cNHEJ could explain the unusual compaction of this animal's genome, perhaps in concert with the adaptation of this species to a life cycle of extreme brevity [2]. From the wider appendicularian genome sequencing done by Deng *et al.* [2], which will certainly become a valuable resource for further comparative genomics, it is clear that the larvacean ancestor was able to survive without the cNHEJ machinery, and that the consequent dramatic genome compaction and rearrangement that occurred (with this rearrangement still ongoing) is due to its likely dependence on an aNHEJ-like pathway. A prerequisite for this must have been that this ancestor's genome was presumably already 'primed' to be scrambled without fatally disrupting gene regulation at high frequency. Thus, the precursor to the appendicularian ancestor must already have evolved to a state in which its gene regulatory mechanisms were presumably focused on short-range gene-specific processes rather than the long-range multigenic mechanisms involving topologically associated domains and genomic regulatory blocks more typical of many other animal genomes [15,16]. Why this should be the case still remains an evolutionary mystery, which may be resolved by the burgeoning work on urochordates.

This highlights that not all animal genomes are necessarily working in the same way and that studying a diversity of species is important. Such an increased diversity of study species is extremely valuable [17], improving our capabilities to address pressing issues such as the various aspects of biology centred on genomics. For example, given the importance of DSB repair to understanding not only genome evolution but also human diseases with roots in gene rearrangements such as various types of cancers, having a whole animal study system that has done away with the cNHEJ machinery is likely to prove a valuable resource for the discovery and elucidation of alternative DSB repair pathways. Perhaps then larvaceans could become a useful new model for not only the evolution of development and genome rearrangement, but also oncogenesis, with a role in the search for the mechanisms of aNHEJ/aEJ and the consequent potential biomedical benefits.

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Microbiology: Peeling Back the Layers of Bacterial Envelope Mechanics

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The Gram-negative cell envelope has two important mechanical elements. Whereas the cell wall bears the brunt of the turgor pressure during normal growth, the outer membrane also provides necessary rigidity under physical stress.

In the everyday life of a bacterium, physical insults — in the form of mechanical stresses encountered during motility, in the presence of flow, or during rapid changes in the osmotic environment —constantly challenge the

cell. How do cells prevent themselves from exploding or imploding as the physical world constantly tries to do them in? New work from Rojas and colleagues [1] shows that *Escherichia coli*'s exoskeleton is more sophisticated than previously thought.

The outer shell, or envelope, of a Gram-negative cell consists of three layers: the inner membrane that compartmentalizes the cytoplasm, a mesh-like cell wall, and the outer membrane that defines the periplasmic space. The cell wall has traditionally been thought to supply the mechanical integrity of the cell based on the observation that rod-shaped cells such as *E. coli* become round 'spheroplasts' when the wall is removed. Although the inner membrane provides a chemical barrier between the highly concentrated cytoplasm and the environment, it is unable to prevent lysis due to the large osmotic pressure, or turgor, which is estimated to be at least 30 kPa [2]. This pressure pushes the inner membrane up against the cell wall, whose elastic deformation then balances the force of turgor. In this picture, the outer membrane's role is to provide a second chemical barrier to define the periplasmic space, but it is not thought to contribute mechanically.

Using a combination of imaging, atomic force microscopy, and chemical and genetic perturbation, Rojas *et al.* [1] demonstrate that both the cell wall and outer membrane act together in a mechanical fashion to provide a very robust response to different kinds of perturbation. Under normal growth and turgor pressure, the cell wall is expanded to nearly twice its unstressed surface area and bears the brunt of the pressure, whereas the outer membrane is under little if any stress. However, if the pressure becomes larger, then the outer membrane begins to bear part of the load and the cell hardly expands at all. If the pressure becomes less and eventually negative, as during an osmotic upshock, the outer membrane mechanics kick in again and prevent the wall from shrinking all the way to its unstressed size.

The outer membrane is a unique structure, comprised of phospholipids in the inner leaflet, lipopolysaccharides in the outer leaflet, and a large number of embedded proteins [3–5]. Although the presence of all of these components almost certainly increases its stiffness relative to a pure lipid bilayer, the outer membrane acts as a fluid with very low bending energy, as evidenced by the observation that wall-less spheroplasts are round. If the cell wall can hold its shape under turgor and the outer membrane cannot, how can they be of comparable stiffnesses and both contribute a mechanical resistance to increases in pressure? The answer lies in the way in which the outer membrane is linked to the cell wall. Although the outer membrane is indeed fluid, its stiffness comes from its resistance to stretching forces. The key here is that the outer membrane is essentially glued to the cell wall by a number of proteins, including Lpp, OmpA, and the Tol-Pal complex. Indeed, when Rojas *et al.* [1] genetically removed the Pal lipoprotein, they found that the outer membrane was no longer strongly attached to the cell wall and the system became much more compliant. Estimates of the abundance of Pal indicate that it numbers in the tens of thousands, implying a spacing of roughly one Pal every hundred nanometers throughout the periplasm [6]. By rigidly attaching the outer membrane to the cell wall, these two seemingly separate layers can act as one mechanical unit, and thus stretching necessarily imposes elastic strain on both materials, and indeed the linkers between them.

Because the unstressed surface areas of the outer membrane and cell wall are different, they act in different ways. A growing cell wall experiences a constant large strain. But the outer membrane is unperturbed due to its larger surface area. In a mechanism that is not fully understood, new cell-wall material is made and inserted into the peptidoglycan mesh in a largely stretched state. The outer membrane, on the other hand, is produced with the surface area of the expanded cell so that, under normal circumstances, it is under very little tension. Thus, the cell-wall mesh serves to bear the turgor necessary to keep a large concentration of small molecules inside the cell while the rigidity of the outer membrane protects the cell from further expansion and maintains cell size during osmotic shock.

These results open up a number of interesting avenues of investigation. How do all the different kinds of molecules in the outer membrane interact to produce a stiff and resilient, but fluid sheet? Are the arrangement and spacing of the proteins in the outer membrane — and in particular the proteins that link the outer membrane to the cell wall — important for the mechanical system? The dynamics measured in the osmotic-shock experiments reveal a complex rheology. On what length and time scales is the outer membrane elastic and when does a viscous behavior dominate? Does the cell use mechanical feedback to ensure that the outer membrane and cell wall are made with the correct surface areas to ensure proper loading? Biophysical measurements combining results from intact cells and purified cell-wall and outer-membrane material on multiple scales will be needed to shine further light on these questions.

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