Spying on proteins in the mycomembrane

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Corynebacteriales encompasses species like *Corynebacterium glutamicum*, which has been harnessed for industrial production of amino acids, as well as *Mycobacterium tuberculosis*, which cause Tuberculosis (TB), a devastating infectious disease for humans. Emerging challenges in TB therapy rely on the discovery of new molecular scaffolds able to penetrate the highly impermeable bacterial cell wall or drugs that target cell wall-associated proteins, which may be more accessible [1].

The mycoloyl-arabinogalactan-peptidoglycan (mAGP) complex is a distinctive component of the *Corynebacteriales* cell envelope, representing 40 % of the dry mass of the bacterium and playing crucial roles in cell viability and virulence. In the last few years, our group has made a number of significant discoveries on the structure and function of cell wall-associated proteins from *C. glutamicum* [2-4]: (i) identification and localization of unusual post-translational modifications including the O-mycoloylation (ii) sub-cellular localization of the proteoforms (iii) role of O-mycoloylation in targeting proteins to the mycomembrane (iv) structural characterization of non-acylated and multi-acylated mycomembrane proteins by using top-down mass spectroscopy and solution NMR. These data support the idea that the mycolic-acid environment of mycomembrane proteins plays a critical role in their structure, assembly and functions.

Here we report a new strategy consisting of 1) dissecting the *C. glutamicum* cell envelope, 2) exploring major molecular components in intact mycomembrane at atomic resolution and 3) building reconstituted systems that are relevant for subsequent structural and functional analysis in the native environment. As a proof of concept, I will show recent data obtained on O-mycoloylated PorA/PorH proteins, which form cation-selective channels in the mycomembrane of *C. glutamicum*.

References

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