A new factor controlling cell envelope integrity in Alphaproteobacteria in the context of cell cycle, stress response and infection

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MicroCommentary

A new factor controlling cell envelope integrity in *Alphaproteobacteria* in the context of cell cycle, stress response and infection

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Summary

The bacterial envelope is a remarkable and complex compartment of the prokaryotic cell in which many essential functions take place. The article by Herrou and collaborators (Herrou *et al.*, in press), by a clever combination of structural analysis, genetics and functional characterization in free-living bacterial cells and during infection in animal models, elucidates a new factor, named EipA, that plays a major role in *Brucella* spp envelope biogenesis and cell division. The authors demonstrate a genetic connection between *eipA* and lipopolysaccharide synthesis, specifically genes involved in the synthesis of the O-antigen portion of lipopolysaccharide (LPS). Beyond its crucial role in *Brucella* physiology, the conservation of EipA in the class *Alphaproteobacteria* urges microbiologists to pursue future investigation of its homologs in other species belonging to this important group of bacteria.

Cell envelope and division

Bacteria are relatively simple organisms when compared to eukaryotes, in which cells are highly compartmentalized by the presence of internal membranes and organelles. However, at a deeper molecular level, bacteria present a complex physiology in which spatially localized functions can take place thanks to organized distribution of proteins within or along different regions of the cell membrane.

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Gram-negative bacteria present two membranes comprising a periplasmic space, in which selectively secreted proteins are involved in multiple functions, such as the transport of various substrates, energy production, and also cell growth and division. A *plethora* of proteins predicted to be involved in the physiology of the periplasmic space remains undefined or unstudied. In the article by Herrou and collaborators (Herrou *et al.*, *in press*), an important new protein, named EipA, has been characterized in *Brucella* species which are members of the Gram-negative class *Alphaproteobacteria*. The authors provide evidence for a model in which *Brucella* EipA, O-polysaccharide biosynthesis and cell division are connected.

The class of *Alphaproteobacteria* comprises several important species including plant symbionts (e.g. *Rhizobia*) (Andrews and Andrews, 2017) and pathogens of plants (*Agrobacterium*) (Gelvin, 2017) and animals (e.g. *Rickettsia*, *Brucella* and *Bartonella*) (Di Russo Case and Samuel, 2016). Other well-studied model species belonging to the alphaproteobacterial class include the bacterial cell cycle and differentiation model *Caulobacter crescentus* (Lasker *et al.*, 2016), the photosynthetic bacterium *Rhodobacter sphaeroides* (Porter *et al.*, 2008) and the magnetotactic bacterium *Magnetospirillum magneticum* (Uebe and Schüler, 2016).

As outlined above, the cell envelope of Gram-negative bacteria has inner and outer membranes that are separated by a space called the periplasm and a protective layer, the cell wall, mainly composed of peptidoglycan (Silhavy *et al.*, 2010). The external part of the outer membrane presents a lipopolysaccharide layer (LPS), which is composed of a glycolipid component and a saccharide component that provides a major contact point between the cell and the environment. The LPS saccharide has a variable core structure and a variable, often dispensable, outer component (named O-specific), whose presence defines what is known as 'smooth' LPS. A lack of this O-specific polysaccharide results in an

LPS that is designated 'rough' (De Castro *et al*., 2008). Lipopolysaccharide O-polysaccharide is a protective layer against several stressing agents such as reactive oxygen compounds and cationic peptides (Lerouge and Vanderleyden, 2002). Clearly the biogenesis of each layer of the envelope, including LPS, is interconnected and a complete overview of envelope biogenesis and its relation to cell division still requires the discovery and characterization of new factors.

Besides transport and energy production, cell division represents one of the most important functions carried out by the cell envelope. It is obvious that division requires that all layers of the cell envelope are properly duplicated upon the completion of cell cytokinesis. This complex process requires recruitment at mid-cell of the conserved protein FtsZ, a homolog of eukaryotic tubulin, while the protein FtsA is required for the recruitment of a large complex of division proteins (i.e. the divisome) that drives neosynthesis of the cell wall peptidoglycan (Lutkenhaus *et al*., 2012; Xiao and Goley, 2016; Woldemeskel and Goley, 2017).

EipA at the crossroads of many important functions

Envelope integrity protein A (EipA) factor belongs to a family of previously uncharacterized proteins (Pfam PF06577 or DUF1134) that are conserved in *Alphaproteobacteria* (Kainth and Gupta, 2005), suggesting its function is connected to distinct biological features found in this phylogenetic class, including its asymmetrical cell differentiation or cell cycle regulation (Hallez *et al*., 2004; Brilli *et al*., 2010; De Bolle *et al*., 2015; Poncin *et al*., 2018). Working primarily in two *Brucella* species, Herrou and colleagues have characterized EipA function for the first time. They demonstrate that EipA folds into a compact beta-barrel structure that is secreted in the periplasmic space. Its expression is directly regulated by the alphaproteobacterial master cell cycle regulator, CtrA, which plays an essential role in the coordination of at least three major cell cycle functions: cell division, DNA replication and cell differentiation (Laub *et al*., 2002). In *Brucella abortus*, CtrA has been implicated in the control of outer membrane composition (Francis *et al*., 2017; Poncin *et al*., 2018). The control of EipA expression by CtrA expands our understanding of the essential role of CtrA in controlling cell duplication and the development of cellular structures in *Alphaproteobacteria*.

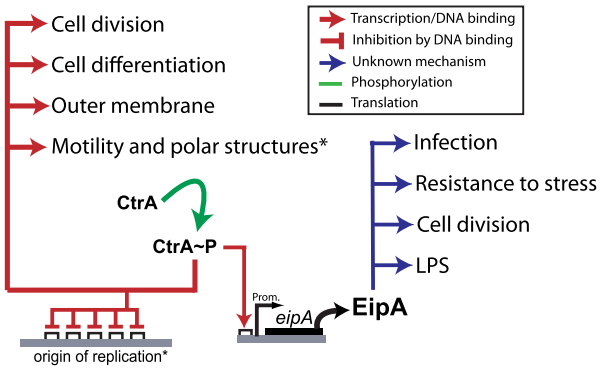

Fig. 1. Circuit of regulation of cell cycle and *eipA* in *Brucella*/*Alphaproteobacteria*. CtrA plays a major role in the control of cell cycle by activating several essential functions and blocking the replication of DNA (asterisks correspond to functions that have been demonstrated in *Caulobacter crescentus*).

O-polysaccharide is an important determinant of stress survival, infection and virulence in many bacteria, and the synthetic lethal genetic relationship between *eipA* and multiple O-polysaccharide synthesis genes in *B. abortus* suggests that the compromised infection and stress response phenotypes of an *eipA* deletion strain may be related to defects in the LPS O-chain (Herrou *et al*., in press). The characterization of EipA opens the possibility for new and interesting roles of periplasmic proteins in the coordination of different layers of envelope biogenesis and functionality. EipA has an apparent role in the maintenance of OM integrity, but can influence the process of cell division, as clearly demonstrated by experiments in which EipA is depleted in *Brucella ovis*. In this naturally 'rough' species, *eipA* is essential, and its depletion results in a dramatic formation of cell chains that are compromised in division.

In summary, Herrou and collaborators have uncovered a new factor, *eipA*, that is controlled by the master regulator of cell cycle in *Alphaproteobacteria,* CtrA. EipA coordinates several important functions in *Brucella* spp., including cell division, response to stress and maintenance of spleen colonization in a mouse model of infection. The authors demonstrate a clear genetic link between *eipA* and the biosynthesis of LPS O-polysaccharide. The connection between *eipA* and LPS is likely directly related to the reported *eipA* deletion phenotypes (Fig. 1). The compact beta-barrel structure of EipA suggests a functional/evolutionary link to outer membrane proteins, although it is presently difficult to predict how EipA structure related to its function. Further studies will be necessary to understand EipA function from a more detailed mechanistic context, but the discoveries reported here provide a remarkable starting point for future investigations.

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