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The Enigmatic Esx Proteins: Looking Beyond Mycobacteria

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Abstract

Bacteria export proteins across membranes using a range of transport machineries. Type VII secretion systems (T7SSs), originally described in mycobacteria, are now known to be widespread across diverse bacterial phyla. Recent studies have characterized secretion components and mechanisms of type VII secretion in pathogenic and environmental bacteria. A variety of functions have been attributed to T7SS substrates, including interactions with eukaryotes and with other bacteria. Here, we evaluate the growing body of knowledge on T7SSs, with focus on the non-mycobacterial systems, reviewing their phylogenetic distribution, structure and function in diverse settings.

Type VII Secretion: Discovery in Mycobacteria

Bacteria possess a range of transport mechanisms that export proteins across the cell envelope. Bacterial secretion systems that mediate protein translocation across the bi-layered Gram-negative cell envelope have been classified into six numbered types, Types I to VI [1]. In monoderm Gram-positive bacteria, the Sec, Tat and flagellar secretion systems were initially thought to account for all protein export, until the discovery of a new specialised secretion system, ESX-1, in *Mycobacterium tuberculosis.* This system first generated attention because it is responsible for the export of an abundant immunogenic protein, ESAT-6 (early secreted antigen target 6; also called EsxA) [2-4]. Additional substrates and components of ESX-1 have since been identified and characterized, including multiple EccC family ATPases [5], leading to the development of models that incorporate structure, function and regulation of this complex secretion apparatus [6, 7]. In addition to ESX-1, *M. tuberculosis* possesses paralogous ESX systems, ESX-2 to ESX-5, which have broadly similar features [5, 8]. The ESX-1 system plays an important role in *M. tuberculosis* virulence and a number of ESX-1 effectors interfere in host cellular and immune functions, primarily by modulating host macrophage and T cell responses [9]. However, the precise evolutionary and functional context for these activities remains uncertain.

The numerical nomenclature for secretion systems was initially restricted to Gram-negative bacteria. However, as it became clear that the mycobacterial cell envelope also possessed a diderm structure analogous to the Gram-negative cell wall, the nomenclature was extended to incorporate the

mycobacterial ESX systems, which were renamed type VII secretion systems (T7SSs) [10].

Shortly after the discovery of type VII secretion in mycobacteria, genome analyses suggested the existence of similar systems in diverse groups of Gram-positive bacteria [11]. However, these T7SSs appeared to be less complex than their mycobacterial counterparts, each with just a single ATPase from the EccC family that contains several FtsK/SpoIIIE domains, together with a handful of secretion substrates. These simpler systems were given the name type VIIb secretion systems to distinguish them from their more complex mycobacterial relatives [12, 13]. Mycobacterial type VII secretion has been reviewed extensively elsewhere [5, 13-15]. Here, we will focus instead on type VIIb secretion systems, summarising new developments in their phylogenetic distribution, structure and function.

Type VII Secretion Occurs Widely Outside the Mycobacteria

Analysis of the *M. tuberculosis* genome showed that EsxA is a member of a family of paralogous proteins [16]. A small-scale comparative genome analysis revealed that gene clusters encoding *esxA* homologues were also found in several close relatives of *M. tuberculosis* within the Actinobacteria, including *Corynebacterium diphtheriae* and *Streptomyces coelicolor* [8]. Predicted proteins representing EsxA homologues were subsequently identified in several members of the other predominantly Gram-positive phylum, the Firmicutes, including bacterial species with roles as pathogens, model organisms or in biotechnology, such as *Staphylococcus aureus,*

Bacillus anthracis, Streptococcus agalactiae, Bacillus subtilis (Figure 1)*, Rhodococcus equi,* and *Clostridium acetobutylicum* [11, 17-20]. As sequence conservation is centered on a WXG motif, and most of these EsxA homologs were around 100 amino acids in length, the term “WXG100 proteins” was coined to describe the family. In the Firmicutes, genes for WXG100 proteins were located in gene clusters that also encoded a conserved ATPase, which contained several FtsK/SpoIIIE domains and which was predicted to power type VII secretion.

In a later study, Jang *et al.* showed that an uncharacterized protein, Hp0062, from the Gram-negative pathogen, *Helicobacter pylori,* shared unexpected but unequivocal structural homology with WXG100 proteins from *M. tuberculosis* and *S. aureus* [21]. Subsequently, Sutcliffe examined the phylogenetic distribution of the WXG100 proteins and reported their occurrence in four phyla with a Gram-negative or diderm cell architecture, the Proteobacteria, the Cyanobacteria, Lentisphaerae, Verrucomicrobiae as well as in the monoderm phylum, Chloroflexi [22].

An up-to-date phylogenetic survey (Table 1) reveals the presence of WXG100 proteins in several additional bacterial phyla or sub-phyla (Table 1), including all five sub-divisions of the Proteobacteria (incorporating several important pathogenic species including *Pseudomonas aeruginosa*, *Vibrio cholerae* and *Stenotrophomonas maltophilia*) together with the Fusobacteria, the Planctomycetes and the Tenericutes.

Scrutiny of the entry for the WXG100 domain in the PFAM database (PF06013) provides a ready guide to the various protein architectures that house this feature (http://pfam.xfam.org/family/PF06013#tabview=tab1). In the majority (>96%) of cases, this domain occurs in a simple one-domain architecture, where the WXG100 domain occupies the entire protein. However, there are now several examples of this domain within a multi-domain protein, typically linked to an enzymatic domain. Most of these domains are linked to toxin-antitoxin systems (e.g. PT-TG, Toxin-deaminase, Colicin-DNase, Tox-HNH-EHHH, DUF2974, CHAP, Ntox44, and Ntox28). In many cases these domains appear to be important participants in conflicts between the cells that carry them and other cells. However, some of these enzymatic domains may play other roles: for example, the lytic transglycosylase activity of the PT-TG domain cleaves the cell wall peptidoglycan making space for the assembly of membrane spanning structures such as secretion systems and flagella [23]. Colicins and pyocins [24, 25] contain HNH domains with DNase activities, acting specifically against *Escherichia coli* and *P. aeruginosa* cells respectively, eliciting cell death through random cleavage of chromosomal DNA [26].The targets of these systems are diverse including action against eukaryotic hosts, against other bacterial species and against members of the same bacterial species fighting for a common niche [25, 27-31]. Additional evidence for a link between T7SSs and toxin-antitoxin systems comes from a wide-ranging bioinformatics survey that reports fusions of WXG100 domains to toxin domains and the presence of toxin and immunity proteins in many T7SS gene clusters [27].

Interestingly, a recent analysis from Das *et al.* [32] revealed an association of the putative EsxA homologue, HP_0062 [21], with an FtsK/SpoIIIE ATPase in at least 21 complete *H. pylori* genomes. Besides the typical WXG proteins, the cluster contains HP_0063, which has an HNH/colicin domain [27], as well as a protein (HP_0064) that belongs to the SUKH superfamily prototyped by the *Saccharomyces cerevisiae* protein Smi1/Knr4, which is responsible for conferring resistance to the killer toxin produced by competing yeast (Figure 1). Nevertheless, experimental evidence is required to corroborate the suggestions of putative T7SS from *H. pylori* and other Gram-negative bacteria.

The WXG100 proteins have been classified into three subfamilies based on their similarity to the heterodimeric mycobacterial proteins EsxA (ESAT-6) or EsxB (CFP-10), which are encoded in bicistronic operons [17, 33], or to the homodimeric non-mycobacterial type VIIb effector EsxA, which is typically encoded in a monocistronic operon [17, 34, 35]. A key conserved feature across the WXG100 protein families is a C-terminus consensus sequence HxxxD/ExxhxxxH, where ‘H’ is a highly conserved hydrophobic residue and ‘h’ is a less conserved hydrophobic residue [17]. In mycobacteria, a YxxxD/E motif is the C-terminal secretion signal required for general targeting of type VII substrates, including WXG100 proteins, and for the modulation of substrate binding and activity of a central ATPase [6, 36, 37]. Similarly, the C-terminal tail is important for secretion of type VIIb effectors in *S. aureus* and *B. subtilis* [35, 38, 39]. In *B. subtilis*, substrate export requires a composite

secretion signal formed by both the WXG100 and C-terminal motifs, each contributed by individual subunits of the substrate dimer [35]. Given the conservation of these motifs across type VII substrates, they likely play a crucial role in controlling type VIIb secretion [6].

Despite the now-abundant evidence from sequence-based analyses that type VII secretion occurs outside the mycobacteria, type VIIb secretion systems have been analysed experimentally only in a handful of bacterial genera, including *Staphylococcus* and *Bacillus*.

The Staphylococcus aureus T7SS

Among the type VIIb secretion systems, the best characterized is that of the opportunistic human pathogen, *S. aureus*. The system, termed Ess (for ESAT-6 secretion system), is required for virulence in murine abscess, pneumonia and skin infection models [40-42] and is linked to the establishment of persistent infection [40, 43]. The *ess* genes showed high levels of upregulation during chronic *S. aureus* infection in cystic fibrosis patients [44], suggesting a similar role in persistent infection in humans.

The Ess machinery comprises six core components [40, 41] (Figure 2), including one extracellular protein, one cytoplasmic protein and four membrane proteins. The most abundant component is EsxA, a secreted WXG100 protein that forms a homodimer [34, 41, 45]. EsxA displays a high degree of sequence conservation across *S. aureus* strains [45] and is required for the secretion of other Ess substrates [38, 40, 41], strongly

implicating it as a core extracellular component. The second soluble protein is EsaB, which harbours a ubiquitin-like fold [46] and appears to regulate the Ess machinery [41, 43].

The four key membrane proteins include EssA, EssB, EssC and EsaA. While EssA is required for substrate secretion, the requirement for EsaA appears to be strain-dependent [41, 43]. X-ray structures are available for soluble domains of EssB [47, 48] and EssC [6, 49]. EssB, has a cytoplasmic domain with a pseudokinase fold, but it lacks ATP-binding signature motifs and is unable to bind ATP-analogues [47].

EssC, the predicted FtsK/SpoIIIE-like motor protein, contains three C-terminal P-loop ATPase domains (D1-D3). Deletion analysis suggests that all three are essential for secretion [49]. Similarly, a transposon insertion in the N-terminal region of D1 abolishes EsxA and EsxB secretion, but curiously, transposon mutants with insertions in the last two domains remain capable of secretion [40]. The reason for this discrepancy is not clear.

Proteins of the FtsK/SpoIIIE family, including $EccC_1$, are known to form hexamers [6]. Although no structures have yet been obtained for the *S. aureus* EssC ATPase domains, homology modelling has provided fresh insights into this phenomenon. X-ray structures of the C-terminal P-loop domains (D2 and D3) have been obtained from the EccC homologue from *Geobacillus thermodenitrificans*. These structures show that D2 has ATP bound whereas D3 exists in two conformations; ATP binding is occluded in

one of these [6, 49]. Modelling the X-ray structure of the C-terminal domains of the *G. thermodentrificans* EssC on to the known hexameric structure of FtsK suggests that EssC possesses an internal pore (diameter~30 Å) wide enough to accommodate EsxA dimers [49].

EssC proteins from type VIIb secretion systems have tandem forkhead-associated (FHA) domains at their N-termini, unlike the related mycobacterial EccC proteins [49, 50]. FHA domains typically bind phospho-threonine peptides and are known to mediate phosphorylation-dependent protein–protein interactions [51]. However, no function has yet been assigned to the FHA domains in EssC, apart from contributing to overall protein stability [49]. *In vivo* crosslinking analysis indicated that Ess membrane proteins homo-multimerise [52] Although multimerisation of *Thermomonospora curvata* EccC is controlled by substrate interactions, EssC multimerisation was observed in a strain of *S. aureus* lacking core Ess components and substrates.[52]. Thus, even if these ATPases show significant sequence homology, there appear to be differences in the control of their assembly and activity.

Ess secretion has been studied in several *S. aureus* strains, including Newman [40, 43, 53], USA300 [38], RN6390 and COL [41]. Genomic analysis shows that the organisation of the *ess* loci in these strains is highly similar although there is some transcriptional heterogeneity between the strains [41]. Transcription of *esxA*, the first gene of the cluster, is under control of the alternative sigma factor σ^B [54]. EsxA is required for bacterial virulence and was recently shown to suppress host cell apoptosis during intracellular

infection [39, 41, 43]. In addition to EsxA, four substrates of the secretion system have been described: EsxB, EsxC, EsxD and EsaD.

EsaD is the only substrate to which a function has been assigned. Initial analysis of an *esaD* mutant strain [32] indicated that EsaD was an accessory factor required for the secretion of other Ess substrates. However, more recently it has been shown that EsaD is a large type VII-secreted anti-bacterial toxin, with a nuclease domain at its C-terminus that targets the DNA of sensitive strains of *S. aureus* [31]. Two further *ess*-encoded proteins are essential for EsaD biogenesis. EsaG is an EsaD anti-toxin, which binds to the nuclease domain of EsaD, protecting the producing cell from self-intoxication. Introduction of EsaG into a sensitive *S. aureus* strain offers significant protection against EsaD-mediated killing. A second protein, EsaE, binds to the non-nuclease part of EsaD and appears to target the EsaDG complex to the secretion machinery [31], analogous to the chaperone EspG that interacts with large PE/PPE substrate proteins in pathogenic mycobacteria [55, 56]. EsaG is probably stripped from EsaD during secretion as it is not detected in the supernatant, although co-secretion of EsaE with EsaD has been noted [31].

EsxB, EsxC and EsxD are all small secretion substrates, around 100 amino acids in length, but only EsxB belongs to the WXG100 family [38, 40, 43]. The functions of these proteins are not clear. The genes encoding EsxB and EsxC are required for virulence in a mouse abscess model. However, as deletion of

these genes affects secretion and/or stability of other substrates, it is not clear whether their requirement for virulence is direct or indirect [40, 43].

Analysis of 153 *S. aureus* genome sequences revealed that the *ess* locus falls into four distinct clusters [45], three of which do not encode EsxB, EsxC, EsxD or EsaD. Instead, each of these has a distinct set of genes downstream of the core *ess* genes, indicative of discrete repertoires of substrates. The complete repertoire of Ess-secretion substrates in the *S. aureus* pan-genome remains to be elucidated, but it is likely that substrates will include further antibacterial toxins and proteins that contribute to virulence.

Type VII secretion in Bacillus

Type VII systems are present in both pathogenic and non-pathogenic species of *Bacillus*. In the model organism *B. subtilis,* the core functional components of an ESX secretion system are encoded by the *yuk/yue* locus that contains *yukE, yukD, yukC, yukBA*, *yueB and yueC* (Figure 1) [57, 58]. YukBA is an FtsK/SpoIIIE family ATPase that mediates the export of YukE, a member of the WXG100 protein superfamily. YukE secretion and stability is dependent on phosphorylated DegU, the response regulator of the DegU-DegP two component system [57]. YukD is a small ubiquitin-like protein with a very short C-terminal tail [46]. YukD is homologous to the *S. aureus* EsaB, which is essential for secretion in some *S. aureus* strains [41, 43], while YukC is similar to the *S. aureus* structural protein EssB [59]. YueB is a surface protein that has been reported to accumulate at the bacterial poles, as observed for the mycobacterial ESX-1 proteins [60, 61]. YueB is essential for phage SPP1

binding and the *yuk* operon, particularly YukE, contributes to phage infection [59]. Recently, YueB was also implicated in conjugative DNA transfer [62].

Interestingly, unlike other type VII systems, YukE appears to be the only substrate associated with the *Bacillus* system. Although a second potential WXG100 substrate YfjA was identified in *B. subtilis* genome [10], there is no evidence that the associated protein is exported in a YukBA-dependent fashion [57, 58]. Utilizing this simple *Bacillus* system Sysoeva *et al* [35] demonstrated that YukE was secreted as a homodimer, and that the WXG100 motif and a C-terminal sequence are required for its secretion. Intriguingly, YukE was secreted as an intact dimeric complex and a bipartite signal formed by the two motifs, each contributed by individual subunits of the dimer, was required for its export [35]. As these motifs are typically present in type VII substrates, this novel mechanism of substrate recognition and protein translocation may be conserved across other type VII systems.

The ATPase domain requirements for substrate translocation in *Bacillus* were recently dissected in a study of domain-specific nucleotide binding and hydrolysis [63]. Only one of the three ATPase domains of YukBA was required for secretion in *Bacillus*, similar to that observed in ATPases from some *S. aureus* strains [40]. This is similar to the mycobacterial EccC ATPases such as the $EccC_5$, where only ATP binding to the D1 domain is necessary for secretion [64], but in contrast to the mycobacterial split ATPase $EccC_1$, where nucleotide binding is required at all three domains [6, 63].

In the pathogen *B. anthracis,* six WXG100 proteins were discovered by genome analysis: EsxB, EsxL, EsxP, EsxQ, EsxV and EsxW (Figure 1) [65]. EsxB and EsxW have been detected from culture filtrates *in vitro,* while EsxW, EsxB and EsxP are expressed only during guinea pig infection [65]. It is possible that *B. anthracis* Esx substrates are induced only under specific yet-to-be-identified conditions.

As with *S. aureus*, an FtsK SpoIIIE-type ATPase, designated *B. anthracis* EssC is required for EsxB secretion. Deletion of EsxB specifically affected secretion of EsxW, and yeast-2-hybrid analysis showed that the two proteins interact, suggesting that EsxB is required for the stability and secretion of EsxW (although not the other way around) [65]. The crystal structure of EsxB revealed a helix-loop-helix hairpin, which allows the protein to associate into two distinct helical bundles [66]. This structural duality may enable formation of homo and hetero-oligomeric helix bundles with other Esx substrates, supporting the idea that EsxB could serve as an adaptor for secretion.

Type VII Effectors and Host-Pathogen Interactions

Type VII effectors from bacteria with intracellular lifestyles, particularly mycobacteria, are adept at modulating host cell pathways. T7SS substrates can interfere with or induce cellular functions in numerous ways, enabling the bacterium to survive within the host [15] (Figure 3, Table 2).

Host Cell Modulation

The ESX-1 locus was originally discovered because loss of the locus was associated with loss of virulence in the attenuated vaccine strain BCG [67]. ESX-1 effectors are required for the virulence of *M. tuberculosis* and impact on several host functions including apoptosis, necrosis, induction of interferons, cytolysis and granuloma formation [68-70]. EsxA directly interacts with host cell membranes causing host cell lysis and phagosomal rupture, which then results in the translocation of bacterial DNA and initiation of cytosolic surveillance pathways leading to ubiquitination, autophagy and interferon responses [71-73] (Figure 3).

In the facultatively intracellular pathogen *S. aureus*, Ess is required for virulence and bacterial persistence during infection [40, 41, 43]. Interestingly, EsxA delays host cell apoptosis, and EsxA and EsxB together facilitate release of intracellular *S. aureus* from human epithelial cells [39]. While the cellular mechanisms underlying the modulation of cell death need further investigation and there are probably differences in the pathways targeted, it is intriguing that Esx proteins from two diverse pathogens, *S. aureus* and *M. tuberculosis* have been recruited to exploit the host cell as a niche for survival. By contrast, the ESAT-6 homologue, EsxA, from the intracellular pathogen, *Listeria monocytogenes* appears to play no role in intracellular survival or virulence [74]. Furthermore, a recent study reported that *L. monocytogenes* lacking EsxA demonstrated increased virulence, suggesting a detrimental effect of ESX-1 during infection [75].

Immune Modulation

The effectors of ESX-1, ESX-3 and ESX-5 are potent activators of CD4+ and CD8+T cells [76-78]. Type VII effectors such as EsxA and EsxH have become prime anti-tuberculosis vaccine candidates because of their strong immunogenic properties [78]. Similarly, the EsxA and EsxB proteins from *S. aureus* are being considered as vaccine candidates because they induce a strong protective antibody response in murine infection models [79]. However, it is not clear whether staphylococcal Esx proteins induce cell-mediated immune responses.

Bacterial Functions of Type VII Systems

The conservation of the T7SS across diverse phyla indicates that these proteins may play important roles in bacteria. The type VII effectors have been associated with a number of fundamental bacterial pathways, in both pathogenic and non-pathogenic species (Table 2, Figure 3).

DNA Transfer

Soon after its discovery, ESX-1 was shown to be essential for conjugative DNA transfer in the environmental mycobacterium, *Mycobacterium smegmatis* [80, 81]. ESX-1 effectors are thought to influence DNA transfer by chaperoning proteins specific to donors or recipients, thereby inhibiting or promoting donor-recipient cell contact. Remarkably, ESX-1 was recently shown to encode a switch in mating identity from recipient to donor in transconjugants. The mating identity (*mid*) locus was mapped within the *esx-1 (Ms0069-0071 and Ms0076-0078)* and was shown to have orthologs in other environmental mycobacteria [82]. ESX-1 may be required for DNA transfer

simply because it mediates secretion of the Mid proteins which dictate the mating identity. In *B. subtilis*, YueB, is also known to play a role in conjugative transfer of the plasmid pLS20. However, the core T7SS ATPase, YukBA, was not directly involved in DNA transfer [62]. The mechanism of YueB-mediated plasmid transfer remains unknown.

Metal Uptake

The mycobacterial ESX-3 T7SS has been implicated in the uptake of metal ions [83, 84]. In *M. smegmatis* and *M. tuberculosis*, expression of ESX-3 proteins is upregulated under iron- or zinc- depleted conditions. Although it is not clear precisely how ESX-3 effectors mediate iron uptake, they appear to influence uptake of mycobactins, molecules that bind iron, and deliver it to the mycobacterial cell [83]. ESX-3 was reported to be essential for growth in pathogenic mycobacteria, although a recent study suggests that these proteins are non-essential when iron is provided in an accessible form [85, 86]. However, some ESX-3 substrates may have iron-independent functions in virulence [86]. It remains unclear whether type VII secretion plays a role in metal uptake in non-mycobacterial systems.

Cell Envelope Integrity

Recent work has indicated that the type VII effectors localise to the capsule of the fish pathogen *Mycobacterium marinum* [87]. ESX-1 and ESX-5 effectors also have an impact on the integrity of the mycobacterial capsule and the stability of the cell wall [88, 89]. These studies suggest that T7SS has a key function in bacterial cell envelope homeostasis, a fundamental requirement for

bacterial survival. Such cell envelope maintenance functions have thus far been limited to mycobacteria.

Sporulation and Development

The type VII system of the plant pathogen *Streptomyces scabies* does not have a role in virulence, but rather surprisingly plays a role in development and sporulation [90]. Defective spore formation has also observed in T7SS effector mutants of the non-pathogenic *Streptomyces coelicolor* [91]. Interestingly, deletion of the ATPase (EccC) responsible for effector secretion does not produce similar phenotypes, suggesting an intracellular role for these effectors, perhaps in nucleoid restructuring, which could in turn influence the developmental programme [90]. Twinned with the role of Esx proteins in DNA transfer in mycobacteria, these observations suggest that regulation of DNA interactions may be a recurrent theme in type VII secretion. In *B. subtilis*, no sporulation defects have been reported for the *yuk/yue* deletion mutants. However, YukE is controlled by phosphorylation of DegU, a protein that accumulates in stationary phase and that has been associated with cell differentiation in bacterial populations [57, 92].

Interbacterial Competition

As described earlier, the EsaD nuclease produced by *S. aureus* serves to kill competitor bacteria, while the cells producing the toxin produce antitoxins to protect themselves [31]. Over half of the *S. aureus* strains analysed encode EsaD, suggesting that it plays an important role in the ecology of this species. In addition, the sequence of the nuclease domain is highly polymorphic,

consistent with a role in intra-species competition. *S. aureus* strains also accumulate *esaG* homologues in strains that do not encode *esaD*, suggesting a molecular arms race where strains accumulate anti-toxins to protect themselves from polymorphic EsaD variants [31]. Along with recent evidence from bioinformatics surveys, these observations suggest that interbacterial competition represents a function common to many T7SSs. It also highlights the parallels in versatility between type VI and type VII secretion, in that both systems are capable of targeting eukaryotes and bacteria.

Concluding Remarks

A decade or more after its discovery, type VII secretion represents a continuing, intriguing and important puzzle. Although there are meaningful homologies visible in the T7SS apparatus and effectors across huge phylogenetic distances, it has been hard to shoehorn their diverse features into a simple one-size-fits-all functional framework. They target membranes, proteins and DNA and have functions within and outside of the bacterial cell. They govern interactions between bacteria and their conspecific competitors, but also mediate host-pathogen interactions. They provide us with novel vaccine and drug targets, as well as new tools for diagnosis. In closing, it is worth stressing that much remains to be discovered (see Outstanding Questions), as so far we have characterised just a few of these systems in the laboratory. As bioinformatics surveys suggest, most of the vast landscape of type VII remains uncharted and untraveled.

Figure Legends

Figure 1. Distribution of WXG100 Domains, Key Components of Type VII Secretion. Genomic organization of the type VII secretion system clusters in selected species representative of Actinobacteria: *M. tuberculosis* & *S. coelicor*; Firmicutes: *S. aureus*, *L. monocytogenes*, *S. agalactiae*, *B. subtilis* & *B. anthracis*; Gammaproteobacteria: *H. pylori*. All genetic loci contain a member of the FtsK/SpoIIIE family (yellow) and at least one gene belonging to the WXG100 superfamily (red). Shown in blue are genes associated with toxin-antitoxin systems. Arrows represent direction of transcription and relative length of the genes.

Figure 2. The Staphylococcal Type VII Secretion System, Ess. A model showing the core Ess secretion machinery, substrates and accessory proteins. Membrane-associated proteins (blue), substrates (yellow), cytoplasmic proteins (green) and FtsK/SpoIII ATPase (red) are shown.

Figure 3. Biological Functions of T7SS. A model illustrating roles for T7SS in bacterial cellular functions, intra/inter-bacterial interactions and host-bacterial interactions.

Table 1. Distribution of WXG100 proteins in Diverse Bacteria[a]

Phylum or Subphylum	Species
Bacteroidetes	*Bacteroides cellulosilyticus , Bacteroides intestinalis, Prevotella bryantii*
Chloroflexa	*Chloroflexus* sp MS-G*, Roseiflexus castenholzii*
Cyanobacteria	*Arthrospira, Arthrospira maxima, Arthrospira platensis, Arthrospira sp. TJSD091, Cyanobacterium aponinum, Geminocystis herdmanii, Leptolyngbya sp. PCC 7376, Lyngbya sp. PCC 8106, Pseudanabaena biceps*
Fusobacteria	*Fusobacterium periodonticum*
Lentisphaerae	*Lentisphaera araneosa*
Planctomycetes	*Gemmata obscuriglobus, Gemmata sp. IIL30, Gemmata sp. SH-PL17, Isosphaera pallida, Phycisphaera mikurensis, Phycisphaerae bacterium SM23_30, Pirellula sp. SH-Sr6A, Pirellula staleyi, Pirellula staleyi DSM 6068, Planctomyces sp. SH-PL14, Planctomyces sp. SH-PL62, Rhodopirellula baltica WH47, Rhodopirellula europaea 6C, Rhodopirellula islandica, Rhodopirellula maiorica, Rhodopirellula sallentina, Rhodopirellula sp. SWK7, Schlesneria paludicola, Singulisphaera acidiphila, Zavarzinella formosa*
α-Proteobacteria	*Acetobacter persici*
β-Proteobacteria	*Kingella denitrificans, Delftia acidovorans, Bordetella petrii*
δ-Proteobacteria	*Desulfatibacillum aliphaticivorans*
ε-Proteobacteria	*Helicobacter bizzozeronii, Helicobacter felis, Helicobacter heilmannii, Helicobacter trogontum, Sulfurimonas gotlandica*
γ-Proteobacteria	*Aeromonas bestiarum, Alcanivorax pacificus, Halomonas hydrothermalis, Methylomonas methanica, Pseudomonas aeruginosa, Pseudomonas lundensis, Pseudomonas stutzeri, Pseudomonas taeanensis, Pseudomonas veronii, Stenotrophomonas maltophilia, Vibrio cholerae, Vibrio halioticoli*

[a]This tabulation draws on the taxonomic distribution of the WXG100 PFAM domain PF06013 and on PSI-BLAST searches of the NCBI non-redundant sequence databases performed with the EsxA and Hp0062 sequences from *M. tuberculosis* and *H. pylori.*

Table 2. Summary of Type VII Effectors and Their Functions[a]

Type VII Effector[a]	Species	Roles
EsxA	*M. tuberculosis* *M. marinum*	Cell membrane lysis [2], activation of interferon responses [69], T-cell activation [76], induction of cell apoptosis [70], virulence [3, 4]
	M smegmatis	DNA transfer [80]
	S. scabies *S. coelicolor*	Spore formation [90, 91], cell development [91], phage resistance [90]
	S. aureus	Modulation of host cell apoptosis [39], virulence [41, 43], protective antibody response [79]
	L. monocytogenes	Unknown
EsxB	*M. tuberculosis* *M. smegmatis*	Cell membrane lysis [2], virulence[3]
	S. scabies *S. coelicolor*	Spore formation [90, 91], cell development [91], phage resistance [90]
	S. aureus	Virulence [43], protective antibody response [79]
	B. anthracis	Unknown
EsxG	*M. tuberculosis*	Iron and zinc uptake [83, 84]
	M. smegmatis	Iron uptake [83]
EsxH	*M. tuberculosis*	Iron and zinc uptake [83], intracellular growth, phagosome maturation [93] T-cell immunogen
	M. smegmatis	Iron uptake [83]
EspA	*M. tuberculosis*	Virulence, cell wall integrity* [88]
PE19	*M. tuberculosis*	Virulence, cell wall permeability [77, 94]
PE5	*M. tuberculosis*	Iron uptake
PPE4	*M. tuberculosis*	Iron uptake
PPE-10	*M. marinum*	Capsule integrity [89]
EsaD	*S. aureus*	Interbacterial competition [31]
EsxC	*S. aureus*	Bacterial persistence [43]
EsxD	*S. aureus*	Unknown
YukE	*B. subtilis*	Unknown
EsxP	*B. anthracis*	Unknown
EsxW	*B. anthracis*	Unknown

[a] Mycobacterial substrates EsxM, EsxN, EspB, EspC, EspE, EspF, EspJ, EspK, PE15, PPE20, PE35, PPE68, PE25 and PPE41, all of unknown function, have not been included in this table.* EspA does not play a role in cell wall integrity in all *M. tuberculosis* strains [95]

References

1. Costa, T.R. et al. (2015) Secretion systems in Gram-negative bacteria: structural and mechanistic insights. Nat Rev Microbiol 13 (6), 343-59.
2. Hsu, T. et al. (2003) The primary mechanism of attenuation of bacillus Calmette-Guerin is a loss of secreted lytic function required for invasion of lung interstitial tissue. Proc Natl Acad Sci U S A 100 (21), 12420-5.
3. Stanley, S.A. et al. (2003) Acute infection and macrophage subversion by *Mycobacterium tuberculosis* require a specialized secretion system. Proc Natl Acad Sci U S A 100 (22), 13001-6.
4. Guinn, K.M. et al. (2004) Individual RD1-region genes are required for export of ESAT-6/CFP-10 and for virulence of *Mycobacterium tuberculosis*. Mol Microbiol 51 (2), 359-70.
5. Houben, E.N. et al. (2014) Take five - Type VII secretion systems of *Mycobacteria*. Biochim Biophys Acta 1843 (8), 1707-16.
6. Rosenberg, O.S. et al. (2015) Substrates Control Multimerization and Activation of the Multi-Domain ATPase Motor of Type VII Secretion. Cell 161 (3), 501-12.
7. Ohol, Y.M. et al. (2010) *Mycobacterium tuberculosis* MycP1 protease plays a dual role in regulation of ESX-1 secretion and virulence. Cell Host Microbe 7 (3), 210-20.
8. Gey Van Pittius, N.C. et al. (2001) The ESAT-6 gene cluster of *Mycobacterium tuberculosi*s and other high G+C Gram-positive bacteria. Genome Biol 2 (10), RESEARCH0044.
9. Stoop, E.J. et al. (2012) Tubercle bacilli rely on a type VII army for pathogenicity. Trends Microbiol 20 (10), 477-84.
10. Bitter, W. et al. (2009) Systematic genetic nomenclature for type VII secretion systems. PLoS Pathog 5 (10), e1000507.
11. Pallen, M.J. (2002) The ESAT-6/WXG100 superfamily -- and a new Gram-positive secretion system? Trends Microbiol 10 (5), 209-12.
12. Bottai, D. et al. (2016) Type VII Secretion Systems in Gram-Positive Bacteria. Curr Top Microbiol Immunol 10.1007/82_2015_5015.
13. Abdallah, A.M. et al. (2007) Type VII secretion--mycobacteria show the way. Nat Rev Microbiol 5 (11), 883-91.
14. Simeone, R. et al. (2009) ESX/type VII secretion systems and their role in host-pathogen interaction. Curr Opin Microbiol 12 (1), 4-10.
15. Groschel, M.I. et al. (2016) ESX secretion systems: mycobacterial evolution to counter host immunity. Nat Rev Microbiol 10.1038/nrmicro.2016.131.
16. Tekaia, F. et al. (1999) Analysis of the proteome of *Mycobacterium tuberculosis in silico*. Tuber Lung Dis 79 (6), 329-42.
17. Poulsen, C. et al. (2014) WXG100 protein superfamily consists of three subfamilies and exhibits an alpha-helical C-terminal conserved residue pattern. PLoS One 9 (2), e89313.
18. Callahan, B. et al. (2010) Conservation of structure and protein-protein interactions mediated by the secreted mycobacterial proteins EsxA, EsxB, and EspA. J Bacteriol 192 (1), 326-35.
19. Desvaux, M. et al. (2005) Genomic analysis of the protein secretion systems in *Clostridium acetobutylicum* ATCC 824. Biochim Biophys Acta 1745 (2), 223-53.

20. Letek, M. et al. (2010) The genome of a pathogenic rhodococcus: cooptive virulence underpinned by key gene acquisitions. PLoS Genet 6 (9), e1001145.
21. Jang, S.B. et al. (2009) Crystal structure of hypothetical protein HP0062 (O24902_HELPY) from *Helicobacter pylori* at 1.65 A resolution. J Biochem 146 (4), 535-40.
22. Sutcliffe, I.C. (2011) New insights into the distribution of WXG100 protein secretion systems. Antonie Van Leeuwenhoek 99 (2), 127-31.
23. Scheurwater, E. et al. (2008) Lytic transglycosylases: bacterial space-making autolysins. Int J Biochem Cell Biol 40 (4), 586-91.
24. Russell, A.B. et al. (2014) Type VI secretion system effectors: poisons with a purpose. Nat Rev Microbiol 12 (2), 137-48.
25. Cascales, E. et al. (2007) Colicin biology. Microbiol Mol Biol Rev 71 (1), 158-229.
26. van der Wal, F.J. et al. (1995) Bacteriocin release proteins: mode of action, structure, and biotechnological application. FEMS Microbiol Rev 17 (4), 381-99.
27. Zhang, D. et al. (2012) Polymorphic toxin systems: Comprehensive characterization of trafficking modes, processing, mechanisms of action, immunity and ecology using comparative genomics. Biol Direct 7, 18.
28. Russell, A.B. et al. (2011) Type VI secretion delivers bacteriolytic effectors to target cells. Nature 475 (7356), 343-7.
29. Holberger, L.E. et al. (2012) A novel family of toxin/antitoxin proteins in *Bacillus* species. FEBS Lett 586 (2), 132-6.
30. Christensen, G.J. et al. (2016) Antagonism between *Staphylococcus epidermidis* and *Propionibacterium acnes* and its genomic basis. BMC Genomics 17, 152.
31. Cao, Z. et al. (2016) The type VII secretion system of *Staphylococcus aureus* secretes a nuclease toxin that targets competitor bacteria. Nat Microbiol 2, 16183.
32. Das, C. et al. (2016) In silico dissection of Type VII Secretion System components across bacteria: New directions towards functional characterization. J Biosci 41 (1), 133-43.
33. Renshaw, P.S. et al. (2005) Structure and function of the complex formed by the tuberculosis virulence factors CFP-10 and ESAT-6. EMBO J 24 (14), 2491-8.
34. Sundaramoorthy, R. et al. (2008) Structure of *Staphylococcus aureus* EsxA suggests a contribution to virulence by action as a transport chaperone and/or adaptor protein. J Mol Biol 383 (3), 603-14.
35. Sysoeva, T.A. et al. (2014) Dimer recognition and secretion by the ESX secretion system in *Bacillus subtilis*. Proc Natl Acad Sci U S A 111 (21), 7653-8.
36. Daleke, M.H. et al. (2012) General secretion signal for the mycobacterial type VII secretion pathway. Proc Natl Acad Sci U S A 109 (28), 11342-7.
37. Champion, P.A. et al. (2006) C-terminal signal sequence promotes virulence factor secretion in *Mycobacterium tuberculosis*. Science 313 (5793), 1632-6.
38. Anderson, M. et al. (2013) Secretion of atypical protein substrates by the ESAT-6 secretion system of *Staphylococcus aureus*. Mol Microbiol 90 (4), 734-43.
39. Korea, C.G. et al. (2014) Staphylococcal Esx proteins modulate apoptosis and release of intracellular *Staphylococcus aureus* during infection in epithelial cells. Infect Immun 82 (10), 4144-53.

40. Burts, M.L. et al. (2005) EsxA and EsxB are secreted by an ESAT-6-like system that is required for the pathogenesis of *Staphylococcus aureus* infections. Proc Natl Acad Sci U S A 102 (4), 1169-74.
41. Kneuper, H. et al. (2014) Heterogeneity in ess transcriptional organization and variable contribution of the Ess/Type VII protein secretion system to virulence across closely related *Staphylocccus aureus* strains. Mol Microbiol 93 (5), 928-43.
42. Wang, Y. et al. (2016) Role of the ESAT-6 secretion system in virulence of the emerging community-associated *Staphylococcus aureus* lineage ST398. Sci Rep 6, 25163.
43. Burts, M.L. et al. (2008) EsaC substrate for the ESAT-6 secretion pathway and its role in persistent infections of *Staphylococcus aureus*. Mol Microbiol 69 (3), 736-46.
44. Windmuller, N. et al. (2015) Transcriptional adaptations during long-term persistence of *Staphylococcus aureus* in the airways of a cystic fibrosis patient. Int J Med Microbiol 305 (1), 38-46.
45. Warne, B. et al. (2016) The Ess/Type VII secretion system of *Staphylococcus aureus* shows unexpected genetic diversity. BMC Genomics 17, 222.
46. van den Ent, F. and Lowe, J. (2005) Crystal structure of the ubiquitin-like protein YukD from *Bacillus subtilis*. FEBS Lett 579 (17), 3837-41.
47. Zoltner, M. et al. (2013) Characterization of *Staphylococcus aureus* EssB, an integral membrane component of the Type VII secretion system: atomic resolution crystal structure of the cytoplasmic segment. Biochem J 449 (2), 469-77.
48. Zoltner, M. et al. (2013) The architecture of EssB, an integral membrane component of the type VII secretion system. Structure 21 (4), 595-603.
49. Zoltner, M. et al. (2016) EssC: domain structures inform on the elusive translocation channel in the Type VII secretion system. Biochem J 473 (13), 1941-52.
50. Tanaka, Y. et al. (2007) Crystal structure analysis reveals a novel forkhead-associated domain of ESAT-6 secretion system C protein in *Staphylococcus aureus*. Proteins 69 (3), 659-64.
51. Pallen, M. et al. (2002) Bacterial FHA domains: neglected players in the phospho-threonine signalling game? Trends Microbiol 10 (12), 556-63.
52. Jager, F. et al. (2016) Membrane interactions and self-association of components of the Ess/Type VII secretion system of *Staphylococcus aureus*. FEBS Lett 590 (3), 349-57.
53. Anderson, M. et al. (2011) EsaD, a Secretion Factor for the Ess Pathway in *Staphylococcus aureus*. J Bacteriol 193 (7), 1583-1589.
54. Schulthess, B. et al. (2012) Opposing roles of sigmaB and sigmaB-controlled SpoVG in the global regulation of esxA in *Staphylococcus aureus*. BMC Microbiol 12, 17.
55. Daleke, M.H. et al. (2012) Specific chaperones for the type VII protein secretion pathway. J Biol Chem 287 (38), 31939-47.
56. Ekiert, D.C. and Cox, J.S. (2014) Structure of a PE-PPE-EspG complex from *Mycobacterium tuberculosis* reveals molecular specificity of ESX protein secretion. Proc Natl Acad Sci U S A 111 (41), 14758-63.
57. Baptista, C. et al. (2013) High levels of DegU-P activate an Esat-6-like secretion system in *Bacillus subtilis*. PLoS One 8 (7), e67840.

58. Huppert, L.A. et al. (2014) The ESX system in *Bacillus subtilis* mediates protein secretion. PLoS One 9 (5), e96267.
59. Sao-Jose, C. et al. (2004) *Bacillus subtilis* operon encoding a membrane receptor for bacteriophage SPP1. J Bacteriol 186 (24), 8337-46.
60. Carlsson, F. et al. (2009) Polar localization of virulence-related Esx-1 secretion in mycobacteria. PLoS Pathog 5 (1), e1000285.
61. Jakutyte, L. et al. (2011) Bacteriophage infection in rod-shaped gram-positive bacteria: evidence for a preferential polar route for phage SPP1 entry in *Bacillus subtilis*. J Bacteriol 193 (18), 4893-903.
62. Rosch, T.C. et al. (2014) The presence of conjugative plasmid pLS20 affects global transcription of Its *Bacillus subtilis* host and confers beneficial stress resistance to cells. Appl Environ Microbiol 80 (4), 1349-58.
63. Ramsdell, T.L. et al. (2015) Linked domain architectures allow for specialization of function in the FtsK/SpoIIIE ATPases of ESX secretion systems. J Mol Biol 427 (5), 1119-32.
64. Ates, L.S. et al. (2015) Essential Role of the ESX-5 Secretion System in Outer Membrane Permeability of Pathogenic Mycobacteria. PLoS Genet 11 (5), e1005190.
65. Garufi, G. et al. (2008) ESAT-6-like protein secretion in *Bacillus anthracis*. J Bacteriol 190 (21), 7004-11.
66. Fan, Y. et al. (2015) EsxB, a secreted protein from *Bacillus anthracis* forms two distinct helical bundles. Protein Sci 24 (9), 1389-400.
67. Pym, A.S. et al. (2002) Loss of RD1 contributed to the attenuation of the live tuberculosis vaccines *Mycobacterium bovis* BCG and *Mycobacterium microti*. Mol Microbiol 46 (3), 709-17.
68. Davis, J.M. and Ramakrishnan, L. (2009) The role of the granuloma in expansion and dissemination of early tuberculous infection. Cell 136 (1), 37-49.
69. Stanley, S.A. et al. (2007) The Type I IFN response to infection with *Mycobacterium tuberculosis* requires ESX-1-mediated secretion and contributes to pathogenesis. J Immunol 178 (5), 3143-52.
70. Aguilo, J.I. et al. (2013) ESX-1-induced apoptosis is involved in cell-to-cell spread of *Mycobacterium tuberculosis*. Cell Microbiol 15 (12), 1994-2005.
71. Watson, R.O. et al. (2012) Extracellular *M. tuberculosis* DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. Cell 150 (4), 803-15.
72. Lerena, M.C. and Colombo, M.I. (2011) *Mycobacterium marinum* induces a marked LC3 recruitment to its containing phagosome that depends on a functional ESX-1 secretion system. Cell Microbiol 13 (6), 814-35.
73. Wassermann, R. et al. (2015) *Mycobacterium tuberculosis* Differentially Activates cGAS- and Inflammasome-Dependent Intracellular Immune Responses through ESX-1. Cell Host Microbe 17 (6), 799-810.
74. Way, S.S. and Wilson, C.B. (2005) The Mycobacterium tuberculosis ESAT-6 homologue in *Listeria monocytogenes* is dispensable for growth in vitro and in vivo. Infect Immun 73 (9), 6151-3.
75. Pinheiro, J. et al. (2016) *Listeria monocytogenes* encodes a functional ESX-1 secretion system whose expression is detrimental to in vivo infection. Virulence DOI: 10.1080/21505594.2016.1244589, 0.
76. Brodin, P. et al. (2006) Dissection of ESAT-6 system 1 of *Mycobacterium tuberculosis* and impact on immunogenicity and virulence. Infect Immun 74 (1), 88-98.

77. Sayes, F. et al. (2012) Strong immunogenicity and cross-reactivity of *Mycobacterium tuberculosis* ESX-5 type VII secretion: encoded PE-PPE proteins predicts vaccine potential. Cell Host Microbe 11 (4), 352-63.
78. Hoang, T. et al. (2013) ESAT-6 (EsxA) and TB10.4 (EsxH) based vaccines for pre- and post-exposure tuberculosis vaccination. PLoS One 8 (12), e80579.
79. Bagnoli, F. et al. (2015) Vaccine composition formulated with a novel TLR7-dependent adjuvant induces high and broad protection against *Staphylococcus aureus*. Proc Natl Acad Sci U S A 112 (12), 3680-5.
80. Flint, J.L. et al. (2004) The RD1 virulence locus of Mycobacterium tuberculosis regulates DNA transfer in *Mycobacterium smegmatis*. Proc Natl Acad Sci U S A 101 (34), 12598-603.
81. Derbyshire, K.M. and Gray, T.A. (2014) Distributive Conjugal Transfer: New Insights into Horizontal Gene Transfer and Genetic Exchange in Mycobacteria. Microbiol Spectr 2 (1).
82. Gray, T.A. et al. (2013) Distributive conjugal transfer in mycobacteria generates progeny with meiotic-like genome-wide mosaicism, allowing mapping of a mating identity locus. PLoS Biol 11 (7), e1001602.
83. Siegrist, M.S. et al. (2009) Mycobacterial Esx-3 is required for mycobactin-mediated iron acquisition. Proc Natl Acad Sci U S A 106 (44), 18792-7.
84. Serafini, A. et al. (2013) The ESX-3 secretion system is necessary for iron and zinc homeostasis in Mycobacterium tuberculosis. PLoS One 8 (10), e78351.
85. Sassetti, C.M. et al. (2003) Genes required for mycobacterial growth defined by high density mutagenesis. Mol Microbiol 48 (1), 77-84.
86. Tufariello, J.M. et al. (2016) Separable roles for *Mycobacterium tuberculosis* ESX-3 effectors in iron acquisition and virulence. Proc Natl Acad Sci U S A 113 (3), E348-57.
87. Sani, M. et al. (2010) Direct visualization by cryo-EM of the mycobacterial capsular layer: a labile structure containing ESX-1-secreted proteins. PLoS Pathog 6 (3), e1000794.
88. Garces, A. et al. (2010) EspA acts as a critical mediator of ESX1-dependent virulence in *Mycobacterium tuberculosis* by affecting bacterial cell wall integrity. PLoS Pathog 6 (6), e1000957.
89. Ates, L.S. et al. (2016) The ESX-5 System of Pathogenic Mycobacteria Is Involved In Capsule Integrity and Virulence through Its Substrate PPE10. PLoS Pathog 12 (6), e1005696.
90. Fyans, J.K. et al. (2013) The ESX/type VII secretion system modulates development, but not virulence, of the plant pathogen *Streptomyces scabies*. Mol Plant Pathol 14 (2), 119-30.
91. Akpe San Roman, S. et al. (2010) A heterodimer of EsxA and EsxB is involved in sporulation and is secreted by a type VII secretion system in *Streptomyces coelicolor*. Microbiology 156 (Pt 6), 1719-29.
92. Verhamme, D.T. et al. (2007) DegU co-ordinates multicellular behaviour exhibited by *Bacillus subtilis*. Mol Microbiol 65 (2), 554-68.
93. Mehra, A. et al. (2013) Mycobacterium tuberculosis type VII secreted effector EsxH targets host ESCRT to impair trafficking. PLoS Pathog 9 (10), e1003734.
94. Ramakrishnan, P. et al. (2016) *Mycobacterium tuberculosis* resists stress by regulating PE19 expression. Infect Immun 84 (3), 735-46.
95. Chen, J.M. et al. (2013) Phenotypic profiling of *Mycobacterium tuberculosis* EspA point mutants reveals that blockage of ESAT-6 and CFP-10 secretion *in*

vitro does not always correlate with attenuation of virulence. J Bacteriol 195 (24), 5421-30.

Figure 1

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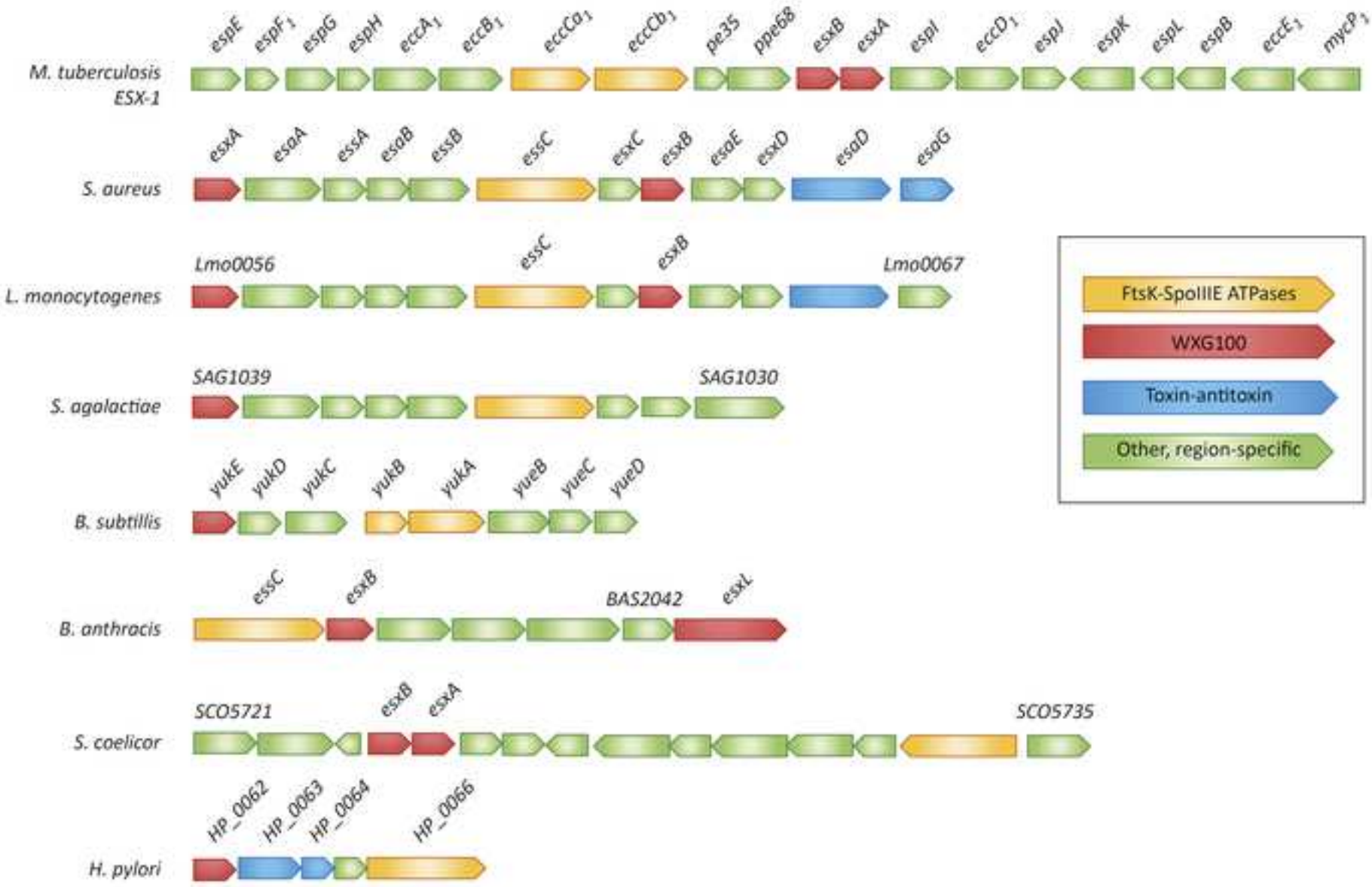

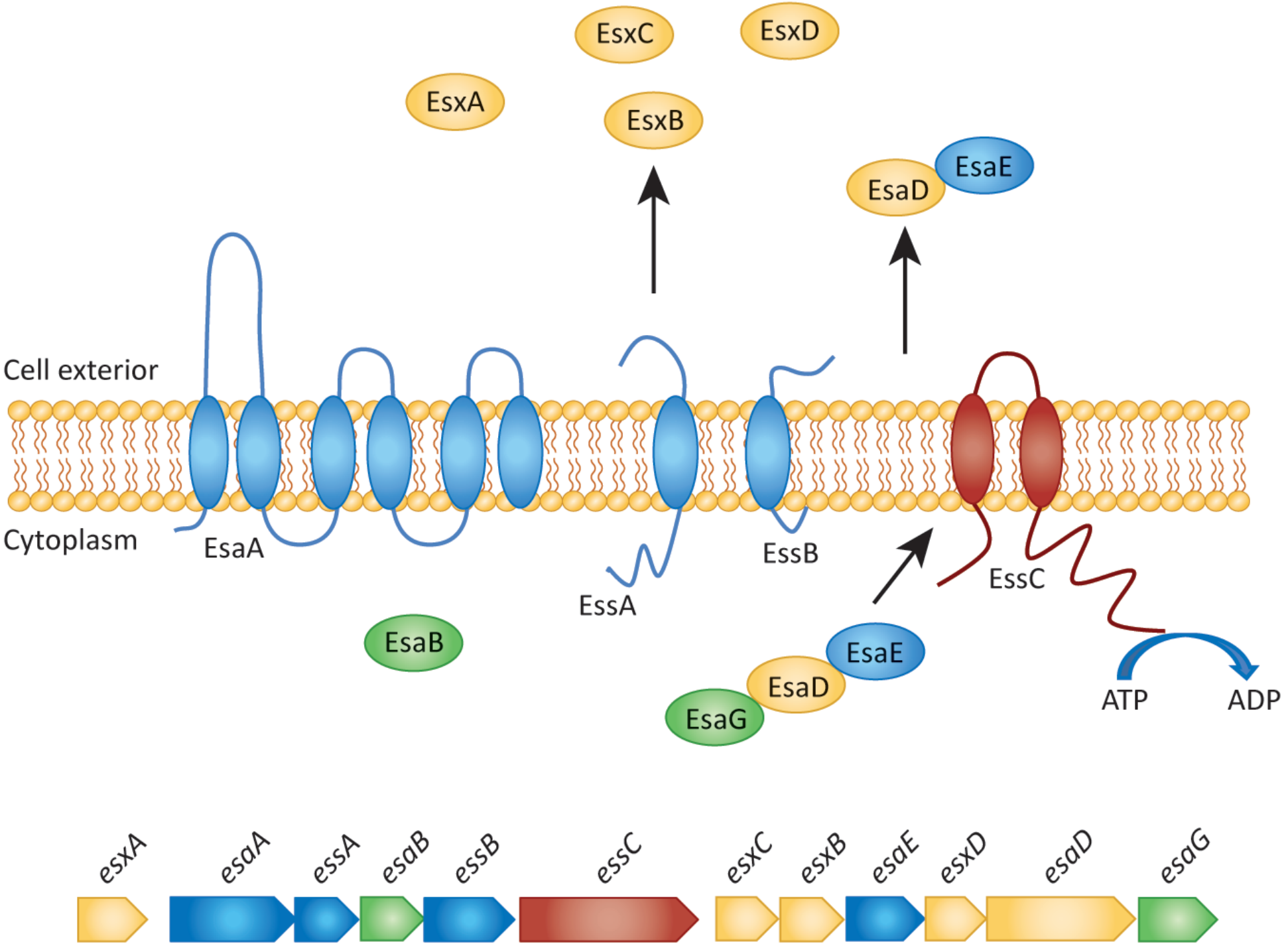
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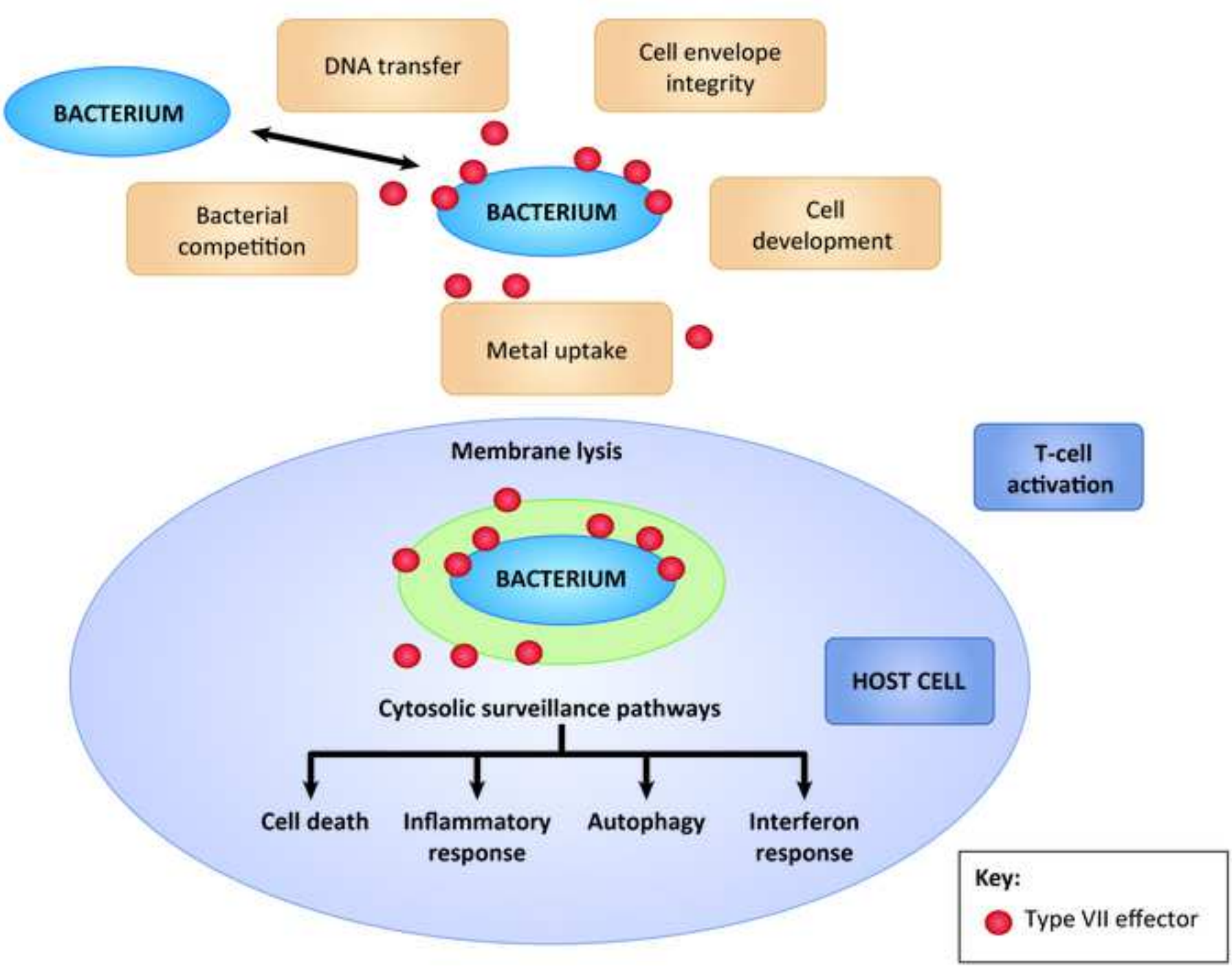
Figure 3



Trends

- T7SSs are more widespread among bacteria than previously thought; unlike other bacterial secretion systems, they are present across diverse bacterial phyla, in both Gram-positive and Gram-negative bacteria.

- Non-mycobacterial T7SSs have distinct secretion machinery and mechanisms of secretion compared to their mycobacterial counterparts.

- Bioinformatics surveys have highlighted an association between toxin-antitoxin modules and type VII secretion in a number of bacterial species and a recent report provides experimental evidence of a role in intra-bacterial interactions in *S. aureus.*

- Type VII effectors display a range of species-specific cellular functions, and can modulate interbacterial interactions as well as interactions between bacteria and eukaryotic cells.

Outstanding Questions

1. What is the structure of the T7SS apparatus?
2. What role does the T7SS play in Gram-negative bacteria? Are substrates targeted to the periplasm or are they exported from the cell? If they leave the cell, how do they cross the outer membrane?
3. Is there crosstalk between T7SSs and other secretion systems?
4. Are there common functions for the T7SSs across pathogenic and environmental bacteria?
5. How do the Esx toxin-antitoxin modules influence interactions within bacterial communities in the environment or during colonisation and infection?
6. Which host proteins do Esx proteins interact with during intracellular infection?
7. What is the role of staphylococcal T7SS effectors during persistent infection?