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Review

Rewiring the Pneumococcal Cell Cycle with Serine/Threonine- and Tyrosine-kinases

Christophe Grangeasse[1,*]

Over the past decade, *Streptococcus pneumoniae* (the pneumococcus) has gained prominence as a model for studying the bacterial cell cycle. This important human pathogen possesses a characteristic diplo-ovococcal cell shape and produces a protective polysaccharide capsule required for virulence, and it has been used to investigate natural genetic transformation. Recent advances have demonstrated that the pneumococcus has evolved phosphorylation-dependent regulatory mechanisms dedicated to controlling cell division and ensuring the concealment of the newborn cells by the capsule. In this review, I survey the role of the only two serine/threonine- (StkP) and tyrosine-kinases (CpsD) of the pneumococcus and discuss the existence of interconnected phosphorylation networks coordinating cell division and morphogenesis with key aspects of the cell cycle.

Trends

The tyrosine-kinase CpsD is a ParA-like protein.

CpsD coordinates capsule production at mid-cell with chromosome segregation.

The eukaryotic-like serine/threonine-kinase StkP finely tunes peptidoglycan synthesis.

MapZ positively regulates the positioning of the division site.

StkP and CpsD are potentially involved in interconnected regulatory networks.

Streptococcus pneumoniae (the pneumococcus): From a Pathogen toward a Bacterial Model for Deciphering the Role of Serine/Threonine- and Tyrosine-kinases

S. pneumoniae is a Gram-positive bacterium that was first isolated in 1881 by Louis Pasteur and George Sternberg [1,2]. The pneumococcus gained prominence 50 years later with the works of Griffith and Avery on its genetic transformation of DNA [3–5], leading to the birth of molecular genetics [6].

While the pneumococcus is usually found as a commensal in healthy adults and children, it does have the potential to become pathogenic and can cause community-acquired diseases [7–9]. These range in severity from otitis media to pneumonia or meningitis. Despite the availability of antibiotics and vaccines, pneumococcal infections still have high mortality rates, and there are more than 1.2 million infant deaths per year [9]. Because the pneumococcus can resist antibiotics and evade vaccines by developing variability in its **polysaccharide capsule** (see Glossary), there has been a lot of effort to understand its physiology. In this respect, processes such as cell division and morphogenesis, as well as capsule synthesis and export, are viewed as areas of untapped potential for finding new therapeutic targets and new antibiotics with novel mechanisms [10,11]. The capsule and cell-division machineries occur as complex webs of protein–protein interactions. However, much remains to be discovered about the nature of these machineries, including the precise molecular function of most of their components and the mechanisms by which these components are assembled.

Over the past 10 years, **protein phosphorylation** on serine, threonine, and tyrosine has been shown to play a role in these processes. The enzymes catalyzing these post-translational

[1]Molecular Microbiology and Structural Biochemistry, UMR 5086, Université Lyon 1, CNRS, IBCP building, 7 passage du Vercors, 69367 Lyon Cedex 07, France

*Correspondence: c.grangeasse@ibcp.fr (C. Grangeasse).

modifications, namely, the so-called bacterial tyrosine-kinases (**BYKs**) and the eukaryotic-like serine/threonine-kinases (**eSTKs**) are widespread among bacterial species but their distribution is highly variable [12–15]. They are involved in the regulation of many cellular processes, including gene expression, extracellular polysaccharide production, DNA metabolism, cell division, and virulence [16–18]. Only one eSTK and one BYK, StkP and CpsD respectively, are encoded by the pneumococcus. The BYK CpsD is crucial for the synthesis and transport of the polysaccharide polymer whereas StkP participates in several aspects of cell division and morphogenesis. The pneumococcus thus represents a very simple model for tackling the regulatory networks involving both BYKs and eSTKs, as attested by the progress made over the last 5 years in our understanding of StkP and CpsD functions. In this review, I present current knowledge on the regulation of pneumococcal cell division and capsule synthesis by StkP and CpsD. I also discuss the possibility that phosphorylation regulatory networks coordinate several other aspects of the pneumococcal cell cycle.

Tyrosine Phosphorylation and Polysaccharide Capsule Production

Capsular polysaccharides are essential components of the bacterial cell surface. They play important functions in the interaction of the cell with its environment, and are important virulence factors in many pathogens. Most of the proteins required for capsule synthesis and export are grouped in a large operon. Capsule assembly starts in the cytoplasm with the synthesis of capsule subunits. Then, these subunits are flipped across either the inner- and outer-membrane or the cytoplasmic membrane of Gram-negative and Gram-positive bacteria, respectively, through a complex assembly machinery. The composition of this machinery varies among bacterial species and it encompasses either an ABC transporter, a synthase, or a Wzy-type polymerase, that assembles the nascent polysaccharide polymer [19,20]. Importantly, the Wzy-polymerase mechanism also involves a BYK that regulates the functioning of the capsule-assembly machinery.

The Capsule-Assembly Machinery

The biosynthesis and export of the pneumococcal polysaccharide capsule is achieved by the Wzy-mediated pathway in which the integral membrane protein CpsH polymerizes the polysaccharide repeat units [21,22]. The current model (Figure 1) proposes that the repeat unit is assembled in the cytoplasm onto the **undecaprenyl-diphosphate lipid** carrier by the UDP-glycosyl transferase CpsE. Then, the lipid-linked repeat unit is flipped across the membrane by the lipid-flippase CpsJ. Once flipped, the polymerase CpsH polymerizes the polysaccharide polymer that is eventually transferred onto **peptidoglycan** by the phosphotransferase CpsA [23,24]. Both CpsJ and CpsH localize at the division septum, indicating that capsule production occurs exclusively at mid-cell [25]. The membrane protein CpsC is required for the localization of the cytoplasmic BYK CpsD at mid-cell and likely acts as a scaffold, organizing CpsE, CpsJ, CpsH, and CpsA [25,26] (Figure 1). Indeed, deletion of *cpsC* induces CpsH delocalization. Moreover, CpsC triggers CpsD kinase activity, allowing autophosphorylation on its C-terminal tyrosine cluster [25]. Phosphorylated CpsD can, in turn, be dephosphorylated by its cognate phosphatase CpsB [27,28]. Cyclic phosphorylation/dephosphorylation of CpsD triggered by CpsC and counterbalanced by CpsB could potentially regulate the activity of the capsule-assembly machinery. However, the underlying molecular regulatory process remains unclear.

The CpsB/CpsC/CpsD Phosphoregulatory System

Based on the structural characterization of the active and inactive forms of the BYKs CapB of *Staphylococcus aureus* and Wzc and Etk of *Escherichia coli*, one can propose that the same applies for CpsD. Hence, CpsD likely forms a ring-shaped octamer in which the tyrosine cluster of one monomer fits into the active site of the neighboring monomer [29–32]. Upon interaction with CpsD, the C-terminal and cytoplasmic end of CpsC complements the nucleotide-binding site of CpsD and promotes ATP binding, allowing phosphorylation on the C-terminal tyrosine

Glossary

BYK: tyrosine-kinases idiosyncratic to bacteria. They structurally differ from eukaryal tyrosine-kinases and are classified in the P-loop ATPase/GTPase protein family. They possess a Walker A and B ATP binding site in their catalytic domain.

Divisome: protein complex formed by all the proteins involved in cell division. The divisome allows the remodeling and the incorporation of new peptidoglycan and the invagination of the membrane at the division septum leading to the separation of the two daughter cells.

eSTK: universal serine- and threonine-protein-kinases found in eukaryotes, bacteria, and archaea. Their catalytic domain is structurally conserved and possesses several conserved amino acid sequences known as the Hanks-signatures.

MapZ: the mid-cell-anchored protein Z is a membrane protein that acts as a molecular beacon to identify the site of division and to positively regulate the positioning of the Z-ring at mid-cell in the pneumococcus. It is conserved in most Lactobacillales.

ParB: a protein widely conserved (but not present in *Escherichia coli*) that binds *parS* DNA sequences located near the chromosomal origin of replication. Together with the protein ParA, ParB forms a system dedicated to plasmid partitioning and chromosome segregation in many bacteria.

PASTA: derived from PBP and serine/threonine kinase-associated domain. This protein domain is found in penicillin-binding proteins (PBPs) that crosslink peptidoglycan and some membrane eSTKs involved in the control of cell morphogenesis, division, and some developmental processes. Their structural fold is conserved, and they can bind to β-lactam rings.

Peptidoglycan: the main component of the cell wall that confers mechanical resistance and keeps the integrity of the cell shape. It consists of carbohydrate chains of alternating *N*-acetylglucosamine and *N*-acetylmuraminic acid that are covalently crosslinked to one other by short peptide chains.

Polysaccharide capsule: the most external structure of the bacterial envelope; it is firmly attached to the cell wall. It consists of polymeric carbohydrates formed by strain-