Trends in Microbiology

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Forum

Bacterial Cell Mechanics Beyond Peptidoglycan

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The bacterial cell envelope plays essential roles in controlling cell shape, division, pathogenicity, and resistance against external stresses. In *Escherichia coli*, peptidoglycan (PG) has long been thought to be the primary component that conveys mechanical strength to the envelope. But a recent publication demonstrates the key contribution of the lipoprotein Lpp in defining the stiffness of the cell envelope and its sensitivity to drugs.

Bacterial Cell Mechanics

The mechanical properties of bacteria are important for cellular function, physiology and disease [1,2]. Importantly, the stiff cell surface also provides a protection against antibiotics, which, together with the continuous rise in drug resistance, makes bacterial pathogens more and more difficult to eradicate. A grand challenge in mechanomicrobiology is to identify the molecular players defining bacterial cell mechanics, and to understand how the cells sense and respond to mechanical stress [2,3]. Today, newly developed nanotechniques offer a wealth of opportunities to tackle these problems (Figure 1; see later for details).

Gram-negative bacteria harbor a three-layered cell envelope (Figure 2A) [4]. The inner layer is a fluid inner membrane (IM) composed of a phospholipid bilayer. The outermost layer is an asymmetrical outer membrane (OM) made of phospholipids in its inner leaflet and lipopolysaccharides in its outer leaflet. Both membranes are separated by an aqueous space, the periplasm, which contains a PG mesh with covalently cross-linked stem peptides. Besides playing a major role as a barrier between the inner side of the cell and its environment, the Gram-negative cell envelope provides mechanical strength for defining cell shape, stiffness, and permeability. For years, PG has been assumed to be the main actor controlling cell shape and mechanical rigidity, the IM and OM functioning essentially as chemical barriers [5]. However, evidence is accumulating that the OM and its connections to PG are also involved in cellular mechanics [6]. In particular, the lipoprotein Lpp, also known as Braun's lipoprotein, the numerically most abundant protein in *E. coli*, provides the only covalent crosslink between the OM and the PG (Figure 2A). The Lpp bridge defines the size of the periplasm, allowing the efficient transfer of stress signals across the envelope [7] and indirectly controlling the length of the flagellum rod. Yet, how exactly Lpp contributes to cell mechanics has remained mysterious.

Nanotools for Cell Mechanics

Atomic force microscopy (AFM) techniques are being increasingly used to probe cell mechanics in relation to function [3] (Figure 1). The most popular approach, nanoindentation, consists in locally pushing the tiny AFM probe onto the sample, and recording the mechanical deformation as a function of the applied force (Figure 2B). Recording indentation curves across the surface makes it possible to map mechanical properties with nanoscale spatial resolution. Force curves on Gram-negative bacteria usually enable the extraction of two mechanical parameters, the Young's modulus (*E*) and the spring

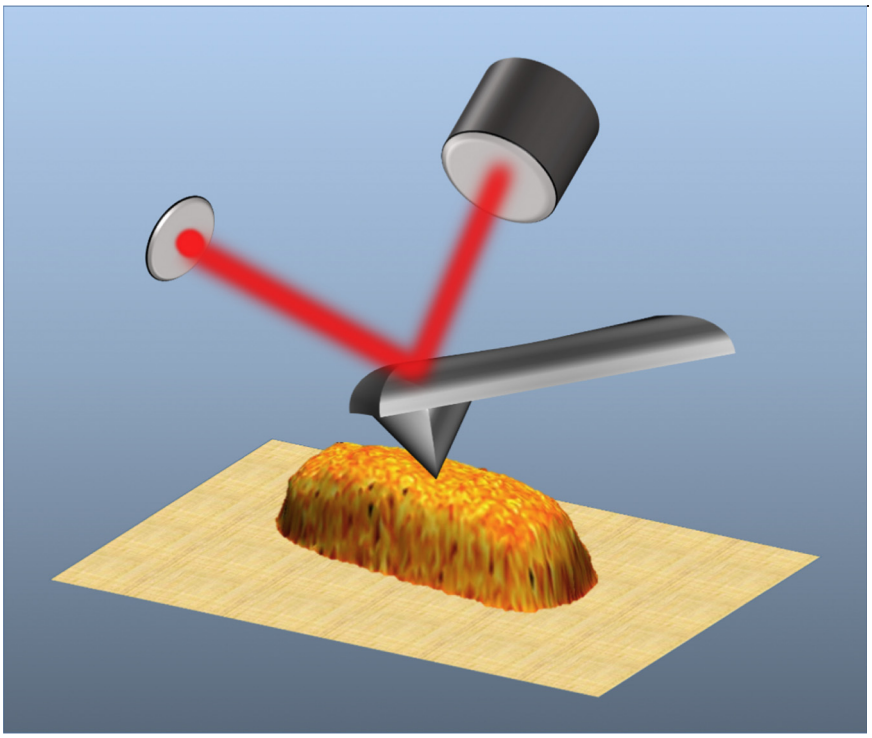

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Figure 1. Atomic Force Microcopy (AFM), a Unique Window into Bacterial Cell Mechanics. AFM setup showing a nanoscale tip simultaneously probing the 3D topography and mechanical properties of a single living *Escherichia coli* cell.

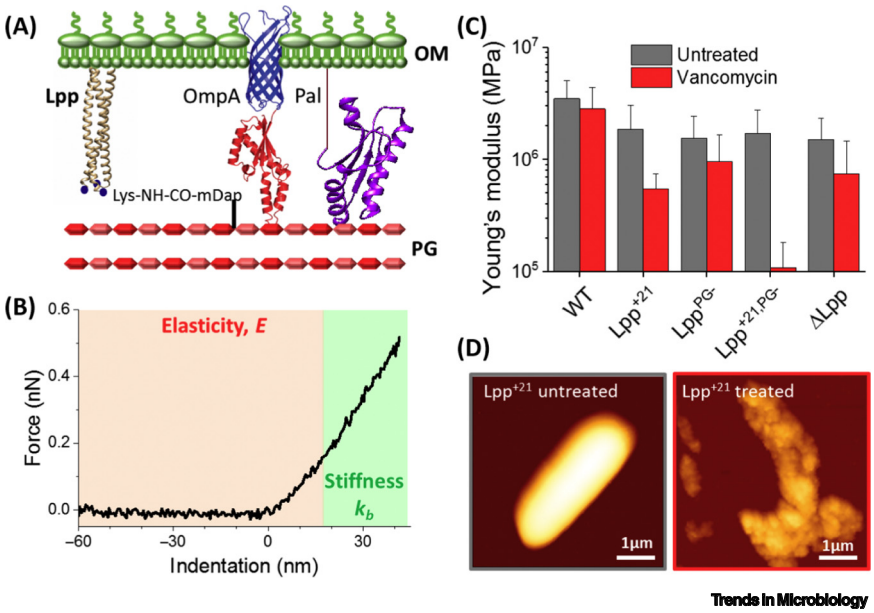

Figure 2. Unraveling the Molecular Details of Lpp-Dependent Cell Envelope Mechanics. (A) Scheme for the multilayer structure of the *Escherichia coli* cell envelope emphasizing the presence of proteins from the outer membrane (OM, green) connecting it to peptidoglycan (PG, red). (B) Plot of a representative indentation curve recorded on a living bacterium, showing the two distinct regimes from which the bacterial Young's modulus *E* and the spring constant k_b are extracted. (C) Mechanical properties of *E. coli* as a function of Lpp mutations before and after vancomycin treatment: wild-type (WT) cells, cells with Lpp elongated by 21 residues (Lpp^{+21}), cells lacking the Lpp covalent connection (Lpp^{PG-}), cells where Lpp is elongated and not crosslinked to the PG ($Lpp^{+21,PG-}$), and cells lacking Lpp (ΔLpp). Shown here are the mean *E* values from 10 to 15 different cells. (D) Atomic force microscopy (AFM) height images of Lpp^{+21} cells before and after vancomycin treatment illustrating dramatic morphological damage associated with the cell softening. Adapted with permission from [12].

constant (k_b). Over the years, AFM has enabled quantification of the mechanical properties of a variety of bacterial samples, from murein sacculi [8] to whole cells [9]. Interestingly, the impact of drugs on cell mechanics can be tracked, as exemplified by lysostaphin, an enzyme that specifically cleaves the *Staphylococcus aureus* PG [9]. Lysostaphin was found to dramatically alter the bacterial cell structure and stiffness, due to PG digestion, eventually leading to the formation of osmotically fragile cells. More recently, newly developed multiparametric imaging modes have enabled simultaneous mapping of the structure, adhesion, and elasticity of *E. coli* [10] and *S. aureus* [11] at high spatiotemporal resolution.

Role of Lpp in *E. coli* Mechanics and Drug Sensitivity

Combining indentation experiments with genetic manipulations, Mathelié-Guinlet *et al.* [12] unveiled an intricate link between Lpp structure and cell mechanics (Figure 2C). Their strategy was to compare the properties of wild-type (WT) *E. coli* bacteria with those of bacteria with Lpp functional mutations: Lpp elongated by 7, 14, or 21 residues, Lpp lacking the covalent connection to PG, and cells lacking Lpp. By quantifying the stiffness of all strains they discovered that, compared with WT cells (E = 3.5 MPa; k_b = 0.01 $N.m^{-1}$), mutant cells were on average twice as soft. This means that the cell envelope rigidity is critically controlled by the structural and chemical nature of the Lpp-dependent OM–PG connection, that is, molecular length and covalent bonding.

The authors [12] also found that the Lpp-dependent cell mechanics has a major impact on antibiotic sensitivity (Figure 2C,D). Lpp functional mutations increased the efficacy of vancomycin, an antibiotic targeting the PG precursors that is normally unable to cross the OM of Gram-negative bacteria. While the morphology and stiffness of WT cells, and of cells lacking Lpp, were hardly altered by the drug, Lpp mutants were drastically susceptible to vancomycin, exhibiting a much softer envelope and a damaged shape and ultrastructure. So the structural and chemical nature of the Lpp bridge is more important in defining drug susceptibility than the presence of the lipoprotein.

Concluding Remarks

Combining nanoindentation experiments with genetic manipulation offers unprecedented opportunities to understand the molecular basis of bacterial cell mechanics in relation to function, pathogenicity, and drug efficiency. We believe that targeting the *E. coli* Lpp connection may lead to the discovery of new antibacterials capable of structurally and functionally altering the cell envelope. Destabilizing the OM–PG connection might be a strategy of choice to not only lead to the shutdown of the bacterial stress response [7] but also to lower cell stiffness and increase permeability, especially in the presence of antibiotics [12]. Further exploration of the mechanical role of Lpp and other proteins, such as OmpA and Pal, could lay the ground for clinically relevant agents. For instance, the relative distribution and exposure of these proteins along the bacterial cell surface remains unclear, a question that could be largely answered by AFM and other nanoscopy tools. Whether Lpp shows the same mechanical role in other Gram-negative bacteria remains to be shown.

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