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Structural insight into the formation of lipoprotein-β-barrel complexes

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The β-barrel assembly machinery (BAM) inserts outer membrane β-barrel proteins (OMPs) in the outer membrane of Gram-negative bacteria. In Enterobacteriacea, BAM also mediates export of the stress sensor lipoprotein RcsF to the cell surface by assembling RcsF–OMP complexes. Here, we report the crystal structure of the key BAM component BamA in complex with RcsF. BamA adopts an inward-open conformation, with the lateral gate to the membrane closed. RcsF is lodged deep within the lumen of the BamA barrel, binding regions proposed to undergo outward and lateral opening during OMP insertion. On the basis of our structural and biochemical data, we propose a push-and-pull model for RcsF export following conformational cycling of BamA, and provide a mechanistic explanation for how RcsF uses its interaction with BamA to detect envelope stress. Our data also suggest that the flux of incoming OMP substrates is involved in the control of BAM activity.

The vast majority of proteins inserted in the outer membrane of Gram-negative bacteria adopt a β-barrel conformation. Their assembly depends on the activity of the conserved BAM, whose core component is the OMP85-family protein BamA[1,2]. BamA is an outer membrane. 16-stranded β-barrel with a large periplasmic extension consisting of five polypeptide transport-associated (POTRA) domains at its N terminus[1]. Structures of BAM have shown that BamA can adopt two conformations: an outward-open conformation[3,4], in which the β-barrel domain opens between strands β1 and β16 to form a lateral gate to the membrane, and an inward-open conformation[5,6], in which the lateral gate is sealed while a periplasmic entry pore to the barrel lumen is open. In the bacterium *Escherichia coli*, four accessory lipoproteins (BamB–E) complete BAM, forming a pentameric holocomplex[7,8]. BamBCDE are anchored to the outer membrane by a lipid moiety but reside in the periplasm. BamB and BamD directly bind the POTRA domains of BamA while BamC and BamE bind BamD[1,2]. Although all components are required for efficient assembly of *E. coli*'s diverse set of OMPs, only BamA and BamD are essential and conserved throughout Gram-negative bacteria[1,2]. Despite important structural and functional insights during 15 years of intense scrutiny due to the essential activity of BAM in generating and maintaining the outer membrane, crucial questions remain unsolved regarding the mechanism of this molecular machine. In particular, the functional importance of BamA cycling between the outward-open and inward-open conformations remains unclear, as are the respective contributions of the various BAM components to OMP assembly[9].

The primary function of BAM is the assembly of OMPs and, when necessary, the translocation of their associated extracellular domains across the outer membrane. More recently, BAM has also been implicated in export of the outer membrane lipoprotein RcsF to the cell surface[10,11] via the assembly of complexes between this lipoprotein and three abundant OMPs (OmpA, OmpC and OmpF)[10,11]. Support for the involvement of BAM in RcsF export comes from in vivo crosslinking experiments in which a complex between RcsF and BamA, considered to be an intermediate in the formation of RcsF–OmpA/C/F complexes, was trapped[10,11]. Further, in cells lacking BamB and BamE, RcsF accumulates on BamA and causes a lethal block to BAM-mediated OMP assembly, suggesting that OMPs and surface-exposed RcsF exploit at least partially overlapping assembly routes[12,13].

RcsF functions as an envelope stress sensor capable of mounting a protective response when damage occurs in either the peptidoglycan or outer membrane[14,15]. Interestingly, we previously determined that movement of RcsF to the surface is part of a cellular strategy that enables RcsF to detect damage in the cell envelope. Under stress conditions, newly synthesized RcsF molecules fail to interact with BamA[10]: they are not exported to the surface and remain exposed to the periplasm, which allows them to trigger the Rcs signaling cascade by reaching the downstream Rcs partner in the inner membrane[16]. Thus, surface exposure is intimately linked to the function of RcsF. However, the molecular details of BamA–RcsF interaction, how BAM orchestrates the export of RcsF with OMP assembly and what prevents RcsF from interacting with BamA under stress conditions remain unknown. Here we sought to address these questions by obtaining structural information about the interaction between BamA and RcsF.

Results

RcsF can be purified with the BAM complex. In a series of exploratory experiments, we co-overexpressed RcsF with either the BamAB subcomplex or the BamABCDE holocomplex; both BamAB–RcsF and BamABCDE–RcsF could be detergent extracted from the membrane and purified via affinity chromatography using

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