Identification of new cell division proteins in *Mycobacteriales* by proximity proteomics in living cells

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Bacterial cell division is an essential and highly regulated process directed by two multiprotein complexes: the elongasome and the divisome, responsible for elongation and division, respectively. These systems have been extensively characterized in model bacteria such as *Escherichia coli* and *Bacillus subtilis*. However, in bacteria of the order *Mycobacteriales*, the asymmetric polar growth and the complexity of their cell envelope confer unique structural and functional features, and many of the homologous components of the classical divisome are absent from their genomes. Although the central protein FtsZ is conserved, the proteins responsible for anchoring the Z-ring to the membrane remain unknown. Moreover, in this group, protein phosphorylation by Ser/Thr kinases emerges as a key mechanism regulating cell division.

This work aimed to identify the proteins that fulfill the missing roles of the divisome in *Mycobacteriales* and to explore the role of phosphorylation in this process. To this end, a proteomic strategy based on proximity-dependent biotinylation in living cells using the enzyme APEX2 was developed and validated, employing *Corynebacterium glutamicum* as a model organism. This approach allowed us to characterize the proteomic environment of FtsZ under physiological conditions and under different phosphorylation states. A set of FtsZ-neighboring proteins was identified, including known cell division proteins, validating

the strategy, as well as new membrane proteins with septal localization, proposed as potential divisome components.

Finally, analysis of a mutant strain lacking a Ser/Thr kinase revealed morphological alterations and changes in the composition of the FtsZ interactome, demonstrating that phosphorylation regulates both the architecture and function of the divisome. Altogether, this work introduces a robust tool for studying protein–protein interactions in *Mycobacteriales* and expands current knowledge of the molecular mechanisms that control bacterial cell division.