Structural biology

How lipopolysaccharide strikes a balance

Russell E. Bishop

Bacteria with two membranes must regulate the production of a surface molecule known as lipopolysaccharide. The structure of an essential signal-transduction protein now reveals how lipopolysaccharide controls its own synthesis. **See p.479**

Feedback inhibition occurs when the product of a metabolic pathway diminishes its own production by triggering a decrease in the activity of a key enzyme in the pathway. Such inhibition controls the production of lipopolysaccharide (LPS) molecules, which are an integral part of the outer membrane of some bacteria. It has long been suspected that the feedback signal responsible for regulating LPS biosynthesis is either LPS itself, or one of its precursors[1]. But, on page 479, Clairfeuille *et al.*[2] add to a flurry of recent work[3–5] showing that the membrane protein PbgA is the long-sought LPS signal transducer in the bacterium *Escherichia coli*. The current study extends our understanding of PbgA by providing a high-resolution structure of the protein bound to LPS.

E. coli has two distinct membranes: the inner membrane, which is a phospholipid bilayer; and the asymmetric outer membrane, in which LPS lines the external surface, and a single layer of phospholipids forms the internal surface. LPS provides a barrier to greasy antibiotics and detergents that are encountered in the gut of mammalian hosts. The ratio of phospholipid to LPS is crucial for membrane function — too much LPS is toxic to the inner membrane and too little compromises the outer membrane (reviewed in ref. 1).

LPS assembly starts on the internal surface of *E. coli*'s inner membrane. The rate of assembly is controlled by the enzyme LpxC. Before LPS generation is completed, the lipid is flipped to the external surface of the inner membrane for further modification. The completed LPS is then transported to the external surface of the outer membrane by means of a protein bridge that connects the membranes (reviewed in ref. 1).

Investigations[3–5] published this year of how this pathway is regulated have produced a model in which PbgA on the inner membrane modulates the activity of LpxC by interacting with LapB — a protein that guides the enzyme FtsH to degrade LpxC (ref. 1). So when levels of LPS are low, PbgA inhibits the interaction between LapB and FtsH in the inner membrane, stabilizing LpxC and promoting LPS biosynthesis (Fig. 1a). When the number of LPS molecules exceeds a threshold in the outer membrane, LPS transport across the bridge ceases[6]. LPS accumulates on the external surface of the inner membrane, which can cause the formation of potentially lethal irregular membrane structures[3]. By sensing the accumulated LPS, PbgA can relax its inhibition of LapB–FtsH. LpxC can be degraded, thus diminishing LPS biosynthesis and restoring the phospholipid–LPS balance (Fig. 1b). Clairfeuille and colleagues' work now points to the same mechanism for LPS sensing, adding weight to this emerging model.

The authors corroborated the finding[3–5] that *E. coli* strains carrying truncated forms of PbgA (which lack extracellular and linker domains that normally connect to its essential transmembrane domain) remain viable, but are chronically deficient in LPS (Fig. 1c). In these mutants, phospholipids migrate into the external surface of the outer membrane to create mixed membranes containing patches of phospholipid bilayer scattered among the zones of LPS–phospholipid membrane. The phospholipid bilayer patches allow greasy antibiotics and detergents to enter the cell, and transient defects at the boundaries between the two different lipid phases allow leakage of large soluble molecules[7].

Previous work has shown that a greasy functional group called palmitate is incorporated into LPS when phospholipids are present at the external surface[8]. Clairfeuille *et al.* demonstrate the presence of palmitate in the outer-membrane LPS of a PbgA mutant.

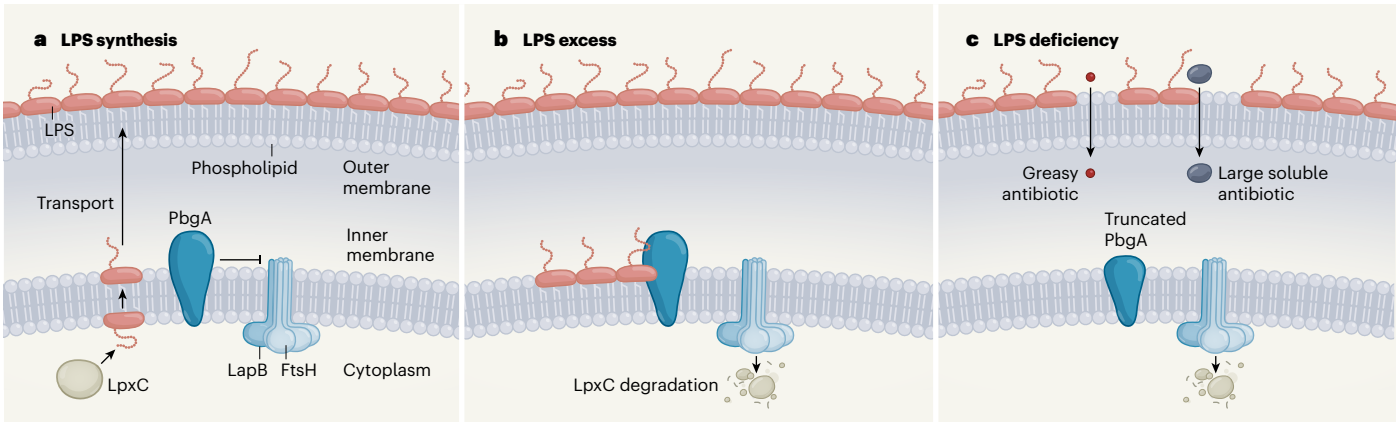

Figure 1 | Feedback inhibition regulates lipopolysaccharide biosynthesis. The bacterium *Escherichia coli* has an inner membrane comprising two phospholipid layers and an outer membrane, which has one layer of phospholipids and one layer of lipopolysaccharide (LPS) molecules. **a**, The enzyme LpxC controls the biosynthesis of LPS from precursors in the cell cytoplasm. After being flipped to the external surface of the inner membrane, the mature LPS is then transported to the outer membrane. The FtsH enzyme, guided by interactions with LapB protein, degrades LpxC — but Clairfeuille *et al.*[2] and others[3–5] show that the protein PbgA inhibits LapB–FtsH activity, and so promotes LPS biosynthesis. **b**, When excess LPS accumulates on the external surface of the inner membrane, it binds to PbgA. The protein relaxes its control on LapB–FtsH, allowing degradation of LpxC to restore normal LPS levels. **c**, A PbgA truncation mutation leads to chronic depletion of LPS, presumably because the mutant only weakly inhibits LapB–FtsH. Phospholipids fill the gaps left by LPS in the outer membrane, enabling greasy antibiotics and detergents to penetrate at local phospholipid bilayers, and large soluble compounds to leak through transient boundary defects where the LPS and phospholipid phases meet.

Such palmitate incorporation has also been reported in bacteria carrying mutations in components of the transport systems that move LPS towards the outer membrane[9] and phospholipids away from it[10,11]. What can these observations tell us about the function of PbgA? They could fit with the proposal[12,13] that PbgA is a transport protein for the phospholipid cardiolipin. However, directly blocking LPS biosynthesis can also lead to LPS depletion, and to incorporation of palmitate in outer-membrane LPS[14,15]. As such, PbgA's apparent influence on cardiolipin transport seems to be a secondary consequence of its role in regulating LPS biosynthesis. In support of this idea, Clairfeuille *et al.* confirmed the finding[16] that PbgA was required for the outer membrane to retain its integrity, whereas eliminating cardiolipin had no effect.

Clairfeuille and colleagues' key advance was to analyse the structure of PbgA at a resolution of 1.9 ångströms, using a technique called X-ray crystallography. They found that PbgA belongs to a family of enzymes that also includes EptA — a protein that adds a phospholipid-derived molecular modification to the lipid A domain of LPS[17]. Lipid A is made of two phosphorylated sugars. By modifying these phosphate groups, EptA provides cells with resistance to antibiotics that bind to lipid A, called polymyxins.

The authors showed that the external surface of PbgA was tightly bound to an LPS molecule. They then re-evaluated a lower-resolution structure of PbgA[13] and — on the basis of the distance between its phosphate groups — verified that it was bound to the lipid A domain of LPS. Although a phospholipid partially occupies a site near the bound LPS, PbgA has lost the amino-acid side chains used by EptA to catalyse LPS modification. Whether or not PbgA retains enzymatic activity remains to be determined.

The picture of PbgA that emerges from Clairfeuille and colleagues' structure is of a protein that has been adapted as a receptor to sense LPS at the external surface of the inner membrane. The structure supports the model that a PbgA–LapB–FtsH–LpxC regulatory circuit acts as a control mechanism, modulating LPS biosynthesis to meet the physical demands of the cell's interconnected double membranes. Indeed, the researchers also confirm the finding[4] that a direct physical interaction occurs between PbgA and LapB in membranes. But how LPS–PbgA binding relaxes the inhibition that PbgA exerts on the LapB–FtsH interaction remains unknown.

Clairfeuille and co-workers' structure reveals that PbgA binds the lipid A moiety through a linker domain, using an amino-acid sequence that has not been reported in any other LPS-binding protein. Mutations in this LPS-binding motif compromised PbgA function. In a final set of experiments, the authors demonstrated that a synthetic peptide based on this sequence could bind LPS and inhibit bacterial growth. Through rational design, they improved the peptide's antibiotic spectrum and potency.

The polymyxins bind lipid A by interacting with both of its phosphorylated sugars[18], but PbgA binds to just one. The polymyxin antibiotic colistin is used as a last resort for treatment of infections in the clinic, but it can also increase outer membrane permeability, thereby sensitizing bacteria to more-effective antibiotics[18]. Clairfeuille and co-workers' show that the PbgA-derived peptide also sensitizes bacteria to other antibiotics, acts in synergy with colistin, and is not hampered by the LPS modifications catalysed by EptA.

PbgA was one of the few essential proteins in *E. coli* without a well-characterized function[4]. The discovery that PbgA is the LPS signal transducer provides insights for antibiotic development, in addition to illuminating a remarkable lipid balancing act in the bacterial membrane.

Russell E. Bishop is in the Department of Biochemistry and Biomedical Sciences, and at the Michael G. DeGroote Institute for Infectious Disease Research, McMaster University, Hamilton, Ontario L8S 4K1, Canada.
e-mail: bishopr@mcmaster.ca

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This article was published online on 12 August 2020.

Electronics

One-way supercurrent achieved in a polar film

Toshiya Ideue & Yoshihiro Iwasa

Diodes are devices that conduct electric current mainly in one direction. An electrically polar film that acts as a diode for superconducting current could lead to electronic devices that have ultralow power consumption. **See p.373**

An essential process in modern electronics is rectification, whereby bidirectional electric current is converted to unidirectional current. Electronic devices that enable rectification are called diodes and are widely used to transform alternating current into direct current, protect electric circuits from excess voltage and detect electromagnetic waves. Extending this concept to a superconducting current, which flows with zero resistance, is a fascinating challenge from both fundamental and technological viewpoints. On page 373, Ando *et al.*[1] report the achievement of this superconducting diode effect and its magnetic control in an electrically polar film that is non-centrosymmetric — lacking symmetry under a transformation known as spatial inversion. The authors' findings demonstrate that charge can be transported in a single direction without energy loss.

In a conventional diode, rectification is realized using a heterojunction (an interface between two different semiconductors), such as a p–n junction (Fig. 1a). For a p–n junction, one of the semiconductors is p-type, containing an excess of positively charged electron vacancies called holes, and the other is n-type, containing an excess of negatively charged electrons. Electric current flows easily only from one side of the interface to the other[2]. Although such a structure is a fundamental component of many devices today, it is difficult to achieve the superconducting-diode effect by this strategy because a non-zero electrical resistance at the junction is inevitable.

Non-centrosymmetric conductors can exhibit an intrinsic rectification effect, even if they are uniform and junction-free