

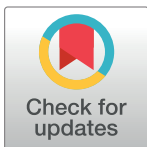
PEARLS

Bacterial secretion systems: Networks of pathogenic regulation and adaptation in mycobacteria and beyond

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Introduction

Bacteria transport proteins to diverse intracellular or extracellular locations to ensure proper function. Proteins are localized intracellularly to the cytoplasm or the periplasm or embedded in membranes. Extracellular proteins are localized to the bacterial surface, secreted into the environment, or directly transported into bacterial or host cells. Protein transport systems can be general or specialized. Recently, there have been several advances in our understanding of specialized protein secretion in pathogenic mycobacteria. Here, we will review broad themes across bacterial secretion systems, highlighting that, although gram-negative and Mycobacterial/Gram-positive specialized secretion systems are unrelated, they share conserved themes of regulation and environmental sensing.

1. Protein transport is essential for bacterial physiology

General secretion systems, including the Sec and twin arginine translocation (Tat) systems, are universal in bacteria. Sec and Tat systems export proteins across the cytoplasmic membrane [1]. Proteins exported into the periplasm by Sec or Tat can be secreted extracellularly by specialized secretion systems in a 2-step mechanism. Some specialized systems secrete protein substrates in a single step from the cytoplasm out of the cell, independently of Sec and Tat [2].

Gram-negative bacteria have 2 membranes (diderms). Proteins and solutes transit across both membranes [3]. Gram-negative specialized secretion systems include Type 1 to 6 and Type 8 to 10 systems that transport proteins across membranes [4–6]. Porins promote solute transport across the outer membrane (OM). Gram-negative bacteria have unique assembly machinery (β -barrel assembly machinery, BAM) that reside within and localize porins to the OM [7].

Mycobacteria are also diderms, but have a unique OM compared to gram-negative bacteria [8–10]. The mycobacterial OM (MOM) requires distinct mechanisms to maintain impermeability while allowing protein, metal, and solute transport. Mycobacteria do not have obvious BAM complexes, and the pathogenic species lack porins. Mycobacteria have Type VII specialized secretion systems (T7SS) [11,12]. Mycobacterial T7SSs (ESX or ESAT-6-systems) are genetically unrelated to specialized gram-negative systems. Interestingly, although gram-positive bacteria are monoderms, T7SS are widespread and are the topic of study in several species [13].

The T7SS that transits the cytoplasmic membrane has been well described [14–17]. The T7SS protein transporter spanning the MOM has not been identified. Similar to T3SS, T4SS and T6SS, several studies suggest that there are extracytoplasmic components of T7SS that

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may include known proteins transported by these systems. Recent work illustrated that PE/PPE proteins, which are known T7 substrates localized to the MOM, likely function in transport [18–20]. Several studies suggest PE/PPE proteins could function similar to BAM complexes for protein localization or like porins for solute transport, providing a bidirectional route of transport across the MOM [19–21]. Another family of T7-dependent secreted proteins, called WXG proteins [22], are also widely associated with ESX systems and may likewise play a role in protein transport. Accordingly, WXG proteins associated with T7SS are often required for the secretion of other substrates [11,23–26].

2. Specialized secretion systems drive pathogenesis in diverse ways

Secretion systems are a critical interface between host and bacterial pathogen. Some secretion systems promote adaptation to the host environment, while others promote immune evasion. Despite the functional diversity of secretion systems, many are structurally homologous [27]. Some pathogens encode for a single specialized secretion system that serves multiple roles required for pathogenesis. Type IV systems are classified into 2 distinct subtypes based on the type of substrate they secrete (DNA or protein). *Legionella* and *Coxiella* pathogenesis requires a single genomically encoded T4BSS which, through the secretion of numerous protein substrates, supports bacterial replication and causes host cell death [28–32]. This phenomenon appears to be specific to intracellular pathogens with the T4BSS, as T4ASS promote nonpathogenic processes including conjugation [33]. However, in the absence of a functional T4BSS, the T4ASS in *Legionella* is sufficient for pathogenesis under conditions mimicking aquatic environments [34]. Other bacteria have multiple copies of a single type of secretion system encoded in the genome or on plasmids. Each secretion system serves a specific role during infection. For example, *Salmonella* has 2 T3SS that mediate bacterial invasion and intracellular survival, respectively [35–38]. Pathogenic *Mycobacterium* have up to 5 genome encoded T7SS, each with discrete roles in phagosomal escape, phagosomal repair, and macrophage toxification [23,24,39–43].

3. Some specialized secretion systems are essential

While many specialized secretion systems are required for survival in the host, some specialized secretion systems are also essential for general bacterial physiology. Several mycobacterial T7SS are also essential for mycobacterial survival during in vitro growth because they are required for iron uptake and maintaining envelope impermeability [19,42–46].

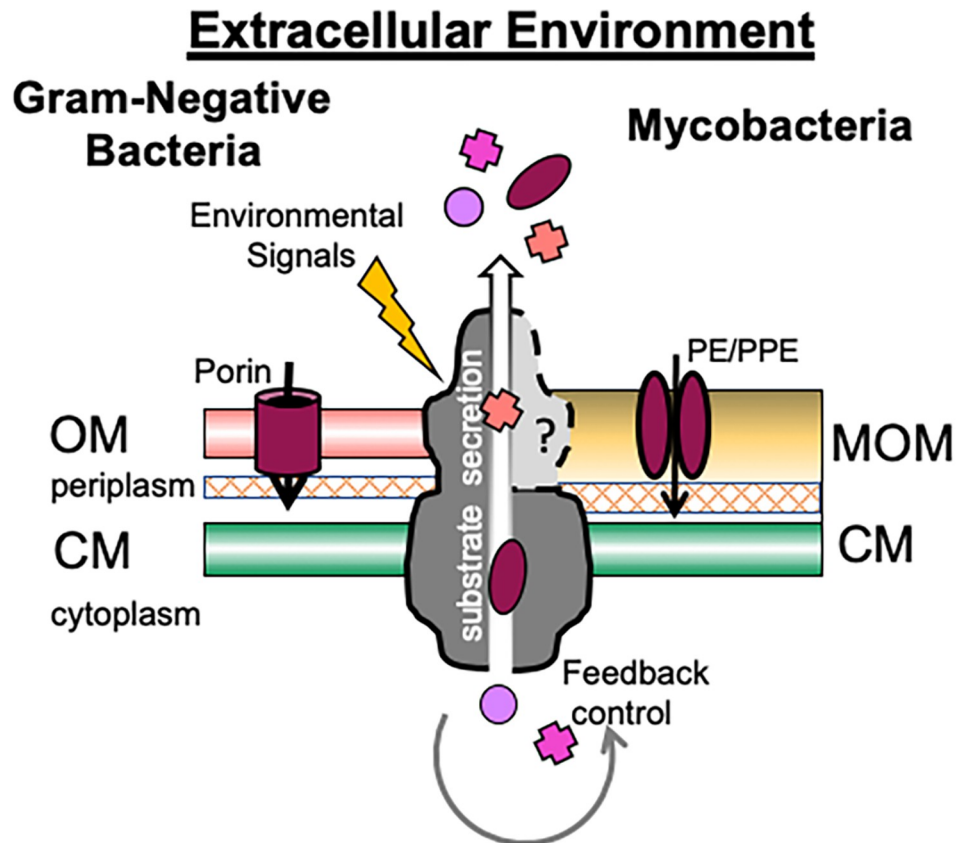
An opposing example of this phenomenon is the accessory Sec system, SecA2, of mycobacteria and some gram-positive bacteria [47]. The Sec system is essential for cell viability because it mediates extracytoplasmic protein localization [1]. The accessory system SecA2 is dispensable for bacterial viability in vitro but is essential for virulence [48]. Across the bacterial kingdom, secretion systems that are specialized or general, essential, and nonessential fill pathogenic and physiological niches necessary for survival.

4. Secretion is a controlled and regulated process

Although secretion systems differ in structure and function, they employ common regulatory themes at the molecular level. Protein transporters are made up of secretion system components that are transcriptionally regulated in gram-negative bacteria [49,50]. Likewise, networks of transcriptional regulation modulate the amount of substrate production. Transcriptional control of substrate levels for T3SS in several pathogens depends upon feedback regulation that responds to several intrinsic secretory cues including substrate concentration, localization, and protein transporter assembly or function [51–58]. Feedback regulation was recently

discovered in the T7SS (ESX-1) of pathogenic mycobacteria. At least 2 ESX-1-associated transcription factors control substrate gene expression in response to the presence or absence of a protein transporter in the cytoplasmic membrane [54–58]. These regulatory systems likely assure that appropriate levels of substrates are maintained during active secretion and prevent production and accumulation of protein substrates when there is no functional pathway for export [59].

In addition to feedback regulation, protein secretion systems, for example, T3SS and T7SS, are often controlled by extrinsic regulators including two-component systems and other transcription factors [53,60,61].



Common themes in sensing & regulation

- Some specialized systems are essential
- Some systems function at discrete points during infection
- Some secretion systems serve multiple pathogenic roles
- Secretion systems sense the status of components and substrates and trigger changes in gene expression

Fig 1. Conserved themes in protein secretion. Gram-negative bacteria and mycobacterial secretion systems also have conserved sensing and regulatory mechanisms (bulleted list). Secretion systems of gram-negative bacteria and mycobacteria both employ feedback regulation (gray arrow) to control the amount of substrates that are produced or secreted (gradient arrow). Some gram-negative bacteria sense and respond to their external environments (lightning bolt). Gram-negative bacteria use porins for solute transport, while mycobacteria use PE/PPE proteins for solute transport. No transporter has yet been identified in the MOM of mycobacteria. CM, cytoplasmic membrane; OM, outer membrane; MOM, mycolic acid outer membrane.

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Secretion systems are also controlled posttranslationally. For example, in Type III secretion injectisome systems (T3SS) of gram-negative pathogens, sequential transport of proteins—or “secretion hierarchy”—is well documented [62]. In hierarchical secretion, secreted components of the protein transporter and substrates are transported sequentially. Substrates are localized to the protein transporter via varying affinities to the transporter or secretion chaperones, which determines secretion hierarchy [62]. Disruptions in secretion hierarchy via loss of individual proteins from machinery or substrates results in a loss of translocon pore formation and a functional secretory apparatus. Work in mycobacteria has shown that loss of individual ESX-1 substrates results in a continuum of secretion defects [25], suggesting substrates may be secreted hierarchically.

5. Secretion systems sense and respond to their environments

Bacteria encounter and adapt to a variety of extracellular environments. Bacteria use secretion systems to sense and respond to specific extracellular stimuli that relay information to the bacteria about their environmental context. For example, Type VI secretion systems (T6SSs) in *Vibrio cholerae* sense and respond to the host environment, including bile acids [63]. Environmental sensing by T7SS of pathogenic mycobacteria has not yet been observed, but responses to environmental cues may explain the discrete spatiotemporal functions of ESX secretion systems during infection. Considering that T7SSs function in a variety of niches within the macrophage, it is likely that they are attuned to the distinct microenvironments of the phagosome and the cytoplasm. Indeed, the ESX-1 system functions in the phagosome to promote cytoplasmic access for pathogenic mycobacteria [64]. Since the ESX-1 system controls gene expression, this T7SS may indeed serve to sense and respond to cytoplasmic exposure [54,55,57].

Conclusions

Bacterial secretion systems serve a wide variety of functions. From general cellular processes to specialized pathogenic functions, these secretion systems are critical for pathogenic regulation and adaptation. Despite system conservation and structural similarities, these machines have slight variations in composition and regulation that result in unique signaling mechanisms, adding specificity to function (Fig 1). Each system is one component of a network of secretion systems that are intertwined and interdependent. The complexity of each system adds to bacterial adaptation and pathogenesis. Ultimately, secretion systems provide the bacteria with several inputs of environmental context, ensuring that environmental signals are integrated into the global network for adaptation, enhancing survival and pathogenesis.

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