

MS12 Biophysical characterization and crystallization

Chairs: Rob Meijers, Bernadette Byrne

MS12-O1 The magnesium transporter A, a bacterial P-type ATPase dependent on cardiolipin and selectively sensitive to free magnesium *in vitro*

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Three classes of Magnesium transporters have been identified in Bacteria and Archaea; CorA, MgtE and MgtA/MgtB (Groisman et al., 2013). CorA and MgtE are constitutively expressed. CorA and MgtE are both magnesium efflux transporter. Influx is believed to be mediated by MgtA. The magnesium transporter A (MgtA) is a specialized P-type ATPase, that import Mg^{2+} into the cytoplasm. In both *Salmonella typhimurium* and *Escherichia coli*, the virulence determining two-component system PhoQ/PhoP regulates the transcription of the *mgtA* gene by sensing Mg^{2+} concentrations in the periplasm, along with low pH and the presence of cationic peptides. This study demonstrates, for the first time, that MgtA is highly dependent on anionic phospholipids and in particular, cardiolipin, the *in vitro* kinetic experiments performed on detergent solubilized MgtA suggest that cardiolipin act as a magnesium chaperone. We further show that MgtA is highly sensitive to free Mg^{2+} (Mg_{free}^{2+}) levels in the solution. MgtA is activated when the Mg_{free}^{2+} concentration is reduced below 10 mM and is strongly inhibited above 1 mM, indicating that Mg_{free}^{2+} acts as product inhibitor. Furthermore, colocalization studies confirm that MgtA is found in the cardiolipin lipid rafts in the membrane. Combined, our findings indicate that MgtA may act as a sensor as well as a transporter of Mg^{2+} (Subramani et al., 2016). Initial crystals have been obtained, using the Hilide method for membrane protein crystallization (Gourdon et al., 2011). The approach of Hilide method will be discussed in this context.

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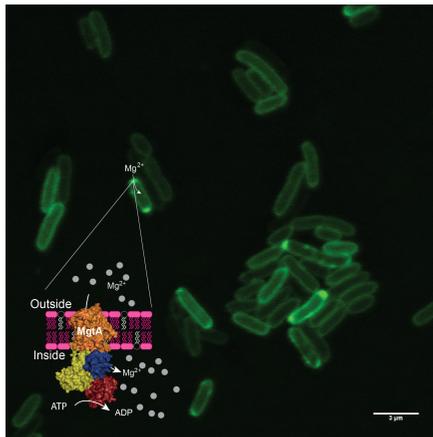


Figure 1. Overexpressed MgtA-GFP in *E. coli* is visualized in the background. The fluorescence intensity indicates polar localization. The cartoon describes ATP dependent magnesium (grey) uptake through the P-type ATPase MgtA. Cardiolipin (pink) is believed to be co-localized in the poles and activate MgtA.

Keywords: MgtA, P-type ATPases, Hilide, Magnesium homeostasis, cardiolipin, membrane protein