Infectious diseases retain a prominent position as a major worldwide cause of morbidity and mortality. This problem has worsened with the emergence of multi-antibiotic resistant bacteria and the failure of the pharmaceutical industry to design antibiotics with novel modes of action. We therefore need both new concepts and new techniques in knowledge-based drug discovery. Inhibition of pathogenesis by targeting bacterial virulence represents a promising alternative for currently available antimicrobial therapies. A central requirement of bacterial virulence is the ability to express subsets of genes in response to signals that are specific for a particular environment. Therefore, inhibition of the regulatory systems controlling the expression of virulence genes will prevent bacterial virulence. Gene expression in bacteria is mainly controlled at the level of transcription initiation, the process in which a particular segment of DNA (gene) is copied into RNA (mRNA) by the enzyme RNA polymerase (RNAP). The RNAP recognizes a specific DNA sequence known as promoter, and modification of the promoter recognition by the RNAP is the first step in the regulation of gene expression. A dissociable sigma (σ) subunit that forms part of the RNAP is the subunit that confers promoter specificity to the enzyme (1). All bacteria contain a primary σ factor that controls expression of essential genes required for the general maintenance of the bacterial cells. In addition, most bacteria contain several alternative σ factors that activate expression of genes required only under specific circumstances (2). By modulating the use of primary and alternative σ factors, bacteria are able to adequately regulate general cell functions as well as the responses to specific signals. Therefore, the versatility and adaptability of bacteria is to a large degree reflected by the number of alternative σ factors they produce. The most abundant and diverse group of alternative σ factors is the so-called extracytoplasmic function σ factor (σ^{ECF}). The activity of σ^{ECF} is controlled by anti-σ factors that bind to and keep sequestered the σ^{ECF}, which is only released and activated in the presence of an inducing signal in the bacterial environment. Given their abundance and widespread nature, σ^{ECFs} are considered the third fundamental mechanism of bacterial signal transduction (3). However, since σ^{ECF} were discovered only 20 years ago (4), many aspects of the σ^{ECF} regulation are still poorly understood. With a main focus on the human pathogen *Pseudomonas aeruginosa* as a model, we have analysed σ^{ECF} regulation in this project. *P. aeruginosa* is an opportunistic pathogen of high clinical relevance since it causes severe hospital-acquired infections, especially in patients with cancer, cystic fibrosis and burn wounds, and its high degree of antibiotic resistance often makes the infections difficult to treat (5-7). This bacterium contains 19 σ^{ECF} that play a key role in the regulation of important bacterial processes such as stress responses, iron uptake and virulence (8). Most σ^{ECF}/anti-σ pairs of *P. aeruginosa* are associated with a surface-exposed receptor. Together, these three proteins form a signal transfer system known as cell-surface signalling (CSS). Presence of the CSS inducing signal in the bacterial environment (including the host during infection) is sensed by the receptor which transduces the signal to the anti-σ factor producing the activation of the σ^{ECF}. Upon activation, the σ^{ECF} can bind to the RNAP and initiates transcription of a specific set of genes, including in some cases virulence genes. Most *P. aeruginosa* CSS systems are expressed in iron-starvation conditions and control iron uptake funtions, which is a very important process during infection. The virulence of pathogens usually depends on their ability to get iron from the host, since one of the innate immune responses to fight an infection is to reduce the amount of iron available for invading microorganisms by for example increasing the synthesis of iron-scavenging proteins that chelate free iron (9, 10). To overcome this problem, pathogens produce and secrete compounds that have high affinity for iron (siderophores) and are also able to use host-iron complexes as source of iron (i.e. heme, haemoglobin, lactoferrin, transferrin) (11, 12). The transport of these

iron-containing compounds into the pathogen is often regulated by CSS. Moreover, *P. aeruginosa* contains at least two CSS directly involved in the regulation of virulence functions, including the PUMA3 system, which has been analysed within this project. For long time, activation of σ^{ECF} by CSS was thought to occur via conformational changes of the CSS proteins in response to the CSS signal. Importantly, the results obtained within this project indicate that such activation in fact occurs through a complex proteolytic cascade that processes the anti-σ factor component and liberates the σ^{ECF} (13-15). We have elucidated the molecular mechanism responsible for transduction of the signal from the bacterial surface to the cytosol via CSS, which has allowed us to refine the initial CSS model (8). These findings have considerably advanced the knowledge of how *P. aeruginosa* senses and responds to the environment and the host, and have provided new strategies to deregulate these circuits and therefore prevent or enhance important bacterial functions, including pathogenicity. Moreover, we have determined that another environmental signal, phosphate starvation, which is also encountered during the infection process, is necessary for expression of the PUMA3 CSS system of *P. aeruginosa* (16). The knowledge of how pathogens interact with the host can be exploited for the rational design of molecules targeting crucial host-pathogen interaction pathways. In fact, we have identified potential targets to inhibit such interactions, and therefore bacterial virulence. This has important clinical implications as it may significantly impact the way bacterial infections in general and *Pseudomonas* infections in particular can be treated in the future.

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