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Spotlight

Game of Trans-Kingdom Effectors

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TplE, a type VI secreted (phospho) lipase, has been identified as the third trans-kingdom effector of *Pseudomonas aeruginosa*, targeting both prokaryotic and eukaryotic hosts. Indeed, TplE triggers the killing of bacterial competitors and promotes autophagy in epithelial cells once localized to the endoplasmic reticulum.

Type VI secretion systems (T6SSs) are present in more than 25% of Gram-negative bacteria, making T6SS one of the most widespread protein-secretion systems [1]. It functions as a molecular crossbow that mainly delivers toxic effectors into bacterial target cells to antagonize and eliminate competitors in close proximity [1]. Besides the benefits in the environmental habitat, bacteria can use a T6SS in the context of a eukaryotic host by two divergent routes [1]. On the one hand, T6SS can directly target eukaryotic cells through delivery of effectors into their cytosol, hijacking host cellular pathways or triggering toxicity. On the other hand, pathogens which are in close contact with the commensal microbiota while infecting a host, can attack or resist T6SS-dependent attack from commensal microbiota, in order to colonize and/or persist within the host [2,3]. Altogether, T6SSs are key new players in complex host–pathogen–microbiota interactions, through both anti-eukaryotic and antibacterial activities.

The T6SS was the first secretion system shown to mediate interactions with prokaryotic and eukaryotic hosts through dedicated and diverse toxins [1]. The case of *P. aeruginosa* is unique in this trait since three T6SS-dependent effectors are capable of targeting both types of host cells, making them the first examples of trans-kingdom effectors [4,5]. Besides being responsible for chronic lung colonization of patients with cystic fibrosis, *P. aeruginosa* is one of the leading causes of hospital-acquired infections. Its genome encodes three independent T6SSs. H1-T6SS is so far exclusively dedicated to antibacterial activity through injection of at least seven different effectors, whereas H2-T6SS and H3-T6SS have a dual role allowing targeting of both prokaryotic and eukaryotic cells [6]. Even though it is considered an extracellular pathogen, *P. aeruginosa* is able to actively invade nonphagocytic cells through injection of effectors PldA and VgrG2b via H2-T6SS [4,7] and PldB via H3-T6SS [4], among other factors. PldA and PldB bind the Akt kinase, whose activation together with the recruitment of the PI3K (phosphoinositide 3-kinase) pathway is further required for actin polymerization at the bacterial binding site leading to bacterial invasion [4]. VgrG2b promotes a microtubule-dependent internalization of *P. aeruginosa* through interaction with the γ-tubulin complex, demonstrating the spectacular ability of *P. aeruginosa* T6SSs to hijack host cellular functions to its own advantage [7].

In a recent report from Jiang and colleagues, TplE was identified as the third T6SS trans-kingdom effector of *P. aeruginosa* [5]. Similar to PldA and PldB, TplE (formerly called Tle4) belongs to the large antibacterial type VI lipase effector family (Tle) [8]. In agreement with this, its structure exhibits the characteristics of a canonical α/β-hydrolase fold [9]. Interestingly, Jiang and colleagues [5] demonstrated that TplE harbors lipase and phospholipase A1 activities *in vitro*. The authors first confirmed that TplE mediates antibacterial activity by producing TplE and a serine catalytic variant in the periplasm of *Escherichia coli*. Importantly, only wild-type TplE led to bacterial growth inhibition, while the catalytic variant of TplE was not toxic. As previously proposed [8], and consistent with a crab claw-like conformation of TplEi (formerly Tli4) [9], the product of the downstream gene *tplEi* (TplE immunity), neutralized the TplE toxicity. Indeed, to protect from self or sister-cell killing, bacteria produce immunity proteins to counteract their cognate T6SS antibacterial effectors. The authors further demonstrated that TplE lipolytic activity contributes to intra- and interspecies competition, once delivered into target bacteria via the H2-T6SS machinery, and that TplEi provides protection against TplE.

Furthermore, the role of TplE during interaction with eukaryotic target cells was also characterized in this report, by demonstrating the H2-T6SS-dependent translocation of TplE into epithelial cells upon infection with *P. aeruginosa* [5]. Interestingly, TplE contains a eukaryotic PGAP1 (post-GPI attachment to proteins 1)-like domain present in proteins localized at the endoplasmic reticulum. The targeting of TplE to this compartment was further demonstrated with a combination of *ex vivo* assays (transfection and infection). One can note that the enzymatic activity of TplE was not required for endoplasmic reticulum localization. Nevertheless this targeting led to endoplasmic reticulum disruption, which in this case, involves the lipolytic activity of TplE. The authors also observed that, in response to this endoplasmic reticulum stress, TplE activated the unfolded protein response via the XBP1 pathway and triggered autophagy, as shown by LC3 conversion and the increase of autophagosomes and autolysosomes. The integrity of the TplE active site was a determinant for these two processes. Autophagy is a conserved destructive process allowing degradation of unnecessary, dysfunctional or undesirable cellular components, including organelles (such as the endoplasmic reticulum) or intracellular pathogens. In the latter case, autophagy is considered an innate immunity defense mechanism of infected cells against invading bacteria, but intracellular pathogens have evolved mechanisms to modulate this host response to their

advantage. The autophagy response activated by *P. aeruginosa* highlights the transient but real intracellular lifestyle of this pathogen, still largely considered an extracellular pathogen.

Whereas the competitive advantage towards other bacteria conferred by TplE, PldA, and PldB is quite simple, how could their actions towards eukaryotic cells be integrated in a common model? PldA, PldB, and VgrG2b contribute to *P. aeruginosa* internalization into epithelial cells that can be viewed as a strategy to avoid innate immunity. However, once intracellular, the destiny of *P. aeruginosa* is still unclear. The pathogen may exploit and benefit from autophagy to acquire nutrients from endoplasmic reticulum degradation and thus promote its own intracellular replication similar to *Francisella tularensis* [10]. Consistent with this hypothesis, the H2-T6SS is not cytotoxic towards epithelial cells [4,7]. The use of autophagy modulators may thus be envisioned as a new therapeutic strategy in the fight against *P. aeruginosa* infections. The findings of Jiang and colleagues [5] provide new and important insights in the pathogenesis of *P. aeruginosa* that acts as a bacterial competitor as well as infecting eukaryotic cells in a game of trans-kingdom effectors. Moreover, because TplE, PldA, and PldB belong to the superfamily of type VI lipase effectors present in various pathogens [8], one can ask whether other trans-kingdom effectors may be discovered in the future.

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Spotlight

Color Me Infected: Painting Cellular Chromatin with a Viral Histone Mimic

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Viruses manipulate cellular chromatin to create a favourable milieu for infection. In several cases, virally-encoded proteins structurally mimic cellular histones to molecularly rewire the host cell. A recent study identified a novel mechanism whereby adenovirus protein VII, a viral histone, binds and manipulates host cell chromatin to suppress inflammatory signalling.

As obligate intracellular parasites, viruses often adopt features or functions of their hosts to gain an evolutionary advantage. Mimicry of host proteins by virally-encoded counterparts is a widespread strategy utilized to promote infection. One emerging paradigm of viral mimicry is the existence of viral proteins that functionally or structurally mimic cellular histones.

Histones are small basic proteins that are tightly associated with DNA in eukaryotic cells. They are the most abundant chromatin-associated proteins, and they functionally compact the genome into nucleosomes [1]. Histone post-translational modifications (PTMs) provide a mechanism for the control of gene expression, but additional regulation depends on other nonhistone, chromatin-associated proteins. Given its central role in nuclear function, chromatin is a common target for manipulation by viruses as they commandeer control of the infected cell.

Currently, only a few distinct examples of viral mimicry of cellular histones have been described. These include a virally-encoded histone H4 orthologue in the insect virus *Cotesia plutellae* bracovirus, which competes with endogenous cellular H4 for incorporation into chromatin [2], and a short orthologous sequence present in the NS1 protein of some influenza strains that mimics the N terminus of histone H3 and sequesters factors required for transcriptional elongation [3]. Interestingly, both of these examples demonstrate a recurring functional theme related to the suppression of antiviral inflammation.