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Streptococcus pneumoniae two-component regulatory systems: The interplay of the pneumococcus with its environment

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ABSTRACT

Streptococcus pneumoniae is a human pathobiont possessing a diverse array of multifunctional proteins essential for bacterial fitness and virulence. Gene expression is tightly controlled by regulatory components and among the pneumococcal sensorial tools, two-component regulatory systems (TCS) are the most widespread and conserved. This review aims to provide a comprehensive analysis of original studies on pneumococcal TCS on a functional level. Despite a rather chaotic nomenclature, the current available information on pneumococcal regulation by these systems can be conveniently addressed, according to the regulation of pathophysiological cell processes and the responses to detectable environmental signals. Pneumococcal pathophysiological processes driven by TCS can be further categorized into competence and fratricide, bacteriocin production, and virulence factors expression. Conversely, detectable environmental signals by pneumococci can be grouped into antibiotics and cell wall perturbations, environmental stress, and nutrients acquisition. This review summarizes the state of the art on pneumococcal TCS based on an integral approach and thus providing insights into the regulatory network(s) of *S. pneumoniae*.

1. Introduction

Pneumonia is a common pulmonary disease, whose main etiological agent is *Streptococcus pneumoniae* (the pneumococcus). These pathobionts are Gram-positive, lancet-shaped, facultative anaerobic diplococci and normally found as commensals in the upper respiratory tract of healthy individuals (Austrian, 1986; Cartwright, 2002). However, they are also important human pathogens responsible for a high burden of diseases worldwide (WHO fact sheet, 2016) (Barocchi et al., 2007; Gillespie, 1989; Kilian et al., 2008). Under special circumstances the pneumococcus is able to circumvent the human immune system, causing a broad spectrum of human diseases ranging from non-invasive and localized infections like otitis media and sinusitis, to more aggressive and life-threatening diseases like community-acquired pneumonia, bacteraemia or meningitis (Cartwright, 2002; Henriques-Normark and Tuomanen, 2013). The pneumococcus is a highly adapted human pathogen, capable of efficiently colonizing and invading its host by displaying a wide array of adhesins, virulence factors, and other proteins with diverse functions such as nutrient scavengers, DNA uptake, oxidative stress resistance, and biological traits acquisition, among many others (Atkinson et al., 2009; Gámez and Hammerschmidt, 2012; Hammerschmidt, 2006; Kadioglu et al., 2008; Kietzman and Rosch, 2015; Siemieniuk et al., 2011; van de Beek et al., 2006).

The *sine qua non* virulence factor of *S. pneumoniae* is the capsular polysaccharide (CPS), masking the diplococci to evade the human immune response (Garcia et al., 2000; Sanchez et al., 2011). According to their biochemical composition, more than 95 different pneumococcal capsule types have been identified to date, conferring the pneumococcus a high degree of variability (Geno et al., 2015; Henrichsen, 1995; Kolkman et al., 1998; Varvio et al., 2009). The CPS undergoes a phase shift along an infection process, in which its density changes and exposes its arsenal of surface proteins (Hammerschmidt et al., 2005; Weiser et al., 1994). Pneumococci are equipped with different classes of surface proteins, which are involved in pneumococcal biological processes and participate in fitness, virulence, antibiotic resistance,

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oxidative stress, among others, and are essential for a successful colonization of its host (Bergmann and Hammerschmidt, 2006; Kohler et al., 2016). Overall, pneumococcal surface proteins are expressed to respond to specific conditions and facilitate a successful adaptive response to the given physiological situation (Kietzman and Rosch, 2015; Nguyen et al., 2015a). However, the forces driving the expression of these virulence factors, by means of the pneumococcus interplay with its host, remain unknown.

Sensing and responding to environmental signals during growth is an innate bacterial feature, necessary for adapting to changing habitats, ensuring metabolic resources, optimizing fitness, and increasing chances for survival (Duan et al., 2009; Harapanahalli et al., 2015; Hibbing et al., 2010; Lozada-Chavez et al., 2006). Specialized proteins involved in these complex mechanisms, also referred to as bacterial sensing systems, detect and process a variety of inputs into efficient output responses (Hoch, 2000; Lozada-Chavez et al., 2006; Miller and Bassler, 2001; Stock et al., 1990). Acquiring nutrients, detoxifying the cell, detecting the presence of neighbouring bacteria, regulating the expression of virulence factors and integrating new DNA material into the cell are among the most common sensing processes, where bacteria interplay with their environments (Duan et al., 2009; Miller and Bassler, 2001). Bacterial sensing systems can be triggered by chemical or mechanical stimuli (El-Sharoud, 2008; Harapanahalli et al., 2015), and chemical sensing relies on the identification of molecules present in the bacterial surroundings like metal traces, oxygen, nutrients and toxins (Duan et al., 2009; Harapanahalli et al., 2015; Miller and Bassler, 2001). Sensing alterations in the concentration of such molecules leads to activation of membrane proteins such as transporters and porins (Cai and Inouye, 2002; Duan et al., 2009; Silhavy et al., 2010). Moreover, bacterial regulatory systems can also be divided into four principal groups:

1) *Quorum sensing systems* are involved in cell-cell signalling and population density response utilizing secreted molecules denominated as autoinducers to sense different environmental conditions. These autoinducers have been reviewed elsewhere and they vary between Gram-positive and Gram-negative bacteria and can generate a crosstalk between species (Miller and Bassler, 2001). The most representative and intensively studied example is *Vibrio fischeri* and its control of bioluminescence via quorum sensing. This Gram-negative bacterium use acyl-homoserine lactone (AHL) as an autoinducer molecule to generate bioluminescence (Eberhard et al., 1981). In *S. pneumoniae* and Gram-positive bacteria in general, quorum sensing mechanisms involve two-component systems in tandem with their autoinducer molecules (Ji et al., 1995; Pestova et al., 1996; Solomon et al., 1996). The signal molecules in pneumococci are secreted peptides known to activate a regulatory response for competence or bacteriocin production (de Saizieu et al., 2000; Havarstein et al., 1995a; Pestova et al., 1996; Reichmann and Hakenbeck, 2000).
2) *Stand-alone regulators* are all in one molecules comprised of signal recognition domains and DNA binding modules. They exert an autonomous and dedicated control of the expression of specific operons. The mechanism on how these regulators recognize their signals is mostly unknown. An example among streptococci is the stand-alone regulator Mga of group A *Streptococcus* (*Streptococcus pyogenes* or GAS). Mga is involved in the exponential phase of growth of GAS and controls the expression of genes involved in the early stages of GAS colonization (McIver and Scott, 1997). In *S. pneumoniae*, several stand-alone regulators have been described. As a representative example, CcpA and MgrA (an orthologous of Mga from GAS) control the expression of genes involved in metabolism and virulence factors, respectively. They are stand-alone regulators that have been studied in detail and most of their regulatory targets have been elucidated in *S. pneumoniae* (Giammarinaro and Paton, 2002; Hemsley et al., 2003; Iyer et al., 2005).
3) *Regulatory RNAs* play a significant role in transcriptional and translational regulation in eukaryotes and bacteria (Morris and Mattick, 2014). Different classes of regulatory RNAs have been identified in bacteria, which modulate the response to specific conditions in the environment and the cell (Storz et al., 2011; Waters and Storz, 2009). For instance, riboswitches and small RNAs (sRNAs) are regulatory RNAs interacting directly with mRNAs or proteins. The interaction with their target molecule facilitates the stability and expression of specific genes and modulation of protein activity (Waters and Storz, 2009). While riboswitches have been described to exert their regulation in cis by binding a ligand and changing their structural conformation, sRNAs act by base pairing in cis or trans with their target mRNA (Storz et al., 2011; Waters and Storz, 2009). *Staphylococcus aureus* RNAIII, one of the largest sRNAs identified in bacteria, is the effector molecule of the accessory genome regulator (*agr*), and is responsible for the regulation of most of the genes under the control of the AgrCA TCS (Morfeldt et al., 1995; Novick et al., 1993). In pneumococci a set of 5 non-coding sRNAs have been described to be regulated by the CiaRH two-component system and are involved in the control of competence and autolysis resistance (Halfmann et al., 2007; Laux et al., 2015). Overall, all the different classes of regulatory RNAs are involved in the response of eukaryotes and bacteria to environmental signals and are connected to other regulatory systems as well.
4) Finally, the *two-component regulatory systems* (TCS) are the most widespread regulatory systems in bacteria (Mitrophanov and Groisman, 2008). TCS are recognized by their two-main protagonists: the histidine kinase (HK) and their cognate response regulator (RR) (Grebe and Stock, 1999; Stock et al., 2000). Both TCS protein components are composed of specific and well-characterized domains, imprinting unique features into their signal input and output, as well as the biological function they accomplish in a given organism (Hoch, 2000; Stock et al., 2000). Classical histidine kinases are membrane proteins anchored to the cell membrane via transmembrane domains (TMD) (Bhate et al., 2015; Mascher et al., 2006b). The TMD facilitate the capture and transfer of a stimulus signal originated in the extracellular milieu or in the intramembrane space (Bhate et al., 2015; Khorchid and Ikura, 2006; Mascher et al., 2006b). A classical HK presents 3 modules for its biological function: (i) a sensing component, traditionally located in the N-terminal end of the protein as an exposed loop located according to their signal origin, (ii) an intermediate linker region connecting the periplasmic N-terminal region to the (iii) C-terminal cytoplasmic domain. The latter module contains the transmitter domain featuring a dimerization and histidine phosphotransfer system (DHp) (Casino et al., 2009; Ferris et al., 2012; Grebe and Stock, 1999; Mascher et al., 2006b). The HK functions as the signal sensing antenna, autophosphorylating itself in trans or cis in response to a specific stimulus, and transferring the phosphate group to a conserved aspartate residue of its cognate RR partner (Bhate et al., 2015; Gao and Stock, 2009; Goulian, 2010; Hoch, 2000; Mascher et al., 2006b; Stock et al., 2000). The RR in turn receives the phosphate group and undergoes a conformational change allowing it to interact with its regulation target (Galperin, 2006; Gao et al., 2007; Goulian, 2010; Hoch, 2000; Stock et al., 2000). Canonical response regulators possess two defined domains connected by a linker region (Martinez-Hackert and Stock, 1997; Walthers et al., 2003). The N-terminal pole contains the receiver domain with a conserved aspartate residue (Asp) for the phosphotransfer from the phosphate donor (Galperin, 2006; Stock et al., 2000). When the Asp residue is phosphorylated a conformational change occurs in the C-terminal section of the RR, depicting an output domain, thus defining the final interaction partner for the regulation process (DNA, RNA or protein) (Barbieri et al., 2010; Gao et al., 2007; Gao and Stock, 2009; Kojetin et al., 2003). The system can work in a loop and can be reset by the dephosphorylation of the RR (Gao and Stock, 2009; Huynh and Stewart, 2011; Jung et al., 2012).