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Cell wall polymers in Archaea (Archaebacteria)

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Abstract. The distribution of the various cell wall and cell envelope (S-layer) polymers among the main lineages of the domain Archaea (Archaebacteria) and the chemical composition and primary structure of polymers forming rigid cell wall sacculi is described. Differences between bacteria and archaea in their sensitivity to antibiotics which inhibit cell wall synthesis in bacteria are discussed.

Key words. Archaea (Archaebacteria); cell walls; pseudomurein; methanochondroitin; glutaminylglycan; S-layers; chemical structure; biosynthesis; antibiotics; evolution.

Introduction

The distinction between the domains Bacteria and Archaea (Archaebacteria) is based mainly on the different types of ribosomal RNA and the chemical nature of the membrane lipids: diacyl D-glycerol diester in bacteria versus isoprenoid L-glycerol diethers or di-L-glycerol tetraethers in archaea [1]. In addition, all archaea lack murein, a peptidoglycan with numerous chemical variations [2] that forms rigid cell wall sacculi in almost all taxa of bacteria with only a few exceptions, such as *Mycoplasma*, *Planctomyces* and *Chlamydia*.

In the absence of murein, polymers of diverse chemical natures are found to form rigid cell wall sacculi in the Gram-positive archaea. The majority of the archaea are Gram-negative and possess only proteinaceous or glycoproteinaceous cell envelopes (S-layers), or a reinforcement of the cytoplasmic membrane reminiscent of the glycocalyx of eucaryotic cells [3] (fig. 1).

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The pseudomurein of the Methanobacteriales and of Methanopyrus

Compared with murein, pseudomurein [3], which is found in members of the Methanobacteriales and the genus *Methanopyrus*, is a fundamentally different type of peptidoglycan (fig. 2). Its glycan moiety contains L-talosaminuronic acid instead of muramic acid, and its peptide moiety lacks D-amino acids. The biosynthesis of pseudomurein starts with the concomitant formation of the disaccharide uridine diphosphate-*N*-acetylglucosminyl-*N*-acetyltalosaminuronic acid (UDP-GlcNAc-NAcTalNA) and a UDP-activated pentapeptide [4] (fig. 3). *N*-acetyltalosaminuronic acid is formed from *N*-acetylgalactosamine via *N*-acetylaltrosaminuronic acid. The UDP-activated pentapeptide is derived from N^{α}-phosphoryl glutamic acid by the stepwise addition of four amino acid residues at the expense of adenosine triphosphate (ATP). N^{α}-UDP-glutamic acid is phosphorylated at the γ-carboxyl group, as evidenced by the isolation of γ-phosphoryl-N^{α}-UDP-glutamic acid from cells of *Methanobacterium thermoautotrophicum* [5]. The pentapeptide is linked to the disaccharide. The resulting disaccharide pentapeptide is transferred to undecaprenyl-P and then attached to the growing glycan strand at the lipid stage. The formation of the nucleotide-activated disaccharide and of a nucleotide-activated pentapeptide

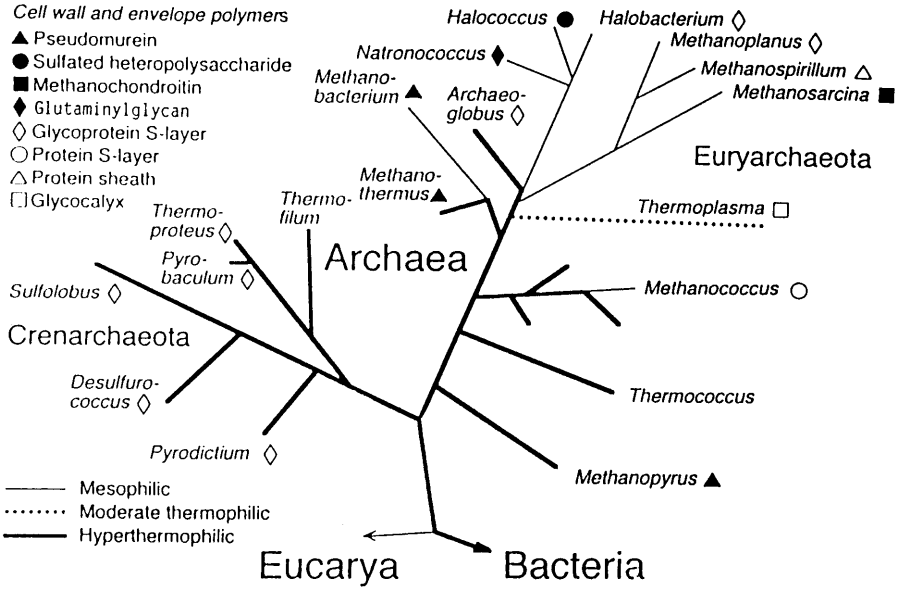

Figure 1. Distribution of cell walls and cell envelope polymers in the domain Archaea (after [3]).

represents novel pathways of oligosaccharide and peptide biosynthesis, respectively.

The methanochondroitin of Methanosarcina

Methanochondroitin, the cell wall matrix of *Methanosarcina* [6], is composed of galactosamine, glucuronic or galacturonic acid and glucose, reminiscent of chondroitin sulphate, a typical polymer of the connective tissue of vertebrates. However, methanochondroitin is not sulphated, and the molar ratio of GalNAc:GlcA is 2:1, not 1:1 as in chondroitin. The biosynthesis of methanochondroitin [7] (fig. 4) proceeds by the repeated addition of the undecaprenyl activated structural repeating unit UDP-PP-GalNAc-GalNAc-GlcA to the growing glycan chain. The repeating unit is formed from UDP-GalNAc and UDP-GlcA via UDP-*N*-acetylchondrosine [7].

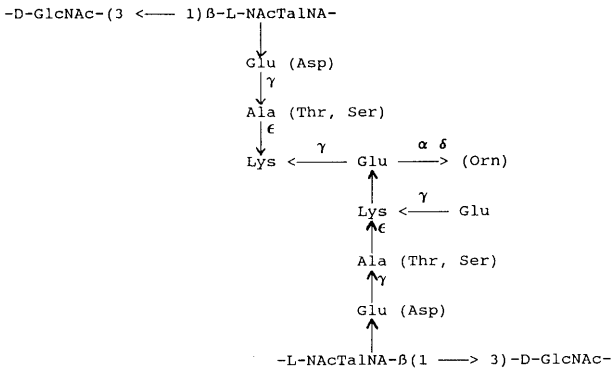

Figure 2. Structure of the pseudomurein of methanobacteria (modifications in brackets) (after [3]).

The heterosaccharide of Halococcus

The rigid cell wall of the extremely halophilic *Halococcus morrhuae* is composed of a highly sulphated heteropolysaccharide [8]. It consists of a mixture of neutral and amino sugars, uronic acids and an aminuronic acid, namely gulosaminuronic acid. It also contains significant amounts of glycine. Glycyl bridges may exist between glucosamine and uronic acid residues of the glycan strands. The primary structure of this polymer has not yet been fully elucidated.

The glutaminylglycan of Natronococcus

Recently, a fourth type of archaeal rigid cell wall polymer was found in the highly alkaliphilic species *Natronococcus occultus* [9]. The cell wall polymer of *Natronococcus* consists of a novel glycoconjugate. Two types of oligosaccharide are linked to a backkbone of poly-γ-L-glutamine via the α-amide group. One oligosaccharide consists of GalNAc and Glc and the other of GlcNAc and GalA (fig. 5).

Evolutionary considerations

The chemical manifold of cell wall and cell envelope polymers in the domain Archaea suggests that these polymers evolved independently from each other within the respective lineages at a rather late stage of evolu-

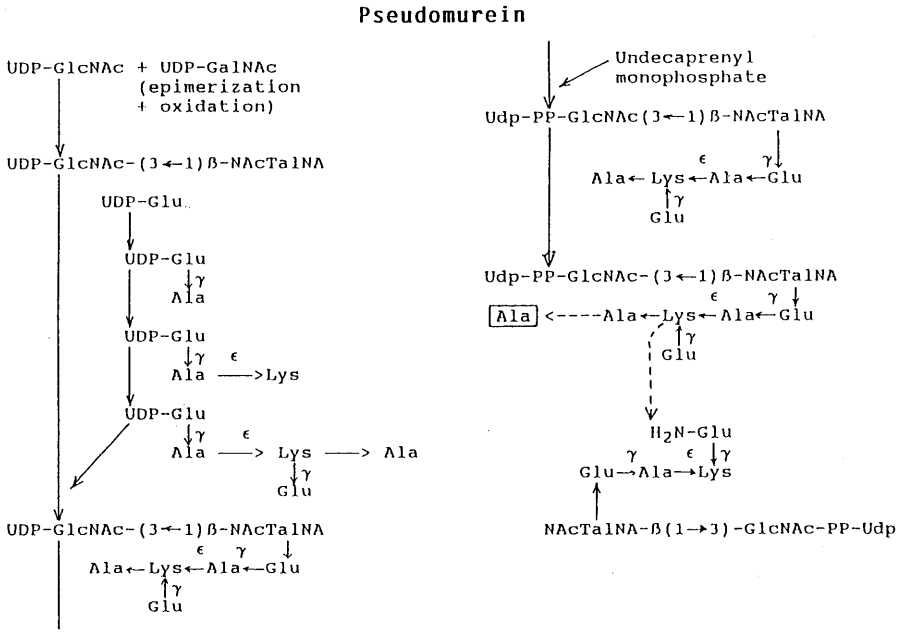

Figure 3. Biosynthesis of the pseudomurein of methanobacteria (after [4]). Udp, undecaprenyl.

tion, when the archaeal protocell had already radiated into numerous diverse lineages. In contrast, the evolution of murein synthesis in the domain Bacteria must have preceded any significant radiation of the bacterial protocell, as indicated by the universal presence of murein in lineage-specific modifications in all lineages. Murein is found even in the two deepest branches of the domain Bacteria, the hyperthermophilic genera Aquifex and *Thermotoga*. The few highly derived mureinless bacterial taxa, mentioned above, are assumed to have lost their murein sacculus during later stages of evolution.

Effect of antibiotics directed against cell wall synthesis

Since cell wall polymers of the various archaeal lineages are chemically unrelated and differ considerably from the bacterial peptidoglycan (murein), no common target for antibiotics directed against the cytoplasmic steps of the cell wall synthesis of all prokaryotes is to be expected. In fact, classical antibiotics directed against

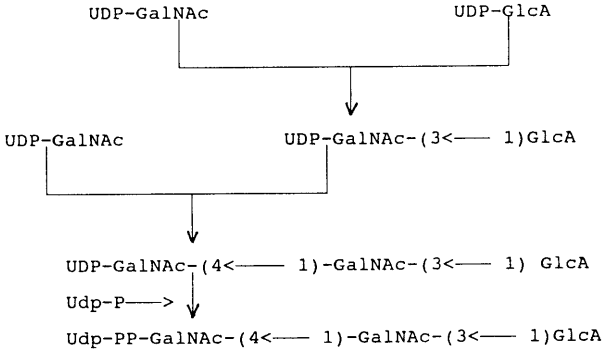

Figure 4. Biosynthesis of the repeating block of the methanochondroitin of *Methansarcina* (after [4]).

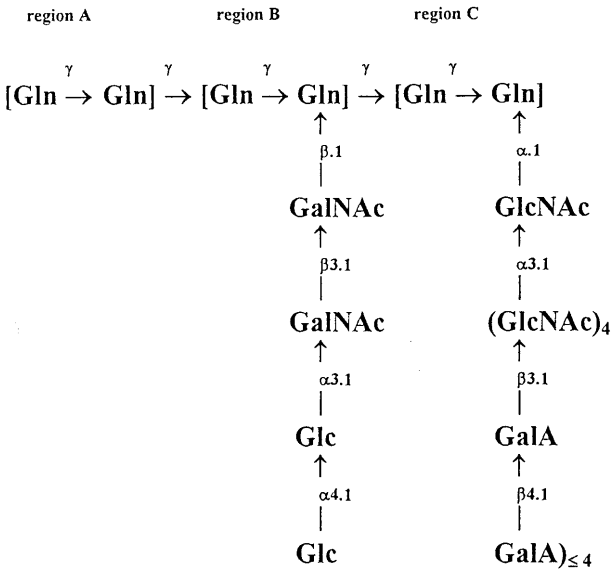

Figure 5. Proposed structure of the glutaminylglycan of *Natrinococcus* (after [9]).

murein biosynthesis, such as fosfomycin, vancomycin and β-lactam antibiotics, have no growth inhibiting effect against archaea [10, 11].

The only known exception is *Methanococcus vannielii*, which is sensitive to D-cycloserine, although this organism possesses no known D-alanine-containing structure, the well-known target of D-cycloserine inhibition [12]. Thus, in this case, the inhibitory mechanism of D-cycloserine is not yet understood.

Antibiotics interfering with the lipid cycle, such as bacitracin and gardimycin, are also inhibitory against different archaea, regardless of whether they possess pseudomurein or other types of cell wall polymers. These antibiotics may interfere with different lipid-bound precursors of various carbohydrate-containing polymers (pseudomurein, heteropolysaccharides, glycoproteins) or with the biosynthesis of isoprenoid diether lipids typical of archaea.

Tunicamycin, which inhibits the transfer of *N*-acetylglucosamine residues in glycoprotein and murein biosynthesis, also prevents the growth of *Methanobacterium thermoautotrophicum* [13], although the transfer mechanism of *N*-acetylglucosamine residues is different in the case of pseudomurein.

In general, our knowledge of the biochemical background of the antibiotic effects in archaea lags far behind that of bacteria. Perhaps archaeal research as a whole will remain the subject of a small circle of 'naturalists' and evolutionists, unless pathogenic archaea (archaebacteria) are discovered and evoke medical interest.

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