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Adapting the neisserial type XI translocon, SLAM, as a molecular multitool in antigen production and delivery

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Background

The type XI secretion system (T11SS), also designated as the Surface Lipoprotein Asssembly Modulator (SLAM), translocates surface lipoproteins (SLPs) from the periplasm across the bacterial outer membrane. This outer membrane translocon has a broad range of substrates including neisserial SLPs: transferrin binding protein (TbpB), lactoferrin binding protein (LbpB), factor-H binding protein (fHbp), and hapto/haemogloblin utilization protein (HpuA). These SLPs are highly conserved and surface-exposed virulence factors, making them excellent targets for vaccine and therapeutic development. We have shown that heterologous expression of SLAM and its cognate SLP in Escherichia coli (E.coli) reconstitutes translocation and surface display. While the molecular mechanism of SLP translocation is unclear, we have identified key elements that enable us to secrete normally anchored SLPs as untethered exoproteins in a SLAM-dependent manner.

Aim/Methods

Herein we describe a novel expression and purification strategy for full-length untethered SLPs that are secreted into the culture supernatant in a SLAM-mediated manner in E.coli. Expression of SLPs natively in Neisseria is often not feasible at levels necessary for structural or immunological studies. Alternatively, cytoplasmic expression in E.coli requires separation of the SLP from lysis debris that complicates purification. These considerations present challenges in SLP-antigen characterization and production with substantial cost, safety, and technical implications. Our T11SS-based system overcomes these difficulties and offers a more efficient and robust method in SLP-antigen production.

Results

We demonstrated that our SLAM-mediated secretion system produces SLPs at scales and purity better than conventional cytoplasmic approaches while requiring far fewer downstream purification steps. As such, the secretion process is stable and efficient, yielding 25 to 75+ mg/L of cell culture for select cognate substrates. We have utilized our system to produce and crystallize full-length meningococcal HpuA, which we resolved to 2.1Å by x-ray crystallography. To extend the utility of our system, we secreted SLPs in fusion with other proteins and tested the limitations of the T11SS to gain molecular insights into the translocation process itself.

Conclusions

Our T11Ss-mediated secretion system is a valuable molecular multitool in advancing SLP-antigen characterization and production. Furthermore, it has also allowed us to gain novel insights into the molecular mechanism in SLAM/SLP translocation.