Structural biology

Two-pore channels open up

Two-pore channels span the membranes of acidic organelles inside cells. A structural and functional analysis reveals secrets of how these channels open to allow passage of ions across the membrane.
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Two-pore channels (TPCs) are an ancient family of ion channels that are unusual in that they are found not at the cell surface, but spanning the membranes of acidic organelles such as endosomes and lysosomes in animal cells and vacuoles in plants[1]. Here, they serve several key organellar functions — releasing calcium ions (Ca^{2+}) into the cytoplasm to control the trafficking of material such as receptors and viruses, for instance, and stabilising junctions with other organelles[1] {PMID: 28199837}. Two-pore channels (TPCs) are increasingly being associated with diseases such as Parkinson's, and are therefore emerging as potential therapeutic targets[1]. Detailed structural information is currently scant, but advances in cryo-electron microscopy are revolutionising our ability to study ion channels. In a paper in *Nature*, She *et al.*[2] use this technique to provide the first detailed glimpse of an animal TPC.

Previous work[3,4] has reported the atomic structure of a plant TPC, which consists of two subunits, each containing two similar transmembrane domains (6-TM I and 6-TM II) connected by a large cytoplasmic linker. 6-TM I and 6-TM II are in turn each made up of six membrane-spanning regions, dubbed S1–S6. The pore through which ions flow is formed by S5-S6 from each of the four like-domains (two per subunit).

She *et al.* resolves the structure of mouse TPC1. Their structure revealed that the overall folding of this channel is, expectedly, similar to plant TPC (Fig. 1). Nonetheless, there is a surprising degree of structural conservation between the linkers, given that animal and plant TPCs have very different amino-acid sequences in this region. In plant TPC, the linker binds Ca^{2+} to help open the channel[3,4]. However, Ca^{2+} binding by mouse TPC1 is unlikely, because amino acids essential for this interaction

are missing. The authors show that the carboxy-terminal domain of mouse TPC1, which is longer than the equivalent domain in the plant protein, forms a horse-shoe-shaped arrangement of four helices that makes direct contact with the linker region. This unique feature of animal TPC probably serves to fine-tune channel activity***.***

Activation of animal TPCs is complex and multifaceted. TPCs were originally identified[5,6] as the targets for a messenger molecule called NAADP, which was known to release Ca^{2+} from acidic organelles {PMID: 12464181}. Subsequent work revealed[7] that TPCs are also activated by the lipid $PI(3,5)P_2$, which is primarily found in acidic organelles. In addition, TPC1 is regulated by changes in voltage across the organelle membrane[8,9]. She *et al.* demonstrated that both $PI(3,5)P_2$ and voltage changes are required to open TPC1; neither alone is sufficient (NAADP was not tested). The authors then resolved structures of TPC1 both in the absence and presence of $PI(3,5)P_2$, giving insight into the structural transitions that occur during channel opening. Their analysis produced two key findings.

First, the group pinpointed the $PI(3,5)P_2$ binding site, which lies within 6-TM I. Mutation of any one of several amino-acid residues in the network that forms this binding site can prevent TPC1 activation by $PI(3,5)P_2$. Interestingly, two of these residues — arginines in a short linker between S4 and S5 — are also required[10] for channel activation by NAADP. This suggests that $PI(3,5)P_2$ likely acts as a cofactor for NAADP action. Comparison of the free and $PI(3,5)P_2$-bound forms of TPC1 revealed a single lysine residue in S6 that mediates conformational changes to the pore in response to $PI(3,5)P_2$ binding, thus directing the first stage of channel opening.

Second, the authors find that changes in voltage are sensed exclusively by arginine residues in 6-TM II. Both 6-TM I and 6-TM II contain sequences in S1-S4 reminiscent of voltage sensors in other channels, but only 6-TM II has a specific helix in S4 that is required for voltage gating. The 6-TM II voltage sensor is in an upward 'activated' form in both structures obtained by the authors — in this form, it can probably mediate changes to the adjacent pore, completing opening of the channel**.**

Taken together, work on TPCs from animals (She et al[2]) and plants[3,4] indicates that both 6-TM I and 6-TM II cooperate to open the channel. 6-TM II is a target for voltage changes in both proteins. By

contrast, 6-TM I is targeted directly by $PI(3,5)P_2$ in animal TPC1, and indirectly by Ca^{2+} in plant TPC. This is a prime example of how evolutionary distant proteins have adapted to conserve a core function.

Which ions pass through animal TPCs once they are opened? Much research suggests these channels are non-selective, similar to plant TPC, but other work indicates they are selective for sodium ions[1,7] (Na^+). She *et al.* found that TPC1 was about 70 times more permeable to Na^+ than to potassium ions (K^+). Their structures reveal that the narrowest part of the pore through which ions are filtered is shaped like an oblong 'coin slot', constricted by specific asparagine residues. The authors provide mutational evidence that these residues allow small Na^+ through, but not larger K^+. This sieve effect is unlikely to explain the authors' data indicating that TPC1 selects for Na^+ over Ca^{2+}, because these ions are about the same size. However, the electrophysiological experiments used by the researchers to determine ion selectivity are performed under conditions quite removed from the situation in live cells, where the permeability of TPCs to Ca^{2+} is readily demonstrable[11].

In sum, She and colleagues' structures provide major insight into how TPCs work. They join recently reported structures in Nature {PMID: 29019983 PMID: 29019979 PMID: 29019981} for a related family of ion channels known as the TRP mucolipins. Like TPCs, they reside within acidic organelles, are activated by $PI(3,5)P_2$ and release Ca^{2+} to control cellular functions such as gene transcription {PMID: 28457591}. The $PI(3,5)P_2$ binding site in TRPMLs is likely in the amino-terminal of the protein and thus very different to that in TPCs, although it has yet to be directly observed. However, a binding site for a synthetic opener of TRP mucolipins has been structurally resolved in a crevice between subunits close to the pore region {PMID: 29019983}.

Looking forward, these rapid advances in the structural biology of organellar ion channels will aid in rationally designing drugs to modulate ion flux through them. This is pertinent as the number of associated diseases grows**.** Mutations in TRP mucolipin 1 cause a lysosomal storage disorder affecting children and TPCs have been implicated in fatty liver disease, Ebola infection as well as a number of neurodegenerative disorders {PMID: 28457591 PMID: 28529827}. In this context, a human TPC structure would be most welcome.

Another challenge is to resolve the structure of TPC2. This protein is regulated by NAADP and $PI(3,5)P_2$, but not by changes in voltage — begging the question of how conformational changes in one TM domain are transmitted to the other to allow channel opening. No doubt, TPCs will further open up and reveal their secrets through forthcoming structures.

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Figure 1. **Structure of animal TPCs revealed**. Depiction of mouse TPC1 showing the two domains and connecting linker of a single subunit (cyan cartoon). The second subunit is shown as a green ribbon. Bound $PI(3,5)P_2$ and arginine residues important for voltage-sensing are marked in red.