RAS and ROS—A Story of *Pseudomonas* Survival

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Some pathogens block generation of reactive oxygen species to evade neutrophil killing, but how that is accomplished is poorly understood. In this issue of *Cell Host & Microbe*, Vareechon et al. (2017) describe ADP-ribosylation of Ras as a strategy to inhibit assembly of neutrophil NADPH oxidase.

Reactive oxygen species (ROS) generated by neutrophil respiratory burst represent a powerful deterrence against microbial invasion of the host and are a major tactical focus of successful pathogens. Microbes rely on two general strategies to combat ROS. They secrete molecular scavengers and antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase, glutathione-reductase, catalase, and peroxiredoxins to transform ROS into less toxic products. Additionally, several pathogens have been shown to disrupt assembly of the NADPH-oxidase complex to block ROS production, but how that is achieved is not well understood. In neutrophils, respiratory burst is initiated by Ras-induced activation of phosphoinositide 3-kinase γ (PI3K), which leads to the assembly of the NADPH-oxidase complex (Pacold et al., 2000). Specifically, activated PI3K leads to the phosphorylation of Akt and protein kinase C (PKC), which in turn phosphorylate the cytosolic components of the NADPH complex, $p47^{phox}$ and $p40^{phox}$ (Chen et al., 2003). These, along with $p67^{phox}$, translocate to the membrane, where they interact with activated Rac and $p22^{phox}$/$gp91^{phox}$ to form the active NADPH-oxidase complex required to generate ROS. In this issue of *Cell Host & Microbe*, Vareechon and colleagues report that *Pseudomonas aeruginosa* utilizes an effector injected through a type III secretion syringe to ADP-ribosylate Ras and block PI3K activation and downstream ROS production (Figure 1) (Vareechon et al., 2017).

Of four effector proteins secreted through the *Pseudomonas* type III secretion syringe, the authors showed that two effectors, ExoS and ExoT, independently block ROS production by neutrophils via their C-terminal ADP-ribosyltransferase domain. Consistent with this finding, a *P. aeruginosa* mutant with an inactivated ADP-ribosyltransferase domain in both ExoS and ExoT has reduced survival in a corneal infection model in wild-type (WT) mice but shows no survival defect compared to WT *P. aeruginosa* in $gp91^{phox-/-}$ mice. Furthermore, a type III secretion system null strain also shows a survival defect in WT but not $gp91^{phox-/-}$ mice compared to WT *P. aeruginosa*, suggesting that the type III secretion system promotes survival of *P. aeruginosa* primarily through ROS inhibition mediated by ExoS and ExoT.

Multiple and distinct targets of ExoT and ExoS have been reported, and the study focused primarily on interaction between ExoS and Ras. The authors showed that ExoS ADP-ribosylates Ras at arginine residue 41, and this appears to sterically hinder Ras interaction with PI3K and block downstream phosphorylation of Akt and $p40^{phox}$. Because of a report showing ADP-ribosylation of Ras slowing GDP/GTP exchange (Ganesan et al., 1999), the authors assessed the possibility that ExoS reduces the amount of active Ras but did not find alteration in the level of GTP-bound Ras. Additionally, ExoS and ExoT have been reported to induce neutrophil apoptosis and block phagocytosis (Frithz-Lindsten et al., 1997), but ROS inhibition under the relatively short assay condition is shown to be independent of these processes. To verify that ADP-ribosylation of Ras at arginine 41 is critical for inhibition of ROS production, the authors introduced into neutrophils a Ras protein with a mutation at arginine 41, which cannot be ADP-ribosylated by ExoS. Infection of those neutrophils leads to significant though not fully restored ROS production and the killing of *P. aeruginosa*. The authors suggested that incomplete restoration of ROS could be a consequence of endogenous Ras or ExoS interaction with other targets to block ROS, for example, binding to Ezrin-Radixin-Moesin proteins, which regulate phagosome maturation (Erwig et al., 2006).

Overall, the study strongly supports the role of ExoS in blocking ROS production by interfering with Ras activation of PI3K and provides insight on how the extracellular bacterium *P. aeruginosa* inhibits NADPH oxidase assembly. Infection with *Coxiella burnetii* or *Neisseria gonorrhoeae*, like *P. aeruginosa* infection, also decreases translocation of the cytoplasmic components $p47^{phox}$, $p67^{phox}$, and $p40^{phox}$ to the phagosome without altering membrane components of the NADPH complex (Siemsen et al., 2009; Smirnov et al., 2014). However, the cytoplasmic targets of these pathogens are not known. In contrast, *Salmonella* uses its pathogenicity island, SPI2, to stop the NADPH oxidase from trafficking to the *Salmonella*-containing vacuole specifically by removing membrane components of the complex, $gp91^{phox}$ and $p22^{phox}$ (Vazquez-Torres et al., 2000).

Comparing the various microbial strategies, blocking ROS production arguably would be more effective than neutralizing individual ROS, since ROS have functions other than inducing microbial damage, including protease release, LC3 recruitment supporting autophagy, apoptosis, and necrosis (Paiva and Bozza, 2014). Therefore, inhibiting ROS production could actually be a multifactorial approach for immune evasion. For several pathogens, ROS inhibition is used in addition to oxidant neutralization mechanisms, corroborating the utility of a multipronged approach to address the host defense. For example, in addition to ExoS and ExoT,

which independently and fully block ROS production in neutrophils, *P. aeruginosa* expresses catalase and superoxide dismutase. Notably, targeting of the PI3K pathway by *P. aeruginosa* is expected to lead to pleiotropic effects on host cells, since the pathway regulates various physiologic functions including survival, growth, immune functions, and intracellular trafficking. For extracellular bacteria, this strategy is presumed to be beneficial, since it has the potential to disrupt multiple cellular defenses without additional expenditure of energy. In comparison, *Salmonella* and presumably some intracellular pathogens rely on precise manipulation of host functions for intracellular survival and therefore likely target more specific host functions. Future studies of ROS inhibition by other pathogens should provide more insight on the various ways to inhibit ROS that are compatible with the lifestyle of the microbes during infection. Irrespective of strategies, finding that ExoS and ExoT have now been linked to an additional *Pseudomonas* virulence function makes these effectors even more compelling targets for antivirulence therapy.

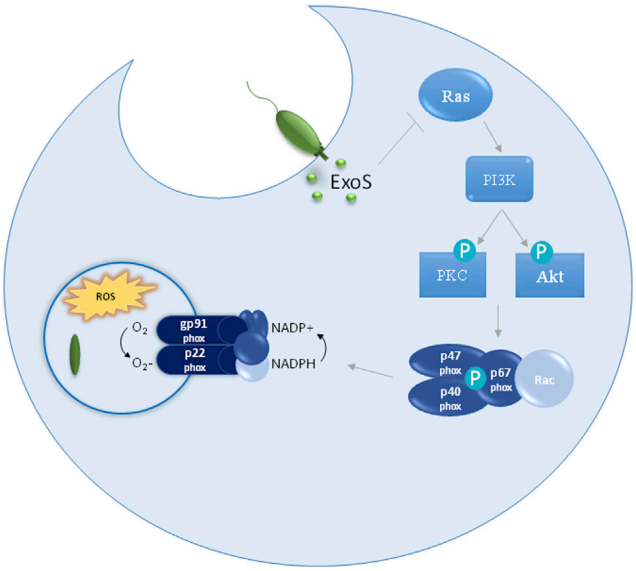

Figure 1. *Pseudomonas aeruginosa* ExoS Targets Ras to Inhibit NADPH-Oxidase Complex Assembly

P. aeruginosa uses a type three secretion syringe to inject effector proteins, including ExoS, to manipulate host cells. ExoS ADP-ribosylates Ras, which physically blocks its interaction with PI3K. This interferes with the activation of NADPH oxidase by Akt and PKC, which inhibits assembly and ultimately ROS production.

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Don't Bite the Hand that Feeds You

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Eukaryotic-bacterial symbioses are ubiquitous in nature. Pathogens and symbionts employ similar machinery, yet symbionts can minimize host damage. In this issue of *Cell Host & Microbe*, Enomoto et al. (2017) demonstrate how quorum sensing regulates expression of virulence genes at appropriate times, thereby enabling symbiont retention throughout the host lifespan.

Bacteria are involved in a multitude of animal relations ranging from pathogenic to mutualistic. This wide continuum of associations often employs common molecular mechanisms for host infection and colonization including the use of flagella, type III secretion systems, toxins, and ureases (Pérez-Brocal et al., 2013). However, why some bacteria inflict harm while others avoid hurting their hosts is mostly unknown. Much research is now dedicated toward understanding the symbiotic and commensal bacteria that live harmoniously within animals. These bacteria have gained recognition for their medical significance by contributing toward various aspects of host health: providing signaling crucial