Microbiology (3)

Bacterial Cell Wall

Transpeptidation

- final step in cell wall biosynthesis — formation of cross-links between muramic acid residues in adjacent glycan chains
- achieved mainly through action of penicillin-binding-protein (PBP) — which catalyses transglycosylation and transpeptidation rxns. (formation of glycosidic & peptide bonds of PG)
  - ↳ FtsI in E.coli (PBP3)
- controlled cutting of cell wall reqd — done by autolysins which can also result in cell death

SEDS (Shape, Elongation, Division, Sporulation)

- widespread family of bacterial cell wall polymerases
- work together as a subcomplex with the transpeptidase PBPs.
  - → RodA — elongasome (responsible for elongation of the cell)
    - moves in a directed and circumferential manner around long axis of cell, polymerising PG

→ FtsW – a homologous enzyme, part of divisome complex, is important for cell division, working with PBPs as well

→ SpoVE – expressed in sporulating cells, resp. for spore cortex PG synthesis. (bac. memb. bound)

→ FtsZ – bacterial homolog of tubulin, in E.coli, initiating cell division.

Key functions of Peptidoglycan

- determines cellular shape that is reproduced from generation to generation.
- serves as attachment sites for virulence factors & adhesins
- aids bacteria in undergoing morphological transformations in response to diff. stress related factors
- in many gram +ve bacteria, PG move outward allowing cellular expansion.

Bacillus subtilis

- has glycan strands up to 5μm, longer than cell
  – part of PG architecture allowing cell growth and division
- inner surface of cell wall has a regular macrostructur with ≈50 nm-wide peptidoglycan cables
  ↳ run across the short axis of cell
- cross striations along each cable are also present
- fundamental cabling architecture is also maintained during

septum development as part of cell division.

How does peptidoglycan get its dynamic, stress-bearing structure?

- glycan is oriented parallely to the plasma memb.
- helical cabling arrangement — of cross striations

Two sets of machinery

To allow cell elongation — To the septum for cell division

- Atomic force microscopy represents cell cylinder cabling architecture — as a result of localisation of PG biosynthetic machinery
  - ↳ MreC reqd. as part of cylinder elongation biosynthetic apparatus reqd. for long glycan strands and cabling architecture.

- It is proposed that during biosynthesis, small no. of glycan strands are polymerised and cross-linked to form a PG rope
- rope is then coiled into a helix with a width of ≈50 nm to form the inner surface cable structures
- nascent helix (cable) is inserted into the cell wall by cross-links b/w two existing cables & the overlying cable interface cleaved by autolysins

- the turgor pressure of the cell causes nascent cable helix made of peptidoglycan ropes to flatten, resulting in cross-striations
- str. may get stabilised by intra/inter glycan cross-links
- helical features brought into relief during Atl hydrolysis
- accommodates very long glycan strands.

Teichoic Acid

- have a backbone of polyol-phosphate usually with sugars and/or D-alanine as substituents
- polyol is usually ribitol (C_5) or glycerol (C_3)
- present in gram +ve bacteria, but similar polymers also occur in gram-negative bacteria (capsule or part of LPS)
- Wall teichoic acid (WTA) and memb. lipoteichoic acid (LTA)
  - WTA → covalently linked to PG → 60% of cell wall
  - LTA → anchored to plasma memb.
- made by tag genes & tar genes
  - tag genes → polyglycerol phosphate
  - tar genes → polyribitol phosphate

Function of teichoic acid:

- regulation of cell morphology and division
- scaffolding roles
- roles in cell elongation and division

- Bacteria lacking WTAs grow slower than wild types
  - clump in solution

- WTAs form a dense network of negative charge on gram -ve cell surfaces — bind cationic groups (mono- & divalent- cations) ↓

  cation homeostasis - reserve for ions close to cell surfaces, important for enzymatic activity

  ⇓

  WTA production is upregulated when metal ↓

- serve as scaffolds for wide range of molecules (e.g, autolysins, in cell growth & div)
  - ↳ grow slower in absence of WTAs

- D-alanylation reduces repulsion b/w WTA acid chains by adding ⊕vely charged amines
  - also increases susceptibility to antibiotics, in absence
  - promotes better adhesion to host tissue & confers resistance to lytic enzymes provided by host

- WTA biosynthetic enzymes associate with protein complexes involved in elongation, and LTA with division
  - ✗ WTA in rod shaped B. subtilis → round cells
    - ↳ increases temperature sensitivity

- ✗WTA → slower growth
  - → clumping
  - → non-uniform thickening of PG
  - → increased cell size
  - → defects in septal positioning & number
  - → aberrant shape
  - → increased temperature & antibiotic sensitivity
  - → decreased adhesion to surfaces
  - → unable to grow in high salt media

Wall teichoic acids in Streptococcus aureus

- PG is highly cross-linked – synthesised by Penicillin-binding protein (PBP-4)
- WTA → attached to PG: act as temporal and spatial regulators of PG metabolism by localising PBP4
- In S. aureus lacking TagO, PBP4 no longer accumulates at the specifically at division septum, but is dispersed through cell membrane, as WTAs are not produced

Important: WTAs themselves are used for localisation of PBP4 and not TagO

WTA acid synthesis starts — signalling for further processing of PG - recruitment of PBP4

Hypothesis:
Bactoprenol carries both WTA and PGN;
When ↓ WTA, more bactoprenol for PGN => more delocalisat
substrate deloc.
[RULED OUT EXPERIMENT]

SUMMARY

Gram-positive cell walls	Gram-negative cell walls

• Thick peptidoglycan
• Teichoic acids
• In acid-fast cells, contains mycolic acid
	• Thin peptidoglycan
	• No teichoic acids
	• Outer membrane
	  • LPS
	  • O - polysaccharide
	  • Lipid A

Teichuronic Acid

- Teichoic acids are phosphate rich, low-P substituent produced in ↓ P environments

- synthesised by Tua operon — Phosphate-free acidic polymer containing GalNAc & D-glucuronic acid
- Functionally interchangeable with teichoic acids??

> Indispensability of teichoic acid in context of host interaction and immune modulation
> *does not contribute significantly to str. integrity & autolysin regulation.
> *less anionic content.

Micrococcus luteus

- TUA made of D-glucose & ManNAcA
- located on cell surface & covalently linked to PG in cell wall through linker.
- synthesised by ① Glucosyltransferase
  ② ManNAcA-Transferase

- Teichuronic acid synthetase (TUAS) enzyme complex
  ↓
  2 glycosyltransferases
  ↳ displays hydrophobic prop. & associated with cytoplasmic memb.

Actinoplanes spp.

characterised by
1. aerial mycelia
2. presence of sporangia
3. spherical and motile spores
4. variety of 2° metabolites
5. gram ⊕ve, soil, riverbed & lake sediments.

Novel teichuronic acid in *Actinoplanes lobatus*

- linear str. of chain and heterogenous repeating units
  - → glycopyranose residues
  - → alternating residues of diaminouronic acids — D-manno, L-gulo & D-gluco config. (6:3:1)
- not found in other gram ⊕ve bacteria

Wall Teichoic Acid	Lipoteichoic acid
• covalently attached to PG layer via disaccharide phosphate residues	• anchored to cytoplasmic membrane through glycolipid moiety & extends into cell wall
• repeating units of glycerol or ribitol phosphate	• conserved backbone of polyglycerol phosphate

• structural integrity of cell wall, cell shape, binding of cations & surface protein, antibiotic resistance	• cell envelope integrity by inhibiting autolysin, host immune modulation (virulence factor)
• binds divalent cations	• contributes to negative charge affects cation binding
• forms biofilm	• maintains biofilm
• maintains localised pH for autolysin func. as well as localises autolysin to regions of division in cell wall	• specific inhibitor of autolysin, → resistance to lysis during stationary phase and bacterial chain formation at lower conc.

Penicilin-binding-proteins (PBPs)

High Molecular Mass (HMM)	Low molecular mass (LMMs)
→ responsible for peptidoglycan polymerisation, cross-linking and insertion into pre-existing cell wall	

↓

Structure (HMMs)

- Topology consists of a cytoplasmic tail, a transmembrane anchor, and two domains joined by β-linker on the outer surface of cytoplasmic memb where PG synthesis takes place

- Structure & catalytic activity of N-terminal domain

  - Class A (glycosyltransferase) → elongation of un-crosslinked glycan chains
  - Class B (cell morphogenesis) → interacts with other proteins involved in cell cycle

- C terminal of both classes has transpeptidase activity → crosslinking b/w two adjacent glycan chains

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LMM PBP → cell separation, peptidoglycan maturation or recycling

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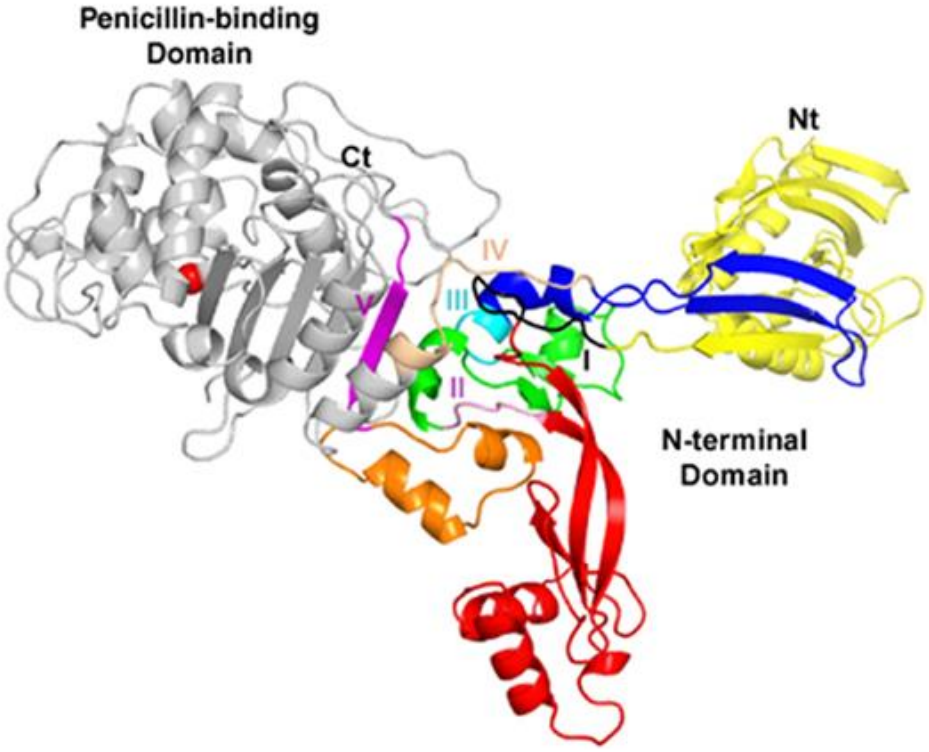
Inside-to-outside growth model for gram ⊕ve bacteria – new material inserted on inner face of wall, adjacent to PBPs.



Bacillus subtilis phosphate starvation

Phosphate starvation

Induction of Pho regulon	Induction of σ^B-dependent general stress regulon
◦ induced by PhoP & PhoR ◦ enables cells to use limiting phosphate resources more efficiently ◦ genes → phoA & phoB (alkaline phosphatase) + phoD (alkaline phosphodiesterase)	

+ pst (phosphate transport operon) + tua (teichuronic acid synthesis operon)	