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Review

Natural transformation and genome evolution in *Streptococcus pneumoniae*

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ABSTRACT

Streptococcus pneumoniae is a frequent colonizer of the human nasopharynx that has the potential to cause severe infections such as pneumonia, bacteremia and meningitis. Despite considerable efforts to reduce the burden of pneumococcal disease, it continues to be a major public health problem. After the Second World War, antimicrobial therapy was introduced to fight pneumococcal infections, followed by the first effective vaccines more than half a century later. These clinical interventions generated a selection pressure that drove the evolution of vaccine-escape mutants and strains that were highly resistant against antibiotics. The remarkable ability of *S. pneumoniae* to acquire drug resistance and evade vaccine pressure is due to its recombination-mediated genetic plasticity. *S. pneumoniae* is competent for natural genetic transformation, a property that enables the pneumococcus to acquire new traits by taking up naked DNA from the environment and incorporating it into its genome through homologous recombination. In the present paper, we review current knowledge on pneumococcal transformation, and discuss how the pneumococcus uses this mechanism to adapt and survive under adverse and fluctuating conditions.

Contents

1. Introduction

The plasticity of bacterial genomes ensures rapid adaptation to changing environmental conditions and enables bacteria to colonize new niches successfully. Molecular mechanisms contributing to genome plasticity include point mutations, genome rearrangements, mobile genetic elements and horizontal gene transfer (Darmon and Leach, 2014). The mutation rate, which is a

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key regulator of adaptability, must strike a balance between genome plasticity and functionality. The optimal balance will at least partially depend on whether the bacterium lives under stable or stressful environmental conditions (Denamur and Matic, 2006; Fonville et al., 2011; Foster, 2007). Under acute antibiotic stress, for instance, hypermutable members of a bacterial population probably have a selective advantage (Gould et al., 2007; Morosini et al., 2003). Horizontal gene transfer (HGT) and recombination helps to disseminate and fix beneficial mutations in the bacterial population. HGT is also responsible for the dissemination of mobile genetic elements and the gain of new genetic information (Boto, 2010; Didelot and Maiden, 2010; Marri et al., 2006). Of the three known HGT mechanism operating in bacteria, natural genetic transformation has been studied in most detail in *Streptococcus pneumoniae*. Bacteria that are competent for natural transformation are able to take up exogenous DNA and incorporate it into their genomes by homologous recombination. Numerous studies have shown that this mechanism is crucial for genetic plasticity in *S. pneumoniae* (Chewapreecha et al., 2014; Croucher et al., 2011; Engelmoer et al., 2013; Hanage et al., 2009). In contrast, it is not known whether phage-mediated HGT is important for generating genetic variation in the pneumococcus. However, judging from the high number of lysogenic phages present in pneumococcal genomes, transduction may contribute significantly to the genetic diversity in this species (Loeffler and Fischetti, 2006; Ramirez et al., 1999; Romero et al., 2009). Several conjugative transposons have been discovered in *S. pneumoniae*. They often carry tetracycline and erythromycin resistance genes (Roberts and Mullany, 2009, 2011; Santagati et al., 2009). In fact, drug resistance determinants are more frequently found on conjugative transposons than on plasmids in the pneumococcus (Montanari et al., 2003).

In this review, we will focus on natural genetic transformation and its impact on genome evolution in the important human pathogen *S. pneumoniae*. Natural transformation has been extensively studied in this species, and the workings of the molecular machine constituting the transformation apparatus is now fairly well understood (for reviews, see Johnston et al., 2014a,b). However, much remains to be learned about the ecology of natural transformation, i.e. the conditions and mechanisms that control gene transfer *in vivo*.

2. Natural transformation in *S. pneumoniae*

Natural transformation was first discovered by Frederick Griffith in 1928 while studying the possibility of developing a vaccine against the pneumococcus (Griffith, 1928). The process was later exploited by Avery et al. (1944) to prove that DNA is the hereditary material. Since then, *S. pneumoniae* has been one of a handful of bacterial species that have served as model organisms for elucidating the molecular basis of natural transformation. In *S. pneumoniae*, the transformation process can be divided into four distinct steps. First, by monitoring internal and external signals the bacterial cell must make the decision to turn on the competent state. Second, to develop the competent state early and late competence proteins must be expressed. Third, competent pneumococci secrete a murein hydrolase that will lyse susceptible neighboring cells. Presumably, the purpose of this is to capture homologous DNA from other pneumococci or closely related streptococcal species sharing the same niche. Fourth, transcription of the competence genes is shut down in order to terminate the competence period. The details of each step are described in Fig. 1 and Sections 2.1–2.4.

2.1. Regulation of competence induction

The ABC-transporter ComAB and the three-component regulatory system ComCDE have a key role in the regulation of competence in *S. pneumoniae* (Håvarstein et al., 1995a; Hui and Morrison, 1991; Pestova et al., 1996). This quorum-sensing-like system functions as a biological switch that integrates a number of internal and external signals, some of which are still poorly characterized (Fig. 1). The *comDE* genes encode a membrane-spanning histidine kinase, ComD, and its cognate response regulator ComE. ComD is the receptor of the competence-stimulating peptide (CSP), which is encoded by the *comC* gene (Håvarstein et al., 1996). CSP is synthesized as a precursor peptide (ComC), which is processed into its mature form and secreted by the ComAB transporter (Håvarstein et al., 1995a,b; Hui and Morrison, 1991). Over the years, a large number of CSP peptides with different primary sequences have been discovered in streptococcal species belonging to the mitis and anginosus phylogenetic groups (Johnsborg et al., 2007; Kilian et al., 2008). Even within the species *S. pneumoniae* several different CSP types (pherotypes) have been identified. Binding of CSP to its associated ComD receptor will result in autophosphorylation of ComD and subsequent transfer of the phosphoryl group to ComE (Martin et al., 2013). ComE binds to a direct repeat motif present in the promoter region of all early competence genes (Ween et al., 1999). Experimental evidence indicates that phosphorylation of ComE promotes the formation of oligomers, and that ComE oligomerization is required for efficient transcriptional activation of the early competence genes. With the exception of the P_{comCDE} promoter, oligomerization does not seem to increase the affinity of ComE-P for its DNA target sequences (Boudes et al., 2014; Martin et al., 2013). Studies show that the *comCDE* genes are constitutively transcribed at a low level from at least two promoters. One of these is the P_{comCDE} promoter, which drives CSP-induced as well as basal ComCDE expression. In addition readthrough across the terminator of the upstream Arg-tRNA gene contributes to expression and maintenance of ComDE in non-competent cells (Martin et al., 2010). The ComABCDE-switch is constructed as an autocatalytic loop. Under competence-permissive conditions (see below), CSP is allowed to accumulate extracellularly. As the direct-repeat motif recognized by ComE-P is present in the promoters of the *comAB* and *comCDE* operons, extracellular CSP will activate the autocatalytic loop resulting in a rapid intracellular increase in ComE-P followed by induction of the competent state (Ween et al., 1999; Martin et al., 2013). The autocatalytic loop will also ensure that the extracellular concentration of CSP increases rapidly, thereby stimulating and coordinating competence development throughout the population. However, in a natural environment communication between pneumococci may be restricted by the specificity of the CSPs they produce. Hence, different pneumococcal strains might co-exist in multispecies biofilms in the nasopharynx without being able to communicate.

So, what are the competence-permissive conditions that allow CSP to accumulate until it triggers the autocatalytic loop that flips the switch? Interestingly, there is now increasing evidence that competence induction in *S. pneumoniae* is a response to some types of stress. Prudhomme et al. (2006) discovered that the antibiotics streptomycin and kanamycin as well as mitomycin C, a DNA-damaging agent, induce natural transformation in the pneumococcus. Building on these results, Stevens et al. (2011) found that the competence-stimulating effect of the above-mentioned antibiotics is caused by their ability to increase the rate of decoding errors during translation. When translation fidelity is high the cell surface protease/chaperone HtrA represses competence development by degrading CSP. However, when accuracy is low, HtrA becomes saturated by misfolded proteins and CSP is allowed to accumulate (Fig. 1) (Cassone et al., 2012; Stevens et al., 2011). More recently, the same group reported that increasing the mutational load by deletion of *mutX*, *hexA* or *hexB* stimulates competence induction in *S. pneumoniae*, suggesting that repair of genetic damage might be the principal function of natural transformation in this species