MS08-O4

Structural and functional studies on a prokaryotic homologue of the mammalian SLC7 cationic amino acid transporters

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The Amino acid/Polyamine/Cationic (APC) superfamily is among the largest of the secondary active transporters. Members of the APC superfamily are responsible for the uptake of amino acids and other substrates using either a proton or sodium gradient to drive substrate translocation across the membrane. Since Amino acids, and their derivatives, are crucial for both prokaryotic and eukaryotic cell biology, the systems responsible for their transport are important targets.

In particular, cationic amino acid transporters (CATs), members of the solute carrier (SLC) family 7, determine the availability of arginine in the cell. Arginine is the precursor for L-ornithine and nitric oxide (NO) synthesis and thus an important cellular signalling molecule as well as a proposed major regulator of the mTORC1 pathway.

Here, the crystal structure of a proton-coupled amino acid transporter closely related to the SLC7 family, from *Geobacillus kaustophilus, Gk*ApcT, is presented. To study the transport mechanism of *Gk*ApcT, liposome-based functional assays were performed to characterise the substrate specificity and kinetics. Additionally, the crystal structure guided a detailed site-directed mutagenesis study, which identified several conserved residues involved in substrate binding and proton-coupling. The current structure served as a template understand proton-coupled amino acid transport in bacteria, and to understand mammalian amino acid transport by the SLC7 family.

Keywords: SLC7, amino acid transporters, membrane protein

MS08-O5

The structure of RVFV fusion protein reveals a lipid binding pocket conserved in class-II fusion proteins

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The Rift Valley fever virus (RVFV), a member of the genus Phlebovirus within the order Bunyavirales, is an arthropod-borne virus (arbovirus) responsible for epizoonoses throughout Africa with devasting economic consequences and causing serious disease in humans. The fact that RVFV can be transmitted to humans by multiple mosquito species, a number of which are spread across the planet, is a source of considerable concern. RVFV virions display two envelope proteins at their surface, Gn and Gc, associated as heterodimers arranged with icosahedral symmetry. The virus enters cells via receptor-mediated endocytosis, with glycoprotein Gc catalyzing the acid-induced membrane fusion reaction with the endosome for entry. The structure of Gc in prefusion conformation showed the characteristic fold of class II membrane fusion proteins, initially identified in other arboviruses, such as those in the genus Flavivirus (zika, dengue and yellow fever viruses) and in the genus Alphavirus (which includes among others the pathogenic chikungunya virus).

We have solved the structure of RVFV fusion protein (Gc) in its post-fusion conformation in complex with a lipid (phosphatidylcholine) bound in a pocket near the fusion loop, which is the region responsible to interact with cellular membranes. Membrane binding experiments showed that Gc requires specific glycerophospholipids (GPL) and cholesterol for binding to target membranes and the X-ray structure, together with site directed mutagenesis and molecular dynamics simulations, reveals the molecular determinants of this specificity [1]. Strikingly, the GPL head group recognition pocket is conserved in the fusion proteins of other arthropod-borne viruses, such as zika, dengue or chikungunya viruses, which have recently caused major epidemics worldwide. Indeed, the membrane-insertion mechanism identified in this work helps us to understand the changes in cholesterol dependence linked to a serious outbreak of Chikungunya virus in 2005-2006 and the phosphatidylserine dependence of Dengue virus for entry. Moreover, the identified pocket will represent a new target for the development of antivirals against a broad range of pathogenic arbovirus (flavivirus, alphavirus and bunyavirus).

References:

[1]. Guardado-Calvo, P., et al., A glycerophospholipid-specific pocket in the RVFV class II fusion protein drives target membrane insertion. Science, 2017. 358(6363): p. 663-667.Keywords: membrane fusion, class-II fusion proteins, bunyavirus

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