

MS8-P7 **Towards the Structural Characterization of MgtA, a Magnesium transporter.** Saranya Subramani, Harmonie Perdreau, Jens Preben Morth. *Centre for Molecular Medicine Norway (NCMM), University of Oslo, Norway*
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The evolvement of sophisticated regulatory pathways allows bacteria to adapt environmental changes. Over the last decade a role for Mg^{2+} homeostasis in *Salmonella* pathogenesis has emerged. *Salmonella* utilizes three different classes of Mg^{2+} transporters to maintain Mg^{2+} homeostasis: CorA, MgtE and MgtA/MgtB. In contrast to the constitutively expressed CorA and MgtE, the expression of MgtA/MgtB is regulated by the two-component system PhoP/PhoQ. MgtB is necessary for the long term survival of pathogen [1] while MgtA have been shown to enhance survival under high temperature [2]. Despite the delineation of basic transport properties and definition of some important intramembrane residues, the mechanism by which Mg^{2+} influx occurs in MgtA and MgtB is not known [3]. It is also not clear whether Mg^{2+} is a primary or secondary substrate. The virulence factor *mgtC* is essential for long term survival of host cell is co-expressed with *mgtB* [4]. The exact function and interacting partners of MgtC are not known. The main objective of our project is to investigate the molecular mechanism of Mg^{2+} transport and homeostasis in pathogenic bacteria from a structural point of view. To accomplish this, MgtA, MgtB and MgtC membrane protein targets were cloned from *Salmonella*. After expression screening using GFP tag, MgtA was selected for overexpression and purification. Crystals were observed after lipidation and addition of a second detergent [5]. Optimization of the crystal condition and standardization of the activity assay for Mg^{2+} transporter is ongoing. Purification and characterization of MgtB and MgtC are also ongoing projects.

- [1] Moncrief MB, Maguire ME. (1999). *J Biol Inorg Chem.*4(5),523-7.
- [2] O'Connor K, Fletcher SA, Csonka LN.(2009) *Proc Natl Acad Sci U S A.* 106(41), 17522-7.
- [3] Maguire ME. (2006). *Front Biosci.* 11, 3149-63.
- [4] Blanc-Potard AB, Groisman EA.(1997) *EMBO J.* 16(17):5376-85.
- [5] Gourdon P., Andersen J.L., Hein K.L., Bublitz M., Pedersen B.P., Liu X-Y., Yatime L., Nyblom M., Claus Olesen C., Müller J.V., Nissen P., Morth J.P. (2011) *Cryst. Growth and Des.* 11, 2098-2106

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MS8-P8 **H^+ -translocating Pyrophosphatase.** Yuh-Ju Sun^a, Shih-Ming Lin^a, Jia-Yin Tsai^b, Chwan-Deng Hsiao^b, Yun-Tzu Huang^a, Chen-Liang Chiu^a, Mu-Hsuan Liu^a, Jung-Yu Tung^a, Tseng-Huang Liu^a, Rong-Long Pan^a
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The membrane embedded H^+ -translocating pyrophosphatases (H^+ -PPases) are active proton transporters that establish a proton gradient across the endomembrane via pyrophosphate (PP_i) hydrolysis. H^+ -PPases are primarily found as homodimers in the vacuolar membrane of plants and the plasma membrane of several protozoa and prokaryotes. Here, we report the crystal structure of a *Vigna radiata* H^+ -PPase (VrH^+ -PPase) in complex with a non-hydrolyzable substrate analogue, imidodiphosphate (IDP), at 2.35Å resolution. VrH^+ -PPase monomer consists an integral membrane domain formed by 16 transmembrane helices. IDP is bound in the cytosolic region of each subunit and trapped by numerous charged residues and five Mg^{2+} ions. A novel proton translocation pathway is formed by six core transmembrane helices. Proton pumping was initialized by PP_i hydrolysis and then H^+ is transported into the vacuolar lumen through a pathway consisting of four acid-base pairs residues.

- [1] Lin, S.-M. Tsai, J.-Y., Hsiao, C.-D., Huang, Y.-T., Chiu, C.-L., Liu, M.-H. Tung, J.-Y., Liu, T.-H., Pan, R.-L. & Sun Y.-J. (2012) *Nature* 484, 399-403.

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