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Channel-transporter complexes: an emerging theme in cell signaling

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Abstract

In a recent edition of *Biochemical Journal,* Mistry and colleagues described discovery of a novel protein complex, formed from the epithelial sodium channel (ENaC) and the sodium chloride cotransporter (NCC) [1]. The importance of these two proteins to regulation of salt balance and blood pressure has long been known, as has their overlapping expression in the distal convoluted tubule (DCT) of the kidney. The new study by Mistry *et al.* now demonstrates their physical interaction in the kidney and when heterologously co-expressed. Furthermore, the authors demonstrate some degree of functional co-dependence between ENaC and NCC, with pharmacological inhibition of the latter diminishing activity of the former when the two are co-assembled. This novel and potentially important interaction adds to a growing number of recently identified channel-transporter ("chansporter") complexes, which together constitute an emerging theme in cell signaling.

Keywords

KCNQ1; SMIT1; SMIT2; ENaC; NCC; CFTR; NIS

The primary difference between true channels versus other membrane proteins capable of moving molecules across the cell membrane is that movement through channels occurs by diffusion, and is driven by the electrochemical gradient of the molecule being moved. The most widely studied examples are the ion channels, which are in many cases highly selective for a specific ion and can facilitate rapid diffusion of, e.g., sodium or potassium ions to create action potentials in excitable cells. Similarly, ion channels including ENaC can regulate fluid homeostasis or other secretory processes in non-excitable, polarized epithelial cells, which has long hinted at the possibility of at least functional, and in some cases physical, interaction with other classes of solute-transporting membrane proteins.

As with true channels, facilitative transporters (also known as uniporters) facilitate diffusion of solutes down their concentration gradient, but unlike true channels, uniporters function by an alternating access model in which conformational changes occur upon substrate binding and there is at no time an open, unrestricted pathway through the transporter that would

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connect the fluid compartments on either side of the plasma membrane – the “alternating access” or “allosteric pump” mechanism [2].

Another class of transporters, the secondary active transporters (a class that includes NCC), rely upon the electrochemical gradient of one or more ions (often Na^+ or H^+) down their electrochemical gradient to power the movement of another up its electrochemical gradient. NCC, encoded by the *SLC12A3* (solute carrier family 12 member 3) gene is an electroneutral cation-coupled co-transporter that uses the sodium ion gradient across the apical membrane of DCT cells to take up both sodium and chloride ions from the tubular fluid [3]. The apically located ENaC, a true channel, also mediates Na^+ uptake into the DCT cells, down its electrochemical gradient [4]. Once inside the DCT cells, a pump (the Na^+/K^+-ATPase) returns Na^+ ions to the bloodstream and Cl^- ions enter the bloodstream by way of the CLCNKB chloride channel [5]. As its name suggests, the Na^+/K^+-ATPase is another type of transporter, termed a pump, which moves ions up their concentration gradient using energy derived from ATP.

Even in this brief snapshot of some of the processes mediating sodium and chloride transport in the DCT, essential for salt reabsorptive pathway, it becomes apparent that ion channels, transporters and pumps must cooperate to ensure ion movement is correctly regulated. Recently, a new concept has emerged in cell signaling, that of ion channel-transporter complexes. This phenomenon moves beyond the various transport proteins merely being localized in the same cells, instead incorporating direct physical interaction. ENaC-NCC complexes in the DCT are the most recently described iteration of this concept, and Mistry *et al* demonstrate not only physical interaction but also a functional co-dependence between the two partners; pharmacological inhibition of NCC with thiazide also reduced the open probability of ENaC, only when the two were co-expressed [1].

Several other recently described functional interactions between channels and transporters, at least some (and possibly all) of which involve formation of ion channel-transporter complexes, include voltage-gated potassium (Kv) channel pore-forming α subunits from the KCNQ (Kv7) subfamily. While studying the mechanistic basis for increased seizure susceptibility in mice lacking the *Kcne2* gene that encodes a ubiquitous Kv channel regulatory (β) subunit, it was found that the *Kcne2* null mice exhibited lower cerebrospinal fluid (CSF) *myo*-inositol concentrations than their wild-type littermates. This led to the finding that KCNQ1-KCNE2 potassium channels form physical complexes with SMIT1, a sodium-dependent *myo*-inositol transporter, in the choroid plexus epithelium – the primary site of CSF production and secretion [6]. Both KCNE2 and KCNQ1 were co-immunoprecipitated with SMIT1, and with one another, from choroid plexus cell lysates - suggesting formation of a detergent-stable, tripartite complex. SMIT1, encoded by the *SLC5A3* gene, is a secondary active transporter requiring downhill movement of Na^+ to facilitate uphill transport of *myo*-inositol, a cyclic polyol important both as an osmolyte and as a precursor for crucial signaling molecules such as phosphatidylinositol 4,5-bisphosphate (PIP_2) [7, 8]. KCNQ1-KCNE2-SMIT1 complexes help modulate CSF *myo*-inositol levels, and this contributes to regulation of neuronal excitability.

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SMIT1 also forms complexes with KCNQ1 when the two are heterologously co-expressed, and KCNE2 is not required for KCNQ1-SMIT1 complex assembly. It is not known whether KCNE2 co-assembles with SMIT1 in the absence of KCNQ1, but KCNE2 does not have overt functional effects on SMIT1 in the absence of KCNQ1 [6]. SMIT1 co-expression enhanced KCNQ1 channel activity ~2-fold when the two were co-expressed in *Xenopus laevis* oocytes. Interestingly, KCNQ1 similarly augmented SMIT1 uptake of *myo*-inositol, but coexpression of both KCNE2 and KCNQ1 strongly inhibited SMIT1 activity. Thus, as for ENaC-NCC, there is functional co-dependence or “crosstalk” within KCNQ1-KCNE2-SMIT1 complexes, although the mechanistic basis for this crosstalk is still unclear. It does not appear as if driving force dictates the ability of KCNQ1 to regulate SMIT1 activity, for example, because a constitutively active KCNQ1 mutant (R231A) strongly inhibited SMIT1, the opposite of what one would expect from a driving force effect (i.e., K^+ efflux hyperpolarizing the cell to facilitate Na+ and thus myo-inositol uptake through SMIT1). Furthermore, KCNQ1 had functionally different interactions with other SLC5 family transporters. SMIT2 (encoded by *SLC5A11*) inhibited KCNQ1 activity, was itself unaffected by wild-type KCNQ1, but was inhibited by R231A-KCNQ1. KCNQ1 activity was augmented by SGLT1 (a sodium-dependent glucose transporter encoded by *SLC5A1*); it should be noted that physical interactions of KCNQ1 with SGLT1 and SMIT2 have yet to be established. However, for both SMIT1 and SMIT2, co-expression with KCNQ1 rendered them sensitive to drugs that inhibit KCNQ1 activity, resulting in inhibition of *myo*-inositol uptake in each case – indicating some functional dependence of the transporter upon the channel [6].

The fact that *myo*-inositol is a precursor for PIP_2 brings an additional twist to the tale, as it was subsequently shown that the PIP_2 generated from *myo*-inositol entering through SMIT1 is sufficient to functionally regulate KCNQ2, a Kv channel best known for generating the M current in mammalian neurons [9]. Like many ion channels, including KCNQ1 (particularly when in complexes with KCNE1), KCNQ2 is highly regulated by PIP_2, a soluble lipid-derived molecule important in cell signaling [10]. SMIT1 overexpression combined with *myo*-inositol supplementation was found to increase intracellular stores of PIP_2, which in turn augmented KCNQ2 activity by shifting its voltage-dependence of activation to more negative membrane potentials. This was of sufficient magnitude to attenuate firing of action potentials in superior cervical ganglion neurons. Although the existence of complexes between SMIT1 and KCNQ2 was not reported to have been examined in the article by Dai and colleagues, it was suggested that complex formation was not required for this effect because it occurred via a soluble molecule (PIP_2) that can diffuse within the cell, and effects on GIRK2 and TRMPM7 channels were also reported. Possible effects of KCNQ2 on SMIT1 function were also not reported. Importantly, the ability of SMIT1 to be upregulated by an extracellular hypertonic solution via the transcription factor tonicity-responsive enhancer binding protein (TonEBP) effectively coupled KCNQ2 electrical activity, and thus membrane excitability, to osmotic potential – suggested to be the physiological role for the functional interaction between KCNQ2 and SMIT1, via a downstream product of SMITI-transported *myo*-inositol, PIP_2 [9]. It will be of interest to investigate KCNQ1-SMIT1 complex formation, and the possible effects of this on SMIT1 activity.

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Even more recently, Park and colleagues found that *Drosophila* (d) SLC5A11 (also named *cupcake)* inhibits the dKCNQ K^+ channel, an ortholog of mammalian KCNQ family channels that exhibits some features similar to those of mammalian KCNQ1 and some more reminiscent of mammalian KCNQ2 [11]. As we found previously for human SMIT2 (SLC5A11) with KCNQ1 [6], dSLC5A11 inhibits dKCNQ activity. Unlike related mammalian SGLT1, dSLC5A11 lacks a consensus sugar binding site and does not generate glucose-dependent currents when expressed in *Xenopus* oocytes, although it passes a large constitutive current that requires sodium and a conserved sodium-binding site. It is hypothesized that dSLC5A11 is a hunger sensor, and that its upregulation in starvation inhibits dKCNQ activity in specific neurons to promote food intake and other hunger-driven behaviors [11]. It is fascinating that despite the functional and sequence differences between mammalian and *Drosophila* forms of the proteins involved, SLC5A11 orthologs have similar effects on same-species KCNQ family isoforms.

Mammalian KCNQ1-KCNE2 channel heteromers also participate in functional interactions with additional transporters, although physical interaction has yet to be reported. Thus, in mice, *Kcne2* deletion causes hypothyroidism, via impaired thyroid iodide uptake through the sodium iodide symporter (NIS), because of disruption of thyroid cell KCNQ1-KCNE2 channels required for optimal NIS activity [12]. NIS, encoded by SLC5A5, is another sodium-dependent secondary active transporter, which uses the sodium gradient to accumulate iodide ions up their concentration gradient in thyroid cells, for thyroid hormone production [13]. KCNQ1-KCNE2 channels and NIS symporters are both localized in the basolateral membrane of thyroid cells, and injection of the KCNQ1--specific antagonist (-)-[3*R*,4*S*]-chromanol 293B (C293B) into mice also impaired thyroid iodide uptake *in vivo,* quantified by positron emission tomography detection of $^{124}I^-$. C293B also inhibited iodide uptake by NIS *in vitro* in the rat thyroid FRTL5 cell line (which expresses endogenous NIS, KCNQ1 and KCNE2) but not in COS cells heterologously expressing NIS in the absence of KCNQ1 and KCNE2 [14].

Several other channel-transporter complexes have been reported. The Orai1 Ca^{2+} channel is best known for being one half of the STIM1-Orai1 complex that mediates store-operated calcium ion influx. In this guise, Orai1 forms the actual pore in the plasma membrane, but its activity is greatly increased when ER-localized, Orai1-interacting STIM1 senses ER Ca^{2+} depletion [15]. Orail can also serve a less well-known, contrasting function – forming channel-transporter complexes with secretory pathway Ca^{2+}-ATPase isoform 2 (SPCA2), a Golgi-localized P-type calcium ion transporter. Orai1-SPCA2 complexes facilitate Ca^{2+} signaling without either STIM1 binding or even SPCA2 pump function, and are considered important in human mammary Ca^{2+} regulation and tumorigenicity [16].

As also referenced by Mistry and colleagues [1], the cystic fibrosis transmembrane conductance regulator (CFTR), a protein that displays properties of both a chloride channel and an ATP-binding cassette transporter, forms complexes with several members of the SLC26A solute transporter family – specifically SLC26A2, A3, A4 and A5 – members of which transport (and exchange) chloride and bicarbonate ions. The functional interaction involves physical interaction between the STAS domain of SLC26A transporters such as DRA (downregulated in adenoma) gene product, and the R domain of CFTR, is facilitated

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by binding of each protein to scaffolding proteins via their PDZ ligands, and activity of both DRA and CFTR is augmented by their interaction with one another. The STAS domain on its own is capable of increasing CFTR open probability more than threefold, and the number of active channels x open probability (nP_o) sevenfold, without altering the total amount of cell surface CFTR protein. CFTR also augments activity of DRA. SLC26A-CFTR complex formation may explain some features of epithelial chloride absorption and bicarbonate secretion incompletely reconciled by CFTR activity alone, especially in the pancreas and salivary glands, and could also impact our understanding and potentially therapeutic approaches to diseases including cystic fibrosis and congenital chloride diarrhea [17, 18].

K_{ATP} channels, which are potassium channels gated by intracellular nucleotides, are formed from an obligate complex between a tetramer of potassium channel a subunits (from the inward rectifier 6, or Kir6, subfamily) and a tetramer of sulfonylurea (SUR) receptor subunits (SUR1, SUR2A or SUR2B). While ATP binding to the Kir6 subunits within the complex closes K_{ATP} channels, MgATP binding to the SUR subunits, and subsequent ATP hydrolysis, can overcome the inhibitory effects of Kir6-bound ATP. K_{ATP} channels thus act as crucial metabolic sensors in the heart, pancreas, and other tissues [19]. SURs belong in the ATP-binding cassette (ABC) transporter superfamily, but, unlike most other ABC proteins, ATP hydrolysis in SURs does not itself provide the energy to actively transport a substrate, but instead induces a conformational change in a co-assembled ion channel to gate the channel. Therefore, K_{ATP} channels can be viewed as chansporter complexes, the transporter component of which at some point in evolution ceased to be a transporter, assuming SURs evolved from true ABC transporters and not the converse. If this is the case, it is interesting to consider whether the integral transporter function of SURs was, in evolutionary terms, lost before or after they began co-assembling with Kir6 subunits.

Finally, physical interaction between a Ca^{2+}-activated K^+ channel (MaxiK) and the GABA transporter 3 (GAT3, a sodium-dependent gamma-aminobutyric acid transporter encoded by *SLC6A11*) was recently found to occur in mouse brain, using a proteomic approach and supported by co-immunoprecipitation and co-localization analysis of the proteins after heterologous co-expression in human embryonic kidney 293 cells [20]. No functional studies of this complex have been reported, but it adds to the growing catalog of ion channel-transporter, or “chansporter” complexes recently recognized in the literature.

As further examples of chansporter complexes are discovered, it will be important to understand whether there are common functional or structural rules that apply between the various complexes, and how widespread is the phenomenon. Are some channel-transporter interactions occurring merely to target each protein to a similar part of the cell, perhaps to decrease the distance over which the ions or other solutes they transport have to travel to reach the partner protein? It is conceivable that in some cases, interaction might occur solely because one of the partners evolves to exploit a targeting motif on the other protein, to end up in a location also favorable to its function. Alternatively, do the majority of chansporter complexes form because the two proteins in the partnership intimately communicate via conformational changes, as may be the case for CFTR-SLC26x complexes and perhaps also for KCNQ1-SMIT1? In addition to understanding their roles and generality in biology, and mechanisms underlying their function, in cases where functional co-dependence is found it

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may be worth exploring strategies with which to exploit chansporter complex formation for therapeutic ends. This could involve, for example, targeting the more easily druggable half of a complex to indirectly modulate the less druggable half, in specific disease states. In these contexts, the recent discovery by Mistry and colleagues of ENaC-NCC chansporter complexes is notable for several reasons. First, as for KCNQ1-KCNE2-SMIT1 and for SLC26A-CFTR, ENaC-NCC represents a channel-transporter complex displaying both physical and functional interactions, and verified in native tissue. Second, it appears to be the first recognized chansporter complex involving a sodium channel, emphasizing the generality of this type of interaction. Third, the discovery may lead to greater understanding of hypertension, a major health issue, and could potentially open up novel therapeutic strategies to prevent, manage or treat this disorder.

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