Regulation of L,D-crosslinks (3-3 CLs) to maintain cell envelope homeostasis in *Escherichia coli*

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Gram-negative bacteria have a unique cell envelope consisting of a lipopolysaccharide-containing outer membrane (OM) that is covalently linked to the thin layer of peptidoglycan (PG). The OM serves as a barrier against toxic molecules including many antibiotics and allows the cells to survive in many environmental stress conditions; more recently it has been shown to contribute to the mechanical integrity of the bacterial cell, a function traditionally assigned to the PG. The growth of OM and PG layers needs to be tightly coordinated. When the OM biogenesis is compromised a PG remodeling program is required to avoid cell lysis. In *Escherichia coli* cells this modification program relies on the activity of LD-transpeptidase (LDTs) family proteins that introduce the non-canonical 3-3 cross-links (CLs) in the PG layer to restore the mechanical strength and the overall stability of the bacterial cell envelope. The LDTs enzymes are members of the YkuD protein family. Six of them have been identified in *E. coli*: LdtA, LdtB, and LdtC that covalently attach Lpp to the PG; LdtD and LdtE that catalyze 3-3 CLs formation. The last identified is DpaA (previously LdtF) that does not catalyze the formation of 3-3 CLs but regulates their formation by modulating the activity of LdtE. Moreover, DpaA is the enzyme that detaches Lpp from PG. Notably, Lpp is the most abundant *E. coli* OM lipoprotein that covalently links the OM to PG. Whereas 3-3 CLs are not required under non stress condition, their formation is highly regulated in the cell during growth. Moreover, they have a central role under envelope stress since the cell ensures the formation of 3-3 CLs by LdtD activation.
Here, we demonstrate that Lpp and its detachment from the PG have an unxpected role in regulating 3-3 CLs formation mediated by LdtE/DpaA. These results shed light on a new function of Lpp, that not only provides structural strength to the cell envelope, but likely regulates the catalysis of 3-3 CLs under normal growth conditions. A possible model on how the dynamics of Lpp attachment and detachment influence the production of 3-3 CLs is dicussed.