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Lifestyle transitions and adaptive pathogenesis of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa acute and chronic infections are of great concern to human health, especially in hospital settings. It is currently assumed that *P. aeruginosa* has two antagonistic pathogenic strategies that parallel two different lifestyles; free-living cells are predominantly cytotoxic and induce an acute inflammatory reaction, while biofilm-forming communities cause refractory chronic infections. Recent findings suggest that the planktonic-to-sessile transition is a complex, reversible and overall dynamic differentiation process. Here, we examine how the Gac/Rsm regulatory cascade, a key player in this lifestyle switch, endows *P. aeruginosa* with both a permissive lifecycle in nature and flexible virulence strategy during infection.

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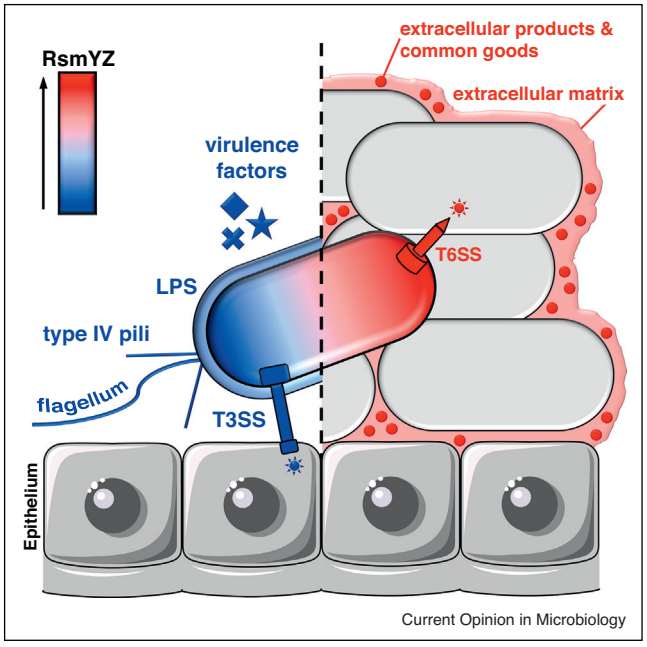
Introduction

Pseudomonas aeruginosa is an opportunistic human pathogen associated with a wide range of infections affecting, among others, skin, ear, eye, urinary tract, heart, airway and lung tissues [1]. The high frequency of *P. aeruginosa* strains causing nosocomial infections, the increasing occurrence of multi-drug resistant strains, and the adaptive antimicrobial resistance displayed by the bacterium during chronic infections, cause a severe threat to human health worldwide [2]. *P. aeruginosa* pathogenesis has been shown to be multifactorial and combinatorial [3]. Some virulence factors have been known for decades, while others have only been identified recently, thanks to whole genome sequencing of environmental and clinical isolates [4]. *P. aeruginosa* infections can be acute or chronic [5]. Interestingly, the type of infection is independent of the pathogen's genotype, but possibly linked to the host health status and the lifestyle adopted by the bacteria when colonising the host [6]. Acute infections are predominantly associated with bacteria adopting a planktonic lifestyle, while biofilm-related functions (attachment, capsule components) play a major role in persistent infections. It has been proposed that a binary regulatory switch controls the transition between the two lifestyles and infection modes of *P. aeruginosa* [6]. This switch model predicts that biofilm-associated functions and virulence factors of acute infection cannot physiologically co-exist in the bacterium. However, recent studies on the formation of biofilms indicate that the lifestyle switch model might be a simplified and static view of a plastic cell differentiation process, where acute and chronic infectious traits can co-exist within a bacterial population. Here, we review the lifestyle/infection model with an emphasis on the role of the Gac/Rsm global gene regulatory network in ensuring behavioural plasticity in *P. aeruginosa*. We propose that it is indeed the permissive lifecycle of the bacterium which is key for its success in the environment as well as for its adaptive pathogenesis during host infections.

The planktonic-to-sessile switch model

The main elements and differences of the two *P. aeruginosa* lifestyles/infection modes are depicted in Figure 1, and are briefly described below. *P. aeruginosa* acute lung infection (pneumonia), occurring mainly in immunocompromised or intensive-care patients, is initiated by bacteria binding at the mucosal barrier through two major adhesins, flagella and retractile type IV pili, which trigger a host inflammatory response [1]. Once in contact with the epithelial cells, bacteria cause significant tissue damage by translocating cytotoxic effectors directly into the eukaryotic cells via their type III secretion system (T3SS), and by secreting another set of virulence factors in the extracellular milieu [7]. The T3SS effectors are also involved in the inhibition of phagocytosis and, together with the LasB protease, eventually cause loss of the endothelial barrier integrity. The disruption of this barrier allows the pathogen to grow and spread rapidly within the host, sometimes resulting in septicaemia [7]. Overall, the process of acute infection leads to a massive mobilisation

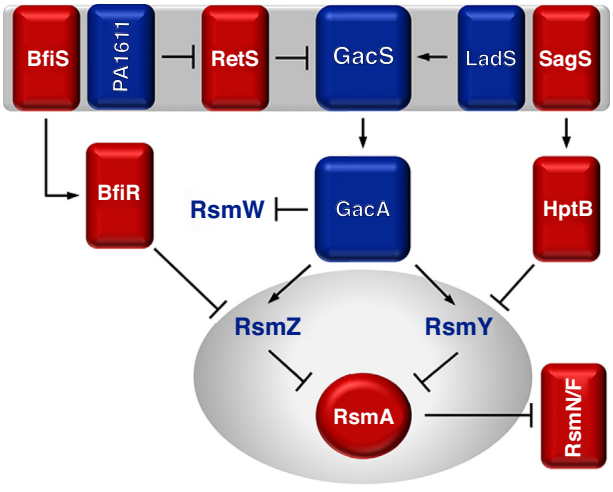
Figure 1



Schematic representation of the lifestyle switch model in *P. aeruginosa*. The levels of the small regulatory RNAs, RsmY/Z, are a major determinant for this transition. When the levels of these regulators are low (blue, left), *P. aeruginosa* cells have a planktonic lifestyle, causing acute infection. Motility, through the expression of type IV pili and flagella, along with the presence of LPS and the release of diffusible virulence factors or T3SS effectors, are crucial in this lifestyle. When the levels of RsmY/Z are high (red/right), the bacterium adopts a sessile lifestyle, causing chronic infection. The extracellular matrix, which encapsulates large numbers of cells (biofilm) and allows productive access to extracellular products and 'common goods' (like siderophores, proteases, elastase, rhamnolipids and HCN), and the presence of the T6SS feature prominently in this lifestyle. During the switch from planktonic to biofilm lifestyle, the LPS is known to undergo reversible structure modifications; this represented by the presence of the classical form of the LPS on the left side of the figure (planktonic lifestyle) which is absent on the right side of the figure (biofilm lifestyle).

Box 1 The Gac/Rsm cascade

The Gac/Rsm global regulatory network comprises several regulatory components and signal transduction pathways, converging on the post-transcriptional regulator RsmA (associated figure). RsmA belongs to the CsrA/Rsm family of RNA-binding proteins which compete with ribosomes for binding to the 5′ untranslated regions of mRNA targets, and possibly affect their stability [32]. Therefore, the main action of RsmA is to repress the translation of target genes, although it has been reported that sometimes it also exerts a positive control on gene expression, either directly or indirectly. In addition to RsmA, the backbone of the Gac/Rsm signalling pathway is composed of the GacS/GacA two-component system, which in *P. aeruginosa* induces the transcription of the genes *rsmY* and *rsmZ* encoding small RNAs. The latter, are regulatory RNAs which have multiple RsmA-binding motifs (GGA motifs), exposed in stem-loops. Upon GacA activation, the small RNAs, abundantly transcribed, sequester RsmA and relieve the target mRNAs from its control. The type of interaction of RsmA with the mRNAs and the resulting levels of RsmY/Z determine the output of the regulatory pathway. The regulatory action of proteins like the sensor kinases RetS, LadS, PA1611 and SagS, the BfiS/R two-component system and the HptB phosphotransfer protein also feed into the core of the Gac/Rsm pathway (grey shaded area) [36]. Finally, additional regulators, like the third RsmA-binding small RNA, RsmW, or the RsmN/F RNA-binding protein, have a supportive role in the absence of backbone components [30]. In the box-associated figure, the main Gac/Rsm regulatory components are illustrated together with their regulatory action (→ activation, ⊣ repression). Blue is used to indicate the components which, when absent, cause an impairment in biofilm formation, while deletion of genes encoding elements in red lead to hyperbiofilm forming strains.



of the immune system, starting with the recruitment of neutrophils and macrophages to the lungs. By contrast, bacteria involved in chronic infections are slow growing, less cytotoxic and immunogenic, and can persist in the host for decades without reaching the bloodstream. Chronic infections are common in the lungs of patients with cystic fibrosis (CF), primary ciliary dyskinesia and bronchiectasis [8]. In chronically infected CF lungs, clusters of *P. aeruginosa* are found encased in a polysaccharide matrix within a thick layer of mucus [9]. Common adaptation routes of *P. aeruginosa* in the CF lungs include the loss of major determinants of the planktonic cells, like motility or a functional T3SS, and the conversion to a mucoid colony phenotype. This has as a result that CF-adapted lineages are usually avirulent in mouse model of acute infection, but unhampered in their ability to establish chronic infections [10]. Altogether, these observations on the lifestyle of the bacterium led to the suggestion of a binary model of *P. aeruginosa* pathogenesis, in which cells in the planktonic state are equipped for the aggressive host-invasion strategy seen in acute infections, while biofilm-forming cells are pre-adapted for long-term persistence and immune evasion, as observed in the airways of chronically infected CF patients [6].

The identification and initial mapping of the Gac/Rsm global regulatory network (Box 1) substantiated this