), cardiac sodium-calcium

exchangers (NCX1), sodium-proton exchangers (NHEx), epithelial sodium channels (ENaC), and ryanodine-sensitive calcium channels (RyR). KCNQ channels, along with hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, are notable among the voltage-gated channels for their robust responses to PIP_2 (Suh & Hille 2008; Hilgemann et al. 2001; G. Loussouarn 2003). PI also plays a critical role in cellular machinery including activation of the Na^+/K^+-ATPase, modulation of catecholamine synthesis by tyrosine hydroxylase, and physical association with alkaline phosphatase and acetylcholinesterase (Holub 1986), and may also play a cooperative role in the actin cytoskeleton (Suh & Hille 2008).

PIP_2 also plays a prominent role as secondary messenger. When a Gq-coupled receptor is stimulated, phospholipase C (PLC) is activated and cleaves PIP_2 into constitutive components: inositol(1,4,5)-triposphate (IP3), and diacylglycerol (DAG) (Figure 2). DAG and IP3 each play a role in activating protein kinase C (PKC) leading to phosphorylation and altered activity of myriad cellular processes. IP3, then released into the cytosol, is free to bind to IP3 receptors, triggering calcium release from intracellular stores.

Of some level of controversy, inositol status has been implicated in various cognitive disorders. Lithium, valproic acid (VPA), and carbamazepine (CBZ) are each potent mood stabilizers which also alter inositol signaling. Lithium treatment reduces MI concentrations in the brain by 30% and concurrently elevates serum MI levels by 50% (Allison & Stewart 1971), and potently inhibits MI synthesis by inhibiting inositol-1 and inositol-4-monophosphatases in a substrate-dependent manner (Harwood 2005). These findings appear to be consistent with later work demonstrating a lithium-induced reduction in both manic symptoms and MI levels in human brain (anterior cingulate cortex) (Davanzo et al. 2001). VPA also inhibits inositol levels in the brain, although by different mechanism. Here, the treatment inhibits *myo*-inositol-1-phosphate (MIP) synthase activity, thereby preventing conversion of D-glucose-6-phosphate (G6P) to *myo*-inositol (Shaltiel et al. 2004). It comes as no surprise, then, that lithium and VPA are sometimes clinically co-administered in treatment of biopolar disorder (Harwood 2005). Interestingly, all three of these mood stabilizers (lithium, VPA, and CBZ) inhibit SMIT1 activity and mRNA transcript levels (Lubrich & van Calker 1999; Willmroth et al. 2007).

Even though inositol can be synthesized by some tissues, dietary restriction or supplementation of inositol raises/lowers inositol concentrations in most tissues, sometimes to deleterious effect (Holub 1986). In the liver, an inositol-deficient diet produces elevated triglycerides and fatty liver (Hayashi et al. 1974). In the intestine, an inositol-deficient high-fat diet produces lipodystrophy and altered gross morphology (Hegsted et al. 1973). Interestingly, each of these syndromes resolved upon addition of inositol to the experimental animals' diets.

Nomenclature of the SMITs can be a source of confusion. Cloned from Madin-Darby canine kidney (MDCK) cells in 1992, SMIT1 was originally known simply as SMIT (Kwon et al. 1992). In 1994 rkST1 was cloned from rabbit (Hitomi & Tsukagoshi 1994), although it was not until nearly a decade later that this protein was renamed to the more suitable "SMIT2" (Coady et al. 2002). Last, SMIT1 is purported to have two splice variants titled "SMIT1-2"

and "SMIT1-3" each of which exclude the 14th transmembrane segment of SMIT1 and possess differing c-termini (Schneider 2015).

In mice, deletion of the *Slc5a3* gene proves fatal shortly after birth, due to hypoventilation, although the phenotype can be rescued by supplementing the drinking water of both pup and dam with additional *myo*-inositol (Berry et al. 2003; Chau et al. 2005). Interestingly, *SLC5A3* shares exon 1 with mitochondrial ribosomal protein subunit 6 (*MRPS6*) with the entirety of the *SLC5A3* coding region contained by *MRPS6* exon 2. Deletion of *SLC5A3* does not alter MRPS6 expression, however. The implications of this exon-sharing are not yet clear, although there is some evidence to suggest the presence of a chimeric SMIT1/MRPS6 protein in human tissue (Buccafusca et al. 2008; Porcellati et al. 1999; Porcellati et al. 1998).

The sodium-dependent iodide symporter—The Na^+/I^- symporter (NIS), encoded by *SLC5A5*, is a large transmembrane protein containing 13 putative transmembrane segments, an extracellular N-terminal, and a cytosolic C-terminal (Figure 1 B). Like other members of the *SLC5A* family, NIS exhibits a high sequence similarity to other members of the *SLC5A* gene family, particularly the sodium-multivitamin transporter (SMVT, *SLC5A6*), with the most distinct sequence differences occurring near the C-terminus. Although the prototypical function of NIS may be associated with the thyroid, the protein is also expressed and functional in the mammary glands, placenta, gastrointestinal (GI) tract (Dohán et al. 2003), and potentially kidney (Vayre et al. 1999; Spitzweg et al. 2001).

In the thyroid, NIS enables active I^- uptake against the electrochemical gradient and into follicular cells - this process is the rate-limiting step in thyroid hormone (TH) biosynthesis. The I^- is then passed through to the colloid by another channel or transporter, the identity of which is still under debate. Once in the colloid, I^- undergoes oxidation by thyroid peroxidase and is incorporated into the thyroglobulin (Tg) molecule to produce monoiodotyrosine (MIT) and diiodotyrosine (DIT) in a process known as organification. The organified Tg is then resorbed by the follicular cells where, when stimulated by thyroid stimulating hormone (TSH), the iodinated Tgs are cleaved to form either thyroxine (T_4), generated from a pair of DIT, or triiodothyronine (T_3), produced from one MIT and one DIT molecule (Figure 3). These THs are critical for nervous system and lung development, as well as normal metabolic function (Morreale de Escobar et al. 2004). Perturbations to NIS function, accordingly, cause hypothyroidism (Carrasco 1993).

Sodium-dependent GABA transporters—In the brain, the inhibitory neurotransmitter GABA is transported across membranes by GABA transporters (GATs) GAT1, GAT2, GAT3 (Figure 1 B), and the betain/GABA transporter type 1 (BGT1). At inhibitory neuronal synapses, removal of GABA from the synaptic cleft by GATs acts to terminate the GABA signaling process and is accordingly a critical component of inhibitory neuron regulation (Jin, Galvan, et al. 2011). GAT3, a 12 transmembrane domain Na^+/Cl^--coupled transporter preferentially expressed in glia (Jin, Paré, et al. 2011; Jin, Galvan, et al. 2011; Borden & Caplan 1996), is here highlighted for its newly-discovered role in ion channel-transporter complex formation (see below) (Singh et al. 2016).

K⁺ channel-SLC transporter interactions

Functional interaction between KCNQ1-KCNE2 and NIS—We first reported the importance of KCNQ1-KCNE2 complexes for efficient thyroid hormone synthesis in 2009 when, together with the Carrasco lab, we discovered that *Kcne2*–/– mice are hypothyroid (Roepke et al. 2009). Using positron emission tomography we found that disruption of KCNQ1-KCNE2 complexes, which we discovered in thyroid epithelial cells, impairs iodide accumulation in the thyroid gland (Roepke et al. 2009). Subsequently, the Lang group confirmed this, using *Kcnq1*–/– mice (Frohlich et al. 2011). We later delineated which of the processes involved in thyroid iodide accumulation (focusing on iodide uptake versus organification) was disrupted by *Kcne2* deletion, discovering it to be iodide uptake by NIS (Purtell et al. 2012).

In the thyroid, I⁻ uptake is mediated by NIS (Figure 3) (Dai et al. 1996). We found that NIS-mediated I⁻ uptake can be strongly inhibited by the KCNQ1-selective antagonist [3*R*,4*S*]-chromanol 293B. This effect was selective to I⁻ transport, without directly altering organification, and occurred both *in vivo* (in the mouse), and *in vitro* (in the rat thyroid cell line, FRTL-5) (Purtell et al. 2012). The effects of chromanol 293B on I⁻ transport are only apparent when KCNQ1-KCNE2 and NIS are co-expressed, providing strong support for KCNQ1-KCNE2 mediation of NIS function.

Regarding the mechanism of KCNQ1-NIS interaction, we do not yet have confirmation of a physical interaction, although this is suspected. The KCNQ1-KCNE2 channel is constitutively active at weakly negative potentials, because KCNE2 modifies the voltage-dependent gating of KCNQ1 such that its current voltage relationship is linear rather than outwardly rectifying (Tinel 2000). The related β subunit KCNE3 achieves a similar effect on KCNQ1 by directly locking its VSD in the activated position via acidic residues in the membrane proximal KCNE3 extracellular segment (Choi & Abbott 2010; Panaghie & Abbott 2007), but this mechanism has not yet been confirmed for KCNQ1-KCNE2 (which is a more difficult channel to study, because KCNE2 also shrinks macroscopic current density). One might predict that the relatively constant outward K⁺ flux that KCNQ1-KCNE2 would be predicted to provide in thyroid cells at weakly negative potentials might facilitate NIS function by enhancing the electrical gradient required for sodium entry (with other pumps etc. redistributing Na⁺ and K⁺ ions to take care of the chemical part of the gradient), but we found that the NIS-related sodium-dependent monocarboxylate transporter (SMCT) was not affected by KCNQ1-KCNE2 status (Purtell et al. 2012), possibly suggesting against the electrical gradient hypothesis. However, it may be that for the outward movement of K⁺ through KCNQ1-KCNE2 to have any effect on sodium-dependent transporters, they need to be localized close to one another, or even tightly coupled; a hypothesis to be tested in the future. Either way, KCNQ1-KCNE2 is critical in the thyroid for its regulation of NIS activity, and an excellent example of biologically important functional interaction between an ion channel and a transporter. Accordingly, disruption of *Kcnq1* or *Kcne2* genes causes hypothyroidism, and in the case of *Kcne2* other phenotypic hallmarks of insufficient THs including dwarfism, alopecia, and cardiomegaly (Roepke et al. 2009; Frohlich et al. 2011).

Physical interaction between KCNQ1-KCNE2 and SMIT1—Additional phenotypic assessment of the *Kcne2* knockout mouse line lead to the discovery that *Kcne2* disruption in mice reduces the seizure threshold and causes behavioral abnormalities (Abbott et al. 2014). We had previously found that in the CNS, KCNE2 is most highly expressed in the choroid plexus epithelium, the primary site of cerebrospinal fluid (CSF) production and secretion (Roepke, Kanda, et al. 2011), where it regulates KCNQ1 and KCNA3 (Kv1.3) channels. We suspected a role for KCNQ1-KCNE2 in regulating CSF composition. After exhausting other hypothesis-based alternatives, we performed unbiased mass spectrometry-based metabolomics analysis of the CSF and found that *Kcne2* deletion altered the concentration of just one identifiable CSF metabolite: *myo*-inositol. We discovered that under normal conditions, KCNQ1-KCNE2 channels physically interact with the sodium-dependent myo-inositol transporter (SMIT1) to form chansporter complexes in which the two protein classes reciprocally regulate one another (Figure 2). KCNQ1 doubled the *myo*-inositol uptake activity of SMIT1, but addition of KCNE2 to the complex had the opposite effect, inhibiting SMIT1. SMIT1 augmented the activity of both KCNQ1 channels and KCNQ1-KCNE2 channels.

Early attempts to determine the mechanism for channel-transporter feedback have yielded interesting but not yet definitive results. Co-expression of KCNQ1 with SMIT1 roughly doubled SMIT1 activity versus SMIT1 alone. One might surmise that the increase in SMIT1 activity is a product of the potassium efflux through KCNQ1 channels, thereby increasing the electrical gradient to favor sodium-coupled *myo*-inositol transport. Consistent with this, we found that when co-expressed with KCNQ1, SMIT1 function was suppressed by XE-991, a pore-binding KCNQ channel-blocker (Abbott et al. 2014). These data provided a basis for targeting transporter activity through a novel drug approach: ion channel manipulation. In addition, they also suggested that K^+ flux through KCNQ1 is seemingly important for augmentation of SMIT1 activity. However, we demonstrated the channel-blocking effect was specific to KCNQ1-SMIT1, because when we co-expressed KCNQ4 and SMIT1 (between which we detected neither complex formation nor reciprocal regulation in oocytes), XE-991 had no effect, even though it inhibits KCNQ4, being a pan-KCNQ blocker. This, then, suggests that merely having K^+ flux through a channel in the same cell is not sufficient to augment SMIT1 activity – arguing that either the electrical gradient hypothesis is incorrect, or (as mentioned in the discussion of KCNQ1-NIS, above) the effect requires close physical proximity between channel and transporter.

Further suggesting that the chansporter inter-subunit cooperation is more complex than merely electrical gradient enhancement, we found unexpected results when studying the KCNQ1-R231A S4 mutant that locks the voltage sensor of the channel into an open, or "on", state, forming a constitutively active channel (Panaghie & Abbott 2007). Following the increased driving force hypothesis, this would be theorized to increase Na^+-linked *myo*-inositol influx, yet the addition of KCNQ1-R231A to SMIT1 greatly *inhibits* transporter function (Abbott et al. 2014). Within the context of the driving force hypothesis these findings are in diametric opposition to each other, and instead suggest the possibility that KCNQ1 voltage sensor conformation, independent of actual conductance, might influence SMIT1 activity. This could also be the reason that KCNE2 inhibits *myo*-inositol uptake of

KCNQ1-SMIT1 complexes, because KCNE2 also holds KCNQ1 open, although KCNE2 also greatly reduces outward current through KCNQ1 despite favoring its open state. Future investigations will be targeted at understanding exactly how KCNQ1 augments SMIT1 and NIS activity and whether there are underlying mechanistic commonalties. Intriguingly, we also found that KCNQ1 and SMIT2 functionally interact, and that SMIT2 inhibits KCNQ1 activity, but we do not yet know the mechanistic basis for these effects either (Abbott et al. 2014).

Physical interaction between MaxiK and GAT3—Very recently, Singh and colleagues uncovered an additional K^+ channel-transporter interaction in the brain (Singh et al. 2016). MaxiK channels – voltage- and Ca^{2+}-activated channels named for their large conductance – were demonstrated to physically interact with GABA transporter 3 (GAT3). As both GAT3 and MaxiK channels are localized in glia, the authors propose that the complexes may serve to limit GABA release, although no functional experiments have been published to date on this very recently discovered chansporter complex. Interestingly, GAT3 is encoded by *SLC6A11* – adding yet another member of the *SLC* superfamily to the list of transporters regulated functionally and/or physically by K^+ channels.

ION CHANNEL-ATPASE INTERACTIONS

Several examples are known of ion channels functionally interacting with transporters in the transmembrane ATPase family (and to our knowledge just one known case of physical interaction). Transmembrane ATPases are primary active transporters that use ATP hydrolysis to provide the energy to transport ions up their electrochemical gradient. In many cell types, ion channels and pumps are involved in activities such as maintaining ion homeostasis, that require one or more of the proteins to support function of one or more of the others. One broad example is the Na^+/K^+-ATPase. This ubiquitous protein eliminates 3 Na^+ ions while bringing in 2 K^+ ions, thus helping to restore the appropriate concentrations of K^+ and Na^+ ions, and also the negative membrane potential, following action potentials in excitable cells so that the cell is ready for future action potentials. Here, however, we will focus on just two contrasting examples. First we discuss facilitation of gastric H^+/K^+-ATPase activity by K^+ channels. As one of many examples of channels cooperating with pumps to ensure favorable cellular/extracellular ion balance or specific ion secretion but probably without the necessity for direct physical coupling, we focus on this example because it involves KCNQ1, which we also discussed above in the context of its ability to co-assemble with the SLC transporters. Second, we cover physical interaction of the unusual Orai1 Ca^{2+} channel with the SPCA2 ATPase – we chose this example because it involves formation of one of the thus-far few known examples of a macromolecular channel-transporter complex.

Functional interactions between K⁺ channels and the gastric H⁺/K⁺-ATPase

Under normal conditions, gastric acid secretion is coordinated by a small symphony of cellular events (Figure 4). In response to gastric parietal cell stimulation, H^+/K^+-ATPase is trafficked to the apical surface of the acid-secreting cells where it can begin the process of H^+ efflux in exchange for K^+ influx. The excreted H^+ becomes the substrate for HCl acid

formation, the intracellular K^+ causes a hyperpolarization of the parietal cell, preventing further acid secretion (Hersey & Sachs 1995). The gastric acid secretion process would end here, as a one-off terminal event, were it not for the presence of potassium-normalizing K^+ channels KCNQ1-KCNE2 (Roepke, Kanda, et al. 2011) and KCNJ1 (*ROMK*) (Vucic et al. 2015). Together, these channels coordinate the "resetting" of intracellular K^+ levels to enable prolonged H^+/K^+-ATPase function and thus further gastric acid secretion.

Thus, in addition to its roles in the thyroid and choroid plexus, KCNQ1-KCNE2 acts as a "team player" within the parietal cells of the gastric glands of the stomach. Here, the KCNQ1-KCNE2 complex acts to counterbalance and functionally reset the high intracellular K^+ levels accumulated by the H^+/K^+-ATPase (Figure 4) (Roepke et al. 2006; Abbott 2014; Lee et al. 2000). Although KCNQ1 channels are typically inhibited by extracellular protons, the formation of the KCNQ1-KCNE2 complex enables constitutive activation of the channel as well as an augmentation of the channel's current and function at low extracellular pH (Heitzmann et al. 2004; Heitzmann et al. 2007). This last attribute is critical to maintaining the function of this channel in the very low pH of the stomach. Gastric acid secretion can be strongly inhibited by application of the KCNQ1 blocker chromanol 293B (Grahammer et al. 2001), thereby inhibiting potassium efflux and maintaining a hyperpolarized parietal cell by way of intracellular potassium accumulation. Also crucial is that KCNE2 helps ensure that KCNQ1 is localized at the apical membrane where it can assist the H^+/K^+-ATPase. Deletion of *Kcne2* results in aberrant localization of KCNQ1 to the basolateral membrane of parietal cells, because in this pathologic state KCNE3 is upregulated and hijacks KCNQ1, taking it to the wrong side of the cell and disenabling it from assisting the H^+/K^+-ATPase. However, even concomitant *Kcne3* deletion does not restore gastric acid secretion in *Kcne2*–/– mice, illustrating the importance of the effects of KCNE2 on KCNQ1 gating and pH sensitivity for the parietal cell function of KCNQ1 (Roepke, King, et al. 2011). It is of no surprise, then, that *Kcne2* and *Kcnq1* knockout mice each display profound hypochlorhydria and altered gastric morphology (Roepke et al. 2006; Lee et al. 2000).

KCNJ1, also known as $K_{ir}1.1$, is an inward-rectifier potassium channel comprised of intracellular N- and C-terminals, two transmembrane segments (M1, M2), and one pore-forming region (H5). Like Kv channels, four of the KCNJ1 proteins must tetramerize to form a membrane protein with a functional pore. KCNJ channels are regulated by multiple pathways, including protein kinase A (PKA), protein kinase C (PKC), intracellular pH, and PIP_2 (Huang 2001). Well-known for its role in potassium recycling in the kidney, KCNJ1 is also expressed in gastric parietal cells (Vucic et al. 2015; Huang 2001). Like KCNQ1-KCNE2, KCNJ1 channels on the parietal cell apical membrane appear to help facilitate gastric acid secretion by recycling K^+ for later participation in ion exchange by the H^+/K^+-ATPase. Selective blockade of KCNJ1 channels by application of K_{ir}-selective blocker tertiapin-Q (TPNQ), and/or application of KCNQ blocker XE-991, to the gastric lumen suggests the requirement of both KCNQ1-KCNE2 and KCNJ1 channel function for maintenance of normal gastric acid secretory responses. These findings are validated in *Kcnj1* knockout mouse studies, which demonstrated reductions in gastric acid secretion independent of any other developmental or morphological changes to the stomach (Vucic et al. 2015). Likewise, we previously showed that although *Kcne2*–/– mice exhibit abnormal parietal cell morphology in addition to achlorhydria, *Kcne2*+/– mice exhibit

hypochlorhydria in the absence of morphological changes to parietal cells, suggesting KCNQ1-KCNE2 channels are directly involved in supporting H^+/K^+-ATPase function rather than their disruption causing achlorhydria indirectly via disruption of cell architecture (Roepke et al., 2006).

H^+/K^+-ATPase is not a member of the *SLC* gene superfamily, and in fact functions as an antiporter – transporting H^+ out of the cell, to participate in gastric acid formation, in exchange for drawing K^+ into the cell. This pump operates in stark contrast to the SLC5A proteins which operate as symporters, drawing both sodium and their substrate (MI, glucose, etc.) into the cell. Although crosstalk between K^+ channels and the gastric H^+/K^+-ATPase has been functionally demonstrated, physical interaction of the transporter with KCNQ1-KCNE2 or any of the channels discussed above has not. In the case of KCNQ1-KCNE2, it is considered unlikely that it operates by direct physical interact with H^+/K^+-ATPase, because while apical membrane expression of the latter is thought to occur only after signaling by secretagogues (which stimulate trafficking of H^+/K^+-ATPase-containing vesicles to the apical membrane), KCNQ1-KCNE2 is thought to reside in the apical membrane at rest – although this has not yet been fully investigated.

Physical interaction between Orai1 and SPCA2

Previous research has demonstrated Orai1 to be a vital component of store-operated calcium channel activation (Soboloff et al. 2006). Orai1 channels (Figure 1 A), which consist of four-transmembrane segment proteins forming a functional hexamer at the plasma membrane (PM), are activated by the depletion of internal calcium stores, generally referred to as a "store-operated" mechanism (Prakriya et al. 2006; Soboloff et al. 2006). The activity of these channels is greatly increased when co-expressed with STIM proteins – endoplasmic reticulum (ER)-bound Ca^{2+} store sensors that possess no channel activity on their own (Soboloff et al. 2006; Mercer et al. 2006). Together, the STIM1-Orai1 complex forms a unique Ca^{2+} sensor-channel pairing between the PM and ER (Soboloff et al. 2006). Perturbations to Orai channel function may cause diverse problems, including immune deficiency (Feske et al. 2006) and myopathy (Nesin et al. 2014).

Secretory pathway Ca^{2+}-ATPase isoform 2 (SPCA2), encoded by (*ATP2C2*) is a P-type calcium transporter localized to the Golgi (Vanoevelen et al. 2005). The SPCA proteins, spanning 10 transmembrane segments, use ATP to transport Ca^{2+} and Mn^{2+} from the cytoplasm and into the Golgi and are critical in their role as Ca^{2+} signal terminators (Durr et al. 1998; Feng et al. 2010). Although SPCA1 is ubiquitously expressed, SPCA2 expression is restricted to brain and secretory and absorptive epithelial tissues (Cross et al. 2013; Xiang et al. 2005) and is highly upregulated in the mammary epithelium during lactation (Feng et al. 2010). Recently, SPCA2 has been highlighted as a critical component in human breast tissue and tumorigenicity, by way of intracellular Ca^{2+} level regulation (Feng et al. 2010). In the same study, SPCA2 was demonstrated to form a complex with calcium channel Orai1 (Figure 5 B) (Feng et al. 2010). When physically associated, SPCA2-Orai1 complexes enable Ca^{2+} signaling independent of Ca^{2+} stores, STIM sensors, or SPCA2 pump activity, revealing a previously unknown mechanism of Orai1 operation (Feng et al. 2010). These

findings provide the first evidence for non-K^+ channel participation in chansporter complex crosstalk or formation.

CONCLUSIONS

It is striking that many of the transporters demonstrated to complex with Kv channels are, to date, from the *SLC* gene families. In addition to SMIT1, we found that SMIT2 and SGLT1 (*SLC5A11* and *SLC5A1,* respectively) exerted effects when co-expressed with KCNQ1 (Abbott et al. 2014), however in-depth characterization remains to be pursued. Further exploration of *SLC* members is warranted and may yield additional Kv-SLC complexes.

The potential connections between SMIT1, states of mania, inositol status, and lithium present a tantalizing cluster of biological phenomena. We know that both SMIT1 mRNA levels and brain inositol levels are acutely responsive to lithium treatment, though the latter is of little surprise given the well-established use of lithium to inhibit IMPase in many *in vitro* experiments. We also know that inositol levels roughly correspond to manic symptom presentation in humans. Together, these data suggest the possibility of altered inositol pathways constituting elements of the etiology of manic disorders, although there have been no pharmaceutical interventions to test or leverage this concept aside from lithium. Given the ability of ion channel-targeted drugs to manipulate co-assembled SMIT1 activity, as described herein, it is interesting to consider the possibility that novel interventions for mania could come in the form of re-purposed, or structurally modified, ion channel drugs. However, it is important to remember that many potassium channels, and KCNQ1 in particular, are widely expressed and fulfil a wide spectrum of physiological roles, therefore targeting even a specific channel isoform with the goal of modulating its associated transporter would present significant challenges with respect to side effects. We have shown that transporter function can be regulated by relatively channel-specific antagonists (Abbott et al. 2014) but can this be therapeutically exploited and/or will it prove to be a gadfly causing unwanted side effects? Increased specificity might potentially be achieved if a drug could target only chansporter complexes rather than non-complexed channels, especially if they could also be tailored for specific transporter-channel-KCNE subunit combinations.

Perhaps most critically, the findings reviewed herein hint at the emergence of a potentially broad paradigm in cell signaling, that of direct channel-transporter crosstalk and co-regulation facilitated by direct physical coupling. There are a number of research directions that should now be pursued to flesh out this idea. First, the scope of chansporter complexes that exist *in vivo* must be catalogued in order to define the generality of this new principle. Second, mechanistic questions must be addressed, including how channels can augment transporter function, what signaling information is exchanged within chansporter complexes, what is the directionality of this information, and why the necessity for direct interaction – is it merely proximity to limit the distance through which ions must diffuse, or are their intimate interactions between the moving parts of channels and transporters that convey information of a higher order than solely ion movement?

Acknowledgments

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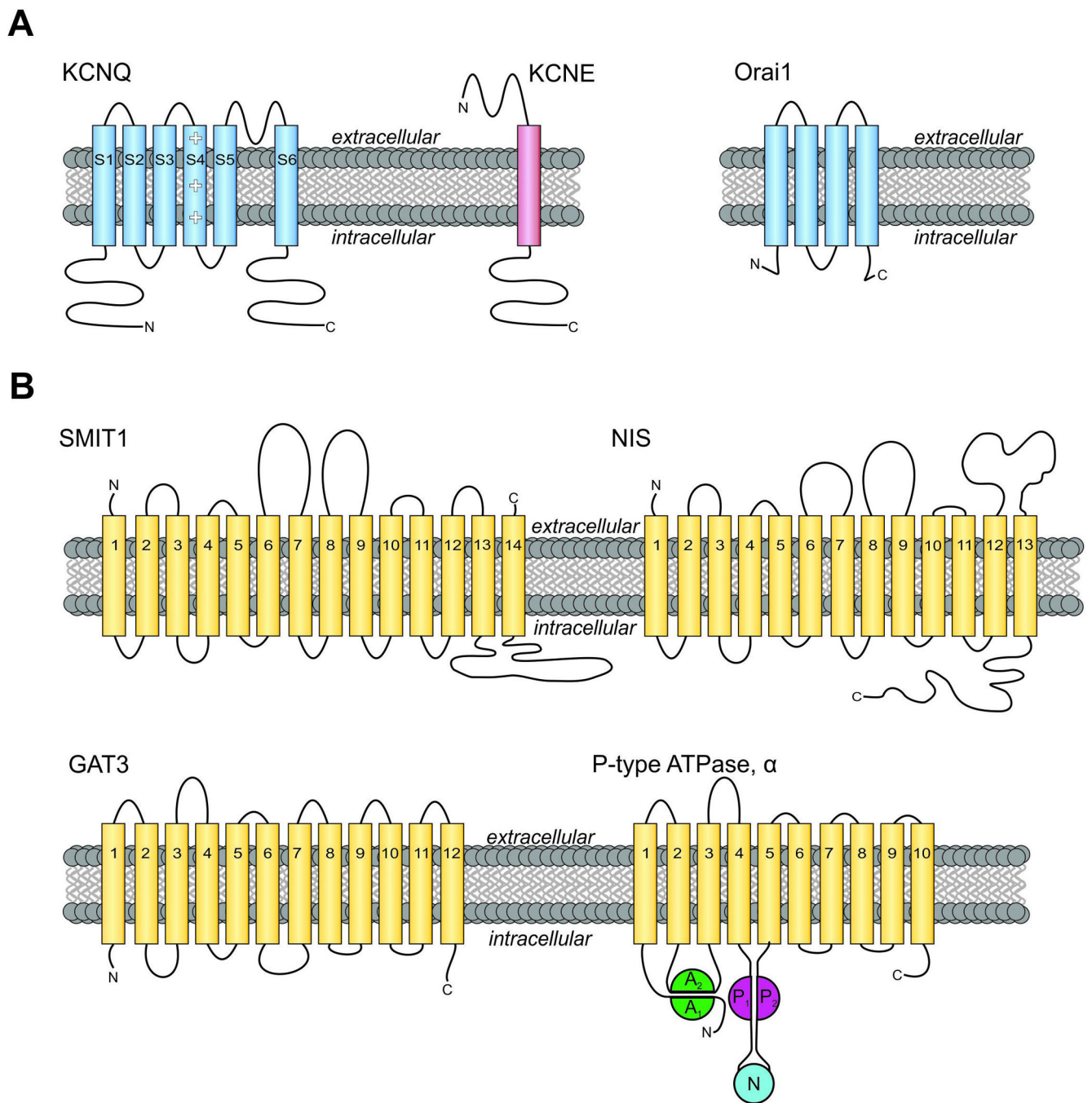
Figure 1. Membrane topology of channel subunits and transporters discussed in this review
A. *Left*, transmembrane topology of KCNQ voltage-gated potassium (Kv) channel α
subunits and KCNE β subunits. Kv pore-forming loop pictured between S5-S6. *Right*,
topology of Orai1. Intracellular N- and C-terminals labeled accordingly.
B. *Upper*, transmembrane topology of two *SLC5A* transporters. (*Left*) Sodium/*myo*-inositol
co-transporter SMIT1, with extracellular N and C-terminals labeled accordingly. SMIT1
features a large intracellular loop between segments 13 and 14, as well as two large
extracellular loops between segments 6–7 and 8–9. (*Right*) The sodium/iodide symporter

NIS. *Lower*, transmembrane topology of GAT3 (*SLC6A11*) and a P-type ATPase α subunit such as the gastric H^+/K^+-ATPase. Extracellular N-terminus and intracellular C-terminus are labeled.

Note that topology maps are shown for clarity and simplicity. High-resolution crystal structures have been solved for Orai (Hou et al. 2012), Kv1.2 (a mammalian relative of KCNQ channels) (Long et al. 2005), and vSGLT (a prokaryotic sodium galactose transporter related to mammalian SLC5A transporters) (Faham et al. 2008). Actual structures may deviate from predicted topologies with respect to, e.g., broken helices and in some cases actual transmembrane disposition.

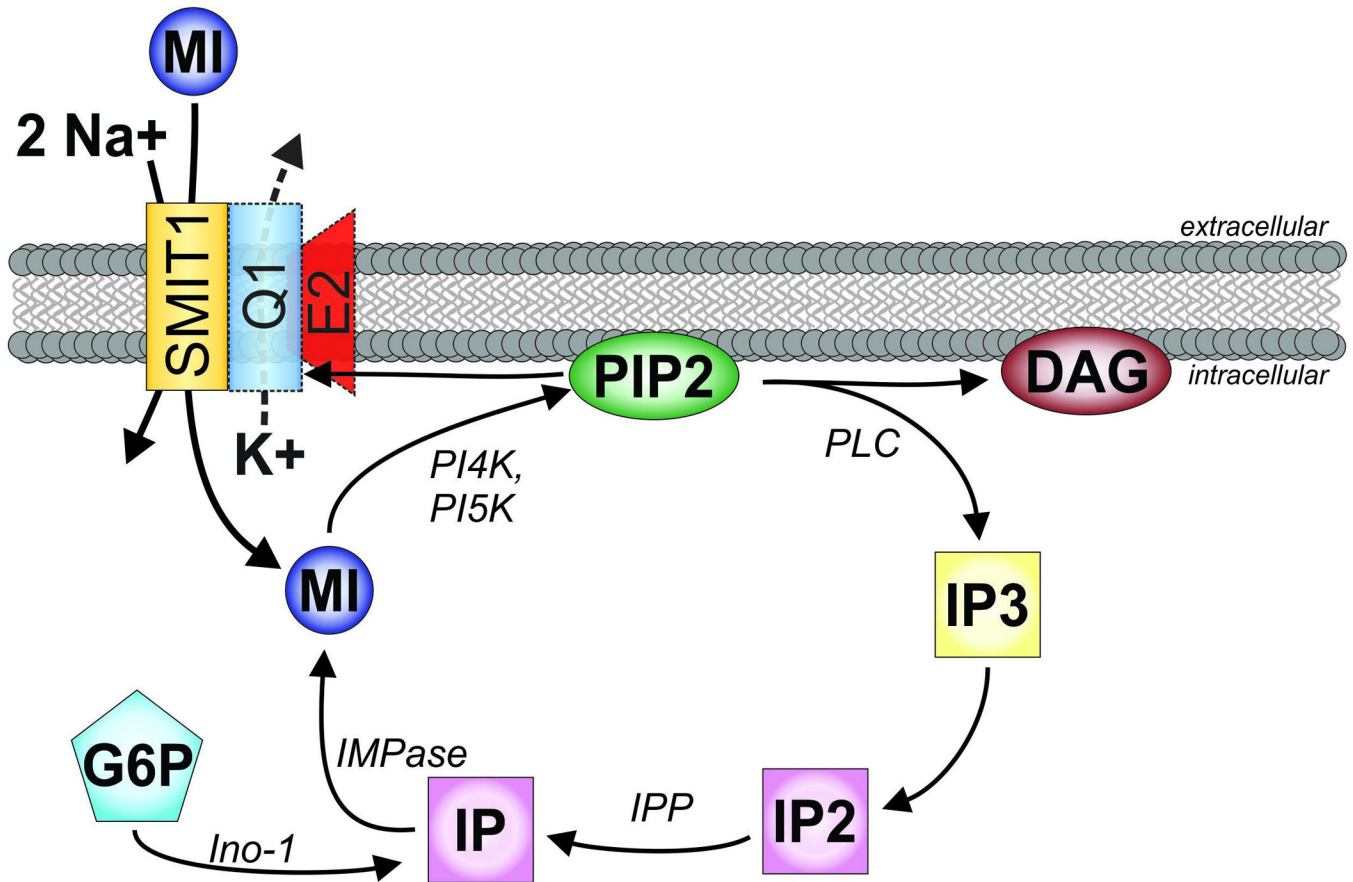A color version of the figure is available online.

Figure 2. The *myo*-inositol pathway in the context of SMIT1 and KCNQ1-KCNE2
SMIT1, orange, transports sodium (Na+) and *myo*-inositol (MI) with a stoichiometry of 2:1. Phosphatidylinositol 4-kinase (PI4K), followed by phosphatidylinositol 5-kinase (PI5K), add phosphates to MI to form phosphatidylinositol 4,5-bisphosphate (PIP_2). When activated, commonly by Gq-coupled receptors, phospholipase C (PLC) cleaves PIP_2 into constituent components diacylglycerol (DAG) and inositol(1,4,5)-trisphosphate (IP3). IP3 is then recycled by dephosporylation to inositol(1,4)-bisphosphate (IP2) and inositol monophosphate (IP) by Inositol-1,4 bisphosphate 1-phosphatase (IPP), and back to (myo)inositol by inositol- 1(or 4)-monophosphatase (IMPase). IP can also be synthesized from glucose 6-phosphate (G6P) by inositol synthase (Ino-1). KCNQ1 (blue) with and without KCNE2 (red) can form complexes with SMIT1, although this interaction is not required for basic SMIT1 function. KCNQ1 gating is heavily regulated by PIP_2.
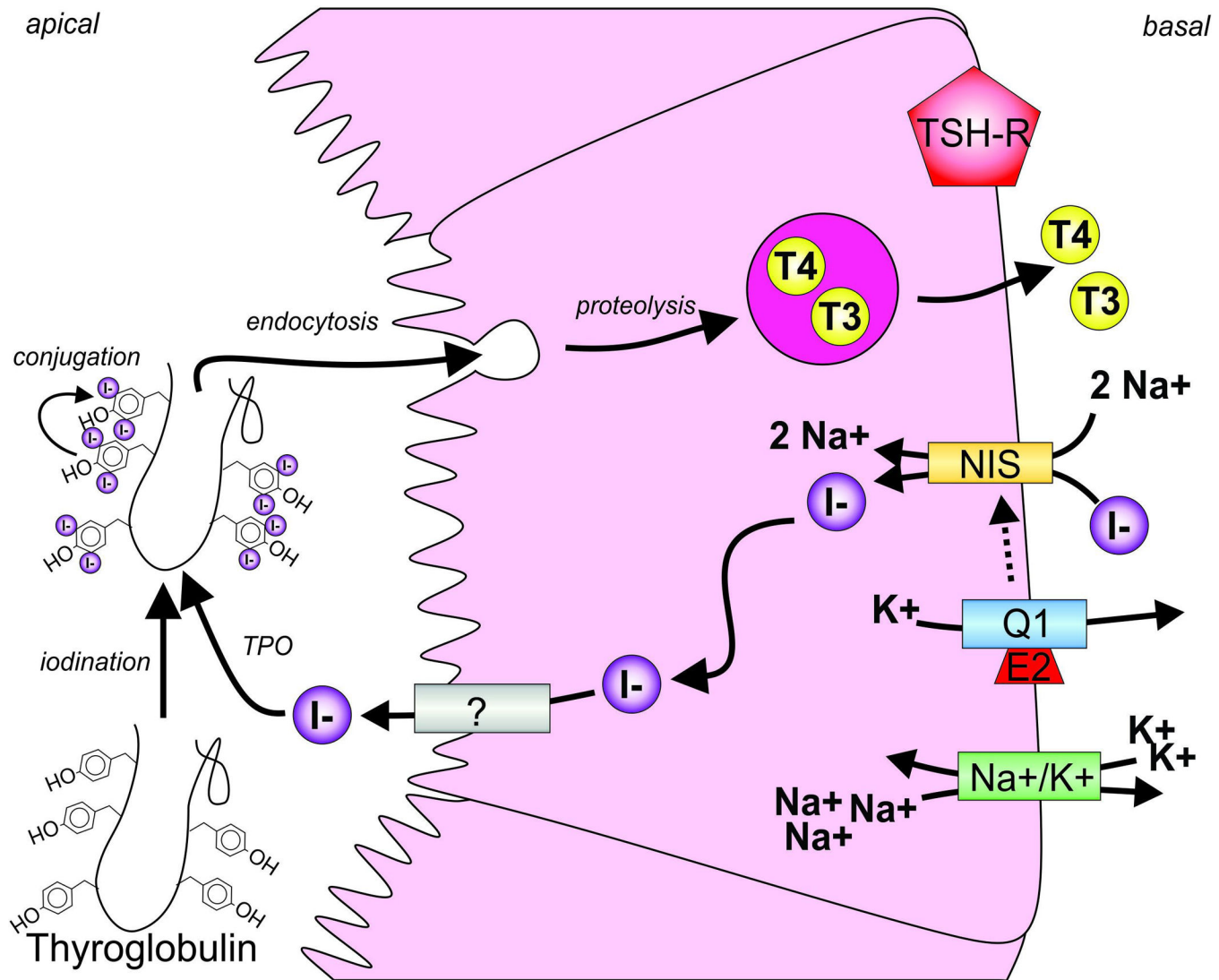A color version of the figure is available online.

Figure 3. Channel-transporter crosstalk in the thyroid

The sodium-iodide symporter (NIS, orange) transports Na^+ and I^- (purple) into the thyrocyte. This process is facilitated, via an incompletely resolved mechanism, by potassium ion efflux through KCNQ1-KCNE2 (Q1, blue; E2, red) channels and also involves active transport by the Na^+/K^+-ATPase (Na^+/K^+, green). I^- is then passed through to the colloid by another protein (?) whose identity is still under debate. I^- undergoes oxidation by thyroid peroxidase (TPO) and is incorporated into the thyroglobulin (Tg) molecule to produce monoiodotyrosine (MIT) and diiodotyrosine (DIT) by organification. The organified Tg is then resorbed by the follicular cells where, when the thyroid stimulating hormone receptor is activated (TSH-R, red), the iodinated Tgs are cleaved to form either thyroxine (T_4), generated from a pair of DIT, or triiodothyronine (T_3), produced from one MIT and one DIT molecule.
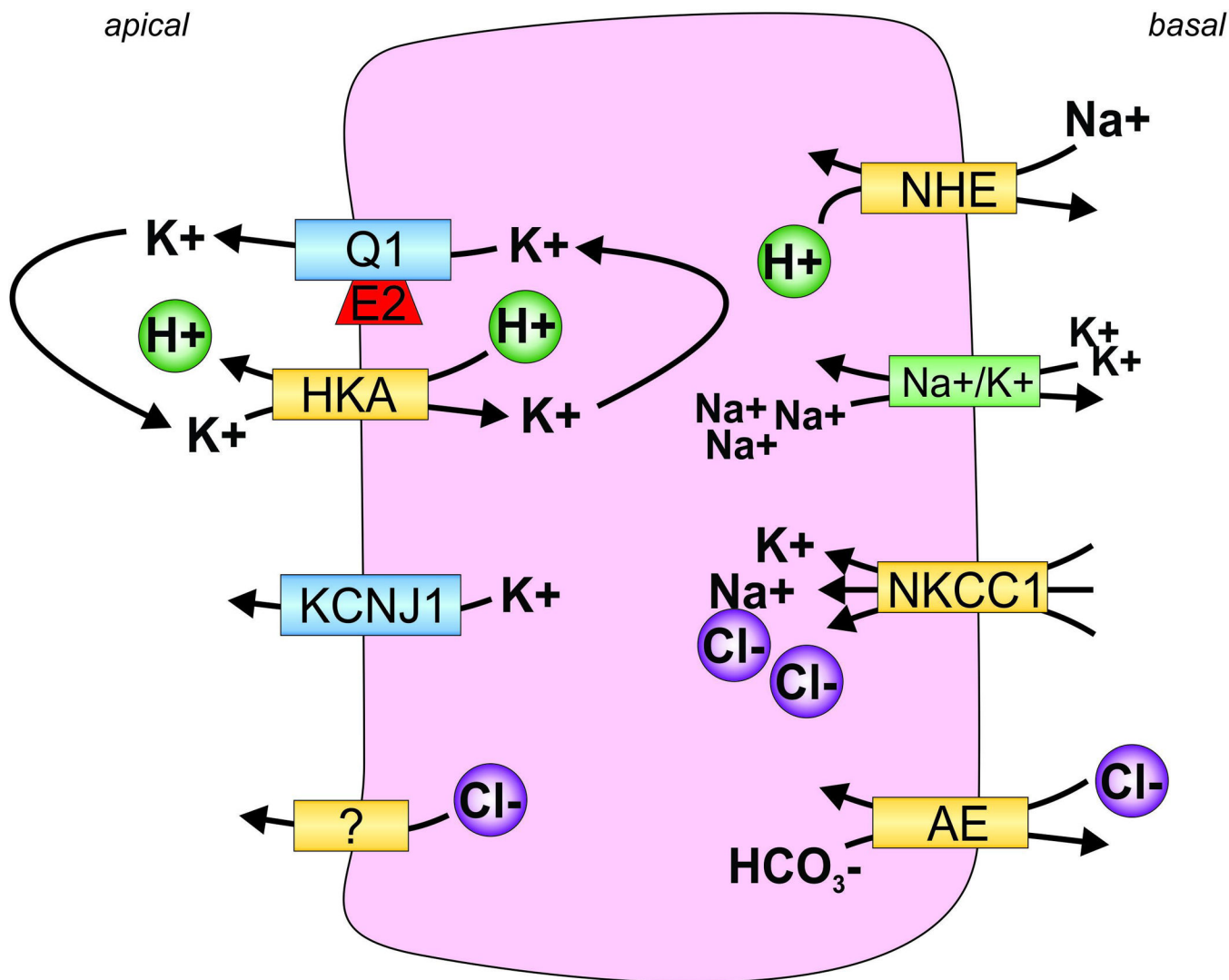
A color version of the figure is available online.

Figure 4. Channel-transporter crosstalk in gastric parietal cells facilitates gastric acid secretion
Sodium-hydrogen exchanger (NHE) draws Na^+ into the cell and expels H^+. The sodium-potassium ATPase (Na^+/K^+) releases Na^+ and draws in K^+. $Na^+/K^+/Cl^-$ cotransporter 1 (NKCC1) draws Na^+, K^+, and Cl^- into the cell. The anion exchanger (AE) exchanges Cl^- and HCO_3^-. H^+/K^+-ATPase (HKA) expels H^+ into the stomach lumen accompanied by an unknown Cl^- channel or transporter to participate in gastric acid formation. KCNQ1-KCNE2 (Q1, E2) and KCNJ channels expel potassium from the cell in order to "reset" the high intracellular K^+ accumulated by HKA activity.
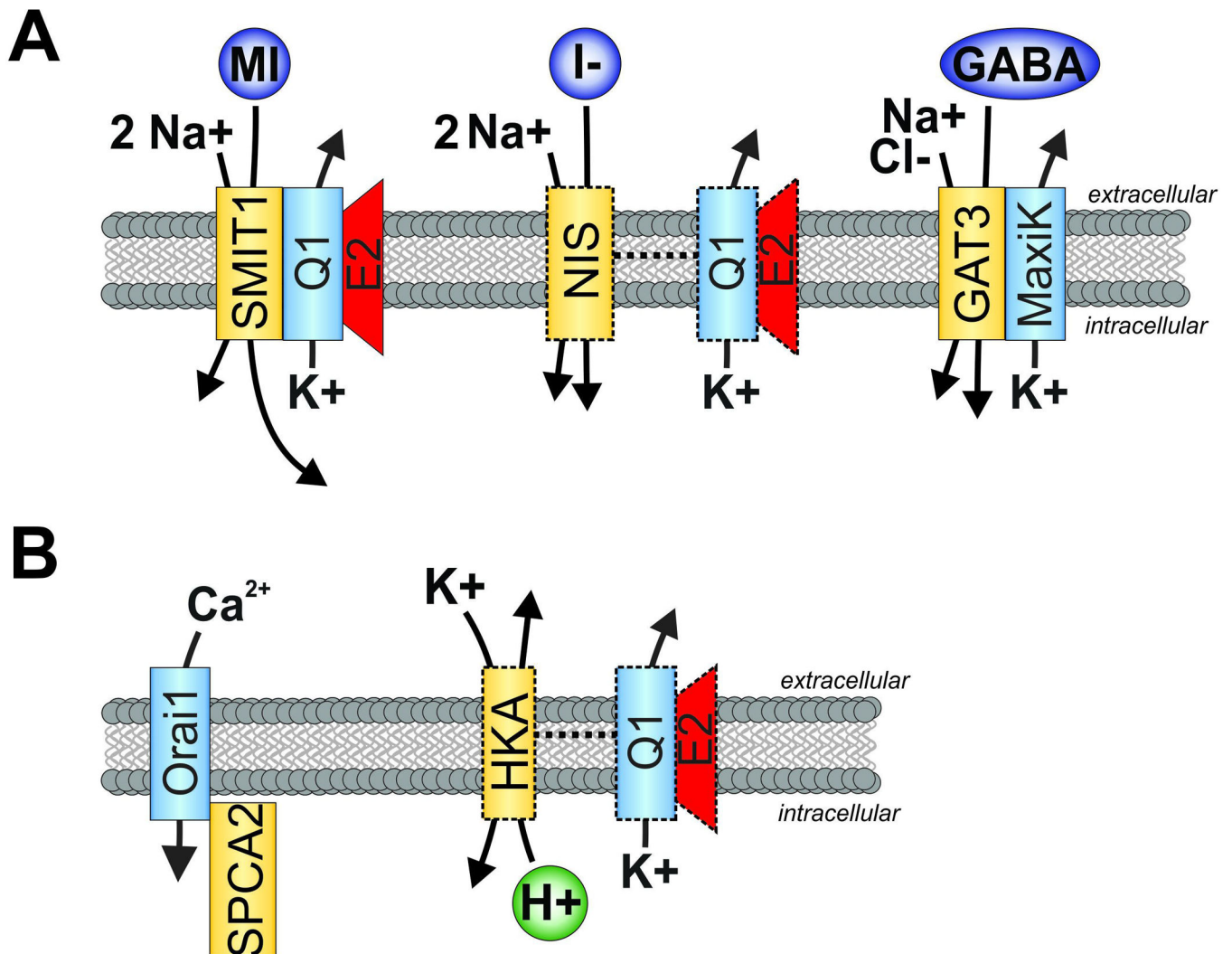A color version of the figure is available online.

Figure 5. Summary of channel-transporter complex formation and functional crosstalk discussed herein

A. K^+ channel interactions with members of the *SLC* superfamily. *Left,* SMIT1 and KCNQ1-KCNE2 form reciprocally regulating, physically interacting complexes in the brain. *Center,* NIS and KCNQ1-KCNE2 exhibit functional crosstalk in the thyroid, although physical interaction remains to be determined (indicated by dotted lines). *Right,* GAT3 and MaxiK form physically interacting chansporter complexes.

B. *Left,* Orai1 and SPCA2 form physically interacting chansporter complexes which enable a novel mode of calcium channel activation, potentially important in tumorigenesis. *Right,* HKA and KCNQ1-KCNE2 exhibit functional crosstalk in the stomach to enable gastric acid secretion. Physical interaction remains to be determined (indicated by dotted lines).

A color version of the figure is available online.