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The cell envelope of diderm bacteria: a unified scaffold, not a stack of layers

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The cell envelope of diderm (Gram-negative) bacteria is a defining structural and functional feature, ensuring mechanical integrity, supporting growth, and enabling adaptation to diverse environments. Traditionally viewed as a stack of discrete layers — the inner membrane, peptidoglycan, and outer membrane — this architecture is now recognized as a mechanically and physiologically integrated system. Recent advances have shown that envelope components are assembled and maintained in a coordinated manner, with physical and functional linkages bridging the different layers. In this review, we examine how these connections contribute to envelope biogenesis, homeostasis, and stress adaptation, using *Escherichia coli* as a reference model. We also discuss the evolutionary diversity of peptidoglycan-outer membrane attachment strategies across bacterial phyla, highlighting their conserved role in maintaining envelope cohesion. Together, these findings support a revised view of the envelope as a unified and dynamic structure, whose integrity depends on the coordinated activity of all its components.

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Introduction

Despite their apparent simplicity, bacterial cells are highly organized, with membranes playing a central role in maintaining integrity and functionality. In diderm (Gram-negative) bacteria, the inner and outer membranes define a peripheral compartment — the periplasm — that contains the peptidoglycan layer. Recent work reveals unexpected features within each layer and their interdependence. Here, using *E. coli* as a model, we review biogenesis and function of the inner membrane, peptidoglycan, and outer membrane, then examine the envelope as an integrated system, emphasizing the connections between the outer membrane and peptidoglycan and their conservation across diderm bacteria. We propose that the envelope, as a whole, acts as a mechanical unit protecting against osmotic lysis.

Peptidoglycan is a central structural and functional hub

Peptidoglycan is a mesh-like polymer of glycan strands cross-linked by short peptides, forming a continuous layer that surrounds the cell [1]. A century ago, Fleming's discovery of lysozyme and penicillin revealed that disrupting this structure causes rapid lysis. Decades of subsequent research established peptidoglycan as the primary barrier against hypoosmotic shock — a sudden drop in external osmolarity that drives water influx, causing cell rupture [2,3]. More recently, live-cell imaging has shown that when peptidoglycan integrity is compromised, turgor pressure forces the inner membrane outward, leading to membrane rupture and cell death [4]. Notably, cells can survive without peptidoglycan if placed in osmoprotective environments, where external osmolarity counterbalances internal turgor; under these conditions, bacteria proliferate as so-called L-forms, which lack a cell wall [5]. Together, these findings have reinforced the view of peptidoglycan as a robust protective shell — sometimes described as the bacterial 'Great Wall'. In this review, we adopt instead the concept of a 'Great Scaffold', in which the peptidoglycan forms a self-retaining cage and the outer membrane serves as its enclosing counterpart, tethered to sustain periplasmic turgor.

Peptidoglycan biosynthesis is a complex process tightly coordinated with other essential cellular pathways governing growth and division [6]. Its assembly relies on the orchestrated activity of inner membrane–associated cytoskeletal elements such as MreB and FtsZ [7], outer membrane–anchored regulators like LpoA and LpoB, and a diverse set of periplasmic remodeling enzymes [8]. Recent studies continue to uncover enzymes involved in peptidoglycan synthesis, recycling, and repair, along

with mechanisms that ensure proper coordination with other envelope components (see below). As a detailed discussion of peptidoglycan biosynthesis is beyond the scope of this review, we refer readers to recent comprehensive overviews [7–10].

More than a barrier: the outer membrane as a load-bearing and adaptive structure

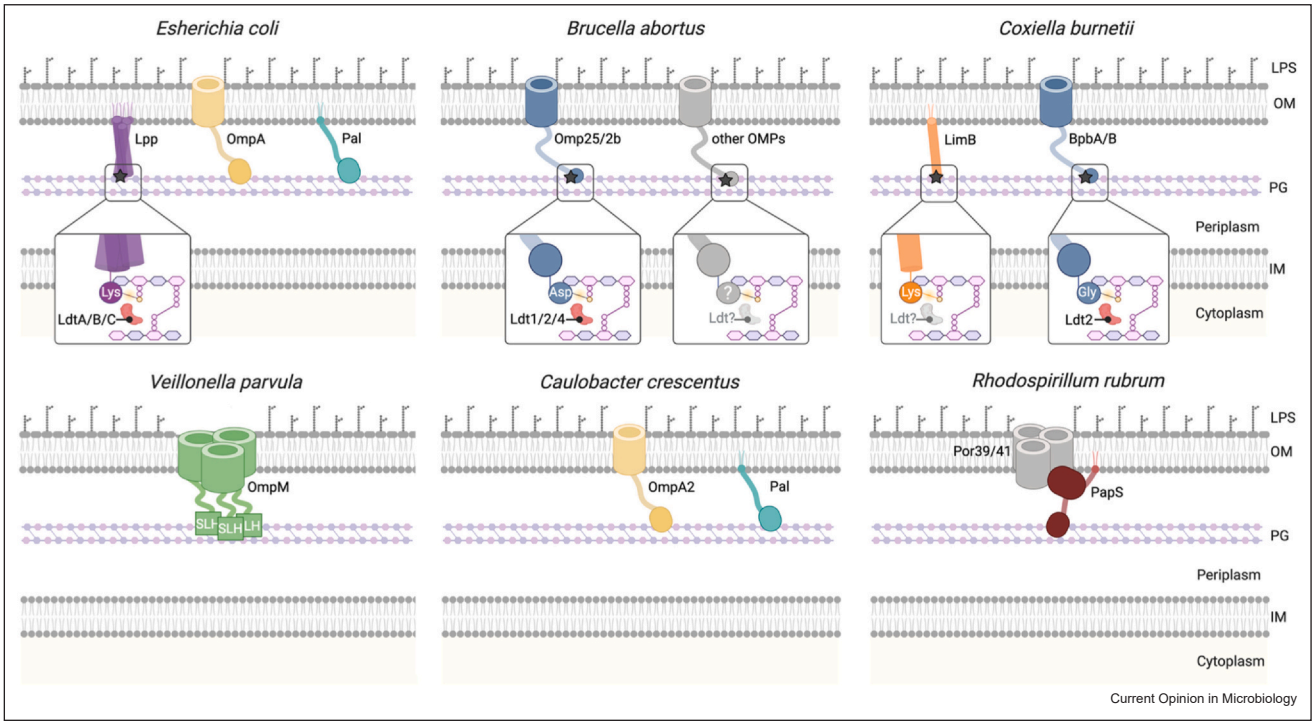
The outer membrane is the hallmark of diderm bacteria, forming the cell's outermost layer. Historically seen as a passive barrier to toxic compounds, it is now recognized as a dynamic structure essential for envelope integrity, morphogenesis, and stress adaptation [11,12]. Rojas et al. [13] demonstrated that compromising outer membrane integrity increases deformation and osmotic lysis, suggesting a direct mechanical role. Computational and experimental work shows that all three envelope layers share the mechanical load [14]. More recently, it was shown [15] that increased outer membrane stiffness can partly compensate for peptidoglycan defects by stabilizing its assembly machinery, highlighting the outer membrane's role in maintaining shape and mechanical stability. While peptidoglycan remains the main determinant of bacterial morphology, the outer membrane complements it as a load-bearing element that supports morphogenesis.

A defining feature of the outer membrane is its asymmetric lipid composition, with lipopolysaccharides (LPS) in the outer leaflet and phospholipids in the inner leaflet [12]. This asymmetry underlies the outer membrane's barrier, selectivity, and mechanical properties [11]. In the outer leaflet, LPS molecules are densely packed, and their negative charges are stabilized by divalent cations bridging adjacent molecules to strengthen the permeability barrier [11,16]. LPS comprises lipid A, a phosphorylated core oligosaccharide, and often an O-antigen. Anionic charge density and divalent-cation (Mg^{2+}/Ca^{2+}) bridging within lipid A and the core region condense the LPS leaflet, increasing packing, stiffness, and barrier tightness; cation depletion loosens packing and increases permeability. O-antigen length/chemistry modulates porin access and surface rheology, thereby tuning selectivity at the membrane surface. Together, charge-mediated cohesion and O-antigen geometry provide the physical basis for the outer membrane's bifunctional role as both a load-bearing shell and a molecular sieve [16,17]. LPS biosynthesis begins in the cytoplasm and is completed at the inner membrane [18]. Its export relies on the Lpt system, an ATP-dependent trans-envelope bridge that transfers LPS to the cell surface, aided by accessory proteins [19–21]. By contrast, phospholipid transport to the outer membrane's inner leaflet remains poorly understood. Recently identified AsmA-like proteins (TamB, YhdP, YdbH) have been proposed to mediate this process [22,23], possibly acting as intermembrane lipid transporters [24]. However, their precise functions, mechanisms, substrate specificity, and interaction partners remain to be fully elucidated. To import nutrients without compromising the barrier, bacteria rely on β-barrel proteins — general porins for passive diffusion and specialized channels with defined size and charge selectivity [25]. β-barrel proteins are assembled and inserted into the outer membrane by the essential BAM complex, which catalyzes their folding and integration into the membrane [26]. Impairing BAM function reduces outer membrane protein (OMP) levels and weakens outer membrane stiffness [27]. Importantly, the outer membrane is laterally heterogeneous, with β-barrel-rich regions forming highly ordered, crystalline-like arrays interspersed with LPS-dense zones [28]. Recent studies have shown that LPS-rich domains exhibit liquid-disordered characteristics, disrupting the rigid packing of β-barrels [29]. This patchwork-like organization is thought to contribute to the mechanical properties of the outer membrane: rigid β-barrel arrays provide structural stability, while more fluid LPS-rich zones enable local adjustments to environmental stress, collectively preserving envelope integrity under dynamic conditions.

Lipoproteins represent another essential class of outer membrane components. Delivered to the membrane by the essential Lol system, they perform a wide range of functions. Some, such as Pal and Lpp, contribute to envelope stability by interacting with the peptidoglycan. Lpp is unique in that it forms the only covalent linkage between the outer membrane and peptidoglycan (see below). Other lipoproteins, such as LpoA and LpoB, regulate peptidoglycan synthesis [8,30], while RcsF and NlpE act as stress sensors [31]. Although most lipoproteins reside in the inner leaflet of the outer membrane, some — including RcsF — are surface-exposed [32,33].

Under stressful conditions, such as exposure to detergents or antimicrobial agents, the outer membrane undergoes rapid remodeling to preserve its integrity and prevent breaches. When LPS molecules are lost from the outer leaflet, the resulting gaps are inappropriately filled by phospholipids, disrupting lipid asymmetry and compromising the permeability barrier [34]. The Mla retrograde transport system acts to retrieve these mislocalized phospholipids and restore membrane homeostasis [34,35]. Recent work has uncovered an additional protective mechanism involving the PhoPQ-regulated lipoprotein SlyB, which oligomerizes around phospholipid accumulations, promotes local phase separation, and stabilizes β-barrels in response to stress [36]. This response is particularly critical under low Mg^{2+} conditions or upon exposure to cationic antimicrobial peptides.

Figure 1



Diversity of molecular mechanisms for outer membrane–peptidoglycan coupling across diderm bacteria. Bacteria employ a variety of molecular linkers to attach the outer membrane to the underlying peptidoglycan. These connectors differ in number, structure, and mode of attachment across species. Linkage can be noncovalent or covalent (indicated by a ★), the latter formed via L,D-transpeptidase-mediated crosslinking. Covalent attachment typically occurs between the meso-diaminopimelic (mDAP) residue at the third position of the peptidoglycan peptide stem and a connector-specific residue. Outer membrane–peptidoglycan interactions may be direct (e.g. in *E. coli*) or mediated through protein complexes (e.g. in *R. rubrum*). LPS, lipopolysaccharides; OM, outer membrane; PG, peptidoglycan; IM, inner membrane; SLH, S-layer homology. Created with BioRender.com.

The inner membrane functions as a central hub for envelope assembly

Often overlooked, the inner membrane is central to envelope assembly. Beyond energy generation, it scaffolds peptidoglycan synthesis: key cytoskeletal elements, such as MreB, a component of the elongasome, and FtsZ, a central player in the divisome, associate with its inner leaflet, while penicillin-binding proteins (PBPs), located at its periplasmic face, catalyze peptidoglycan assembly [8]. Second, the inner membrane drives outer membrane biogenesis: it is the site of the final steps of phospholipid and LPS synthesis, lipoprotein maturation, and the export machineries that deliver these components outward. For instance, the LolCDE complex extracts newly synthesized lipoproteins and transfers them to the periplasmic carrier LolA [31], while the $LptB_2CFG$ complex extracts LPS and hands it to LptA, which then shuttles it to LptDE in the outer membrane [19]. Recent single-molecule tracking revealed that Lpt proteins at the inner membrane alternate between mobile and immobile states. About half form transient periplasm–spanning bridges that last 5–10 s and are stabilized by LPS, suggesting a direct coupling between LPS production and export [37].

Together, these findings underscore the tightly coordinated nature of envelope biogenesis: the inner membrane, peptidoglycan, and outer membrane do not function in isolation, but as interdependent components of a highly integrated system. In the next section, we examine how the outer membrane and peptidoglycan layer are mechanically and physiologically interconnected.

Mechanical and physiological integration of the outer membrane and peptidoglycan

For a long time, the peptidoglycan layer and the outer membrane of diderm bacteria were studied as separate, structurally distinct entities. Yet, we now understand that the reality is more complex. Recent findings show that the assembly of these two layers is tightly coordinated [6]. In *Pseudomonas aeruginosa*, MurA — the enzyme catalyzing the first committed step in peptidoglycan biosynthesis — was shown to interact physically with LpxC, the key enzyme in LPS synthesis [38]. This MurA-dependent activation of LpxC ensures that the biogenesis of the peptidoglycan layer and the outer membrane is coupled from the very beginning of envelope assembly, allowing growth to proceed in a

balanced manner [38]. In *E. coli*, however, LpxC levels are instead regulated by the inner membrane protease FtsH [6], and no direct link between LPS and peptidoglycan synthesis has been identified. Coordination persists at later stages: Mamou et al. [39] demonstrated that sites of nascent peptidoglycan insertion coincide with zones of increased β-barrel protein biogenesis in *E. coli*. This spatial correlation suggests that peptidoglycan synthesis not only supplies the structural scaffold for envelope expansion but also guides outer membrane assembly, highlighting the interdependence of these two layers. While OMP insertion appears to follow zones of nascent peptidoglycan, LPS assembly seems to occur uniformly across the outer membrane [40].

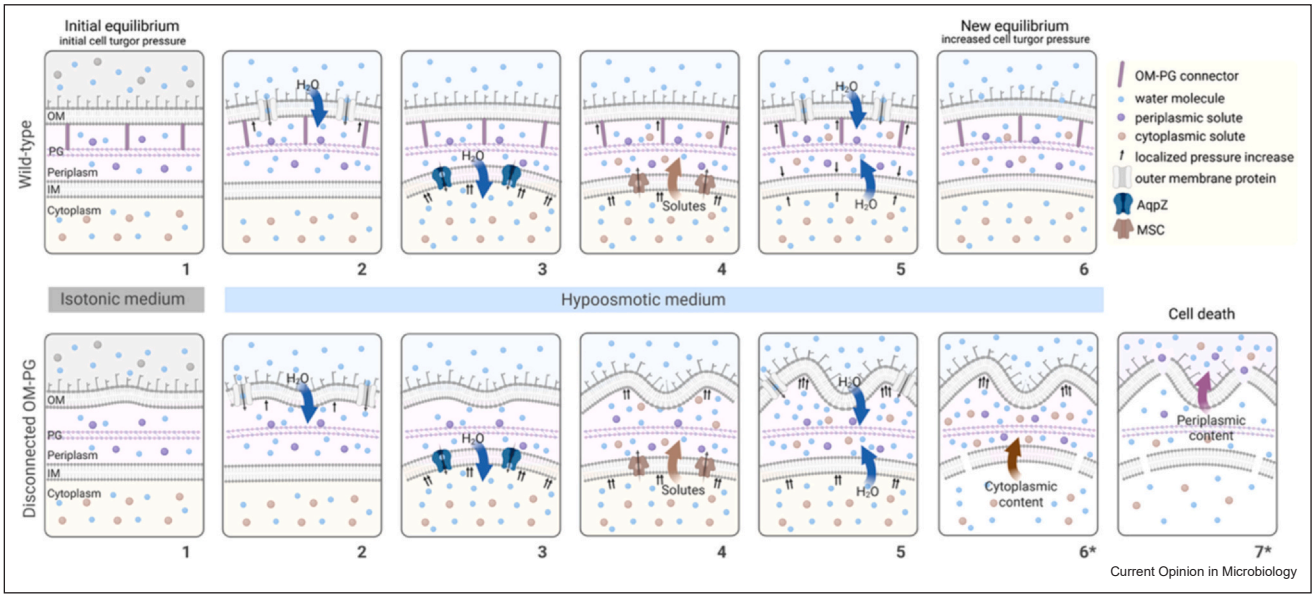
This coupling during synthesis is mirrored at later stages by the physical connectivity that binds the envelope as it expands and remodels. In *E. coli*, three molecular tethers — the lipoproteins Lpp and Pal, and the β-barrel protein OmpA — anchor the outer membrane to the peptidoglycan layer, ensuring structural cohesion as well as the coordinated spatial and temporal control of growth, division, and stress adaptation [41,42] (Figure 1). Lpp, the numerically most abundant protein in *E. coli* [43], is the prototypical connector between the outer membrane and peptidoglycan. It is inserted as a trimer into the inner leaflet of the outer membrane via its lipidated N-terminus and covalently anchored to the peptidoglycan by its C-terminal lysine [44,45] through the action of the L,D-transpeptidases LdtA, LdtB, and LdtC [46] (Figure 1). Although these enzymes perform similar functions, they are present in different amounts [43]. This covalent linkage stabilizes the envelope, prevents membrane blebbing and detachment, and constrains the width of the periplasmic space [47,48] — an architectural feature required for efficient signal transmission between envelope compartments [48]. Approximately one-third of Lpp is crosslinked to the peptidoglycan, and this anchoring is dynamic [49]: Lpp can be released from old peptidoglycan peptide stems by the hydrolytic enzyme DpaA (formerly known as LdtF) and re-attached to new ones, enabling remodeling during growth [50,51]. High-resolution atomic force microscopy imaging of purified sacculi revealed that Lpp–peptidoglycan crosslinks are homogeneously distributed along the cylindrical and polar regions in non-dividing cells but excluded from the division site during septation [52]. This spatial regulation of anchoring likely allows local envelope flexibility required for constriction and septal remodeling. Pal, a component of the trans-envelope Tol–Pal system, also binds the peptidoglycan, but noncovalently [53–55]. During cell division, Pal is recruited to midcell by the inner membrane TolQRA complex, which uses the proton motive force to actively displace the periplasmic protein TolB from Pal, unmasking Pal's peptidoglycan-binding domain and enabling it to stably anchor the outer membrane at the site of constriction [56,57]. This energy-dependent remodeling of the TolB–Pal complex ensures the precise midcell localization of Pal. Strikingly, in the absence of Pal, Lpp is no longer excluded from the division site and accumulates there [52], suggesting that Pal normally prevents Lpp anchoring at the constriction zone. In addition to its mechanical role, Pal contributes to outer membrane lipid homeostasis [58,59] and regulates access to peptidoglycan-modifying enzymes, including PBP1B and various hydrolases, via its interaction with CpoB [60–62]. OmpA provides a different kind of tethering. Embedded within the outer membrane via its N-terminal β-barrel domain, it displays a soluble C-terminal periplasmic domain, structurally homologous to that of Pal, which binds the peptidoglycan noncovalently [63,64]. By limiting lateral diffusion of neighboring β-barrel proteins, OmpA helps organize the outer membrane into functional domains and selectively modulates enzyme activity through specific OMP interactions [65,66]. It also modulates stress signaling by sequestering the lipoprotein RcsF, thereby preventing activation of the Rcs phosphorelay [67]. The absence of OmpA results in increased membrane permeability and spontaneous vesicle formation [68], underscoring its role in maintaining envelope integrity.

Thus, at a structural level, the *E. coli* envelope is physically stitched together by three molecular tethers — Lpp, OmpA, and Pal — each with specific molecular features, ensuring mechanical cohesion and the precise spatial and temporal coordination required for envelope remodeling throughout growth and division.

The periplasm functions as an osmoprotective compartment

Although connections between peptidoglycan and the outer membrane were identified long ago, their functional significance has only recently been fully appreciated. Traditionally seen as a passive buffer for enzymes and transported molecules, the periplasm is now recognized as a mechanically active compartment that accumulates turgor and contributes to envelope cell stability. Recent work from our lab provided compelling evidence that the buildup of turgor pressure in the periplasm is essential for cells to withstand sudden drops in external osmolarity, and this pressure buildup requires a physical linkage between the outer membrane and the peptidoglycan (Figure 2). Using osmotic shock experiments in *E. coli*, we uncovered a mechanistic model in which cytoplasmic and periplasmic turgor dynamically adjust to maintain envelope integrity. Upon sudden hypoosmotic shock, water rapidly enters the cytoplasm via the aquaporin AqpZ, increasing cytoplasmic turgor. This is sensed by mechanosensitive channels in the inner membrane, which open to release solutes into the periplasm, increasing its osmolarity. The resulting water

Figure 2

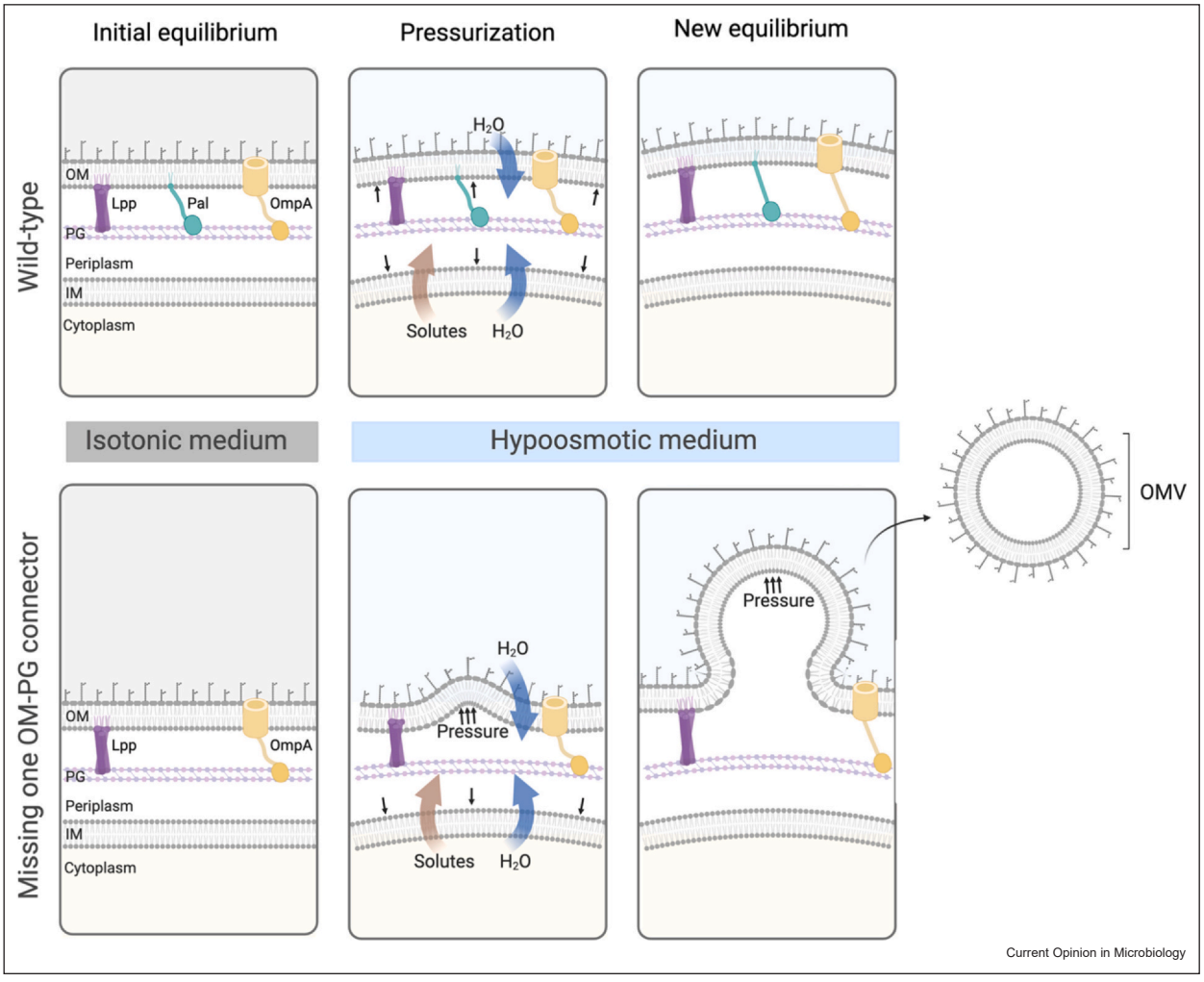


Outer membrane–peptidoglycan tethering is critical for osmoadaptation via periplasmic pressurization in *E. coli*. Upon exposure to a hypoosmotic environment (e.g. water), *E. coli* cells experience rapid water influx, leading to increased internal turgor (steps 2–3). Periplasmic water influx is facilitated by outer membrane proteins, while cytoplasmic water influx, facilitated by the aquaporin AqpZ, generates outward pressure (indicated by arrows) on the inner membrane (steps 2–3). This rise in membrane tension activates mechanosensitive channels (MSCs), triggering the release of solutes into the periplasm (step 4). The resulting accumulation of osmotically active solutes draws additional water into the periplasm, increasing periplasmic turgor (step 5). In wild-type cells, outer membrane–peptidoglycan connectors mechanically constrain periplasmic volume extension, allowing pressure to build within the compartment (step 5). The resulting inward-directed pressure counterbalances cytoplasmic turgor, redistributes mechanical load across the cell, and preserves cell integrity (steps 5–6). In contrast, cells lacking outer membrane–peptidoglycan tethering fail to restrict pressure-driven periplasmic volume expansion. The unopposed pressure exerted on the outer membrane drives it away from the inner membrane, resulting in outer membrane bulging (step 5). Without sufficient counter-pressure, cytoplasmic turgor overwhelms the inner membrane, leading to rupture (step 6*). Cytoplasmic content leaks into the periplasm, further increasing pressure and destabilizing the outer membrane, ultimately resulting in cell lysis and release of intracellular material (step 7*). This model highlights the essential role of outer membrane–peptidoglycan tethering in defining the periplasm as a pressurizable compartment and maintaining envelope integrity under osmotic stress. OM, outer membrane; PG, peptidoglycan; IM, inner membrane. Created with BioRender.com.

influx expands the periplasm, generating turgor that counterbalances cytoplasmic pressure and stabilizes the envelope. This mechanism involves osmoregulated periplasmic glucans (OPGs, also known as membrane-derived oligosaccharides or MDOs), which enable the transient accumulation of solutes released by mechanosensitive channels (MSCs) within the periplasm, thereby promoting pressure buildup. Crucially, this buildup of periplasmic turgor requires a compartment constrained in volume: in mutants lacking peptidoglycan–outer membrane connectors such as Lpp and OmpA, the outer membrane detaches during hypoosmotic shock, the periplasm swells, and pressure fails to accumulate. As a result, these mutants are more susceptible to lysis under osmotic stress. Conversely, reducing solute transfer or strengthening the outer membrane restores envelope pressurization and promotes cell survival. Thus, our findings support a model in which the envelope behaves as a mechanically integrated system: the peptidoglycan and outer membrane must be physically connected to define the periplasm as a pressurizable compartment (Figure 2). The presence of Lpp, OmpA, and Pal imposes volume constraint on the periplasm, while AqpZ, MSCs, and OPGs participate in dynamic osmotic responses. In this model, cytoplasmic and periplasmic pressures adjust dynamically to environmental changes, ensuring cell survival [69].

The mechanical consequences of disrupted peptidoglycan–outer membrane connectivity are further illustrated by the overproduction of outer membrane vesicles (OMVs) (Figure 3). While OMVs play important roles in bacterial communication and pathogenesis, their excessive release reflects structural imbalances in many species [70]. *E. coli* mutants lacking Lpp, OmpA, or Pal exhibit increased vesiculation, with the strongest effects observed in double mutants [56,68,69]. Among the three tethers, Pal appears especially critical: its absence alone is sufficient to trigger massive OMV formation; Pal overexpression can partly suppress vesiculation in *lpp* mutants, but not vice versa [52,54]. Thus, peptidoglycan–outer membrane tethers not only keep the envelope

Figure 3



Disruption of peptidoglycan–outer membrane connectivity promotes OMV formation. Upon exposure to a hypoosmotic environment (e.g. water), *E. coli* cells experience rapid pressurization of internal compartments due to water influx and osmolyte displacement. In wild-type cells, outer membrane–peptidoglycan connectors mechanically constrain periplasmic volume expansion and prevent outer membrane detachment. In contrast, cells lacking one or more major outer membrane–peptidoglycan tethers exhibit localized outer membrane bulging at sites where these linkages are absent, promoting OMV formation. OM, outer membrane; PG, peptidoglycan; IM, inner membrane; OMV, outer membrane vesicle. Created with BioRender.com.

together but also play a mechanical function by preserving periplasmic pressure and protecting the envelope under fluctuating osmotic conditions.

Conserved function, diverse solutions: peptidoglycan–outer membrane connectivity across bacterial phyla

The extensive characterization of peptidoglycan–outer membrane connectivity in *E. coli* has revealed its essential contribution to envelope integrity, particularly under osmotic stress (Figure 2). This raises the broader question of how other Gram-negative bacteria achieve similar mechanical stability. While Lpp plays a central role in *E. coli*, comparative genomics indicates that it is restricted to a narrow clade within the Proteobacteria, implying that other bacterial lineages have evolved distinct molecular solutions [41,45,71]. Recent studies have begun to uncover these alternative systems and to show how diverse species have tailored peptidoglycan–outer membrane connectivity to their specific lifestyles and envelope architectures (Figure 1). In several Alphaproteobacteria, including *Brucella abortus*, envelope integrity relies on covalent linkages between β-barrel proteins and peptidoglycan. These connections, catalyzed by L,D-transpeptidases, tether β-barrel proteins directly to peptide stems and confer resistance to host-induced stress [72] (Figure 1). A related strategy operates in *Coxiella burnetii*, where both β-barrels and

lipoproteins become covalently anchored to peptidoglycan during the stationary phase, reinforcing the envelope in response to environmental and intracellular pressures [73] (Figure 1). In contrast, no covalent peptidoglycan–outer membrane tethers have been identified to date in the Alphaproteobacterium *Caulobacter crescentus* (Figure 1). Pal, however, is essential for envelope maintenance, and its depletion leads to local bulging at the pole and midcell — phenotypes consistent with a loss of periplasmic constraint [74]. In *Pseudomonas aeruginosa*, the Pal homolog OprL is also critical, and mutants lacking either OprL or the OmpA-like protein OprF exhibit increased susceptibility to hypoosmotic stress [69,75]. These observations suggest that Pal-based systems may serve as the primary peptidoglycan–outer membrane connectors in species lacking covalent anchoring. Peptidoglycan–outer membrane connectivity also contributes to morphogenesis. In *Rhodospirillum rubrum*, two porins, Por39 and Por41, form a helical lattice that recruits the peptidoglycan-binding lipoprotein PapS (Figure 1). PapS, in turn, traps elongasome complexes within specific envelope zones, promoting localized peptidoglycan insertion and outward curvature [76]. This noncovalent mode of connectivity illustrates how tethering can spatially direct cell wall growth and shape cell architecture. Outside the Gracilicutes, members of the Terrabacteria clade use an entirely different strategy. In these bacteria, where lipoproteins are absent, β-barrel proteins such as OmpM, which contain an S-layer homology domain, connect the outer membrane to the underlying peptidoglycan [77,78] (Figure 1). Deletion of *ompM* in *Veillonella parvula* causes outer membrane detachment, vesicle formation, and severe envelope instability [71]. It has been proposed that the weakening of this single tethering system enabled the transition from diderm toward monoderm envelopes in Firmicutes [71]. By contrast, in Gracilicutes, the emergence of multiple redundant peptidoglycan–outer membrane connectors such as Lpp, OmpA, and Pal may have stabilized the outer membrane and supported the evolution of more complex, stress-resistant envelopes. Such robust, multicomponent tethering systems likely provide the flexibility required for survival in dynamic and heterogeneous environments, as exemplified by *Enterobacteriaceae* and *Vibrionaceae*, which alternate between host-associated and environmental niches and thus regularly experience osmotic stress. Together, these studies indicate that although the number and molecular nature of peptidoglycan–outer membrane connectors vary across species, their role in ensuring envelope robustness is widely conserved. The capacity to withstand mechanical and osmotic stress appears to be a universal constraint shaping the evolution of the Gram-negative envelope architecture. While much has been learned about peptidoglycan–outer membrane attachment, considerably less is known about how the peptidoglycan layer is positioned relative to the inner membrane. In *Dickeya dadantii*, the inner membrane protein OutB was recently shown to be covalently linked to peptidoglycan by the same mechanism as Lpp, suggesting that additional, yet unidentified, tethering strategies may exist in Proteobacteria [79].

A holistic envelope model and outlook

Taken together, the findings and concepts discussed throughout this review emphasize a more integrated view of the bacterial cell envelope: rather than a passive barrier composed of discrete layers, the bacterial cell envelope functions as a cohesive and dynamic system whose mechanical and physiological integration is central to bacterial life. In *E. coli*, molecular connectors such as Lpp, Pal, and OmpA physically stitch the envelope together, allowing the periplasm to accumulate turgor and protect against osmotic stress (Figure 2). Comparable systems, involving distinct molecular solutions, are now being uncovered across diverse bacterial phyla, underscoring the evolutionary importance of peptidoglycan–outer membrane connectivity (Figure 1). Yet many fundamental questions remain. To what extent do peptidoglycan–outer membrane tethers in other bacteria serve similar dual roles in mechanical cohesion and osmoadaptation? How are these systems regulated, spatially and temporally, across different lifestyles and envelope architectures? Are their functions redundant or specialized, and how do they contribute to ecological success, pathogenesis, or the evolutionary transitions between diderm and monoderm envelopes? In some Terrabacteria, the outer membrane lacks LPS [78] — how then do these envelopes maintain mechanical integrity and permeability control? Addressing these questions will require multidisciplinary approaches across a broader range of bacterial models. From a translational perspective, peptidoglycan–outer membrane connectors may represent promising antimicrobial targets: by weakening the envelope scaffold, their inhibition could synergize with existing antibiotics and broaden the scope of envelope-directed therapies. In embracing the envelope as a 'Great Scaffold,' we gain not only a conceptual framework but also a roadmap for future exploration of bacterial resilience, diversity, and vulnerability.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

None.

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