(1) Submission ID#1526847

Investigating the mechanism of surface lipoproteins translocation by Slam – a type XI secretion system in Neisseria meningitidis

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Background

Surface lipoproteins (SLPs) are peripherally attached to the outer leaflet of the outer membrane in many Gram-negative bacteria, playing significant roles in nutrient acquisition (transferrin binding protein B, TbpB) and immune evasion (Factor H binding protein, FHbp) in the host. The molecular machinery that are involved in the synthesis and delivery of SLPs across the inner membrane are well characterized and recently we identified an outer membrane protein, named Surface lipoprotein assembly modulator – (Slam) that functions as the outer membrane translocon for a handful of SLPs. However, the molecular details of how Slam translocate SLPs across the outer membrane remained unknown, especially how the translocon recognizes and initiates the translocation in the periplasmic space.

Aim/Methods

In this study, we used mass spectrometry to identify periplasmic factors that associate with flag-tagged SLPs while they transit through the periplasm to the outer membrane. We knocked out these factors in N. meningitidis and tested the translocation of SLPs. Finally, we reconstituted the purified outer membrane protein, Slam, into proteoliposomes and examined its function as a translocon in vitro as well as the involvement of the periplasmic factors.

Results

Our reconstitution assay confirmed Slam as an autonomous translocon, working independently from other major translocons such as the BAM complex, to translocate unfolded TbpB into the proteoliposomes. Furthermore, our pulldown assay, coupled with mass spectrometry identified chaperone Skp as a periplasmic factor that interacts with TbpB when the protein is in transit across the periplasm, potentially to protect TbpB in its unfolded state from being digested by periplasmic proteases. The knock-out mutagenesis confirmed that the presence of Skp is required for the display of functional TbpB on the surface of N. meningitidis. Addition of Skp into our in vitro proteoliposomes translocation assay demonstrated the chaperone is essential factor to enhance translocation efficiency.

Conclusions

This study revealed Slam, a recent classifed Type XI secretion system, works as an autonomous translocon to translocate non-folded lipoproteins such as TbpB to the bacterial surface. The periplasmic chaperone Skp was identified as a component that enhances translocation of functional TbpB to the cell surface.