Endo-lysosomal two-pore channels and their protein partners

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Two-pore channels are ion channels expressed on acidic organelles such as the various vesicles that constitute the endo-lysosomal system. They are permeable to Ca^{2+} and Na^{+} and activated by the second messenger NAADP as well as the phosphoinositide, $PI(3,5)P_2$ and/or voltage. Here, we review the proteins that interact with these channels including recently identified NAADP receptors.

1. Introduction

Two-pore channels (TPCs) are ancient members of the voltage-gated ion channel superfamily (1). Structurally, they are dimers with each polypeptide chain consisting of a cytosolic N-terminus, two homologous ion channel domains joined by a cytosolic linker, and a cytosolic C-terminus (2)(Fig. 1A). Each ion channel domain is a modular assembly of six transmembrane (TM) regions where the first 4 TM regions form a voltage sensor and the last two, the pore (3, 4). They display pseudo-fourfold symmetry typical of other members of the family (5-8).

TPCs are ubiquitous and ancient tracing their roots back to basal eukaryotes (9, 10). In mammals, there are typically three isoforms present in the genome of most members (TPC1-3) although TPC3 is lost or degenerated in several species including humans and mice (11-13). TPCs localize predominantly in acidic organelles such endosomes, lysosomes (11, 14) and plant vacuoles (15). They are activated by multiple means including voltage, the Ca^{2+} mobilizing messenger, nicotinic acid adeninine dinucleotide phosphate (NAADP) and the phosphoinositide $PI(3,5)P_2$ in an isoform-selective manner (12, 14, 16-19). A recent work shows that TPC2 (voltage-insensitive) can change its ion selectivity in an agonist-dependent manner (20). Thus, in the presence of NAADP it behaves mainly as a Ca^{2+}-permeable channel whereas in the presence of $PI(3,5)P_2$ it behaves as a Na^{+}-selective channel. This switch can be mimicked by new small molecule agonists. These findings reconcile apparently contradictory results relating to activating cues and ion selectivity (20). Functionally, TPCs regulate numerous processes including many aspects of endo-lysosomal membrane trafficking (21, 22). They are heavily implicated in disease and have emerged as druggable targets (23-26).

Protein-protein interaction is a ubiquitous means to coordinate cellular activity. Many ion channels associate with accessory proteins. Voltage-gated Ca^{2+}, Na^{+} and K^{+} channels for example are macromolecular complexes comprising the pore forming alpha subunits and several other subunits that regulate trafficking and channel characteristics (27). These subunits are considered obligate components of the channel complex although it is becoming increasingly clear that these proteins are promiscuous and regulate other targets (28, 29). Ion channels also associate with numerous additional regulatory proteins including protein kinases. Defining such interactions is key to understanding how ion channels are regulated and how they interface with other signalling pathways (30). This is particularly pertinent for TPCs given that they are regulated by NAADP and indirectly through small molecular weight NAADP binding proteins that associate with the channel to confer NAADP sensitivity (31, 32).

In this Chapter, we summarize current knowledge relating to the proteins that interact with TPCs focussing first on those identified through unbiased approaches, second on associated protein kinases and third on candidate NAADP receptors.

2. TPC interacting proteins identified by unbiased approaches

To date, five interaction data sets for TPCs have been published over the past decade (33-37). The first study employed a yeast two-hybrid assay with cytosolic fragments of TPCs. Subsequent analyses all used mass spectrometry approaches to identify interactors of full length TPC1, TPC2 or both. These screens are summarised in Table 1**.**

2.1 HCLS1 Associated Protein X-1 (**HAX1)**

Using the N-terminus, middle and C-terminus domains of TPC2 as bait, Lam *et al.* (33) performed a yeast two-hybrid screen using a heart cDNA library. The TPC2 C-terminal domain (residues 694-752) produced hits, one of which was identified as Hematopoietic lineage cell-specific protein (HCLS)-associated protein X-1 (HAX1). Further glutathione S-transferase (GST) pull-down and co-immunoprecipitation experiments confirmed direct HAX1 interaction with the C-terminus and full-length TPC2. HAX1 was also isolated in a subsequent TPC2 affinity purification study using the full length protein (see below) (34). Interestingly, the C-terminus of TPC1 also interacted with HAX1 in both yeast two-hybrid and GST pull-down assays (33). The C-termini of TPCs differ significantly. TPC1 C-terminus is substantially longer than that of TPC2 and the sequences diverge downstream of the S6 region in the second ion channel domain. It is currently unknown if the TPC2 C-terminus domain assumes a similar conformation to that of TPC1 because it was not resolved in the recently published high resolution structure of human TPC2 (8) (Fig. 1A). Surprisingly, TPC2 N-terminus (residues 1-85) and central domain (residues 313-435) did not yield any interactants. This may be due to limitations of the assay (interaction takes place in the yeast nucleus) and/or the use of the cardiac tissue library where potential TPC2-binding proteins could be of low abundance.

HAX1 is an ubiquitously expressed, likely multifunctional protein involved in diverse cellular processes ranging from apoptosis and cell migration to granulopoiesis, mRNA processing and calcium homeostasis (reviewed in (38)). Although HAX1 is predominantly found in mitochondria, the endoplasmic reticulum and the nucleus, its subcellular localization is highly variable depending on cell type and activation state and likely underpinned by its interaction with a plethora of protein partners. For example, HAX1 is targeted to the sarcoplasmic reticulum in the heart through its association with phospholamban and the sarco/endoplasmic reticulum Ca^{2+}-ATPase (39, 40). Pathologically, the loss of functional HAX1 protein is associated with severe congenital neutropenia and neurodevelopmental abnormalities, whereas HAX1 upregulation has been observed in various tumours similar to TPCs (see below)(38). The functional significance of the TPC-HAX1 association remains to be elucidated.

2.2 Proteins involved in membrane trafficking

Lin-Moshier *et al* provided the first proteomic characterization of TPC interactors (34). Interaction of 40 proteins/protein families common to both TPC1 and TPC2 were described. Interactants were ranked according to the total number of unique peptides recovered for both isoforms in immunoprecipitation assays. This analysis confirmed an interaction between TPCs and HAX1 (Fig. 1B). Proteins involved in regulating membrane trafficking and organisation were well represented in this interactome (41). These included members of the Rab GTPase family, SNARE proteins (syntaxins, synaptogyrins) and several annexins (reviewed in (42)).

Chief amongst the hits were the Rab family of GTPases and their regulators (the rab regulator protein GDI2) (34). Rab proteins constitute small, monomeric GTP-binding proteins involved in many facets of trafficking, targeting and fusion of vesicles and organelles (43). Individual Rab proteins, localized to the cytoplasmic surface of membranes, coordinate their GTPase cycle with vectorial transport along specific endocytic and exocytic pathways. Given the prominence of TPC interactions with different Rab isoforms, as well as the role of Rabs in many processes where NAADP/TPC activity has been demonstrated (e.g. viral infection, neurite extension, autophagy, melanogenesis and cancer) (43), the structural basis and functional significance of this interaction was explored.

A Rab binding site was mapped to a consensus motif within the N-terminus of TPC2 (residues 33-37), within a region not yet resolved in TPC2 structures (Fig. 1A) (34). Truncations, or point mutations within this region, eliminated Rab7 binding to TPC2, as well as alterations in subcellular trafficking that were dependent on TPC2 expression and function. In cells expressing Rab-binding deficient TPC2 mutants, or in cells incubated in a pharmacological inhibitor of Rab activity, NAADP-evoked Ca^{2+} release and alterations in trafficking dynamics were attenuated (34). These data demonstrate a functional interplay between Rab activity and NAADP-evoked Ca^{2+} release, at least for TPC2. Given the crucial role of TPCs in trafficking both physiological and pathological cargoes through the endo-lysosomal system, there is clearly merit in studying these interactions in more detail. The TPC2 Rab binding motif is not conserved in TPC1, yet TPC1 also demonstrably binds Rab proteins (34). This suggests that TPCs may interact with Rab proteins through distinct sites that are perhaps coupled to distinct trafficking outcomes. The preference of these motifs for different Rab isoforms has also yet to be defined. Clearly, additional work is required to map these interactions and define their functional impact.

Also represented in the original TPC interactome (34) were proteins resident in organelles other than endosomes and lysosomes. These interactors could potentially mediate protein-protein interactions that scaffold membrane contact sites between organelles, where TPC activity is critical for coordinating communication between organelles and thereby whole cell responses (44). One example is the association of TPC1 with Transmembrane protein 33 (TMEM33) (34), an endoplasmic reticulum protein which interacts with polycystin2 to shape both lysosomal and ER Ca^{2+} dynamics (45). Another example is Progesterone Receptor Membrane Component 1 (PGMRC1), a TPC interactor resident in the endoplasmic reticulum which markedly amplifies NAADP-evoked Ca^{2+} signals *via* TPC1 (Gunaratne *et al*, unpublished). Further effort to map these interactions and profile their functional significance for each and every candidate will be needed to understand their roles in the overall TPC complex.

Grimm *et al* focussed on mouse TPC2 interacting proteins (35). Similar to Lin-Moshier et al (34), an interaction between Rab proteins, synaptogyrin and syntaxins were identified (Fig. 1B) with SynGR emerging as their most significant hit. Other common hits included BAG and TMED proteins (Fig. 1B). Physical interaction between recombinant TPC2 and syntaxins 6 and 7 was confirmed by FRET and/or co-immunoprecipitation but neither appeared to affect $PI(3,5)P_2$- or NAADP-mediated TPC2 channel activity (35). Additional interactants included VAMP isoforms and VTIB which are also SNARE proteins. These proteins may function to position TPC2 within the fusion complex.

Castonguay *et al* (36) analysed the interactome of TPC1. Importantly, these authors used validated antibodies against mouse TPC1 to immuno-precipitate *endogenous* TPC1 from mouse kidney. Specificity was determined by comparing interactants using samples from TPC1 knockout mice. Similar to the recombinant interactomes, the SNARE proteins (syntaxins and VTIB) were identified (Fig. 1B) pointing to a fusogenic role for TPC1 similar to TPC2. Other interactants included members of the ESCRT family (IST1 homolog and the Charged Multivesicular Body Proteins, CHMP2B and CHMP3). ESCRT components form a multi-protein complex and regulate membrane remodelling in particular inverse scission events associated with the formation cytosol-laden vesicles (46). This is potentially relevant given the morphological changes in the endo-lysosomal system associated with TPC activity (44) and might link TPCs to processes such as multi-vesicular body formation, membrane repair and nuclear membrane dynamics in which ESCRTs play key roles (46). Such an association might also be relevant in extending the actions of TPCs from viral entry which involve TPCs to viral egress, another important ESCRT-regulated process (46).

Both Grimm *et al* (35) and Castonguay *et al* (36) identified components of the V-type ATPases as TPC interacting proteins (Fig. 1B). The V-type ATPase is a proton pump responsible for generation of the large proton gradients that exist across the various endocytic organelles. This raises the possibility that TPCs may regulate acidification. In this context it is worth mentioning that NAADP

(47) and its mimetic TPC2-A1-N (20) both induce acute alkalisation and that at least in some systems knockout of TPC2 increases vesicular pH (48).

In summary, non-biased approaches to define TPC partners have converged on proteins involved in membrane traffic but which define largely isoform-selective interactomes. See (41) and (42) for additional comparisons of the above data sets.

3. Protein kinases associated with TPCs

Several protein kinases have been shown (or inferred) to associate with TPCs to regulate their activity.

3.1 Leucine rich repeat kinase 2 (LRRK2)

The first protein kinase identified as a TPC interacting protein was LRRK2.

Work by Gomez-Suaga *et al* demonstrated co-immunoprecipitation of recombinant human TPC2 with endogenous LRRK2 in HEK cells (49).

LRRK2 is multi-domain protein possessing kinase as well as GTPase activity (50). This protein has attracted intense interest following identification of mutations in the LRRK2 gene as a cause of autosomal dominant Parkinson's disease (51). Despite such interest, the function of this protein is unclear although it is appears to be linked to endo-lysosomal function (52). Gomez-Suaga *et al* identified autophagic defects in cells overexpressing LRRK2 (49). These defects were phenocopied by NAADP-AM (a cell permeable NAADP analogue) and reversed by LRRK2 kinase inhibitors, Ned-19 (an NAADP antagonist) and a dominant negative form of TPC2 (53), suggesting a role for phosphorylation and NAADP-regulated TPC2 activity in mediating autophagic dysfunction (49). Indeed, a role for TPCs in autophagy had already been proposed (54) and a number of follow-up studies support this idea, although it remained unclear how TPCs exactly regulate autophagy. Additional studies identified lysosomal morphology defects in fibroblasts from people with the G2019S mutation in LRRK2 (24). These effects were again kinase and NAADP-dependent and reversed by TPC2 (but not TPC1) knockdown (24). They were also reversed by chemical blockade of Rab7 consistent with both TPC-Rab (34) and LRRK2-Rab (55) connectivity.

3.2 Mechanistic target of rapamycin (mTOR)

Work by Cang *et al* identified mTOR as a TPC associated protein kinase (56).

Using technically demanding vesicular patch clamp technique, the team found that $PI(3,5)P_2$ induced Na^+ currents through TPC1 or TPC2 were inhibited by ATP (56). Subsequent analyses using chemical inhibitors, knockdown and overexpression showed that ATP inhibition was mediated by mTOR. mTOR co-immunoprecipitated with both recombinant TPC1 and TPC2. mTOR was also recovered in the proteomic screen of Lin-Moshier *et al* (Fig. 1B).

mTOR is a protein kinase associated with the mTORC1 and mTORC2 complexes (57). The former is intimately linked to nutrient sensing and growth. Under nutrient replete conditions, mTORC1 is active at the lysosome surface promoting protein synthesis. Upon nutrient depletion, it is inactivated promoting autophagy. Consistent with regulation of TPCs by mTOR, ATP failed to inhibit TPCs following starvation *in vitro*. TPC knockout mice showed defects in exercise endurance after fasting *in vivo* (56). Furthermore, acute inhibition of mTOR with rapamycin in intact cells appeared to evoke TPC2-dependent Ca^{2+} signals (58) but this required high concentrations of the drug. Interestingly, polymorphic variation in the C-terminal tail of TPC2 (G734) associated with blond hair colour modulated channel regulation by mTOR (59). Might this region of TPC2 at least interact with mTOR? Reciprocally, TPC2 has been proposed to regulate mTOR (60).

3.3 Citron Kinase

Work by Horton *et al* identified citron kinase as a protein kinase associated with TPC1 but not TPC2 (61). They showed that in HEK cells stably expressing human TPC1 or TPC2, the former co-immunoprecipitated with recombinant citron kinase.

Citron kinase is a multi-domain protein that functions during cytokinesis (62). Consistent with this, overexpression of TPC1 but not TPC2 in HEK cells disrupted the cell cycle resulting in multinucleated cells, polyploidy and arrest in the G2/M phase (61). TPC1 (and TPC2) protein levels appeared to fluctuate during the cell cycle and were elevated in several cancers. The antibodies used however were not validated. Overexpression of TPC1 also reciprocally regulated levels of active Rho (up) and phospho-myosin light chain (down) – key regulators of cytokinesis. How the functional effects of TPC1 overexpression relate to association of TPC1 with citron kinase is not so clear although one possibility discussed by the authors is that TPC1 sequesters endogenous citron kinase thus disrupting its normal function during cytokinesis (61).

3.4 Other protein kinases

In work by Jha *et al*, inhibition of TPC2 currents by ATP was also modulated by chemical or molecular manipulation of the mitogen activated protein kinases (MAP kinases) JNK and P38 (63). Thus, small molecule inhibitors of the kinases or expression of dominant negative constructs slowed ATP-dependent inhibition whereas expression of wild type constructs accelerated it. Interestingly, these effects were observed in both vesicular recordings and upon rerouting TPC2 to the plasma membrane. This suggests tight association of the kinases with TPC2 but such an association was not tested. Manipulation of kinase activity through molecular means also revealed negative regulation of NAADP-evoked Ca^{2+} release in intact cells by both JNK and P38. MAP kinases, like mTOR promote cell growth and survival. That both inhibit TPCs suggests TPCs may activate during times of stress.

Cyclic AMP-dependent protein kinase (PKA) has also been proposed to regulate TPC2 (64). Identification of a sequence in TPC2 conforming to the consensus PKA phosphorylation R-R/K-X-S/T site prompted Lee *et al* to examine the effects of phospho-mimetic and phospho-null mutants on the single channel activity of TPC2. This analysis appeared to uncover positive regulation of the channel at this site. Addition of PKA to the cytosolic surface of TPC2 also potentiated activity. But the putative PKA phosphorylation site in TPC2 is deep in the pore of TPC2 at the luminal surface (65). Thus, it is presently unclear if TPC2 is a *bona fide* substrate for this kinase.

In sum, association of TPCs with a number of protein kinases links TPCs to diverse signalling pathways.

4. NAADP receptors associated with TPCs

The Ca^{2+} mobilizing properties of NAADP were first identified over a quarter of a century ago in sea urchin eggs (66). This was soon followed by radioligand binding studies in egg (67) and mammalian cell (68) preparations that clearly pointed to the existence of specific NAADP receptors. The hunt was on to identify those receptors molecularly. The subsequent discovery of TPCs as target channels for NAADP over a decade ago (11, 14, 69) was *not* followed by TPCs emerging as receptors for NAADP. No NAADP binding sites have been identified on TPCs. Rather, photo-affinity labelling studies using NAADP-based probes implicated smaller ~23kDa proteins as high affinity, NAADP-selective targets in mammalian cell lines (70-72). Labeling persisted upon knockout in mice of TPC1 or TPC2 alone (71) or in combination (73) thereby definitively separating NAADP binding and NAADP-evoked Ca^{2+} release. Importantly, in sea urchin egg homogenates, labeled NAADP receptors co-immunoprecipitated with TPCs (70). This led to a generally accepted model ofNAADP regulated TPCs through associated protein(s) that bound NAADP. The hunt to identify NAADP receptors at the molecular level continued resulting in the identification of Jupiter microtubule associated homolog 2 (JPT2) and Sm-like protein 12 (LSM12).

4.1 JPT2

In 2021, two independent groups converged on JPT2 as an NAADP receptor (74, 75). Both isolated this protein using different strategies and differing starting materials but using a common, newly described 'clickable' photo-affinity probe (76). In the work of Gunaratne *et al* (74), JPT2 was purified from red blood cells, which surprisingly showed strong, selective labelling of the ~23kDa NAADP-binding protein. Following photolabeling and click chemistry to enhance recovery of photolabeled proteins, JPT2 was identified as the elusive ~23kDa NAADP binding protein. Recombinant JPT2 bound NAADP with properties similar to the endogenous target. Knockdown of JPT2 in both HEK and U2OS cells reduced photolabeling. Importantly, endogenous JPT2 co-immunoprecipitated with expressed TPC1, less so TPC2. Knockdown of JPT2 showed that JPT2 was also required for endogenous NAADP-evoked Ca^{2+} release.

Roggenkamp *et al* (75) purified and identified JPT2 from Jurkat T lymphocytes. As in the Gunaratne study, recombinant JPT2 bound NAADP (albeit more weakly). In contrast to most other studies, NAADP appears to release Ca^{2+} through activation of RyR1, not TPCs in these cells (but see (77)). Knockout of JPT2 in Jurkat T lymphocytes and in primary T cells reduced local Ca^{2+} signals evoked by antigen stimulation which the authors had previously assigned to activation of RyR1 by NAADP (78). Consistent with this, JPT2 part co-localized with RyR1 in the cell periphery and co-immunoprecipitated with it (75). Neither the requirement for JPT2 in NAADP-evoked Ca^{2+} release nor interaction with TPCs was reported.

Little is known about JPT2. The gene encoding JPT2 also known as HN1L together with a homologue (JPT1/HN1) has been described in several higher vertebrates (79). A single JPT gene is present in most other animal phyla (74). Functionally, JPT2 has been implicated in various forms of cancer (80-83). This is significant because TPCs have also been implicated in cancer (reviewed in (84)). In *Drosophila* (which lacks TPCs), JPT encodes a microtubule binding protein (85) whereas in the silkworm, JPT functions as an anti-apoptotic protein during infection with nucleopolyhedrovirus (86). The latter is again potentially significant given an emerging role for TPCs in virus trafficking in mammalian cells (reviewed in (25)). Indeed, similar to TPC knockdown, knockdown of JPT2 but not JPT1 reduced SARS-CoV-2 pseudo-virus infection (74). Such isoform specificity implies JPT1 might not be an NAADP receptor.

4.2 LSM12

Is the molecular hunt for the NAADP receptor over? Photo-affinity labels tag more than one species (70-72). Could these be JPT2 isoforms, or additional gene products that are NAADP targets? Work published in preprint form at the time of writing strongly suggests the latter.

Zhang *et al* (37) reported interactomes for recombinant human TPC1 and TPC2 expressed in HEK cells. A total of 40 interactants for each TPC isoform were identified. These interactomes appeared more divergent than previously published ones (Fig. 1B). Common TPC1 interactors identified were PGRMC1 and ATP1A as reported by Lin-Moshier *et al* (34), RTN4 as reported by Grimm *et al* (35) and LAMP2 as reported by Castonguay *et al* (36). No common TPC2-interacting proteins were identified (Fig. 1B). Nevertheless, comparison of the TPC1 and TPC2 interactomes acquired in parallel identified 8 common hits. Zhang *et al* also defined proteins in cells expressing TPC1 and TPC2 that bound to immobilized NAADP. These NAADP interactomes revealed 17 proteins in common but they were very distinct from the TPC interactomes. However, there was a single protein present in all data sets i.e. one that interacted with both TPC isoforms *and* NAADP. This protein, LSM12, was characterized in detail.

Like JPT2, recombinant LSM12 bound NAADP selectively and with nanomolar affinity similar to the findings of Gunaratne *et al* (74). Knockout of LSM12 in HEK cells reduced Ca^{2+} release by and channel activity of recombinant TPC2 in response to NAADP, and this could be rescued by re-expression of LSM12 or injection of recombinant protein. Knockout also prevented interaction of TPCs with immobilized NAADP. Further experiments showed that the LSM domain was required for interaction of LSM12 with NAADP and TPC2, and its ability to rescue NAADP-evoked Ca^{2+} release.

Endogenous NAADP-evoked Ca^{2+} release was compromised in MEFs derived from LSM12 knockout mice lacking a short peptide in the LSM domain. And analysis of the corresponding deletion mutant showed that it bound NAADP but neither interacted with TPC2 nor rescued Ca^{2+} release or channel activity of TPC2. Collectively, these multiple lines of evidence identify LSM12 as a key TPC-interacting protein required for NAADP activation.

LSM12 is a member of the Sm protein family comprising the founding member (Sm) and a number of 'like-Sm' (LSM) isoforms. These proteins form oligomers and play roles in RNA processing. Relatively little is known about LSM12. Like other Sm proteins it harbours an LSM domain that is known to bind RNA. In addition, it is characterized by an extended C-terminus harbouring an anticodon-binding domain (87). Similar to JPT2, it has been implicated in cancer (88, 89). It also regulates circadian rhythms in Drosophila (90) and nuclear protein transport in mammalian cells (91) suggesting possible NAADP involvement in these processes.

In sum, the last year has seen significant advances in the molecular identification of NAADP receptors with two understudied proteins (JPT2 and LSM12) validated as prime candidates.

5. Outlook

Overall, the TPC interactomes are demonstrably large but more validation of the interactions and the functional consequences is required. Bindings sites of channels and partners need to be identified in order to selectively manipulate interactivity. This is particularly important for NAADP receptors which appear to perform redundant functions in conferring NAADP sensitivity to TPCs. TPCs are regulated by protein kinases and some of them associate with TPCs. But is regulation mediated by direct phosphorylation of TPCs? Plant TPCs are evidently phosphorylated in both the N- and C-termini (92) but direct evidence that animal TPCs are phosphoproteins aside from annotation in public databases is currently lacking. Do all partners regulate channel activity or might they act as scaffolds to recruit effectors? This is underexplored. Further work is therefore needed to fully understand the relationship between TPCs and their partners.

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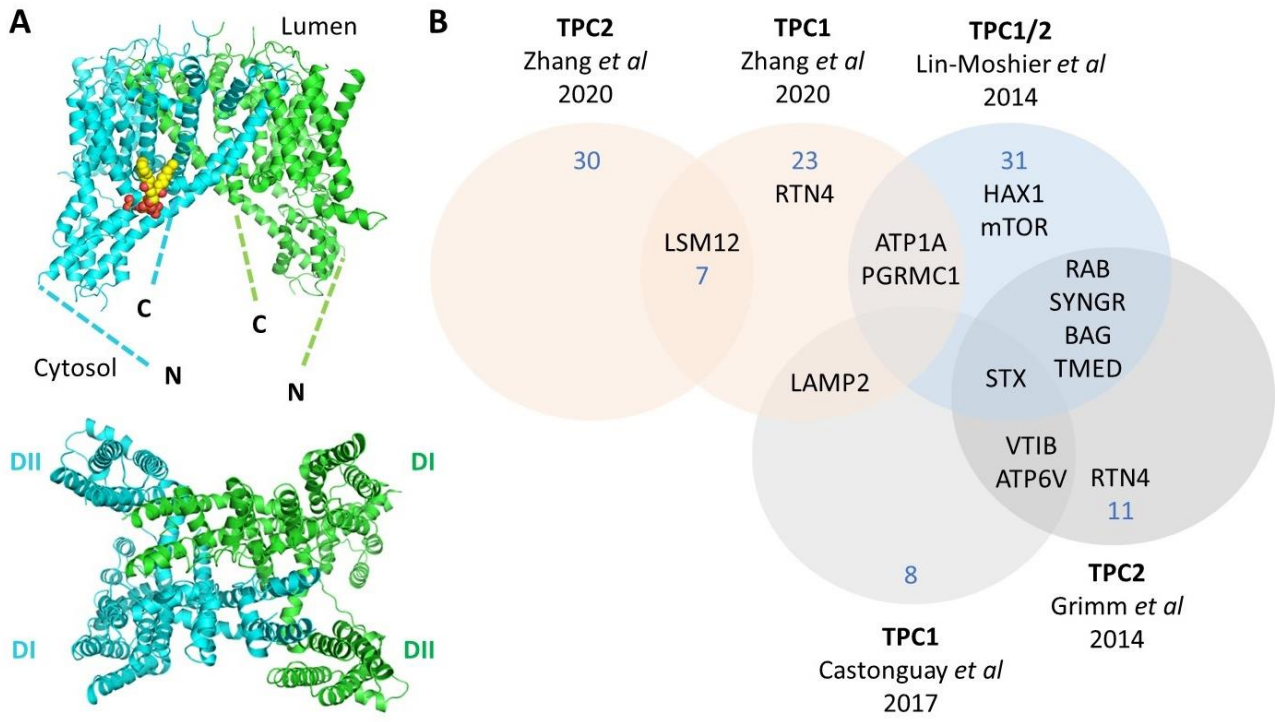

Figure 1. TPCs and their protein partners. A, Structure of TPCs. Cryo-EM structure of human TPC2 (pdb: 6NQ0) viewed from the plane of the membrane with PI(3,5)P_2 bound (top, Yellow = C, Red = O atoms, Orange = P) spheres) and viewed from the lysosome lumen (bottom). N- and C-termini not resolved in the structure are represented by the dashed lines. DI and DII refer to the two ion channel domains. Each monomer is depicted in a different colour. **B**, Common interacting proteins. Venn diagram of TPC-interacting proteins identified in the indicated studies. Comparisons are based protein families (e.g. syntaxins; STX) not individual isoforms (e.g. STX 6,7,8,12,16,18) which may differ between data sets. Numbers refer to unique interacting families.

Table 1 Summary of TPC interaction studies. Listed are the TPC isoform studied, the nature of the tag (if applicable), the source of cells/tissues and the experimental method used to identify TPC partners. Abbreviations: Y2H, yeast two-hybrid; SILAC (stable isotope labelling by amino acids in cell culture); IP (immunoprecipitation); STrEP tag (a ~2.5kDa tag with high affinity for a specific type of streptavidin).

TPC isoform	Tag	Source	Method	Reference
Human TPC1+TPC2	-	Human heart	Y2H	(33)
Human TPC1+TPC2	STrEP	HEK+SKBR3 cells	IP	(34)
Mouse TPC2	GFP	HEK cells	SILAC	(35)
Mouse TPC1	Endogenous	Mouse kidney	IP	(36)
Human TPC1+TPC2	GFP-FLAG	HEK cells	SILAC	(37)

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