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All in—Multiple parallel strategies for intracellular delivery by bacterial pathogens

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ABSTRACT

Microbial pathogens have developed intriguing molecular strategies to modulate and/or control host cell functions to ensure their own survival and replication. During this molecular interplay between microbes and their respective hosts especially secreted virulence factors play a major role. These factors not only include a plethora of cytotoxins but also sophisticated effector proteins targeting intracellular decision points leading to inhibition of defense responses - and/or even to cell death. To be effective, most of these secreted factors have to get across the cytoplasmic membrane and reach their targets in the cytoplasm. Apparently, pathogens use multiple mechanisms to deliver virulence factors to their cytoplasmic destination. Here, we exemplarily discuss the recently emerging scenario of parallel strategies for the intracellular deployment of toxins and effector proteins of Gram-negative pathogens with a special focus on pathogenic *Escherichia coli*. These pathogens employ various nanomachines such as the type III secretion system (T3SS), cell-penetrating effector proteins (CPE), and the wrapping of virulence factors in outer membrane vesicles (OMV) for protection and parallel delivery. As intracellular delivery remains a major problem in drug development, potential therapeutic applications based on these bacterial strategies will be briefly discussed.

1. Introduction

Enteropathogenic and enterohemorrhagic *Escherichia coli* (EPEC and EHEC), *Salmonella* and *Shigella* are major foodborne pathogens, which are responsible for severe outbreaks of diarrhea in adults and particularly in young children foremost in underdeveloped areas (Dos Santos et al., 2018; Eng et al., 2015; Frankel et al., 1998; Kotloff et al., 2018; Levine, 1987; Lima et al., 2015; Pearson et al., 2016; Santos and Finlay, 2015; Zhang et al., 2018). Shiga toxin-producing *E. coli* (STEC) or EHEC is the main cause of the hemorrhagic-uremic syndrome (HUS), which may lead to kidney failure in young children (Karch et al., 2006, 2012; Karpman et al., 2017). These pathogens use a plethora of secreted virulence factors to secure their survival by tampering with the host's immune system, thereby protecting their respective niches (Costa et al., 2015; Portaliou et al., 2016) (Fig. 1). For this process, pathogens have developed various ways to overcome the cytoplasmic membrane of host cells to reach their specific targets in the cytosol. In recent years the various secretion systems have attracted particular interest, as these represent intriguing nanomachines, which act by secreting and/or directly injecting virulence factors across the host's cell membrane into its cytosol (Chang et al., 2014; Costa et al., 2015; Galán and Waksman, 2018; Green and Mecsas, 2016; Walker et al., 2017; Xu and Liu, 2014; Zhuang et al., 2017). Of the seven currently known secretion systems (T1SS-T7SS), however, only the T3-, T4- and T6SS inject their effector proteins directly into the host cell's cytoplasm. T1- T2- and T5SS, and in some cases also T3SS (Chamekh et al., 2014; Shi et al., 2016; Walker et al., 2017), secrete their substrates into the extracellular space and therefore will not be discussed here. Despite intense research efforts the T7SS, first found in *Mycobacteriaceae* and later also in Gram-positive pathogens such as *Staphylococci*, has remained somewhat elusive and is just beginning to be unraveled (Abdallah et al., 2007; Xu and Liu, 2014). As there are quite a few recent excellent reviews covering the directly injecting T3-, T4- and T6- secretion systems, these will only be discussed briefly in this review (e.g. Alteri and Mobley, 2016; Costa et al., 2015; Grohmann et al., 2018; Ho et al., 2014; Journet and Cascales, 2016; Pinaud et al., 2018).

Recently, cell-penetrating bacterial effector proteins (CPE) have been identified as an innovative strategy to modulate and/or inhibit particularly immune signaling pathways in host cells – not only for a potential benefit of the microbes but also as a putative therapeutic application directed against overwhelming immune responses (Grabowski et al., 2017; Rüter and Hardwidge, 2014; Rüter and Schmidt, 2017). We will give a short overview of this exciting emerging topic.

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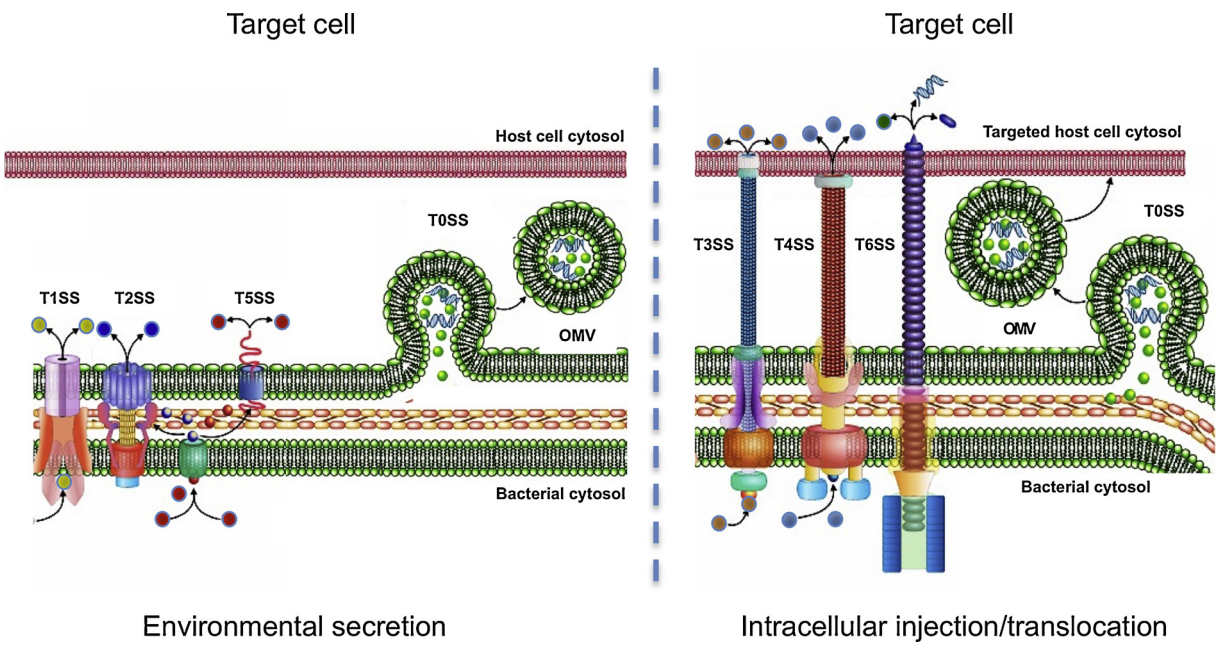

Fig. 1. Bacterial secretion systems targeted for environmental secretion (left) and intracellular secretion/ injection and delivery into target cells (right). OMV: outer membrane vesicles; TXSS: type x secretion system (modified according to Guerrero-Mandujano et al., 2017 with permission).

Furthermore, outer membrane vesicles (OMV) shed by Gram-negative pathogens including EPEC and EHEC but also probiotics such as *E. coli* Nissle 1917 have re-emerged in the focus of research due to their additional role in the protected secretion of packaged virulence factors into the cytoplasm of targeted host cells (Fig. 1).

Due to their remarkable characteristics these secretion systems are being investigated as possible delivery systems for biomedical applications and for the development of innovative therapeutic strategies (e.g. Bai et al., 2018; Grabowski et al., 2017; Rüter and Schmidt, 2017).

2. Injection of effector proteins by type III secretion systems (T3SSs)

The T3SS is a complex secretion system requiring more than twenty proteins for its assembly and correct function, which, mostly after cell contact and dependent on the particular species and strain, injects between six and more than thirty effector proteins into the targeted host cells (Tobe et al., 2006). However, due to its decisive importance for the virulence of a number of major Gram-negative pathogens such as EPEC, EHEC, *Yersinia*, *Shigella*, *Salmonella, Vibrio, Pseudomonas* etc., it is by far the most extensively studied and also the best understood bacterial secretion system (e.g. Burkinshaw and Strynadka, 2014; Connolly et al., 2015; De Nisco et al., 2018; Notti and Stebbins, 2016; Pinaud et al., 2018). As the T3SS has been reviewed extensively (e.g. Barison et al., 2013; Costa et al., 2015; Deng et al., 2017; Galán et al., 2014; Gaytán et al., 2016; Green and Mecsas, 2016; Hu et al., 2017; Izoré et al., 2011; Kendall, 2017; Portaliou et al., 2016; Xu and Liu, 2014) here only a few key characteristics will be mentioned. Representing a prominent virulence property of most Gram-negative pathogens, the T3SS has the ability to inject effector proteins directly into targeted host cells (e. g. Burkinshaw and Strynadka, 2014; Galán et al., 2014; Portaliou et al., 2017). Once inside host cells, these bacterial effector proteins shape different stages of infection and are particularly involved in the manipulation of inflammatory signaling pathways, thereby enabling bacteria to evade the host immune responses (Asrat et al., 2015; Santos and Finlay, 2015). Thus, the T3SS and its secreted effectors are often essential for full virulence of many bacterial pathogens (Coburn et al., 2007; Galán et al., 2014; Rapisarda and Fronzes, 2018). Structurally, T3SSs are very conserved and consist of a basal body, constituted by an inner and outer membrane ring, a periplasmic complex, and a filament, which, dependent on the species, might be rigid and rod-like as in *Shigella* or long and flexible as in EPEC/EHEC (Sekiya et al., 2001). This filament spans from the outer membrane of the bacterium to the eukaryotic cytoplasmic membrane and carries a translocator complex or' translocon' at the tip, which upon cell contact inserts into the host cell membrane and thus connects the bacterial cytoplasm with the cytoplasm of the targeted cell (Portaliou et al., 2017; Sheahan and Isberg, 2015). Although the components of the conserved T3SS nanomachine are very similar among different species, the number and function of the injected effector proteins or' toxins' differ widely. In recent years, the pool of bacterial T3SS-dependent effector proteins has been increasing steadily and currently the 'effectome' lists more than a thousand different effectors (Hu et al., 2017; Rüter and Schmidt, 2017). It appears that the injected effector proteins share similarities in targeting key cellular structures including intercellular junctions, the cytoskeleton, cellular trafficking, and, furthermore, major signaling pathways such as the NF-κB and MAPK pathways involved in immune responses. Interestingly, effectors often mimick host proteins in their functions (Stebbins and Galán, 2001). Altogether these assaults impede host responses, affect cell death and/or cellular homeostasis, and foster bacterial survival. In targeting essential signaling pathways, often at checkpoints or at several proteins in a given signaling cascade, injected effector proteins efficiently modulate/inhibit cellular responses (Pinaud et al., 2018; Zhuang et al., 2017). Interestingly, since various T3SS effectors from different species exhibit functional similarities and often act in concert, it is assumed that effector targeting in the host cells might have been optimized over time and their activities might be a product of convergent evolution (Galán and Waksman, 2018).

3. Type 4 secretion systems – T4SSs

The T4SSs are ancestrally related to bacterial conjugation systems and are hence unique, as besides proteins they are able to mediate also the translocation of DNA into the cytosol of prokaryotic and/or eukaryotic cells (Cascales and Christie, 2003; Christie et al., 2014; Christie, 2016; Costa et al., 2015; Green and Mecsas, 2016; Grohmann et al., 2018, 2017; Voth et al., 2012; Xu and Liu, 2014). T4SSs are versatile multiprotein nanomachines in Gram-negative and Gram-