New insights into gating mechanisms in TPCs: relevance for drug discovery

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Endo-lysosomal two-pore channels (TPCs) have emerged as molecular hubs converging different stimuli to divergent outcomes. Shimomura et al. [1] focused on coactivation of TPCs by voltage and ligands, particularly phosphoinositides, discovering a cooperative effect of the first and second Shaker-like channel domain, which fine tunes channel gating and opens a new way to modulate TPCs Fig. 1.

Discovered in plants and animals, two-pore channels (TPCs) are classified as belonging to the voltage-gated ion channel (VGIC) superfamily with characteristic Shaker-like domains (DI and DII), consisting of 6 transmembrane (TM) units, each. Despite similar structure, they are gated by a range of different factors: plants – Ca^{2+} and voltage, animals – phosphoinositides and NAADP in a voltage dependent (TPC1 and TPC3) or voltage independent (TPC2) manner [2,3]. Animal TPCs are part of the ion transport machinery embedded in endolysosomal structures playing important roles in virus trafficking, LDL trafficking and fatty liver disease, Parkinson's disease, melanin production, angiogenesis, EGF/EGFR trafficking and cancer [4–6]. Recent studies have identified JPT2 and LSM12 proteins as key intermediaries in mediating NAADP activation of TPCs [7,8] and functional integration with $PI(3,5)P_2$ in Ca^{2+} signalling by TPC2 [9]. Despite these recent discoveries, stimuli hierarchy and interdependence are still weakly understood.

Experimental cryo-EM 3D structures of mouse TPC1, human (Hs)TPC2, zebrafish (Xt)TPC3 as well as crystal structures of Thale cress (*Arabidopsis thaliana*) TPC1 have provided valuable insights into the mechanisms of voltage sensing, ion selectivity, channel activation, and phosphorylation. However, crystal structures or even structures representing HsTPC2 channel closed versus ligand-bound conformations are not sufficient to cover all relevant protein states, which vary with susceptibility to modulation. To give an example: ligand-bound HsTPC2 channel can exist in open and closed conformations with a ratio of about 3:5 [2,3]. Thus, time and impact on subunit motions are crucial factors for a proper description of the activity of TPCs in response to different stimuli.

Shimomura et al. (2023) [1] used mutagenesis, voltage clamp fluorometry, and the two-electrode voltage-clamp technique to study the previously postulated impact of $PI(3,4)P_2$ binding in S4-S5 linker in DI to the distantly located TM4 in DII of TPC3 (core of voltage-sensor in Shaker like domain II). They inferred an intermediate state of DII-TM4, which is strongly $PI(3,4)P_2$ dependent. This confirmed global and complex integration of TPC3 gating mechanisms and revealed interaction between Shaker like domain I (DI) and Shaker like domain II D(II). Interestingly, stabilization of XtTPC3 DII-TM4 in the intermediate state by mutagenesis essentially converted the predominantly voltage-gated channel into a strong $PI(3,4)P_2$-gated channel type, mimicking TPC2. This suggests that there is a regulatory mechanism hidden in DII-TM4, which defines channel susceptibility to $PI(3,4)P_2$ versus voltage activation.

To gain insight into the molecular behaviour of TPC2, the authors created a HsTPC2 channel version in the stabilized intermediate state of DII-TM4 (mutagenesis+$CdCl_2$). Although TPC2 is considered voltage-insensitive, previous work showed that a number of TCAs activate TPC2 in a voltage-dependent manner [10]. The new work found that desipramine-induced (voltage-dependent) currents in the mutant were blocked, while $PI(3,5)P_2$-induced (voltage-independent) currents remained unchanged, similar to TPC3. Furthermore, desipramine action was assigned to DII-TM4, not DI-TM4, suggesting that TPC2 possesses a ‘cryptic’ voltage sensor, which gets activated when desipramine binds. Indeed, a combination of desipramine and $PI(3,5)P_2$ acted synergistically on wild type TPC2, magnifying channel activity.

Another interesting perspective was provided relating to the mechanism of action of the alkaloid naringenin, which is a blocker of $PI(3,5)P_2$-activated TPC2. The authors found that this compound stimulated voltage-dependent desipramine induced currents. However, it remains unclear if there is an endogenous equivalent mimicking these effects. Nevertheless, the presented mechanism of ligand and voltage gating opens opportunities for new strategies in drug discovery i.e., selective channel activation in a certain voltage range rather than a simple switch on switch off mechanism.

Workers Leaving The Lumière Factory in Lyon (*La Sortie de l'Usine Lumière à Lyon*) is a film produced by Louis Lumière in 1895, often referred to as the ***first real motion*** picture ever made. (Georges Sadoul, Histoire du cinéma mondial: des origines à nos jours, Paris, Flammarion, 1968, 719 p., p.19). This project is considered to be the birth of cinema. In analogy, we propose that the elucidation of the movement of the cryptic voltage sensor in TPC2 may initiate completely new, potentially more selective strategies to modulate TPCs and to treat a range of human diseases such as lysosomal storage, neurodegenerative, inflammatory, infectious diseases or certain types of cancer e.g., melanoma.

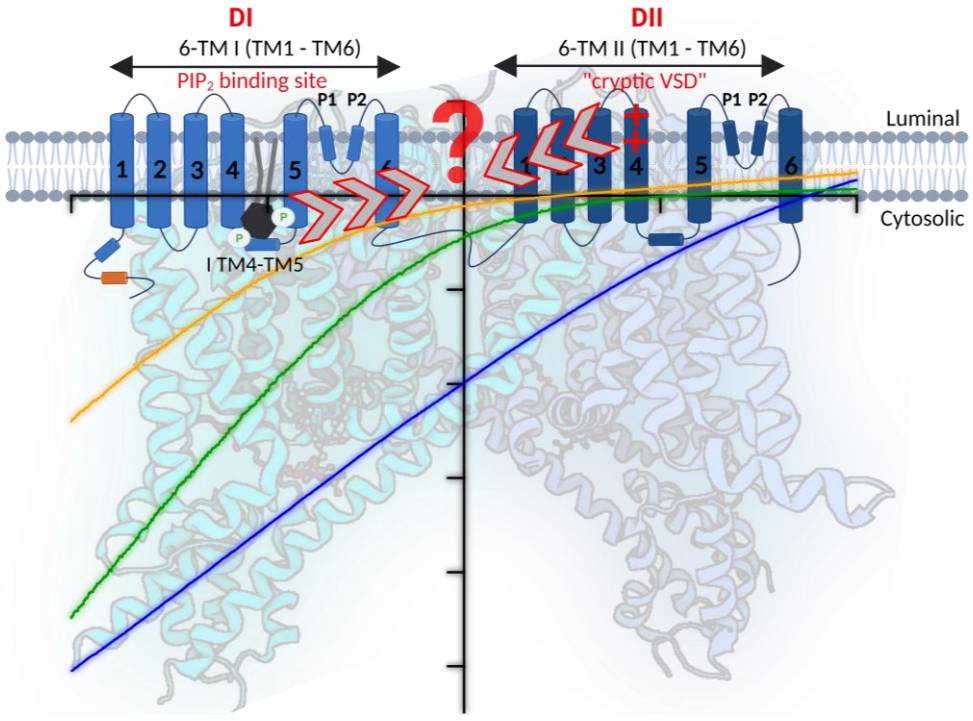

Figure 1. Two-Pore Channel 2 multimodal gating mechanism. Illustrative 2D structure and current-voltage ramp traces (blue – voltage independent evoked by $PI(3,5)P_2$, green and yellow – voltage-dependent in response to TPC2 antagonists such as, e.g., naringenin) superimposed on 3D visualization of TPC topology (PDB accession number 6NQ0) with bound $PI(3,5)P_2$. Marked are Shaker-like domains (DI and DII) spanning through the lipid membrane, each consisting of six transmembrane domains (TM1–TM6), the cryptic voltage-sensing domain (VSD) located in TM4 of the C-terminal Shaker-like domain (DII), the ion-conducting pore-forming loops (P1 and P2) between

segments TM5 and TM6 and the PI(3,5)P_2 binding site near DI TM4-S5. Question mark suggests interaction between locations of possible TPC2 activation (phospholipids in Shaker-like domain I and cryptic voltage sensing domain in Shaker-like domain II). Layout created with BioRender.com.

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