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Structural basis of FtsEX-independent RipA-mediated cell separation in Corynebacteriales

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Abstract

The bacterial cell wall is a multi-layered mesh, whose major component is peptidoglycan (PG), a sugar polymer cross-linked by short peptide stems. During cell division, a careful balance of PG synthesis and degradation, precisely coordinated both in time and space, is necessary to prevent uncontrolled destruction of the cell wall. In *Corynebacteriales*, the D,L endopeptidase RipA has emerged as a major PG hydrolase for cell separation, and RipA defaults have major implications for virulence of the human pathogens *Mycobacterium tuberculosis* and *Corynebacterium diphtheriae*. However, the precise mechanisms by which RipA mediates cell separation remain elusive.

I will present a phylogenetic, biochemical, and structural analysis of the *Corynebacterium glutamicum* homologue of RipA, Cg1735, which we published over the course of my PhD in PNAS [1]. The crystal structures of full-length RipA in two different crystal forms revealed the C-terminal NlpC/P60 catalytic domain obtruded by its N-terminal conserved coiled-coil domain, which locks the enzyme in an auto-inhibited state. We showed that this auto-inhibition is relieved by the extracellular core domain of the transmembrane protein Cg1604 (SteB), which was recently identified as a corynebacterial cell division protein [2]. The crystal structure of SteB revealed a (beta/alpha) protein with an overall topology similar to that of receiver domains from response regulator proteins. The atomic model of the RipA-SteB complex, based on bioinformatical and mutational analysis, indicates that a distal-membrane helical insertion in SteB is responsible for RipA activation.

These findings are clinically relevant, as RipA and SteB are conserved in pathogenic *Corynebacteriales* and involved in *C. glutamicum* tolerance to antibiotics. Our work provides important insights into the fundamental biological processes and specificities that underlie corynebacterial cell division.

[1] Gaday et al. PNAS. 2022 ; [2] Lim et al. PLoS Genet. 2019