

Bacterial shape, size, and chaining are fundamental, evolved properties that determine bacterial survival in different environmental niches. Bacterial shape, size, and chaining are largely determined by processes that regulate the biosynthesis and placement of the semi-rigid peptidoglycan (PG) mesh that forms the bacterial cell wall. Moreover, the surface machines that mediate PG biosynthesis provide important targets for antibiotics and vaccines. *Streptococcus pneumoniae* (pneumococcus) is a commensal inhabitant of the human nasopharynx that can become a serious opportunistic respiratory pathogen, killing millions of people annually worldwide. *Streptococcus pneumoniae* is an ovococcus, prolate-ellipsoid shaped bacterium, whose colonization and capacity to cause invasive diseases depends on genes that determine shape, size, and chaining, including genes that encode penicillin-binding proteins (PBPs) and PG remodeling hydrolases. In this talk, recently published results will be discussed that track the essential PBPs required for septal and peripheral PG synthesis at the midcells of dividing pneumococcal cells. PBP activity and proteins were detected by 2D and high-resolution 3D-SIM microscopy using fluorescent-D-amino acid (FDAA) probes and immunofluorescence approaches. These results demonstrate separation of the adjacent septal and peripheral PG synthesis machines at later stages of cell division. New results based on genetic suppressor analysis will be presented that identify previously unknown, essential genes that mediate pneumococcal PG biosynthesis. The talk will end with a discussion of the roles of PG hydrolases in the turnover of pneumococcal PG and new genetic and structural information about the FtsEX:PcsB remodeling PG hydrolase. Together, findings from this talk and the recent scientific literature strongly support the contention that the mechanisms of PG biosynthesis and placement in ovococcus bacteria are significantly different from those characterized previously in rod-shaped and spherically shaped bacterial species.