Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Microbiology

Role of *Pseudomonas aeruginosa* type III effectors in disease

Joanne Engel and Priya Balachandran

Pseudomonas aeruginosa uses a type III secretion system (T3SS) to directly inject four known effectors into host cells. ExoU is a potent cytotoxin with phospholipase A2 activity that causes rapid necrotic death in many cell types. The biological function of ExoY, an adenylate cyclase, remains incompletely defined. ExoS and ExoT are closely related bifunctional proteins with N-terminal GTPase activating protein (GAP) activity toward Rho family proteins and C-terminal ADP ribosylase (ADPRT) activity toward distinct and non-overlapping set of targets. While almost no strain encodes or secretes all four effectors, the commonly found combinations of ExoU/ExoT or ExoS/ExoT provides redundant and failsafe mechanisms to cause mucosal barrier injury, inhibit many arms of the innate immune response, and prevent wound repair.

Addresses
Departments of Microbiology/Immunology and Medicine, Microbial Pathogenesis and Host Defense Program, Box 0654 UCSF, 513 Parnassus Ave, San Francisco, CA 94143, United States

Corresponding author: Engel, Joanne (jengel@medicine.ucsf.edu)

Current Opinion in Microbiology 2009, **12**:61–66

This review comes from a themed issue on
Host-microbe interactions: bacteria
Edited by Brendan Kenny and Raphael Valdivia

Available online 23rd January 2009

1369-5274/$ – see front matter

DOI 10.1016/j.mib.2008.12.007

Introduction

Pseudomonas aeruginosa, a ubiquitous Gram negative pathogen widespread throughout the environment, is a leading cause of opportunistic infections in humans [1]. In normal hosts, with an intact epithelial barrier, *P. aeruginosa* rarely causes disease. However, in the setting of epithelial damage, as is seen in immunocompromised and/or hospitalized patients, *P. aeruginosa* is a common cause of nosocomial infections. Most of these are acute infections, including sepsis, ventilator-associated pneumonia, and infections in post-operative wound and burn patients. *P. aeruginosa* also chronically colonizes Cystic Fibrosis (CF) patients, leading to severe pulmonary damage and death. Despite treatment with appropriate antibiotics, mortality remains as high as 40% in acute infections, and multi-drug resistant isolates are increasingly reported.

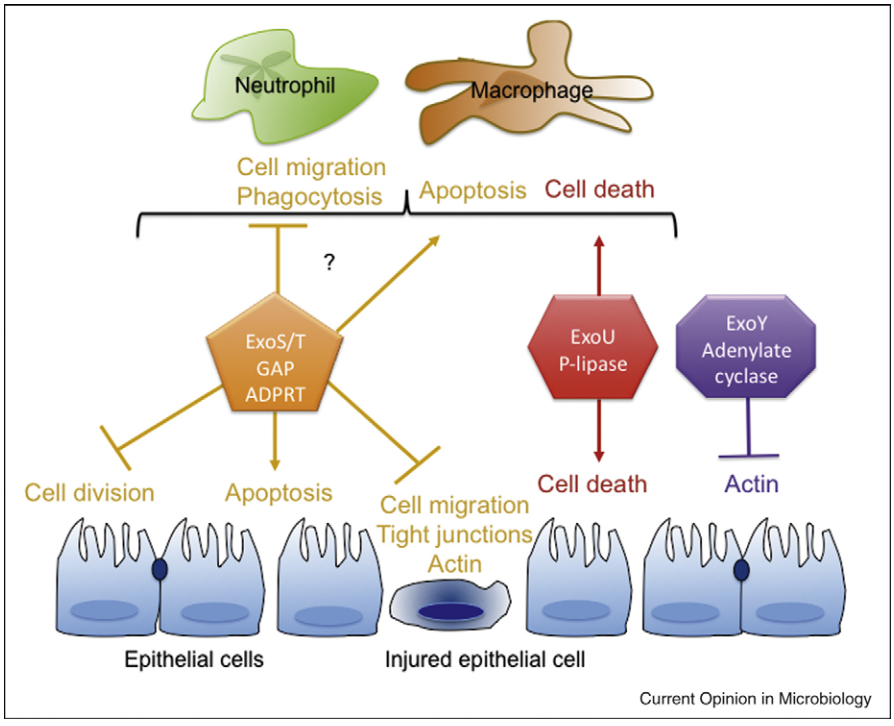
P. aeruginosa has a large armamentarium of secreted virulence factors that rely on specialized export systems, including the type I, II, III, V, and the recently discovered type VI secretion systems [2–4]. The type III secretion system (T3SS), a contact-dependent *sec*-independent protein secretion pathway that forms a conduit for the translocation of bacterial effectors into the host cell, is thought to play a key role in the pathogenesis of acute *P. aeruginosa* infections. The T3SS of *P. aeruginosa* contributes to epithelial cell and macrophage damage *in vitro*, in animal models of disease, and in human infections [5]. This review summarizes exciting progress in recent years in understanding the effectors and in placing these findings in the context of the pathogenesis of this important opportunistic pathogen of humans.

Four T3SS effectors have been identified in *P. aeruginosa*

In contrast to some organisms that encode a multitude of effectors, only four T3SS effector molecules have been identified in *P. aeruginosa* so far: ExoU, ExoS, ExoT, and ExoY (Figure 1). Additional T3SS effectors may be revealed as more strains are sequenced and further analyzed. ExoT and ExoY are encoded by almost all strains, though not all strains produce functional ExoY due to the presence of frameshift mutations. ExoS and ExoU are variably encoded genes and are almost never found in the same strain [5]. Although the T3SS system and its associated effectors were probably acquired by horizontal transmission, only ExoU and ExoS bear characteristic hallmarks of recent acquisition. ExoS is flanked by 10 base pair repeats that are only present as a single copy in non-ExoS-encoding strains, consistent with horizontal gene transfer. Interestingly, ExoS is more similar than ExoT to other bacterially encoded ADPRTs, suggesting that ExoT may have arisen from a gene duplication event. In this scenario, ExoS would have then undergone a deletion event in some strains. Four distinct configurations of the genome region containing ExoU have been identified by comparative sequencing, though no allelic variation was observed in the coding region of ExoU, consistent with relatively recent dissemination of this gene in natural populations. Further analysis suggests that this gene was probably acquired by transposition onto a transmissible plasmid followed by the transfer of the plasmid onto different strains and subsequent integration into the $tRNA^{lys}$ gene, where it acquired insertion sequences and underwent deletions and rearrangements [6].

Most strains examined so far can be divided into two groups. ExoU and ExoT producing strains (including

Figure 1



The T3SS effectors of *P. aeruginosa* have diverse and pleiotropic effects on host cell function. The light blue cells represent the polarized epithelial barrier; the dark blue cell represents an injured, depolarized cell at the epithelial barrier. A typical neutrophil is shown in green and a macrophage is shown in orange. The effects of the T3SS effectors are diagrammed. ExoS induces apoptosis, inhibits cell migration, disrupts tight junctions, and disrupts the actin cytoskeleton in epithelial cells (and probably endothelial cells). ExoT inhibits cell division and cell migration, disrupts focal adhesions, and can induce cell death. Both ExoS and ExoT inhibit bacterial uptake into epithelial and phagocytic cells. They may inhibit neutrophil and macrophage function as well. ExoS may also inhibit vesicular trafficking (not pictured). ExoU possesses phospholipase A2 activity, leading to rapid cell death in many cell types. Finally, ExoY is an adenylate cyclase that may disrupt the actin cytoskeleton. Together, these T3SS effectors efficiently inhibit wound repair and the host innate immune response, perpetuate tissue injury, and render the host susceptible to further colonization and injury by *P. aeruginosa* and other pathogens.

PA103 and PA14) cause rapid necrotic host cell death and are poorly internalized. By contrast, ExoS and ExoT producing strains (as exemplified by PAK and PA01) are more efficiently internalized and result in caspase-dependent host cell death with a slower time course. Interestingly, all four T3SS effectors require a host co-factor for activity; this attribute may prevent them from damaging bacteria before translocation. Both genotypes (ExoS/ExoT and ExoU/ExoT) are associated with acute infections in humans, though ExoU-producing strains are under-represented in persistently infected CF patients [7]. The expression of the T3SS is downregulated in CF isolates from chronically colonized CF patients, consistent with the notion that bacterial persistence requires the downregulation of many virulence factors [8].

ExoU, a potent phospholipase

ExoU possesses phospholipase A2-like activity with broad substrate specificity [9••]. The chaperone for ExoU, SpcU, is encoded in the adjacent gene. Superoxide dismutase (SOD) has been reported to function as the co-factor, though the enzymatic activity of SOD was not required [10]. Interestingly, upon translocation into the host, ExoU rapidly associates with the membrane, which may bring ExoU in close proximity to its substrate phospholipids [11,12]. ExoU has also been reported to be ubiquinated. This post-translational modification does not alter the degradation of ExoU, and its biological significance is as yet unclear [11].

ExoU production and activity are required for virulence in a mouse model of acute pneumonia and are associated with up to 90% of cases of severe disease in human infections [5]. In addition to its cytotoxic effects, ExoU triggers an arachidonic acid-dependent inflammatory cascade *in vivo* and induces expression of inflammatory genes [13–15]. ExoU is predicted to cleave surfactant, a key component of alveoli [16]. In human infections and in mouse models of pneumonia, ExoU may specifically target and kill neutrophils, leading to localized areas of immunosuppression that render the host susceptible to secondary infections, including viral infections [17•,18••].