

DpaA and Lpp affect LD-cross-link formation in *Escherichia coli*

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Peptidoglycan (PG) is an essential component of the cell envelope, required to mechanically sustain the turgor pressure of the cytoplasm. In *Escherichia coli*, the PG consists of glycan chains with attached stem peptides mainly cross-linked via DD-cross-links formed between Alanine (D-Ala⁴) and *meso*-diaminopimelic acid (DAP³) by DD transpeptidases (Vollmer *et al.*, 2008). A small amount of LD-cross-links occurs between two DAP³ residues, formed by LD-transpeptidases members of the YkuD family of proteins (Magnet *et al.*, 2008). Although not essential under standard laboratory conditions, LD-cross-links become crucial for β -lactam resistance, PG homeostasis and survival under stress conditions (Hugonnet *et al.*, 2016, Morè *et al.*, 2019, Alvarez *et al.*, 2024). *E. coli* has two LD transpeptidases, LdtD and LdtE for LD-cross-link formation (Magnet *et al.*, 2008). LdtD is the cell envelope-stress-induced LDT and LdtE is the housekeeping LDT active under non-stress conditions (Morè *et al.*, 2019). Interestingly, the formation of LD-cross-links by LdtE requires DpaA, another YkuD protein family member, which detaches the outer membrane (OM)-anchored lipoprotein Lpp from the PG (Badour *et al.*, 2021; Winkle *et al.*, 2021). Lpp (Braun lipoprotein) is the most abundant OM lipoprotein which is attached to PG via its terminal Lys and a DAP³ residue in the PG. This is the only covalent linkage between the PG and OM and is needed to maintain a robust cell envelope. By analysing the PG composition of wild type and mutants lacking *ldt* genes, *dpaA*, and/or *lpp*, we found that DpaA and Lpp are both required for LD-cross-link formation by LdtE, highlighting the unexpected functional interaction between Lpp detachment dynamics and LD-cross-link formation.