We Need To Break Up – Why Bacteria Produce Potentially Lethal Peptidoglycan Hydrolases & How They Are Regulated

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1 Introduction

Bacteria inhabit nearly every environment on Earth, capable of thriving 10,000 metres below the ocean's surface or more than 40,000 metres up into the atmosphere – in both radioactive waste and within other organisms [1–4]. One feature nearly all bacteria share is a cell wall made of peptidoglycan [5]. This net-like sacculus helps scaffold cell envelope components, maintain cell shape, and withstand turgor pressures up to 5 atm in diderms (Gram-negative bacteria) and as high as 30 atm in monoderms (Gram-positive bacteria). Without a cell wall, these pressurised bacteria would quickly lyse [6, 7].

To expand, divide, and remodel the sacculus, bacteria rely on peptidoglycan hydrolases to break particular bonds within the cell wall. While this is essential for growth, it's also a careful balancing act, as excessive hydrolysis can weaken the peptidoglycan and result in lysis [8].

This review will provide some background on peptidoglycan structure, synthesis, and hydrolysis before discussing the essential roles peptidoglycan hydrolases play in the cell and how their potentially destructive activity is regulated.

2 Background

2.1 Peptidoglycan

2.1.1 Structure

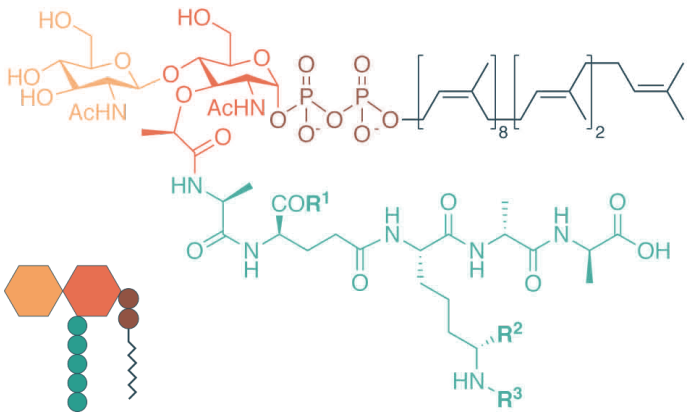
Chemically, peptidoglycan is a polymer of alternating *N*-Acetylglucosamine (GlcNAc) and *N*-Acetylmuramic acid (MurNAc) monomers cross-linked by short MurNAc-bound peptides [9]. Typically, each MurNAc starts off carrying a pentapeptide with the sequence L-Ala-D-isoGlu-(mDAP/L-Lys)-D-Ala-D-Ala (Fig. 1A). Meso-diaminopimeilic acid (mDAP) is found more commonly in diderms, while most monoderms contain L-Lys (Fig. 1B) [10].

Between their inner and outer membranes, diderms usually contain a single layer of peptidoglycan 2.5–6.5 nm thick, while monoderms lack an outer membrane entirely and surround themselves instead with a thicker (15–30 nm), multilayered peptidoglycan wall [6, 11].

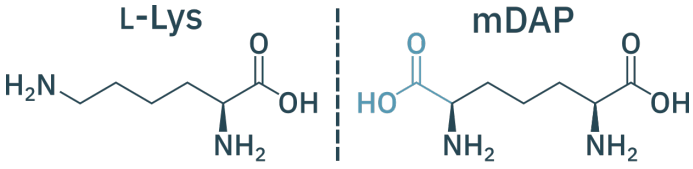
2.1.2 Synthesis

Peptidoglycan synthesis starts in the cytoplasm, where a GlcNAc monomer is converted into a MurNAc-pentapeptide and coupled to a prenyl phospholipid anchored in the cytoplasmic membrane (Fig. 2A). A second GlcNAc is then transferred onto the C4 hydroxyl of the MurNAc to form Lipid II, the basic building-block of peptidoglycan [12]. Depending on the organism, the peptide stem may undergo additional processing before being exported by a Lipid II flippase such as MurJ [10, 13, 14].

Outside the cytoplasmic membrane, Lipid II units are polymerised into glycan chains by glycosyltransferases and cross-linked by transpeptidases, which usually form covalent bonds from the D-Ala in position four of the donor peptide to the side-chain amine of either mDAP or L-Lys in the acceptor peptide (Fig. 2B) [15–17].



(A) The Chemical Structure of Lipid II



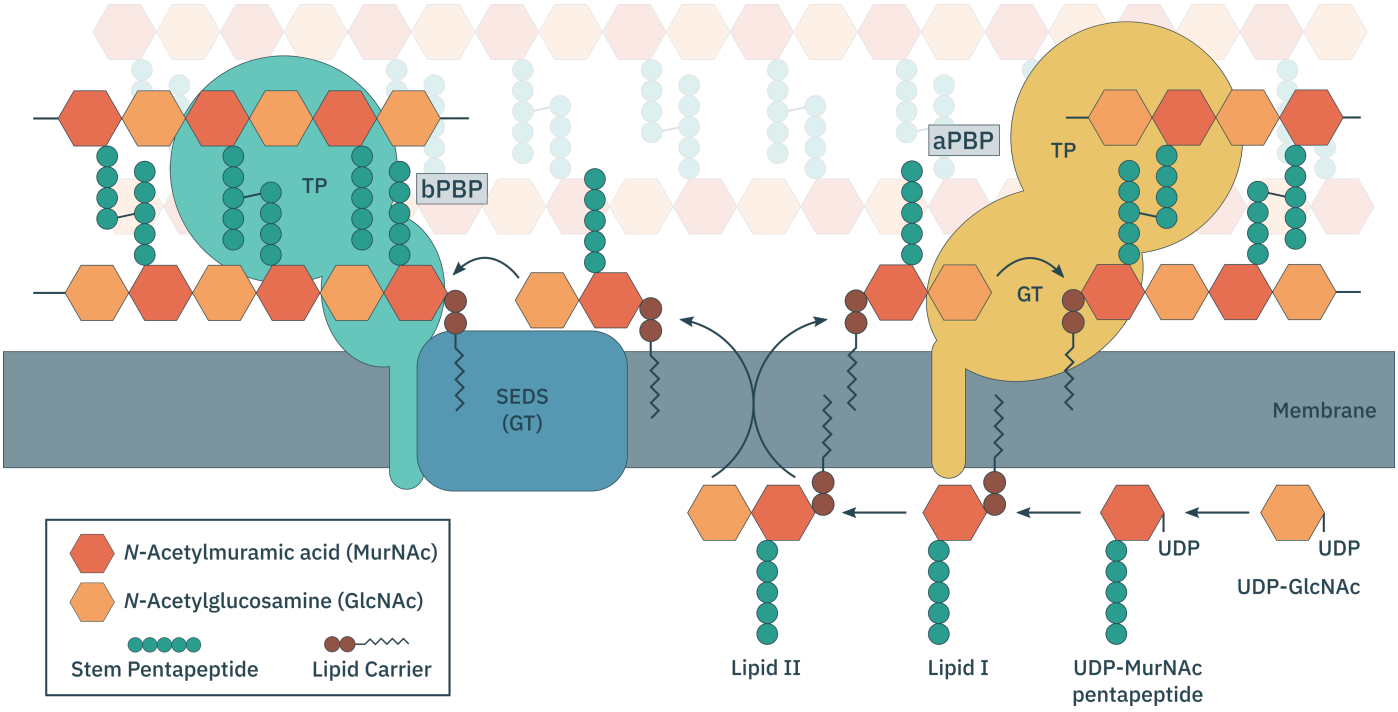
(B) Cross-Link Accepting Amino Acids

Figure 1: Pentapeptide Structure Varies By Species
(A) Lipid II is the basic building-block of peptidoglycan. Variable groups within the stem peptide (shown in teal) have been labelled R^1–R^3. R^1 may be either OH or NH_2 (forming D-isoGlu or D-isoGln), R^2 may be either H or COOH (forming L-Lys or mDAP), and R^3 is where cross-linking occurs (potentially through a poly-Gly or poly-L-Ala linker). Figure adapted from Do, Page, and Walker [10]. **(B)** In the pentapeptide's third position is a diamino acid that acts as a cross-link acceptor. Typically, monoderms carry L-Lys while diderms carry mDAP, a carboxy derivative of lysine. The additional carboxyl group of mDAP has been highlighted in light blue.

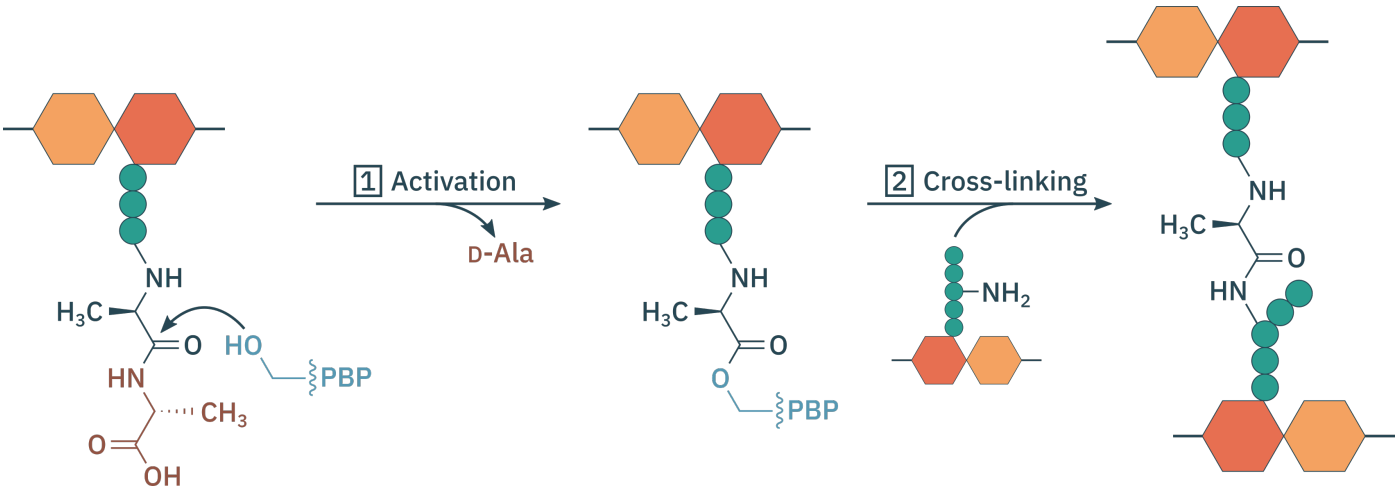
2.2 Peptidoglycan Hydrolases

2.2.1 Classification

For nearly every linkage in peptidoglycan, there exists a hydrolase capable of cleaving it (Fig. 3). Those that cleave



(A) An Overview of Peptidoglycan Synthesis



(B) Transpeptidase Mediated Cross-Link Formation

Figure 2: Peptidoglycan Synthesis Requires Enzymatic Activity in the Cytoplasm and the Periplasm
(A) In the cytoplasm, Lipid II is synthesised and anchored to the plasma membrane. Fully processed Lipid II units are then exported to the periplasm by a flippase and polymerised by a glycosyltransferase, releasing the lipid anchor to be recycled. Following polymerisation, peptide stems are cross-linked by a transpeptidase, connecting adjacent glycan strands. Many bacteria contain class A penicillin binding proteins (aPBPs) exhibiting both glycosyltransferase and transpeptidase activity, while others rely on separate proteins for polymerising glycan (the SEDS family) and forming cross-links (bPBPs) [18–20]. **(B)** To cross-link stem peptides, DD-transpeptidases attack the D-Ala-D-Ala bond of a donor peptide and form a covalent intermediate, releasing the terminal D-Ala in the process. The nucleophilic amine of the incoming acceptor peptide then attacks this intermediate to form a peptide bond between the two stems. Rarer cross-links between two diamino acids in the third position may also be formed by LD-transpeptidases (not shown) and don't rely on the presence of a D-Ala-D-Ala motif [21]. Figures adapted from Do, Page, and Walker [10].

the glycan chain are referred to as glycosidases, and enzymes cleaving within the stem peptides are referred to as peptidases [8].

Glycosidases can be further broken into glucosaminidases (cleaving after GlcNAc), and muramidases (cleaving after MurNAc). Though they aren't strictly hydrolases, lytic transglycosylases cleave the same linkages as muramidases and are generally lumped in with the glycosidases [8].

Peptidases can then be divided into amidases, hydrolysing the bond between MurNAc and L-Ala, endopeptidases, which cleave peptide cross-links or other bonds within the peptide, and carboxypeptidases, which cleave residues from the C-terminal ends of peptide stems. Finally, the endo- and carboxypeptidases can be further classified as DD, LD, or DL peptidases depending on the specific linkages they target [8].

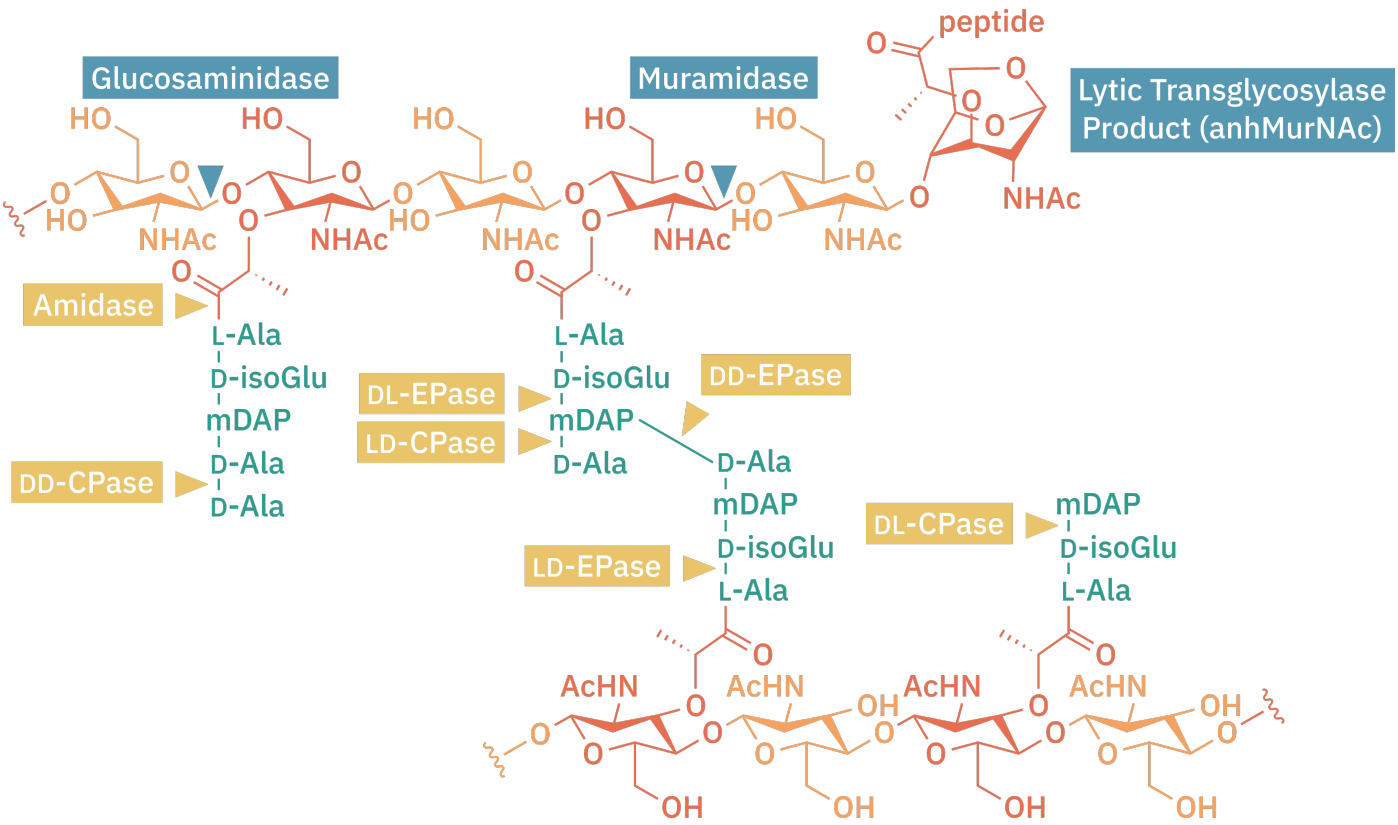
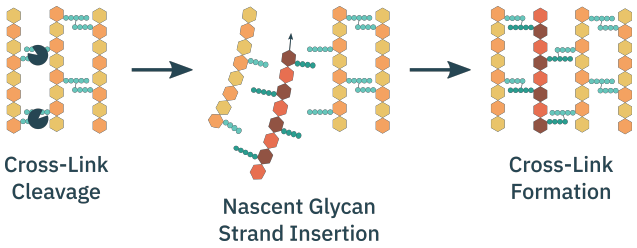


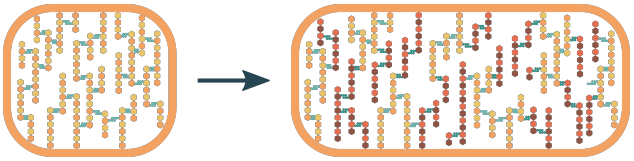
Figure 3: Hydrolases Exist for Nearly Every Linkage in Peptidoglycan
Peptidoglycan hydrolases can be divided into glycosidases (shown in blue, cleaving the glycan chain) and peptidases (shown in yellow, cleaving the stem peptides). The carets indicate the site of hydrolysis for each enzyme. Lytic transglycosylases cut the same bonds as muramidases but aren't true hydrolases – they break bonds without H_2O. When lytic transglycosylases cleave the glycan strand, an intermolecular mechanism results in the formation of anhydro-MurNAc, preventing further extension of the glycan strand. Figure adapted from Vollmer et al. [8] and Do, Page, and Walker [10].

2.2.2 Redundancy & Multifunctionality

It's often quite difficult to assign biological function to particular hydrolases, as many bacteria possess a high number of them with redundant roles, and individual hydrolases can have several distinct functions [8]. *E. coli*, for example, has five amidases, six lytic transglycosylases and three endopeptidases that all contribute to the separation of daughter cells following cell division [22, 23].



(A) Cross-Link Hydrolysis & Nascent Strand Insertion



(B) Sacculus Expansion Following Glycan Strand Insertion

Figure 4: Sacculus Expansion Requires Hydrolysis
(A) To expand the sacculus, hydrolases cleave the cross-links holding old glycan strands together and allow newly synthesised peptidoglycan to be inserted. Once inserted, the nascent strands can be cross-linked to the rest of the cell wall. **(B)** In rod-shaped bacteria, glycan strands are inserted perpendicular to the cell axis, facilitating lateral growth while maintaining a relatively constant diameter. Newly synthesised peptidoglycan is shown in darker colours. Figures adapted from Singh et al. [27].

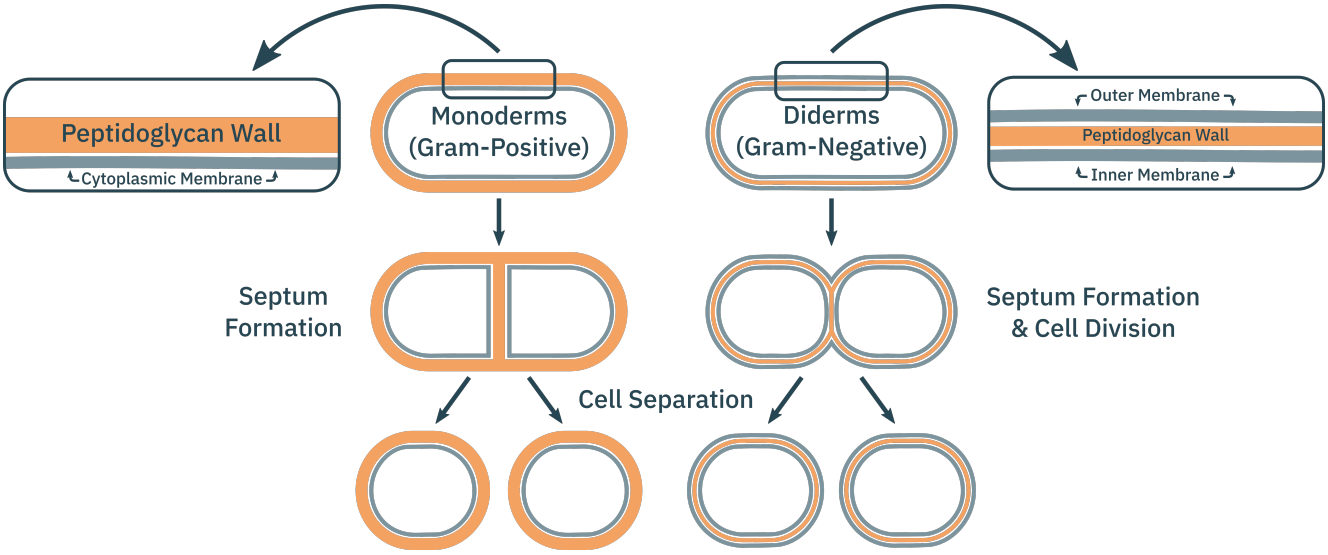
3 Why Hydrolases Are Essential

3.1 Cell Growth

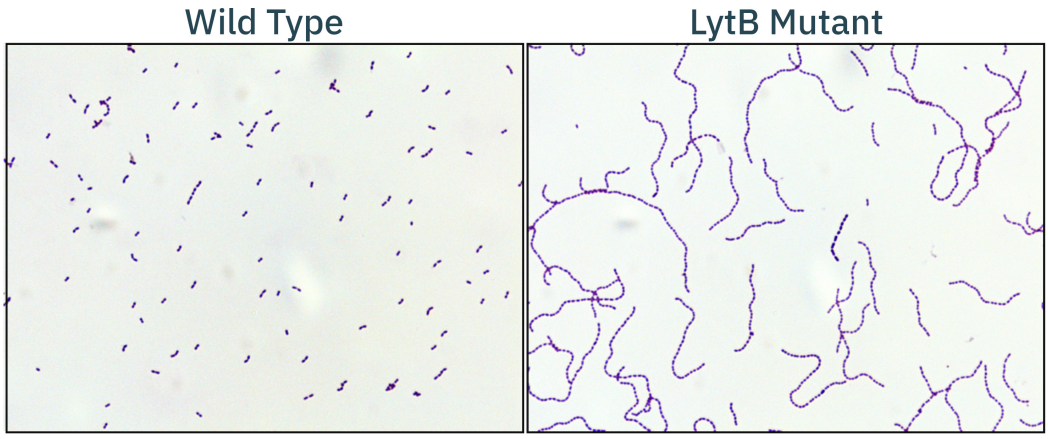
For bacteria to grow, the peptidoglycan sacculus must expand; this requires the rupture of covalent bonds to release tension and make room for new glycan strands to be inserted (Fig. 4) [24, 25]. The exact mechanism likely differs between monoderms and diderms but ultimately results in the release of peptide cross-links (often via endopeptidases). In *B. subtilis*, the LytE and CwlO DL-endopeptidases are essential for growth and double mutants are not viable [26].

3.2 Rigidity Regulation

The careful shortening of glycan chains and reduction of peptide cross-linking can introduce some elasticity into peptidoglycan while maintaining enough rigidity to prevent



(A) Cell Division in Monoderms and Diderms



(B) Hydrolase Mutants Exhibiting a Chaining Phenotype

Figure 5: Hydrolases Are Required for Septum Cleavage and the Separation of Cells
(A) The cell envelope is structured differently in monoderms and diderms, resulting in subtly different methods of division. In monoderms, a septum fully forms mid-cell before being hydrolytically cleaved; but in diderms, septum formation and division occur concomitantly – the "septum" exists only for a moment before the cells separate. **(B)** In the absence of hydrolytic activity, cells can fail to fully separate and may form long chains. Deletion of the essential LytB hydrolase in *Streptococcus gordonii* results in a prominent chaining phenotype. Microscopy image from Arrigucci and Pozzi [31].

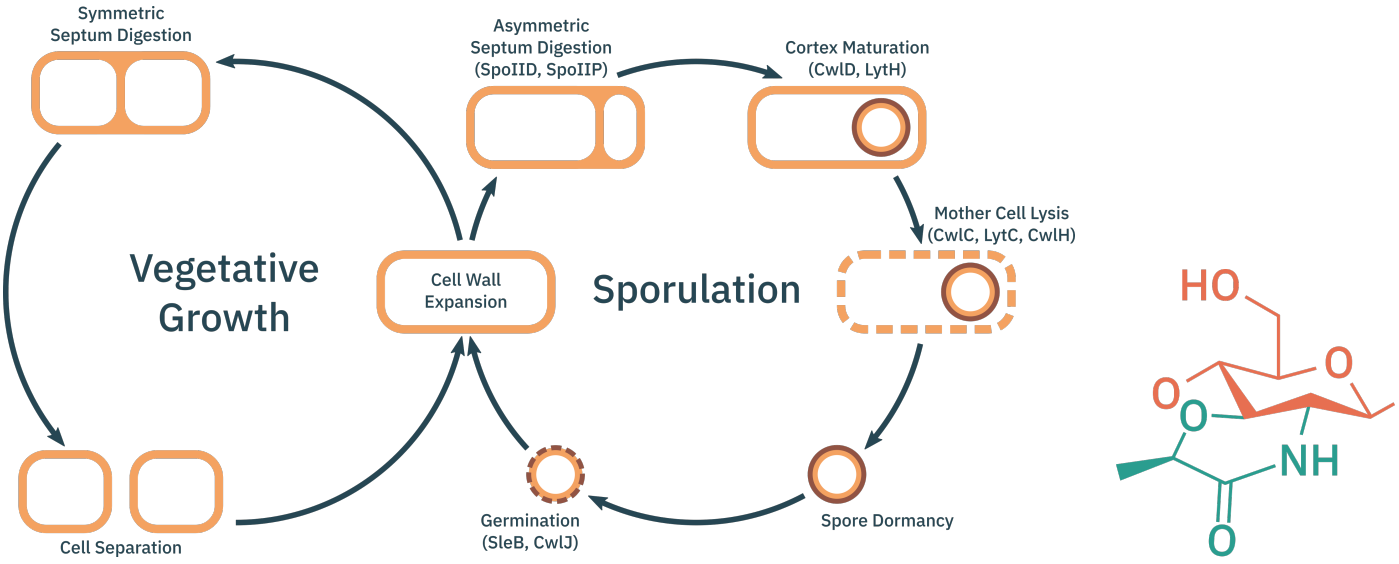
osmotic lysis [10, 28]. Lytic transglycosylases like MltG in *E. coli* both cleave stiff glycan stands and leave an anhydro-MurNAc terminus that prevents further extension (Fig. 3) [29]. Carboxypeptidases can then reduce cross-linking by reducing the number of donor stem peptides. By trimming pentapeptides into tetrapeptides, the D-Ala-D-Ala bond needed to form the DD-transpeptidase donor complex is removed (Fig. 2B) [8].

3.3 Cell Division

In monoderms, cell division requires an intracellular septum to first fully form then be cleaved, but in diderms the sacculus is pinched off gradually during cell separation (Fig. 5A). Regardless of mechanism, hydrolysis is eventually required to separate the daughter cell walls [8]. *E. coli*, for example, recruit a number of hydrolases to the septum; particularly vital are the AmiA/B/C amidases, without which chains of up to 20 cells form. Further hydrolase deletions can extend these chains to 100 cells or more (Fig. 5B) [23, 30].

3.4 Sporulation & Germination

Sporulating species rely on hydrolases during spore formation, maturation, mother cell lysis, and germination (Fig. 6A) [32, 33]. To form an endospore in *B. subtilis*, the cell partially divides to form an asymmetric septum, then reshapes it with the SpoIID and SpoIIP hydrolases, allowing the mother cell to engulf the endospore [34]. As a second layer of cortical peptidoglycan encases the spore, peptide stem removal by the CwlD amidase allows for a unique δ-lactam ring to form within the MurNAc (Fig. 6B) [8, 35]. To release the endospore, the mother cell is lysed by LytC and the sporulation-specific CwlC amidase [32]. Finally, during germination, the cortical peptidoglycan is selectively digested by the SleB and CwlJ hydrolases, freeing the spore to rehydrate and grow [36, 37].



(A) The Life-Cycle of a Sporulating Species **(B)** MurN-Lactam

Figure 6: Hydrolases Are Involved in Spore Formation, Maturation, Mother Cell Lysis, and Germination
(A) Under normal conditions, sporulating bacteria grow like any other bacteria (left), but when exposed to potentially lethal stressors, they can form resistant endospores that lie dormant until conditions improve (right). The hydrolases employed by *B. subtilis* to progress through each stage of the sporulation cycle have been indicated in brackets. The cortical peptidoglycan unique to bacterial endospores is shown in brown. **(B)** During cortex maturation, every other MurNAc within the cortical peptidoglycan has its peptide stem removed and replaced with a δ-lactam ring (shown in teal). This modification vastly reduces cross-linking to give cortical peptidoglycan its resilience and allow for its selective degradation during germination.

3.5 Trans-Envelope Assemblies

Peptidoglycan naturally contains pores that allow proteins up to 100kDa through, but they are too small to permit the assembly of large trans-envelope complexes [6, 38]. To get around this, pili, flagella, and secretion systems are often coexpressed with a glycosidase that can locally enlarge peptidoglycan pores [39, 40]. FlgJ is a multifunctional flagellar protein containing an N-terminal domain involved in rod assembly and a C-terminal domain with pore-enlarging glucosaminidase activity [41].

4 How Hydrolases Are Regulated

4.1 Genetic Control

4.1.1 Two-Component Systems

The LytE and CwlO DL-endopeptidases essential for growth in *B. subtilis* are homeostatically regulated by the WalRK two-component system (Fig. 7) [42]. A drop in the extracellular concentration of DL-endopeptidase cleavage products activates the WalK histidine kinase which goes on to phosphorylate the intracellular WalR response regulator, enabling it to promote the expression of *lytE* and *cwlO*, increasing DL-endopeptidase activity [43].

A buildup of cleavage products, on the other hand, leads to the deactivation of WalK and WalR, halting the transcription of *lytE* and *cwlO* and unrepressing the *iseA* repressor. CwlO has a naturally short half-life (around seven minutes) and quickly degrades, and IseA directly inhibits LytE, overall reducing DL-endopeptidase activity [43].

4.1.2 Coupled Expression

The coupling of FltJ glucosaminidase expression to flagellum assembly is part of a larger trend – VirB1 is a hydrolase expressed as part of *Agrobacterium tumefaciens*'s DNA-transferring secretion system, and PilT makes room for pilus synthesis in *E. coli* [39].

Analogously, as chained cells can't efficiently chemotax, the *B. subtilis* cell-separation hydrolases LytC and LytD are expressed as part of the σ^D "flagella, motility, and chemotaxis regulon"; σ^D is responsible for 70–90% of *lytABC* transcription and 95% of *lytD* transcription [8, 44, 45].

4.2 Hydrolase Localisation

4.2.1 Septum Targeting

In *E. coli*, the AmiB/C amidases are targeted to the septum, focusing amidase activity at the site of cell separation [46]. In preparation for division, FtsZ polymerises midcell to form the Z-ring, and its conserved C-terminal peptide recruits further proteins to form the divisome (Fig. 8). One of these proteins is FtsEX, a transmembrane protein that goes on to bind and localise AmiB/C in the periplasm [47].

4.2.2 Coordinating Synthesis & Hydrolysis

Diderms with thin sacculi can ensure a balance of peptidoglycan formation and degradation via multi-enzyme synthase-hydrolase complexes (Fig. 9A) [50]. *E. coli* synthesis machinery can directly complex with the hydrolases PBP4/7 and NlpI can serve as an adaptor protein connecting further endopeptidases like MepS and MepM to the complex [51].

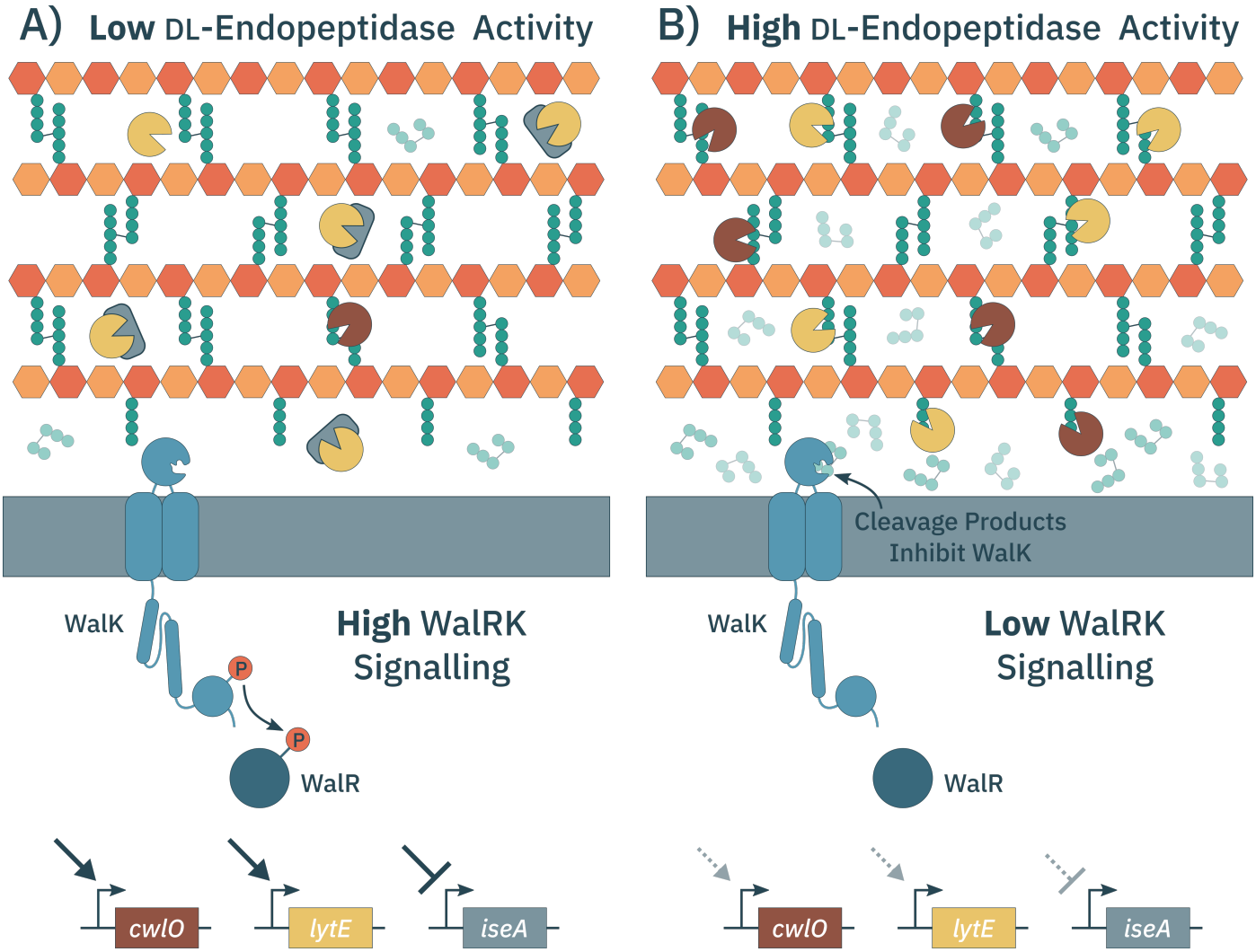


Figure 7: The WalRK Two-Component System Maintains Homeostatic Levels of Endopeptidase Activity
(A) In this scenario, little of the CwlO hydrolase (brown) is present, and the majority of LytE hydrolase (yellow) is being repressed by IseA (grey), resulting in an overall low level of DL-endopeptidase activity. WalK is then active and can phosphorylate the WalR response regulator, priming it to promote *cwlO* and *lytE* while repressing the *iseA* repressor. The net effect is an increase in endopeptidase activity. **(B)** If the rate of hydrolysis exceeds homeostatic levels, DL-cleavage products (shown in faint teal) can build up and repress WalK. This leads to the inactivation of WalR, the repression of the DL-endopeptidases, and expression of IseA, slowing the rate of hydrolysis and restoring homeostasis. Figure adapted from Dobihal et al. [43].

In monoderms, however, this direct coupling approach is not feasible as hydrolysis and synthesis occur on opposite sides of the sacculus [8]. Instead, in *B. subtilis*, actin-like MreB and MreBH proteins assemble into filaments just below the plasma membrane (Fig. 9B). MreB directly localises trans-membrane synthases, and MreBH localises LytE endopeptidases before exporting them into the periplasm at the site of synthesis [52].

4.3 Proteolytic Processing

4.3.1 Hydrolase Degradation

In *E. coli*, MepS is a cross-link cleaving DD-endopeptidase that enables cell growth and is subject to protease-mediated degradation [27, 54]. The NlpI adaptor protein can target MepS for proteolysis by forming a complex with the periplasmic Prc protease, reducing the half-life of MepS from 45 minutes to just 2; this ensures the degradation of excess MepS that is not already associated with a synthesis complex via NlpI [27, 51].

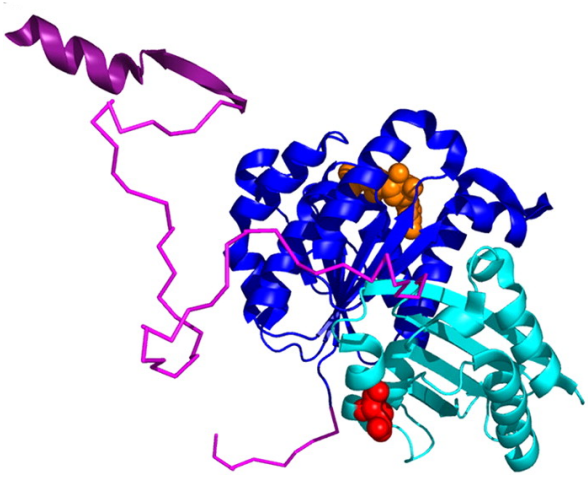
4.3.2 Zymogen Activation

Some hydrolases, like RipA from *Mycobacterium tuberculosis*, are produced as zymogens. RipA contains an N-terminal blocking domain that interferes with the DL-endopeptidase activity of the C-terminal domain, but proteolytic cleavage of the loop linking the N and C-terminal domains reactivates the hydrolase (Fig. 10) [55]. Similarly, Atl in *S. aureus* is produced as a 138kDa proenzyme that's then proteolytically processed into two distinct hydrolases, an amidase and a glucosaminidase [56].

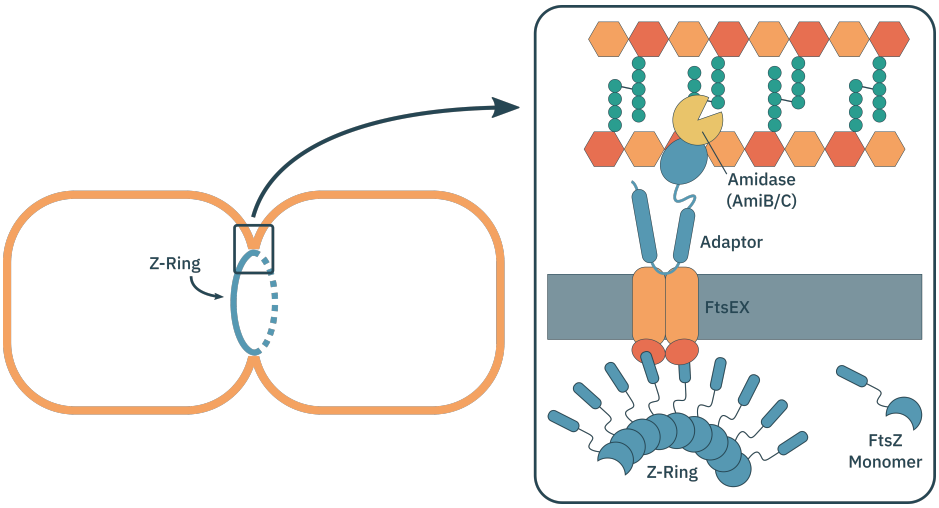
4.4 Substrate Specificity & Modifications

4.4.1 Hydrolysis Resistant Substrates

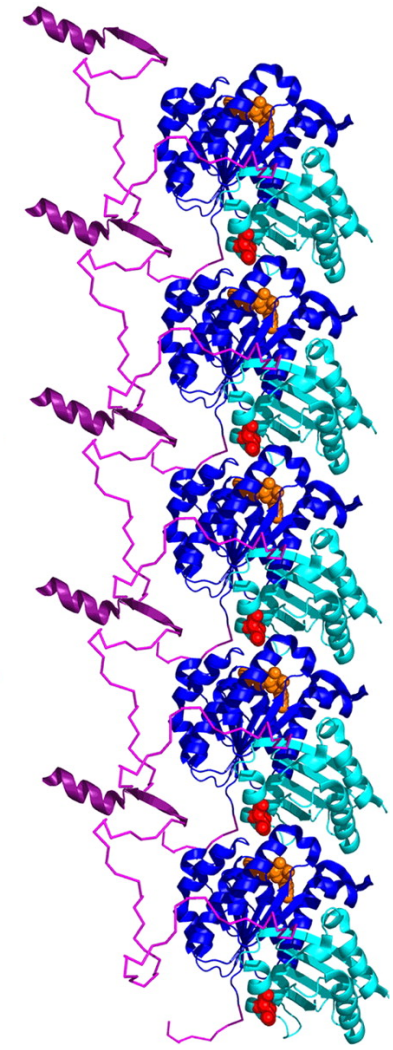
In monoderms and diderms, MurNAc that's been O-acetylated at the C6 hydroxyl protects the glycan chain



(A) The Tubulin-Like FtsZ Protein



(B) Z-Ring Positioning and Hydrolase Localisation



(C) A Filament of Polymerised FtsZ

Figure 8: FtsZ Forms the Z-Ring During Cell Division and Recruits Divisome Proteins via its C-Terminal Peptide **(A)** The two globular sub-domains of FtsZ are shown in blue and cyan. The orange space-fill represents a bound GDP and the red space fill highlights an essential residue of the "synergy" loop, a motif also present in α-tubulin that promotes the hydrolysis of GTP during filament formation. In magenta is the highly conserved C-terminal peptide (15–17 residues) and an intrinsically disordered peptide linker that can be anything from 43 to 95 amino acids in length [48, 49]. **(B)** During cell division, FtsZ polymerises mid-cell to form the Z-ring and its C-terminal peptide recruits a number of divisome proteins – including FtsE of FtsEX. The periplasmic domain of FtsEX can then localise select hydrolases to the septum. **(C)** To form the Z-ring, the tubulin-like domain of FtsZ polymerises into long filaments. Cartoon structures of FtsZ from Erickson, Anderson, and Osawa [48].

from cleavage by lytic transglycosylases, as the C6 hydroxyl plays an essential attacking role in the lytic transglycosylase mechanism [57]. Monoderms can also modify their peptidoglycan with teichoic acids. These anionic polymers help maintain a buffered micro-environment within the peptidoglycan matrix and can sequester positively charged hydrolases, protecting the surrounding peptidoglycan from degradation [58, 59].

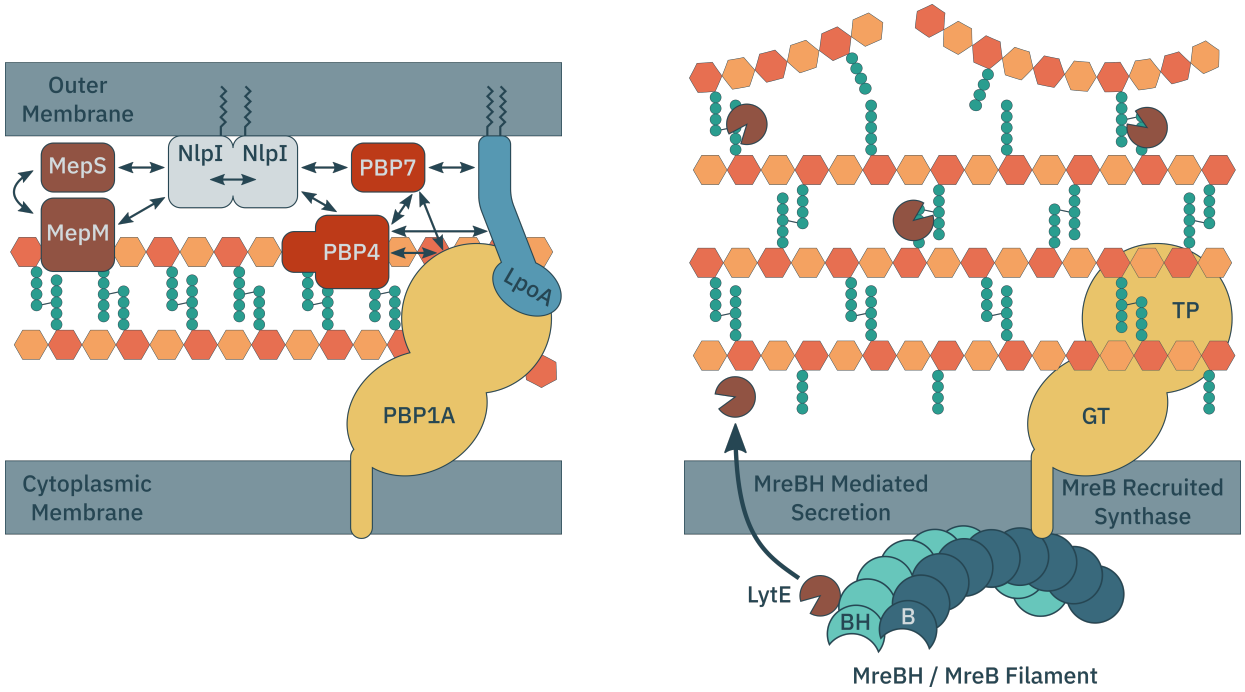
4.4.2 Hydrolysis Sensitive Substrates

In *B. subtilis*, the hydrolases SleB and CwlJ act as germination-specific lytic enzymes (GSLEs) that recognise the unique δ-lactam MurNAc of the spore cortex (Fig. 6B). This specificity allows the cortex to be fully degraded while leaving the inner, primordial layer of peptidoglycan untouched [36, 60]. Hydrolases can also discriminate by molecular weight: those containing a LysM domain, such as AtlA from *E. faecalis*, preferentially target glycan chains four sugars or longer (Fig. 11). Consequentially, AtlA readily digests peptidoglycan in the sacculus but not peptidoglycan found in smaller, two-sugar fragments [8, 61].

4.5 Protein-Protein Interactions

4.5.1 Hydrolase Inhibitors

IseA inhibits the activity of DL-endopeptidases in *B. subtilis*, occluding the active sites of LytE (involved in growth) and LytF (involved in cell-separation) (Fig. 12A). Normally, this dials back hydrolysis during stationary phase, but the overexpression of IseA can titrate out LytF and lead to cell chaining [62, 63]. In bacteria that don't O-acetylate their peptidoglycan, like *E. coli* and *P. aeruginosa*, another inhibitor – Ivy – provides lytic transglycosylase protection by blocking cellular glycosidase activity [64].



(A) Synthase-Hydrolase Complexes in Diderms

(B) Synthase-Hydrolase Colocalisation in Monoderms

Figure 9: Peptidoglycan Synthesis and Hydrolysis Is Coupled in Diderms and Monoderms
(A) The thin peptidoglycan wall of diderms like *E. coli* allows synthesis and hydrolysis machinery to physically interact. Synthase proteins like PBP1A and its activator LpoA straddle the inner and outer membranes, complexing directly with DD-carboxy/endopeptidases like PBP4/7 [53]. NlpI is an adaptor protein localised to the outer membrane that can bridge these PBPs to other DD-endopeptidases like MepS and MepM. Figure adapted from Banzhaf et al. [51]. **(B)** In monoderms like *B. subtilis*, the cell wall is too thick for hydrolases (which act primarily on the outer layers of peptidoglycan) to interact with synthases (embedded in the cytoplasmic membrane). Instead, filaments of MreB and MreBH assemble just below the membrane – MreB directly localises membrane-bound synthases, and MreBH carries LytE the site of synthesis before its secretion into the periplasm.

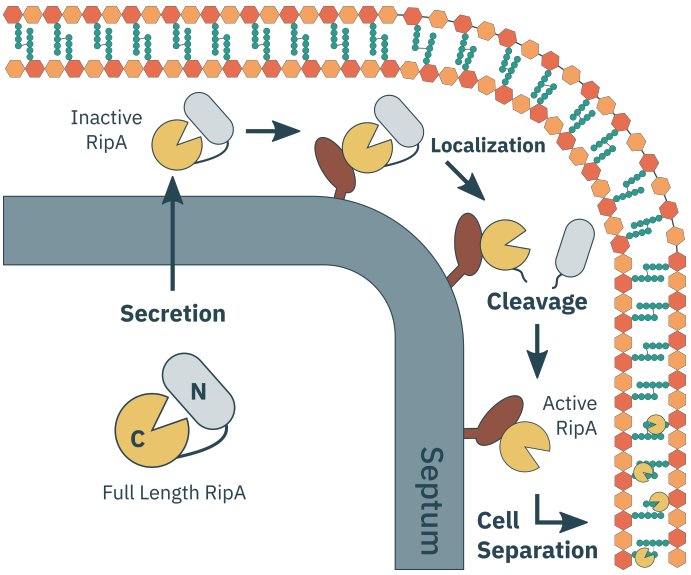


Figure 10: RipA Is Proteolytically Activated
The full-length RipA hydrolase in *Mycobacterium tuberculosis* contains a DL-endopeptidase domain linked to an activity-blocking N-terminal domain. RipA can be safely stored in the periplasm until its needed for cell separation, then localised to the septum where the peptide linking its N and C-terminal domains is cleaved. With the N-terminal blocking domain removed, RipA regains its DL-endopeptidase activity and can contribute to cell separation. Figure adapted from Chao et al. [55].

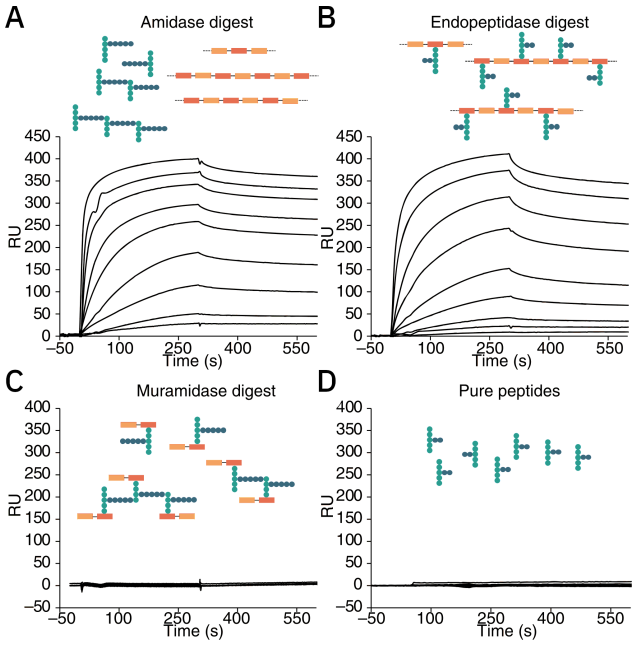
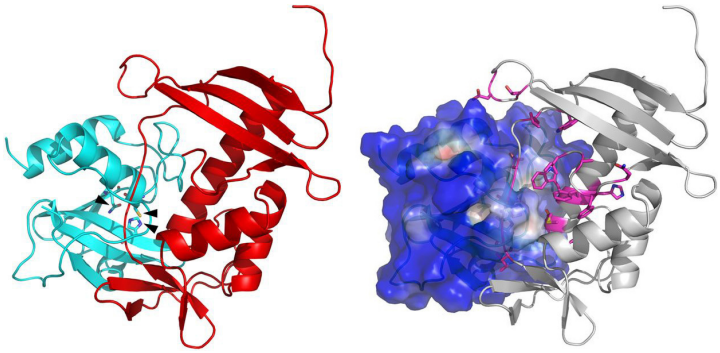
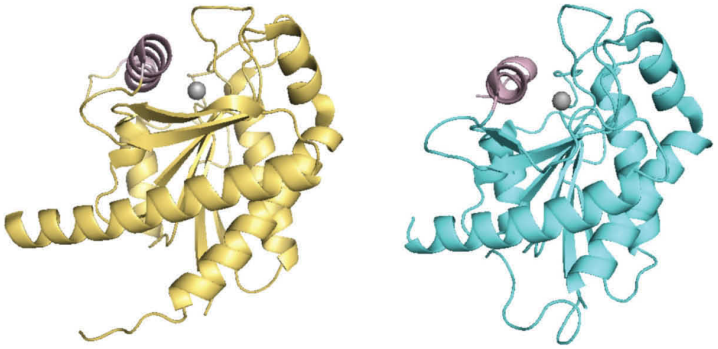


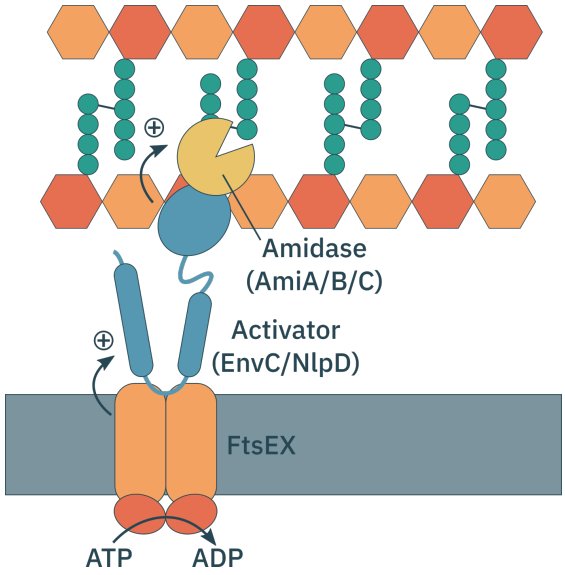
Figure 11: LysM Domains Target the Sacculus
Some hydrolases, like AtlA from *Enterococcus faecalis*, contain LysM domains for binding the cell wall. Mesnage et al. [61] determined via surface plasmon resonance that immobilised LysM bound peptidoglycan fragments containing intact glycan chains as seen in the amidase (A) and endopeptidase (B) digests, but not those containing only GlcNAc-MurNAc dimers (C) or no glycan at all (D). The selective binding of LysM targets AtlA specifically to the long glycan chains present in the sacculus.



(A) IseA Inhibition of LytF



(B) The Auto-Inhibitory Helices of AmiB and AmiC



(C) FtsEX Activation of the Cell-Separation Amidases

Figure 12: Hydrolases Can Be Activated or Inhibited By Protein-Protein Interactions
(A) IseA (shown in red) is an inhibitory protein that occludes the active site of DL-endopeptidases like LytF (shown in cyan) using a hacksaw-like loop region. The black carets on the left indicate the catalytic triad of LytF and its occlusion by IseA. The right shows the same structure, but with the charge surface of LytF calculated and IseA's potentially interacting residues highlighted in magenta. Figure from Arai et al. [63]. **(B)** The cell-separation amidases AmiB and AmiC contain α-helices (shown in pink) that occlude their active site. Figure from Do, Page, and Walker [10]. **(C)** FtsEX is an ABC transporter that uses ATP hydrolysis to induce a conformational change in the coiled-coil domain of a bound regulator; this change allows EnvC to activate AmiA/B and NlpD to activate AmiC. Figure adapted from Do, Page, and Walker [10].

4.5.2 Hydrolase Activators

In *E. coli*, the cell separation amidases AmiA/B/C are activated by EnvC and NlpD, proteins that displace an α-helix occluding the amidases' active site (Fig. 12B) [65–67]. However, before EnvC and NlpD can activate anything, they must themselves be activated by FtsEX (Fig. 12C). FtsEX is an ABC transporter that doesn't actually transport anything but instead uses ATP hydrolysis to drive a conformational change in the coiled-coil domain of a bound regulator, inducing its activity. FtsEX can also directly activate the CwlO elongation hydrolase in *B. subtilis* without the help of an additional adaptor [68, 69].

5 Conclusion

Peptidoglycan is an incredibly dynamic macromolecule, constantly remodelled by synthases and hydrolases. This dynamism allows bacteria to grow and divide but is a delicate balance: too little hydrolysis impairs growth and too much results in lysis. To manage this in a robust way, bacteria have evolved an array of redundant hydrolases and multi-level regulatory mechanisms, eliminating single points of failure and maintaining homeostasis even when whole pathways are disrupted. The LytE and CwlO elongation hydrolases of *B. subtilis* exhibit redundancy, as either hydrolase is sufficient for growth, and multi-level control, being regulated at the level of transcription, localisation, and protein-protein interaction.

Despite the substantial body of literature regarding peptidoglycan hydrolases, the story of their regulation remains incomplete. Only a handful of hydrolases and regulators have had their biological functions fully characterised, and many of the better studied mechanisms just push the question upstream. For example, while RipA is known to be proteolytically activated at the septum of *M. tuberculosis*, it's not clear how that proteolysis itself is regulated [55].

Since the 1940s, antibiotics have saved millions of lives, but their effectiveness has fallen as pathogenic bacteria develop resistance [70]. Given the ubiquity of peptidoglycan, the continued study of its synthesis and hydrolysis could reveal valuable new antibiotic targets – purified hydrolases have even been applied directly as an antimicrobial drug, representing a novel class of antibiotic [71]. Understanding what started as a "paradox", why bacteria produce potentially lethal hydrolases, may end up being the key to staving off antibiotic resistance.

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