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Highlight

Bacterial dynamin as a membrane puncture repair kit

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Dynamin(-like) proteins (DLPs) are widely distributed in bacteria, and both structural and biochemical data have clearly demonstrated that, like their eukaryotic counterparts, these proteins can function in membrane modelling. Although various functions for bacterial DLPs have been hypothesized, a clear function for these proteins within bacteria remained an enigma. A new study by Bramkamp and colleagues (Sawant *et al.*, 2016) provides the first glimpse for a role of bacterial dynamins as a surveillance system that monitors membrane integrity and that counteracts pore formation by phages and antibiotics.

The presence of dynamin homologues in bacteria was noticed over 15 years ago (van der Blik, 1999). As with cytoskeletal proteins, the homology between bacterial and eukaryotic dynamins is very low (~20% sequence identity), but the domain organization is well conserved (Fig. 1). In eukaryotes, dynamins function in a plethora of membrane remodelling processes, such as the scission of clathrin-coated endocytic vesicles, fission of mitochondrial, chloroplast and endoplasmic reticulum membranes and protection against virus infection (Low and Löwe, 2010; Bramkamp, 2012). The molecular details of the membrane fission process are not fully resolved yet (Morlot and Roux, 2013). In a landmark study by Low and Löwe, the first bacterial DLP (BDLP) from the filamentous cyanobacterium *Nostoc punctiforme* was purified, crystallized and characterized (Low and Löwe, 2006). Immediately, it was obvious that BDLP can self-assemble and organize lipid bilayers in tubular structures, which were visualized at high resolution in a later study providing more detailed information on how BDLPs can rearrange the membrane (Low *et al.*, 2009). Even though the BDLP gene has low sequence homology to the eukaryotic counterparts, the molecular architecture between the human dynamin 1 and BDLP is rather conserved (Fig. 1). The domain organization of BDLP seems to be most similar to

the one predicted for mitofusins, which are DLPs with a transmembrane lipid binding domain located between the middle domain and the Guanosine-5'-triphosphate (GTP)ase effector domain (GED), involved in mitochondrial fusion events (Low *et al.*, 2009; Low and Löwe, 2010). Structural data suggest that GTP binding induces a conformational change in the BDLP dimer that facilitates membrane binding through a mobile lipid binding domain termed the 'paddle', and that GTP hydrolysis may be coupled to dissociation from lipids and disassembly of BDLP polymers (Low *et al.*, 2009).

The *Bacillus subtilis* DLP, DynA, was the first DLP that was shown to be capable of *in vitro* membrane fusion (Bürmann *et al.*, 2011). DynA arose from a fusion of two full DLP genes (D1 and D2, Fig. 1), and thus contains two dynamin-like subunits and GTPase domains. Nucleotide binding to both GTPase domains is required to activate GTP hydrolysis, but DynA-mediated liposome fusion does not require GTP hydrolysis, only the presence of Mg²⁺ as a cofactor (Bürmann *et al.*, 2011).

These biochemical and structural studies clearly showed that bacterial DLPs are dynamin homologues that can remodel membranes, yet the cellular role of these proteins remained enigmatic. An obvious function for dynamins could be in membrane remodelling during cell division, but deletion of *B. subtilis* *dynA* did not result in a phenotype (Bürmann *et al.*, 2011). Later, a mild division phenotype was reported, which was exacerbated when the *dynA* deletion was combined with deletions in other genes involved in membrane organization such as *mreB* or *floT* (Dempwolff *et al.*, 2012). Moreover, both DynA from *B. subtilis* and BDLP from *N. punctiforme* localized in punctate patterns along the membrane as well as to the division site, which is not in agreement with a division-exclusive function (Low and Löwe, 2006; Bürmann *et al.*, 2011). Recently, a periplasmic BDLP encoded on a pathogenicity island in enterotoxigenic *Escherichia coli* was shown to function in secretion of membrane vesicles (Michie *et al.*, 2014), which again points to a membrane remodelling function. However, most BDLPs are located in the cytosol so vesicle secretion does not seem a plausible function for all BDLPs.

Enter the new study by Bramkamp and co-workers (Sawant *et al.*, 2016). Noting that eukaryotic dynamin-like

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Fig. 1. Molecular architecture of human dynamin 1, *N. punctiforme* BDLP and *B. subtilis* DynA. Common domains include bundle signalling elements (BSE, cyan, solid, or hatched for potential BSEs), GTPase domains (G, red), the stalk (orange), the Pleckstrin Homology (PH) or paddle domains involved in lipid binding (PH or Pad, green). The proline-rich domain (PRD, yellow) is not present in BDLPS.

proteins also have antiviral functions, the role of DynA under membrane stress conditions was investigated. *Bacillus subtilis*, a ubiquitous soil bacterium, faces several adverse environmental conditions, such as nutrient deprivation and long periods of drought, and the presence of antibiotics and bacteriophages. When the growth of wild-type and *dynA* deletion ($\Delta dynA$) strains exposed to various antibiotics that cause membrane stress (nisin and bacitracin) was compared, it was obvious that the strains without DynA were more sensitive to the antibiotics. Overproduction of DynA in the $\Delta dynA$ strain rescued the sensitivity or even improved growth, compared with that of wild type, in the presence of the antibiotic. Interestingly, ectopically expressed *dynA* was prone to proteolysis, which suggests that the major degradation product of DynA is sufficient to complement the DynA function. Nisin and bacitracin both bind to the peptidoglycan precursor LipidII, which results in membrane stress and in cell wall stress. The sensitivity of cells to the cell wall synthesis antibiotics vancomycin and carbenicillin was unaltered in the $\Delta dynA$ strain, whereas resistance to the membrane targeting antibiotic daptomycin increased on overproduction of DynA, which all points to a role for DynA in a membrane stress response. Antibiotic induced membrane stress is often concomitant with dissipation of the membrane potential. Growth experiments in the presence of gramicidin D, which forms small ion channels in the membrane, revealed that all strains were equally susceptible to gramicidin D. Thus, DynA responds to membrane stress, not dissipation of the membrane potential. Finally, the lack of *dynA* in two different *B. subtilis* strains increased sensitivity of these strains to infection with bacteriophages, resulting in more plaques and a faster execution of the lytic cycle, which could be rescued by overexpression of DynA (Sawant *et al.*, 2016).

How does DynA provide protection against stress caused by membrane punctures? The authors provide hints at a mechanism for DynA function by showing that membrane associated DynA–Green Fluorescent Protein (GFP) is highly dynamic, but localizes to static foci upon

nisin-induced membrane stress. Nisin exposure resulted in membrane deformations in $\Delta dynA$ cells, visualized by accumulations of the membrane dye FM4-64, which were absent from wild-type cells. In cells expressing DynA–GFP, the DynA–GFP foci overlapped with these membrane deformations. This suggests that DynA finds areas of damaged membrane. It should be noted though, that the membrane deformations did not form in wild type or DynA overexpressing cells, whereas they did form when DynA–GFP was produced in a $\Delta dynA$ background, which suggests that the DynA–GFP fusion is not completely functional. Also, the membrane deformations in the $\Delta dynA$ cells only appeared after prolonged exposure to nisin, whereas nisin-induced pore formation and associated cluster formation in the membrane is rapid, even in wild-type cells (Tol *et al.*, 2015). It may be that the nisin-induced damage in wild-type cells can be repaired quickly, whereas prolonged exposure in $\Delta dynA$ results in irreparable damage that shows up as FM4-64 accumulations.

To further look at the influence of membrane-associated DynA on membrane dynamics, the authors determined lipid dynamics in the cell. In an elegant experiment, rhodamine-labelled phosphatidyl-ethanolamine was incorporated in membranes of wild-type and $\Delta dynA$ cells, and the mobility of the lipids was investigated by monitoring both fluorescence loss and recovery after photobleaching. In $\Delta dynA$ cells, lipid mobility was higher than in wild-type (or *dynA* overexpressing) cells. Nisin treatment increased lipid mobility in the wild-type cells but not in the $\Delta dynA$ cells. This observation suggests that DynA, which is localized along the membrane, restricts lipid movement in the membrane. Concentration of DynA in static foci as a result of membrane stress provides membrane space to lipids to diffuse more freely (Sawant *et al.*, 2016).

Combined, these findings provide the first indication for a general function of the widely distributed bacterial dynamins as a membrane surveillance mechanism that provides a rapid response against membrane punctures caused by antibiotics and bacteriophages. Recruitment of DynA molecules to the punctures, followed by DynA–

Fig. 2. A model for DynA functions. DynA (red) is membrane attached and distributed evenly throughout the cell. Membrane stress caused by pore formation through antibiotics like nisin, or bacteriophages, results in the recruitment of DynA to the pores followed by membrane repair through fusion. Similarly, DynA could relieve stresses in the rapidly growing, curved, membranes at the cell division site and possibly aid in membrane fusion during septation.

induced membrane fusion, could quickly repair membrane lesions (Fig. 2), although the molecular mechanisms of this activity still need to be established. This function adds to known membrane stress response systems, which include drug extrusion mechanisms, phage shock response proteins and responses that function over a longer time span such as modifications of the membrane lipids and cell wall (Murinova and Dercova, 2014; Revilla-Guarinos *et al.*, 2014). In retrospect, indications for DLP function in membrane stress surveillance were already present in the literature as the IniA protein from *Mycobacterium tuberculosis*, which was initially thought to be part of an efflux system but later identified as a DLP, conferred enhanced resistance to cell wall targeting antibiotics (Colangeli *et al.*, 2005). Given the extensive synthesis and remodelling of the membrane that occurs during bacterial division, monitoring of membrane stress and regulation of fluidity at the division site may be an extra function of DynA (Fig. 2), in line with the observed perturbations in $\Delta dynA$ cells that are exacerbated in the absence of other membrane-organizing proteins (Dempwolff *et al.*, 2012).

The discovery of a role for bacterial dynamins that explains the widespread presence of these proteins in bacteria is an important step forward. A detectable phenotype in $\Delta dynA$ cells now also enables further studies in the function and properties of bacterial dynamins. An obvious example is the role of GTP hydrolysis in DynA function – whereas a role for GTP hydrolysis in membrane attachment was postulated for *N. punctiforme* BDLP (Low *et al.*, 2009), DynA's membrane-binding and fusogenic activity was nucleotide independent (Bürmann *et al.*, 2011). Also, the role of proteins that interact with DynA, such as RNaseY and the uncharacterized YneK and YwpG (Bürmann *et al.*, 2012) can now be addressed in the context of a phenotype. Combined, this paper fills both

a large gap in our knowledge on bacterial dynamins and opens up the field to further study.

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