Unraveling *corynebacterial* divisome composition by proximity labeling in the living cell

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Bacterial cell division is directed by the divisome, a protein complex whose assembly begins with the polymerization of FtsZ at midcell to form a ring (Z-ring) This structure participates in the recruitment of other divisome proteins, that in the case of the model bacilli (*Escherichia coli* and *Bacillus subtilis*) have been identified and characterized. However, the order *Corynebacteriales* (that includes important human pathogens as *Mycobacterium tuberculosis* and *Corynebacterium diphtheriae*) lacks recognizable homologues for many of these cell division proteins, and the ones fulfilling these missing functions are yet to be identified.

To identify the unknown pieces of the corynebacterial divisome, we developed and optimized a proteomic strategy based on proximity biotinylation in the living cell, using *Corynebacterium glutamicum* as a model organism. We generated a strain expressing FtsZ fused to an engineered ascorbate peroxidase (APEX2). APEX2 catalyzes the oxidation of phenol biotin in the presence of H_2O_2 giving rise to a radical that reacts with amino acids of nearby proteins. This allowed us to label the proteomic environment of FtsZ in the living cell, and its purification and identification by Mass Spectrometry.

We corroborated that APEX2 is active in the biochemical background of *C. glutamicum*, and optimized the labelling strategy to guarantee the identification of physiologically relevant FtsZ neighbours. We identified a confident list of 253 FtsZ neighbors, that includes known cell division proteins as well as an important number of non-characterized proteins, which represents putative new divisome components. We focused on hypothetical membrane proteins, that might mediate membrane anchor of the Z-ring, as most of the proteins fulfilling

this role in *E. coli* and *B. subtilis* are not present in corynebacterial genomes. We generate strains expressing the selected candidates fused to a fluorescent proof to evaluate their subcellular localization and their interaction with FtsZ. The results allowed us to identify new conserved membrane bound components of the corynebacterial divisome. Their precise role in cell division, the molecular details of its interaction with the Z-ring and its regulation by protein phosphorylation are being studied.

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