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Cryo–electron microscopy unveils the gating mechanism of the human Kir2.1 channel

Catherine Venien-Bryan[1], Dr Carlos Fernandes[1], Dr Dania Zuniga[1], Mr Andreas Zoumpoulakis[1], Mr Rafael Veloso[1], Dr Renaud Wagner[2]
[1]IMPMC, UMR7590 Sorbonne University,CNRS,MNHN, Paris, France, [2]IMPReSs Facility, UMR 7242, CNRS University of Strasbourg, Strasbourg, France

Background

Inward-rectifier potassium (Kir) channels are a group of integral membrane proteins that selectively control the permeation of K+ ions across cell membranes. The small outward K+ current through Kir channels controls the resting membrane potential and membrane excitability, regulates cardiac and neuronal electrical activities, couples insulin secretion to blood glucose levels, and maintains electrolyte balance1. All Kir channels are tetramers and share characteristic structural features. They have a canonical pore-forming transmembrane domain (TMD) made of two transmembrane helices separated by a K+ ion selectivity filter and a large cytoplasmic domain (CTD) containing both N and C termini. The CTD extends the ion conduction pathway and provides docking sites for regulatory ions, proteins, and ligands2. The strong inward-rectification mechanism results from a block on the cytoplasmic side of the channels by endogenous polyamines and Mg2+ that plug the channel pore at depolarized potentials. The blockers are then removed from the pore when the K+ ions flow into the cell at hyperpolarized potentials. In addition to being inwardly rectifying, the gating of Kir2.1 channels are selectively activated by the lipid phosphatidylinositol 4,5-bisphosphate (PIP2). The physiological importance of these channels is underpinned by the fact that mutations in these proteins cause a wide range of pathologies. In previous published work, we used cryo–electron microscopy (cryo-EM) combined with image analysis to elucidate the structure of a human Kir channel, Kir2.1 in the closed state3. Furthermore, computational investigations reveal crucial conformational movements, including compaction of the structure and opening movements at the interface between the TMD and CTD, which could facilitate the binding of PIP2. In order to understand the gating mechanism, both structures of Kir2.1 in the closed and open state are needed.

Methods

A total of 9944 micrographs were collected on a Titan Krios G4 microscope operated at 300 kV equipped with Falcon4 and SelectrisX image filter. After a visual inspection to remove poor-quality micrographs, the movies were motion-corrected and dose-weighted (MotionCor2) and contrast function parameters were estimated (CTFFIND4) on 6961 selected micrographs. A total of 781,076 particles were automated picked (SPHIRE-crYOLO). The extracted particles were subject to one round of 2D and 3D classifications (RELION). A map containing 187,153 particles were subjected to 3D Auto-Refine, CTF-refinement and particle polishing. The polished particles were submited to a 3D non-uniform refinement (cryoSPARC)

Results

Here we present the cryo-EM structure of the human Kir2.1 channel complexed to PIP2 in the open state (Fig.). and provide the final cryo-EM map of the Kir2.1/PIP2 complex at 2.85 Å resolution. Preliminary atomic structure of Kir2.1/PIP2 complex was build from the cryo-EM map and revealed the PIP2 binding site.

Conclusion

Comparative structural analysis of the Kir2.1/PIP2 complex (open state) with apo-Kir2.1 (closed state) and in silico studies reveal the structural changes that lead to channel opening. Moreover, the structure of the Kir2.1/PIP2 complex highlights the role of secondary anionic binding site for channel opening. These data will help to understand the pathological mechanisms associated with mutations in the Ki2.1 channel

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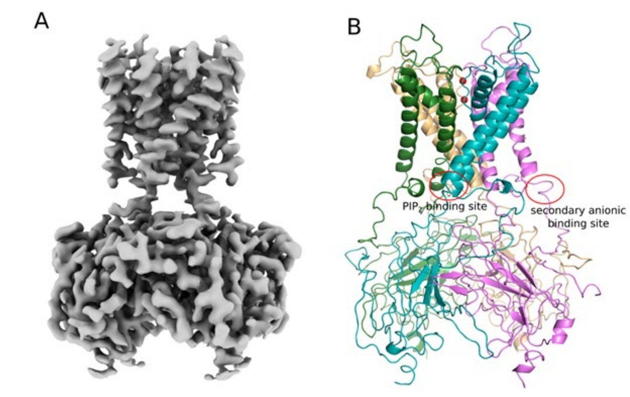
Graphic:



Keywords:

Potassium channel, gating, in silico

Reference:

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