Type IV secretion in Gram-negative and Gram-positive bacteria

Elisabeth Grohmann,[1*] Peter J. Christie,[2] Gabriel Waksman,[3] Steffen Backert[4]*

[1]Beuth University of Applied Sciences Berlin, Life Sciences and Technology, D-13347 Berlin, Germany

[2]Department of Microbiology and Molecular Genetics, The University of Texas Medical School at Houston, 6431 Fannin St, Houston, Texas 77030, USA

[3]Institute of Structural and Molecular Biology, University College London and Birkbeck College, London WC1E 7HX, United Kingdom

[4]Friedrich Alexander University Erlangen-Nuremberg, Department of Biology, Division of Microbiology, Staudtstrasse 5, D-91058 Erlangen, Germany

*Corresponding authors: elisabeth.grohmann@beuth-hochschule.de and steffen.backert@fau.de

Key words: type IV secretion, T4SS, VirB/VirD4, coupling protein, T4CP, ATPase, NTPase, core structure, conjugation, pilus, mating pair formation, *oriT*, relaxase, relaxosome, transfer genes, transfer DNA, T-DNA, 3D-structure, CagA, Dot/Icm, vacuole, intracellular trafficking, anti-apoptosis, apoptosis, killer toxin, integrin, CEACAM, signaling, signal transduction

Abstract

Type IV secretion systems (T4SSs) are versatile multiprotein nanomachines spanning the entire bacterial cell envelope in Gram-negative and Gram-positive bacteria. They play important roles through the contact-dependent secretion of effector molecules into eukaryotic hosts and conjugative transfer of mobile DNA elements as well as contact-independent exchange of DNA with the extracellular milieu. In the last few years, many details on the molecular mechanisms of T4SSs have been elucidated. Exciting structures of T4SS complexes from *Escherichia coli* plasmids R388 and pKM101, *Helicobacter pylori* and *Legionella pneumophila* have been solved. The structure of the F-pilus was also reported and surprisingly revealed a filament composed of pilin subunits in 1:1 stoichiometry with phospholipid molecules. Many new T4SSs have been identified and characterized, underscoring the structural and functional diversity of this secretion superfamily. Complex regulatory circuits also have been shown to control T4SS machine production in response to host cell physiological status or a quorum of bacterial recipient cells in the vicinity. Here, we summarize recent advances in our knowledge of 'paradigmatic' and emerging systems, and further explore how new basic insights are aiding in the design of strategies aimed at suppressing T4SS functions in bacterial infections and spread of antimicrobial resistances.

Introduction

The T4SSs represent a highly diverse superfamily of secretion systems found in many bacterial species. This diversity is represented at a functional level by an astounding collective capacity of T4SSs to i) recognize and translocate single-stranded (ss) DNA substrates (conjugation machines) to bacterial recipients, ii) deliver effector proteins (effector translocator systems) to eukaryotic target cells, iii) exchange DNA with the milieu, iv) contribute to biofilm development, and v) deliver a killing toxin to bacterial neighbors (Figs. 1A-D). Many pathogenic bacteria deploy T4SSs as virulence determinants aiding their colonization and propagation in the eukaryotic host (Fig. 1E). Most if not all T4SS-carrying species alternatively utilize these machines to disseminate mobile genetic elements, often rife with antibiotic resistance genes and other fitness traits, for enhanced survival in clinical and other environmental settings. In this MicroReview, we summarize intriguing advances in studies of the evolution, structure, and function of T4SSs operating in various human pathogens. Our new insights form an important foundation for emerging translational studies aimed at suppressing the action of T4SSs in pathogenic bacteria or repurposing T4SSs for therapeutic ends.

Evolution of the structurally and functionally diverse T4SS superfamily

The T4SSs can be viewed as composite structures of two or more functional protein modules. The large subfamily of conjugation systems in Gram-negative (Gram$^-$) bacteria is composed of four such units: i) the relaxosome responsible for nicking DNA substrates at their origin of transfer (*oriT*) sequences, ii) the type IV coupling protein (T4CP) functioning as a substrate receptor, iii) the cell-envelope-spanning T4SS machine constituting the mating channel, and iv) an extracellular pilus important for establishment of intercellular contacts and robust biofilm development (Alvarez-Martinez and Christie, 2009; Christie, 2016). Over the last few years, phylogenetic studies have focused on delineating the ancestral relationships of several key components of these functional modules. For example, the relaxase enzymes in the relaxosome likely evolved from rolling circle replicases (Garcillan-Barcia *et al.*, 2009), whereas T4CPs and the VirB4 ATPase components of the T4SS channel evolved from ancestral SpoIIIE/FtsK-like ATPases (Guglielmini *et al.*, 2013; 2014). Mating channels likely functioned originally as protein transport systems and evolved as

conjugation systems through recognition by T4CPs of rolling circle replicases as substrates. By tracing the evolutionary paths of the signature T4CP and VirB4 ATPases, the conjugation systems are thought to have emerged first in the diderm ($Gram^-$) species and then expanded to the monoderm Gram-positive ($Gram^+$) species. These systems then diverged on a relatively recent evolutionary time scale to generate the extreme biological diversity of the T4SSs ranging from the widely distributed conjugation machines to dedicated effector translocator systems to various other systems adapted for entirely novel purposes (Bhatty *et al.*, 2013; Guglielmini *et al.*, 2013; 2014).

The T4SSs of $Gram^-$ bacteria have been classified into two broad phylogenetic subfamilies, designated as types IVA and IVB. The *Agrobacterium tumefaciens* VirB/VirD4 T4SS and *E. coli* conjugation apparatuses, encoded by the R388 and pKM101 plasmids, have served as paradigms of the type IVA systems (Chandran Darbari and Waksman, 2015; Christie, 2016). These T4SSs characteristically are composed of 12 subunits, each in multiple copies, termed VirB1 through VirB11 and VirD4 based on the *A. tumefaciens* subunit names as a unifying nomenclature for this secretion superfamily. Of these, VirB2-VirB11 and VirD4 are required for substrate transfer, whereas VirB1 is necessary and VirD4 is dispensable for assembly of the conjugative pilus. The subunits can be grouped according to general function or subcellular location as: i) the cytoplasmic ATPases (VirB4, VirB11, VirD4), ii) components of an inner membrane platform (VirB3, VirB6, VirB8), iii) constituents of an outer membrane core complex (OMCC; VirB7, VirB9, VirB10), and iv) pilus-assembly components (VirB1 transglycosylase, VirB2 pilin, VirB5 pilus-tip protein). As described in more detail below, recent structure - function studies are advancing our mechanistic knowledge of these 'paradigmatic' systems. Two other well-characterized systems, the T4SS encoded by *E. coli* F plasmids and the Cag (Cytotoxin-associated genes) T4SS encoded by *H. pylori*, are composed of orthologs by all the VirB/VirD4 proteins, and thus are classified as type IVA. However, these systems additionally require many F- and Cag-specific subunits for their assembly, and thus likely have novel structural and functional features (Backert *et al*., 2015; Christie, 2016). The type IVB transporters also require many (>25) proteins for their assembly, of which only a few are related to the VirB/VirD4 subunits and over 20 are specific for the IVB machineries. The *L. pneumophila* Dot/Icm (Defective for organelle

trafficking/Intracellular multiplication) system serves as a paradigm for this subfamily (Nagai and Kubori, 2011).

The T4SSs have evolved as highly-specialized nanomachines both in recognition of specific substrate repertoires and in delivery of substrates to specific prokaryotic or eukaryotic cell types. This specialization arose largely through appropriation by the ancestral conjugation systems of novel motifs, proteins or protein subassemblies (Christie, 2016). For example, there is accumulating evidence that many VirD4 substrate receptors have acquired sequence-variable C-terminal extensions (CTEs) that are capable of binding secretion chaperones or adaptors, often required for secretion of associated substrates. These VirD4 CTE - adaptor interactions play important roles in defining the substrate repertoire of cognate T4SSs. Additionally, the VirB6 subunits typically consist of five or more inner membrane-spanning helices, but a large subfamily of these subunits (called extended VirB6's) have acquired large hydrophilic domains shown to extend to the cell surface or into target cells to modulate the bacterial donor-target cell interaction. Similarly, the VirB7 and VirB10 subunits typically form part of the OMCC, yet variants of these subunits carry long variable repeat sequences implicated in specifying host cell recognition or immune evasion (Christie, 2016). Recent genomics studies also have identified redundant but sequence-variable copies of genes encoding VirB2 and VirB5 pilin subunits; these pilins also are thought to assemble as surface-variable structures enabling modulation of host cell binding or persistence in an infection setting (Alvarez-Martinez and Christie, 2009; Gillespie *et al.*, 2009; 2010; Vayssier-Taussat *et al.*, 2010). Finally, there also is increasing evidence that T4SSs have appropriated other bacterial host proteins, e.g., surface-exposed adhesins or outer membrane proteins (OMPs), to promote binding to other bacterial or eukaryotic cell targets as a prerequisite for interbacterial gene or interkingdom effector protein transfer (Bhatty *et al.*, 2015; Javaheri *et al.*, 2016; Königer *et al.*, 2016). This structural and functional diversity is especially evident among the T4SSs deployed by important human pathogens for effector translocation, as highlighted later in this MicroReview.

T4SS architecture and pilus formation

Over the last decade, there has been significant progress in deciphering the structures of T4SS subassemblies from conjugation machines functioning in *E. coli* and from T-DNA transfer in *A. tumefaciens*. These structures represent an architectural blueprint for the IVA transporters that, coupled with results of earlier formaldehyde crosslinking studies in the *A. tumefaciens* VirB/VirD4 system (Atmakuri *et al.*, 2004; Cascales and Christie, 2004), generate a view of how secretion substrates are conveyed through the T4SS to the cell surface. Until now the structures of three T4SSs have been reported, two of isolated machines and one in the native context of the bacterial cell envelope. The best-characterized structures to date have been presented for the Trw T4SS encoded by plasmid R388, achieved by negative-stain and cryo-electron microscopy (cryo-EM) imaging of isolated machines. The largest structure is designated the $VirB_{3-10}$ assembly because it is composed of homologs of the *A. tumefaciens* VirB3-VirB10 subunits (Low *et al*., 2014). This ~3 MegaDalton complex consists of a large outer membrane subassembly called the core complex (Fig. 1F). Core complex structures also have been presented for the plasmid pKM101-encoded T4SS at a high resolution and for the *A. tumefaciens* VirB/VirD4 T4SS at a lower resolution (Chandran *et al*., 2009; Fronzes *et al*., 2009; Rivera-Calzada *et al*., 2013; Gordon *et al.*, 2017). The R388 $VirB_{3-10}$ structure is additionally composed of an inner membrane complex (IMC) of extraordinary design and a slight flexible section (the stalk), connecting the core complex with the IMC (Trokter *et al*., 2014). The IMC is composed of 12 copies each of VirB3, VirB4, VirB5, VirB6, and VirB8, coming together to form a double-barreled structure, each of the barrels protruding in the cytoplasm. These barrel-shaped structures are each made of the VirB4 ATPase, observed here as trimers of VirB4 dimers. Cryo-EM of the pKM101 core complex identified a ring structure of 185 Å in diameter, comprising the VirB7, VirB9 and VirB10 proteins (Fig. 1G, top), each existent in 14 copies (Fronzes *et al*., 2009; Rivera-Calzada *et al*., 2013). In fact, this complex is composed of inner (I) and outer (O) layers. The O-layer is formed by VirB7 and the C-terminal domains of VirB9 and VirB10. The 2.6 Å resolution O-layer structure revealed that VirB10 forms the interior lining of the complex while VirB9/VirB7 forms a protective crown around it. Fourteen VirB10 subunits project each a helical bundle to form a highly unusual outer-membrane channel (Chandran *et al*., 2009). The cryo-EM assembly of

a truncated pKM101 core structure, lacking the N-terminus of VirB10 (Fig. 1G, bottom), was determined at 8.5 Å resolution and provided further details on the structure of the I-layer (Rivera-Calzada *et al*., 2013). The I-layer in the arrangement is composed of 14 VirB9 N-terminal domains and covers the outer wall. Molecular modeling supported the view that these domains represent β-sandwich folds. Remarkable projections from a middle platform tighten the channel, connecting the chambers in the O-layer and I-layer (Fig. 1G, bottom right). This podium is apparently formed by VirB9, with proposed function in effector molecule delivery across the core complex. In addition, three NTPases (VirB4, VirB11 and VirD4) function as hexamers (Yeo *et al*., 2000; Gomis-Rüth *et al*., 2001; Savvides *et al*., 2003; Hare *et al*., 2006; Wallden *et al*., 2012). They face the cytoplasm and are essential for substrate secretion. Two of these NTPases (VirB4 and VirB11) are also essential for extracellular pilus formation. T4SS-pili represent tube-like appendages (Eisenbrandt *et al*., 1999; Wang *et al*., 2009), and stimulate contact and subsequent mating pair formation with the recipient (Dürrenberger *et al*., 1991; Samuels *et al*., 2000; Hospenthal *et al*., 2017). However, the composition of these mating bridges is not fully explored. It appears that conjugative pili function as conduits for DNA transfer and can appear at significant cell-to-cell distances (Babic *et al*., 2008). Interestingly, uncoupling mutations in agrobacterial T4SS proteins blocked pilus biogenesis, but allowed proper DNA transfer (Jakubowski *et al*., 2009; Banta *et al*., 2011). This implied that intact pili are not necessary for substrate transfer. However, production of VirB2 and VirB5 is important for proper T4SS function and host cell interaction (Berger and Christie, 1993; Backert *et al*., 2008). These data together denote the existence of two configurations for the IVA-type T4SSs, a pilus biogenesis-competent form and a secretion-competent form, that may be composed of a pilus structure extending through the chamber of the OMCC (Banta *et al*., 2011).

Several other T4SS-associated structures have been solved, including a recent cryo-EM structure of a relaxase that revealed the molecular basis of DNA unwinding during bacterial conjugation (Fig. 1H-J) (Ilangovan *et al*., 2017). A structure of a VirD4 coupling protein bound to a VirB-type T4SS machinery was also described, providing a view of how secretion substrates might be conveyed through the T4SS (Fig. 1J) (Redzej *et al*., 2017). Structures of the *H. pylori* Cag (Frick-Cheng *et al*., 2016) and *L.*

pneumophila Dot/Icm systems (Ghosal *et al.*, 2017) have been reported, although not yet at the resolution of the R388 $VirB_{3-10}$ structure. Even at this time, however, these structures allow for general comparisons between the 'paradigmatic' VirB/VirD4-like type IVA machines and phylogenetically diverse systems; for example, the IVA and IVB types have a similar architecture consisting of a 14-fold symmetrical core complex mounted through a stalk to a double-barreled IMC. Finally, in addition to the structures solved for plasmid-encoded T4SSs or subassemblies, in the last year atomic models were solved by cryo-EM for two F family pili. Strikingly, these pili are composed of TraA pilin subunits in 1:1 stoichiometric association with phospholipid (Costa *et al*., 2016). These structures provide a molecular basis for understanding the dynamics of F-pilus assembly and retraction (Costa *et al*., 2016). Taken together, these new T4SS structures represent significant breakthroughs in the field of bacterial secretion.

Nature and recruitment of T4SS substrates

Pioneering work on the nature and recruitment of T4SS substrates, with focus on conjugative plasmids from Gram⁻ bacteria, has been performed by the groups of Llosa and Zechner (Fernandéz-Gonzaléz *et al*., 2011; Zechner *et al*., 2012; Lang *et al*., 2014; Gruber *et al*., 2016). All conjugative T4SSs encode relaxases, which initiate substrate processing by a nucleophilic attack of the active site tyrosyl-hydroxyl group of the enzyme on the scissile phosphate group within *oriT*, releasing the bridging oxygen and forming a long-lived ssDNA-protein conjugate. This high-energy bond serves several functions: i) it physically links the ssDNA substrate with the relaxase whose translocation signal (TS) mediates transfer through the T4SS, ii) it protects the phosphate of the ssDNA from nucleophilic attack when it enters the recipient, and iii) it provides the means to rejoin the plasmid ends in the recipient (Zechner *et al*., 2012). T4SS substrates are equipped with TSs that identify them as substrates for secretion (Zechner *et al*., 2012). Redzej and co-workers reported the first structure of a TS in relaxase TraI from plasmid R1 (Redzej *et al*., 2013). The latter TS domain can be divided into three subdomains with striking structural homology to helicase subdomains of the SF1B family. This work provided the first evidence that the TS can be part of larger structural scaffolds, overlapping with translocation-independent activities (Redzej *et al*., 2013).

Before entering the secretion channel, T4SS substrates form complexes with specific cytosolic binding partners, which can act as chaperones or adaptors to mediate substrate contacts with the cognate T4CP (Zechner *et al*., 2012). In the F system, for example, the TraM accessory factor functions in substrate selection by promoting a specific interaction between the F plasmid substrate and the F-encoded TraD T4CP (Wong *et al*., 2012). Other characterized T4SS accessory factors include the VirE1 chaperone, required for translocation of the VirE2 effector through the *A. tumefaciens* VirB/VirD4 T4SS, and the IcmS, IcmW, LvgA adaptors essential for translocation of different subsets of effectors through the *Legionell*a Dot/Icm translocation apparatus (Alvarez-Martinez and Christie, 2009; Kwak *et al.*, 2017). Par-like proteins such as *A. tumefaciens* VirC1 and VirC2 (Atmakuri *et al.*, 2007) and R1 plasmid-encoded ParM and ParR (Gruber *et al.*, 2016) also appear to play a role in promoting the docking of the T-DNA and R1 DNA substrates with their cognate T4SSs.

In Gram$^+$ bacteria, the DNA processing steps prior to conjugative transfer appear to be mechanistically very similar (Zechner *et al*., 2012). One exception to this generalization is that the *Clostridium perfringens* plasmid pCW3 codes for an atypical relaxase in the sense that it carries a catalytic tyrosine residue. Other catalytic residues conserved in tyrosine recombinases are not required for TcpM activity, suggesting that TcpM is not a site-specific recombinase (Wisniewski *et al*., 2016). Also, the first evidence was presented that a T4SS deployed by a Gram$^+$ species functions to deliver effector proteins to eukaryotic host cells during the course of infection (Li *et al*., 2011; Zhao *et al.*, 2011; Jiang *et al*., 2016; Yin *et al*., 2016). This T4SS is encoded by the 89 kb pathogenicity island (called 89K PAI) associated with *Streptococcus suis* and is also found in other pathogenic streptococci, e.g., *S. pneumoniae, S. agalactiae* (Wang *et al*., 2017). It will now be of considerable interest to confirm effector translocation, identify the effector repertoire, and define the nature of the translocation signals required for translocation through this and other possible effector translocators in Gram$^+$ species.

Conjugative transfer systems

Conjugative T4SSs are encoded on conjugative plasmids, integrative and conjugative elements also known as ICEs or conjugative transposons, or genomic PAIs (Fig. 1A). These systems are found in most species of

Gram$^-$ and Gram$^+$ bacteria, and conjugative plasmids also exist in a few species of archaea (Wagner *et al.*, 2017). In bacteria, these mobile genetic elements contribute to the spread of fitness traits and, more problematically from a clinical perspective, multiple antibiotic resistances (Christie, 2016; Grohmann *et al*., 2016). As mentioned earlier, the prototypic systems among Gram$^-$ species include the *A. tumefaciens* VirB/VirD4 T4SS (Christie, 2016), and the *E. coli* conjugative plasmids F, R388, and pKM101 (Lawley *et al*., 2003; Llosa and de la Cruz, 2005; de la Cruz *et al*., 2010; Frost and Koraimann, 2010; Zechner *et al.*, 2012, Arutyunov and Frost, 2013; Koraimann and Wagner, 2014; Cabezon *et al.*, 2015).

Currently, the best characterized T4SSs from Gram$^+$ bacteria are those encoded by the *Enterococcus faecalis* sex-pheromone responsive plasmid pCF10 (Li *et al*., 2012; Clewell *et al*., 2014; Laverde Gomez *et al*.; 2014; Bhatty *et al*., 2015; Whitaker *et al*., 2015; Bhatty *et al*., 2017), *C. perfringens* plasmid pCW3 (Bantwal *et al*., 2012; Porter *et al.*, 2012; Wisniewski *et al*., 2015; 2016; Wisniewski and Rood, 2017) and broad-host-range plasmid pIP501 originally isolated from *S. agalactiae* (Arends *et al*., 2013; Goessweiner-Mohr *et al.*, 2013 a and b; 2014 a and b; Fercher *et al*., 2016; Grohmann *et al.*, 2016; Kohler *et al*., 2017; Laverde *et al*., 2017). The conjugation machines in Gram$^+$ species differ from their Gram$^-$ species counterparts mainly by lacking the outer membrane core complex and the VirB11 ATPase. They also do not produce conjugative pili and instead rely on surface adhesins to mediate donor-recipient cell contacts (Bhatty *et al.*, 2013; 2015). The Gram$^+$ systems also typically employ VirB1-like lytic transglycosylases with two or more catalytic domains, presumably to allow for machine assembly across the thick peptidoglycan layer (Arends *et al*., 2013; Laverde Gomez *et al*., 2014).

With respect to structure - function advances of the Gram$^+$ T4SSs, high-resolution structures of individual components from the pIP501 and pCW3 systems have been solved (Porter *et al.*, 2012; Goessweiner-Mohr *et al.*, 2013a; 2014b; Fercher *et al.*, 2016), although no structures are presently available for larger T4SS machine assemblies. Considerable progress also has been made in defining signaling cascades and regulatory networks governing assembly of several Gram$^+$ systems. In the *E. faecalis* T4SS, pheromone-dependent overproduction of PrgB, otherwise known as Aggregation Substance, induces formation of intercellular aggregates. Interestingly, however, upon overproduction, PrgB confers toxicity on

E. faecalis donors by a mechanism dependent on extracellular DNA (e-DNA) (Bhatty *et al.*, 2015). A gene linked to *prgB* codes for a putative RNA-binding protein termed PrgU, whose synthesis mitigates PrgB-overproduction toxicity by blocking transcription from the upstream *prgQ* promoter (Bhatty *et al.*, 2017). Studies also have deciphered regulatory features and the molecular organization of the ICE*Bs1*-encoded T4SS carried by *Bacillus subtilis* (Carraro and Burrus, 2014; DeWitt and Grossman, 2014; Johnson and Grossman, 2015; Leonetti *et al*., 2015; Auchtung *et al*., 2016). Finally, as noted above, the intriguing recent studies of the 89K PAI from *S. suis* for the first time have supplied evidence that a T4SS encoded by a $Gram^+$ species is capable of translocating effector proteins into human host cells during the course of infection (Li *et al*., 2011; Jiang *et al*., 2016; Yin *et al*., 2016).

DNA export and import systems

The subfamily of contact-independent import/export machines is presently restricted to the *H. pylori* ComB competence system and the *Neisseria gonorrhoeae* DNA release apparatus (Figs. 1B,C). In the ComB system, the T4SS mediates the first step in DNA uptake across the outer membrane (Hofreuter *et al.*, 1998; 2000; Stingl *et al*., 2010, Krüger and Stingl, 2011). This apparatus was identified as the major mediator of DNA transfer between *H. pylori* strains, both in a DNaseI-sensitive (transformation) and DNaseI-resistant (conjugative transfer) manner (Rohrer *et al.*, 2012). The ComB system comprises a nearly complete set of T4SS components, lacking only the homologs of VirB1, VirB5, and VirB11 ATPase (Fernández-González and Backert, 2014). An early study showed that all VirB homologs except for the VirB7-like subunit are required for DNA uptake (Hofreuter *et al*., 2003). In addition to the ComB subunits, the cytoplasmic protein DprA (Smeets *et al.*, 2000a), the secreted protein ComH (Smeets *et al.*, 2000b), and the cytoplasmic channel subunit ComEC (Yeh *et al*., 2003) are essential for DNA import by *H. pylori* (Fernández-González and Backert, 2014). Recently, a two-step DNA uptake mechanism was proposed in which ComB translocates double-stranded (ds) DNA across the outer membrane and delivers the substrate to the ComEC channel for uptake across the inner membrane (Stingl *et al*., 2010; Fernández-González and Backert, 2014). It also has

been suggested that some *comB* genes might contribute to *H. pylori* infection of mammalian host cells (Fernández-González and Backert, 2014).

Neisseria gonorrhoeae is an obligate human pathogen responsible for the sexually transmitted disease gonorrhea. It encodes a plasmid F-like T4SS within the gonococcal genetic island (GGI), which secretes ssDNA directly into the external environment (Pachulec *et al*., 2014). This DNA is effective in transforming gonococci in the population, and may contribute to the high extent of genetic diversity in this species (Kohler *et al*., 2013). Secretion of ssDNA is also required for the initial stages of biofilm formation, presumably helping during colonization (Zweig *et al*., 2013; Obergfell and Seifert, 2015) (Fig. 1B). Sequence comparisons revealed that GGI-like T4SSs are highly conserved units located both on chromosomes and plasmids. The *yaa-atlA* and *parA-parB* gene regions were shown to be essential for DNA secretion (Pachulec *et al*., 2014). In addition, it was postulated that release of DNA occurs through the action of ParA, ParB, TraI, Yea, and TraD proteins. Reminiscent of the VirC1/VirC2 and ParM/ParR systems described earlier, the *N. gonorrhoeae* ParA and ParB are partitioning factors implicated in chromosome and plasmid DNA segregation during replication, but evidently also coordinate early DNA substrate docking reactions with the cognate GGI-encoded T4SSs (Leonard *et al*., 2005; Obergfell and Seifert, 2015).

Host-pathogen interactions

Helicobacter pylori

Helicobacter pylori is a paradigm of persistent pathogens and major risk factor of peptic ulceration and gastric adenocarcinoma in humans (Salama *et al*., 2013). Highly virulent isolates elaborate a T4SS encoded by the *cag* PAI. Machine assembly requires orthologs of all 12 agrobacterial VirB/VirD4 proteins and about a dozen other subunits, making this system clearly distinct from the 'paradigmatic' IVA systems discussed above (Fischer *et al*., 2001; Backert *et al*., 2015). The T4SS core complex was visualized by negative-staining EM, bearing some architectural similarity to the R388-encoded $VirB_{3-10}$ subassembly (Frick-Cheng *et al*., 2016). However, the Cag structure is considerably larger with a cross-section of 41 nm as opposed to 28 nm, and it is composed of five (Cag3, CagM, CagT, CagX, CagY) as opposed to three (VirB7, VirB9,

VirB10) subunits, respectively. The Cag T4SS also was shown to be associated with an extracellular pilus (Backert *et al*., 2015). Another distinction from the canonical systems is that several subunits, including CagL, CagI, CagY and the CagA secretion substrate, are associated with the surface-exposed portion of the pilus. These factors permit binding of the basolateral host receptor integrin $\alpha_5\beta_1$, which is necessary for T4SS function (Kwok *et al.*, 2007; Barden *et al.*, 2013). New studies revealed that T4SS-pilus formation occurred predominantly at basolateral membranes during infection of polarized gastric epithelial cells, and not at apical sites. For this purpose, *H. pylori* secretes the serine protease HtrA, which opens cell-to-cell junctions through cleavage of the junctional proteins occludin, claudin-8 and E-cadherin (Schmidt *et al*., 2016; Tegtmeyer *et al*., 2017a). The only known Cag T4SS effector protein is CagA, and several crystal structures of CagA's N-terminus are now available (Hayashi *et al*., 2012; Kaplan-Türköz *et al*., 2012). After delivery into host cells, CagA undergoes tyrosine phosphorylation by cellular Src and Abl kinases (Mueller *et al.*, 2012). CagA can then interact with about 25 signaling proteins, including Shp2, Grb2, Par1b, PI3-kinase or tumor suppressor ASPP2 (Higashi *et al*., 2002; Mimuro *et al*., 2002; Saadat *et al*., 2007; Selbach *et al*., 2009, Nešić *et al*., 2014; Zhang *et al*., 2015). Through these interactions, CagA interferes with fundamental host signaling cascades such as cell adhesion, polarity, proliferation, anti-apoptosis and inflammation (Tegtmeyer *et al*., 2017b). Functional studies in Mongolian gerbils (Franco *et al.*, 2008) and transgenic mice (Ohnishi *et al*., 2008) have shown that CagA production is necessary and sufficient to stimulate gastric cancerogenesis. However, besides CagA, this T4SS can translocate chromosomal DNA (Varga *et al*., 2016), peptidoglycan (Viala *et al*., 2004) and D-glycero-β-D-manno-heptose 1,7-bisphosphate (Gall *et al*., 2017; Stein *et al.*, 2017; Zimmermann *et al*., 2017) into epithelial cells, which respectively stimulate TLR-9, kinase AKAP and pro-inflammatory transcription factor NF-κB signaling modules. *H. pylori* also exploits host CEACAM (carcinoembryonic antigen-related cell adhesion molecules) receptors via the surface-exposed OMP HopQ, for bacterial adherence and translocation of CagA. The HopQ - CEACAM interaction is necessary for full T4SS function, gastric colonization and pathology (Javaheri *et al*., 2016; Königer *et al*., 2016).

Legionella pneumophila

Several intracellular pathogens including *Legionella, Coxiella, Bartonella* and *Brucella* species produce specialized T4SSs to aid in survival and spread in the human host (Personnic *et al*., 2016; Sherwood and Roy, 2016). *Legionella pneumophila* is an environmental amoeba-adapted parasite that also colonizes human alveolar macrophages, hence triggering severe pneumonia, called Legionnaires' disease (Vogel and Isberg, 1999). To evade killing by the host, the *L. pneumophila* convert phagosomes into a protective compartment termed the "*Legionella*-containing vacuole" (LCV). Formation of this replicative niche requires the Dot/Icm T4SS. EM studies have visualized the Dot/Icm T4SS core as a ring-shaped structure composed of five proteins, DotC, DotD, DotF, DotG and DotH (Kubori *et al*., 2014). More recently, the Dot/Icm core complex was visualized by cryo-electron tomography of *L. pneumophila* cells. This structure more closely resembles the *H. pylori* Cag T4SS in its size (41 nm cross-section) and overall architecture (Ghosal *et al.*, 2017). However, in contrast to the R388-encoded $VirB_{3-10}$ structure, which presents information about the IMC, at present there is no knowledge of how the inner membrane subassemblies of either the Dot/Icm or Cag T4SSs are architecturally configured. Interestingly, the Dot/Icm system assembles at *Legionella* cell poles, and polar translocation of effector proteins appears to be important for virulence (Jeong *et al*., 2017). Remarkably, this T4SS is postulated to translocate over 300 effectors during infection, many of which have been shown to target host cellular pathways controlling membrane transport processes (Sherwood and Roy, 2016). *Legionella* LCVs are designed to escape fusion with lysosomes but comprehensively interact with various endosomal and secretory vesicle trafficking cascades (Isberg *et al*., 2009; Personnic *et al*., 2016; Sherwood and Roy, 2016). The LCVs move along microtubules in the host cell and finally merge with the endoplasmic reticulum (ER) (Horwitz *et al*., 1983; Lu and Clarke, 2005; Robinson and Roy, 2006). In this scenario, translocated effector proteins deregulate crucial factors of host signaling including various phosphatidylinositol lipids (Weber *et al*., 2006; 2014; Ragaz *et al*., 2008; Brombacher *et al*., 2009; Hsu *et al*., 2012; Toulabi *et al*., 2013), autophagy components (Choy *et al*., 2012), H^+-ATPase (Xu *et al*., 2010) as well as the small GTPases Rab1 (Machner and Isberg, 2006; Murata *et al*., 2006; Schoebel *et al*., 2010; Itzen and Goody, 2011), Arf1 (Nagai *et al*., 2002) or Ran (Rothmeier *et al*., 2013; Simon *et al*., 2014). The

composition of LCVs formed in *Dictyostelium discoideum* was shown by proteomics to involve >560 host cell proteins (Brombacher, 2009; Urwyler, 2009; Hilbi *et al*., 2011). In addition, depletion of the *D. discoideum* OCRL ortholog Dd5P4, encoding an inositol polyphosphate 5-phosphatase, stimulated the intracellular replication of *L. pneumophila* (Weber *et al*., 2009, Finsel *et al*., 2013). Both catalytically active OCRL and Dd5P4 enzymes co-localize with LCVs and enhance the quantity of phosphatidylinositol 4-phosphate accessible for binding of other Icm/Dot proteins (Weber *et al*., 2009). Taken together, *L. pneumophila* utilizes a sophisticated T4SS, manipulating intracellular trafficking machineries for growth and a functional retrograde transport pathway restricts the replication of the pathogen.

Coxiella burnetii

Coxiella burnetii is the causative agent of the zoonosis Q-fever in humans. *Coxiella* replicates effectively within a lysosome-like compartment called the "*Coxiella*-containing vacuole" (CCV). Similar to *L. pneumophila*, *C. burnetii* encodes a Dot/Icm-like T4SS whose function is to modify the host endocytic transport systems and generate the CCV replicative niches (Segal *et al.*, 2005; Voth and Heinzen, 2009). The *C. burnetii* T4SS orthologs DotH, IcmV and IcmT localize at the bacterial cell poles in infected Vero cell, as shown by immunofluorescence microscopy (IFM) and EM combined with immunogold labeling (Morgan *et al.*, 2010). Although the CCVs exhibit lysosomal capabilities, they display specific features such as homotypic fusion and a cholesterol-enriched limiting membrane, in addition to robustly interacting with autophagosomes (Howe and Heinzen, 2006; Kohler and Roy, 2015). Compelling evidence for the functionality of the *Coxiella* Dot/Icm subunits came from swapping experiments in *L. pneumophila* (Chen *et al.*, 2010; Carey *et al*., 2011). In addition, axenic growth and methods for genetic manipulation were achieved for *Coxiella*, enabling proof that the Dot/Icm T4SS is essential for growth in CCVs and ultimately identified >130 translocated effector proteins (Zamboni *et al*., 2003; Zusman *et al*., 2003; Pan *et al*., 2008; Beare *et al*., 2011; Carey *et al*., 2011; Moffatt *et al*., 2015). Computer modeling coupled with a validation approach also has facilitated the identification of T4SS secretion signals that may prove useful for discovering novel effector proteins in *Legionella* and *Coxiella* (Lifshitz *et al*., 2013). The endosomal nature

of CCVs is reflected by the accumulation of late endosomal markers LAMP-1/-2, vasodilator-stimulated phosphoprotein (VASP), as well as the V-ATPase (Voth and Heinzen, 2007; Colonne *et al*., 2016). Genome-wide gene silencing screens using siRNA identified additional host factors including the retromer complex (McDonough *et al*., 2013). Prominent effector proteins include Cig2, which promotes fusion of autophagosomes with the CCV to maintain this compartment in an autolysosomal maturation stage (Kohler *et al*., 2016). Another translocated effector, Cig57, co-opts clathrin-mediated trafficking to facilitate the biogenesis of the fusogenic CCVs (Latomanski *et al.*, 2016). In addition, the ankyrin repeat (Ank) family member AnkG was found to interact with the host protein p32, regulating an anti-apoptotic pathway, required for *Coxiella*'s adaptation to mammalian hosts (Lührmann *et al*., 2010). Finally, *C. burnetii* inhibits caspase-mediated activation of the NLRP3 inflammasome in macrophages by the effector protein IcaA (Cunha *et al*., 2015). Thus, *Coxiella* appears to dampen the inflammasome machinery to avoid clearance by the host immune system.

Bartonella henselae

Bartonalla henselae is a zoonotic parasite colonizing cats and humans (Dehio, 2005; Regier *et al*., 2016). Clinical outcomes range from cat scratch disease to persistent bacteremia and vascular tumors. *Bartonella* exhibits a tropism towards endothelial cells and erythrocytes (Eicher and Dehio, 2012). Binding to the extracellular matrix by adhesins and the activity of a VirB/VirD4-type T4SS by *B. henselae* induces a massive rearrangement of the host cytoskeleton, which leads to uptake of the bacteria into endothelial cells. Bacterial entry into erythrocytes is mediated by a second T4SS designated Trw, which is followed by intracellular *Bartonella* growth and persistence. The infection process is aided at different steps by the *Bartonella* effector proteins (Bep's). All known Bep's carry a C-terminal BID (Bep intracellular delivery) domain acting in part as a T4SS translocation signal (Schulein *et al*., 2005). Many Beps also have an enzymatic N-terminal FIC (filamentation induced by cAMP) module that facilitates the AMPylation of host cell proteins (Siamer and Dehio, 2015). This AMPylation activity typically triggers the inactivation of yet unknown host cell proteins of 40–50 kDa (Palanivelu *et al*., 2011). In addition, three effectors (BepD, BepE

and BepF) harbor tyrosine phosphorylation motifs, which are targeted by host cell kinases similar to CagA described above (Schulein *et al*., 2005). A proteomics-based screen identified 8 cellular interaction partners (Grb2, Grb7, Shp1, Shp2 and others) of the Bep's, which subvert host cell signaling with roles in pro-inflammatory responses by activation of NF-κB, anti-apoptosis, cell proliferation and others (Selbach *et al*., 2009). Finally, more recent work established that the VirB/VirD4 T4SS functions as a delivery system for DNA both to other bacteria and human cells (Fernández-González *et al.*, 2011; Schröder *et al.*, 2011). Understanding the functions associated with interkingdom transfer of protein effectors, and potentially DNA substrates, will shed new light on the molecular bases underlying *Bartonella* - host cell interactions.

Brucella abortus

Brucella abortus is the causative agent of the zoonosis brucellosis and primarily infects phagocytes (Celli, 2015). The intracellular replication cycle proceeds within the *Brucella*-containing vacuole (BCV), which initially traffics along the endocytic pathway, acquiring the cellular markers early endosome antigen-1 (EEA-1), Rab5 and Rab7 (Pizarro-Cerdá *et al*., 1998; Chaves-Olarte *et al*., 2002; Celli *et al*., 2003; Starr *et al*., 2008; Lee *et al*., 2013). These BCVs fuse rapidly with lysosomes, which provides physicochemical cues for elaboration of the VirB T4SS (Pizarro-Cerdá *et al*., 1998; Boschiroli *et al.*, 2002; Celli *et al*., 2003; Starr *et al*., 2008, Smith *et al*., 2016). Initially of endosomal origin, BCVs are converted through various VirB-dependent steps into organelles derived from the ER that support bacterial proliferation, suggesting these events require the delivery of T4SS effector proteins. Presently, ~15 T4SS effector proteins have been identified (de Jong *et al.*, 2008; Marchesini *et al*., 2011; Myeni *et al*., 2013; Ke et *al.*, 2015), although only a few are reported to play a clear role in *Brucella* pathogenesis. Inflammation and IL-6 production triggered by *B. abortus* infection induces significant ER stress via the T4SS effector protein VceC (Keestra-Gounder *et al*., 2016). This process is receptor NOD1/2-, TRAF2- and RIP2-dependent. The association of NOD1 and NOD2 with pro-inflammatory responses induced by the IRE1α/TRAF2 signaling pathway provides a novel link between innate immunity and ER-stress-induced inflammation. Further studies elucidating the functions of *Brucella* effector proteins will help clarify the molecular roles of the VirB T4SS during infection.

Anaplasma phagocytophilum and *Ehrlichia chaffeensis*

T4SS nanomachines also have been encountered in obligatory intracellular human pathogens such as *A. phagocytophilum* and *E. chaffeensis*, which respectively cause human granulocytic anaplasmosis and monocytic ehrlichiosis (Ohashi *et al.*, 2002). Both species are transmitted from tick vectors to mammalian hosts where they survive and proliferate in membrane-bound inclusions that lack lysosomal markers and components of NADPH oxidase. These intracellular pathogens depend mostly on host-synthesized nutrients, as they have only a limited number of genes for biosynthesis and metabolism. The first described T4SS effectors were AnkA and Ats-1 from *A. phagocytophilum* (Lin *et al.*, 2007; Niu *et al.*, 2010), and since then several additional effectors [AM185, AM470, AM705 (AnkA), AM1141] were identified in *Anaplasma marginale* (Lockwood *et al*., 2011). To promote their intracellular survival, *Anaplasma* and *Ehrlichia* modulate host cell apoptosis by secreting proteins that interfere with this cell death pathway. Strikingly, early studies showed that *A. phagocytophilum* Ats-1 translocates across the bacterial cell envelope, host cell membrane, and ultimately into mitochondria where it interferes with apoptosis induction (Niu *et al.*, 2010; Niu and Rikihisa, 2013; 2014). More recently, in *E. chaffeensis*, a T4SS effector similarly was shown to block mitochondrion-mediated host cell apoptosis (Rikihisa, 2015). *Ehrlichia chaffeensis* infection was further shown to depend on the translocated ehrlichial translocated factor-1 (Etf-1), which induces Rab5-regulated autophagy to provide host cytosolic nutrients to the pathogen. The role of Etf-1 in host cell autophagy and infection was confirmed by mutagenesis (Sharma *et al*., 2017). Etf-1-mediated manipulation of Rab5 is a simple strategy to avoid destruction of the pathogen in lysosomes, obtain membrane components, and establish a homeostatic intra-host cell environment for proliferation (Rikihisa, 2017).

Conclusions and Outlook

The recent structures of purified T4SS machine subunits and subassemblies continue to generate important molecular details about the paradigmatic systems. Furthermore, cryo-electron tomography yielding the first *in situ* image of a T4SS represents a promising new direction for structural definition of T4SSs in their native membrane environments (Ghosal *et al.*, 2017). Equally importantly, new T4SSs are being described that

further highlight the biological diversity of this secretion superfamily. This has been exemplified by discovery that *Xanthomonas citri* employs a T4SS to kill competing bacteria in the close vicinity in a contact-dependent manner, reminiscent of the type VI secretion killing systems (Souza *et al*., 2015). An update of this system was presented at a T4SS conference held last December 2016 in Schloss Hirschberg, Germany (www.t4ss-conference.de). This T4SS translocates effectors bearing C-terminal translocation signals, whose bacteriolytic activities degrade peptidoglycan in target cells, but in the donor cell can be neutralized by the synthesis of cognate immunity proteins (Souza *et al.*, 2015; 2016). Intriguingly, more than one thousand *Xanthomonas* T4SS effectors showing only very limited homology to each other or other proteins were found in protein databases (Souza *et al.*, 2016). This T4SS appears to be widely dispersed among *Xanthomonas* and related species, making this a possible paradigm for an emerging new family of T4SS-killing machines in bacteria.

In the course of ongoing high-throughput genome sequencing projects, hundreds of putative T4SSs have been identified in obligatory intracellular and other pathogens as well as endosymbionts, e.g., *Anaplasma, Rickettsia, Orientia,* and *Wolbachia spp.* (Gillespie *et al*., 2009; Sonthayanon *et al*., 2010; Gillespie *et al*., 2016; Ramirez-Puebla *et al*., 2016). Most strikingly, the *virB/virD4*-like genes are often distributed in clusters around the genomes, and the *virB2* and *virB6* gene families have undergone unprecedented expansions. How these T4SSs contribute to establishment of pathogen or symbiont - host relationships remain ripe areas for further study. Similarly, the accumulating evidence for effector protein transfer by the 89K PAI-encoded T4SS in *S. suis* raises intriguing questions about the extent to which $Gram^+$ species deploy T4SSs for interkingdom effector translocation during infection.

As our knowledge of T4SS structures and mechanisms of action deepens, the field is poised to develop effective therapies aimed at suppressing T4SS functions in clinical settings. Indeed, several studies already have targeted the conserved VirB8 homologs as potential drug targets. By screening of a small-molecule library using *Brucella* VirB8 as a target, compounds were found that inhibited VirB8 dimerization (Paschos *et al*., 2011). These also were active against VirB8 from plasmid pKM101, resulting in disruption of VirB8 dimerization and inhibition of conjugation (Casu *et al*., 2016). A different approach was employed

for the inhibition of TraM, the VirB8-homolog associated with the pIP501-encoded T4SS as well as other $Gram^+$ conjugation machines. Anti-TraM antibodies directed against the VirB8-homolog from plasmid pIP501 considerably reduced the survival of clinical *E. faecalis* and *S. aureus* strains harboring a putative T4SS *in vitro* and in an *in vivo* mouse infection model (Laverde *et al*., 2017). A recent study also established the value of testing the efficacy of small molecule inhibitors shown to block the production of phylogenetically unrelated pilus assembly or secretion systems for effects on T4SS biogenesis or function. For example, compounds containing a ring-fused 2-pyridone peptidomimetic fragment that previously had been shown to block the *E. coli* chaperone-usher pilus pathway also impaired *H. pylori* Cag pilus production, *A. tumefaciens* T-DNA transfer, and DNA transfer through the pKM101 and R1-16-encoded conjugation machines (Shaffer *et al.*, 2016).

Finally, recent work by the Llosa and Dehio groups has shown that T4SSs also are viable vectors for delivery of potentially therapeutic DNA into human cells. *Escherichia coli* and *B. henselae* donors successfully transfer DNA to human cells where the translocated DNA is stably integrated into the human genome (Schroder *et al.*, 2011; Llosa *et al.*, 2012; Alperi *et al*., 2013; Gonzalez-Prieto *et al*., 2017). TrwC-relaxase mediated site-specific DNA integration into the human genome also has been demonstrated, albeit with very low efficiency compared to random integration. TrwC might stabilize the plasmid DNA in the nucleus of the human cell by promoting recircularization of the transferred strand, thus considerably increasing the chances for integration of the DNA by the host machinery (Gonzalez-Prieto *et al*., 2017). The implementation of state-of-the art metagenomics analyses, cell imaging, and ultrastructural approaches - along with the development of translational applications - promises a bright future for the T4SS field.

Acknowledgements

We thank the many scientists in the T4SS field for their valuable contributions, and we apologize for omissions of published work due to constraints on page limitations. The recent T4SS conference in Schloss Hirschberg, Germany (www.t4ss-conference.de) served as a foundation for this MicroReview. Work in the Backert lab is funded by the German Research Foundation (SFB-1181, project A04), the Christie lab by NIH

grants R01GM48476 and R21AI105454, the Waksman lab by Wellcome Trust grant 098302, and the Grohmann lab by DLR grant 50WB1466. We thank Adam Redzej from the Waksman lab for assembling the Graphical Abstract picture.

References

Alperi, A., Larrea, D., Fernández-González, E., Dehio, C., Zechner, E.L., and Llosa M. (2013) A translocation motif in relaxase TrwC specifically affects recruitment by its conjugative type IV secretion system. *J Bacteriol* **195:** 4999–5006.

Alvarez-Martinez, C.E., and Christie, P.J. (2009) Biological diversity of prokaryotic type IV secretion systems. *Microbiol Mol Biol Rev* **73:** 775-808.

Arends, K., Celik, E.-K., Probst, I., Goessweiner-Mohr, N., Fercher, C., Grumet, L., *et al*. (2013) TraG encoded by the pIP501 type IV secretion system is a two domain peptidoglycan degrading enzyme essential for conjugative transfer. *J Bacteriol* **195:** 4436-4444.

Arutyunov, D., and Frost, L.S. (2013) F conjugation: back to the beginning. *Plasmid* **70:** 18-32.

Atmakuri, K., Cascales, E. Burton, O.T., Banta, L.M., and Christie, P.J. (2007) *Agrobacterium* ParA/MinD-like VirC1 spatially coordinates early conjugative DNA transfer reactions. *EMBO J* **26:** 2540-2551.

Atmakuri, K., Cascales, E., and Christie, P.J. (2004) Energetic components VirD4, VirB11 and VirB4 mediate early DNA transfer reactions required for bacterial type IV secretion. *Mol Microbiol* **54:** 1199-1211.

Auchtung, J.M., Aleksanyan, N., Bulku, A., and Berkmen, M.B. (2016) Biology of ICE*Bs1*, an integrative and conjugative element in *Bacillus subtilis*. *Plasmid* **86:** 14-25.

Babic, A., Lindner, A.B., Vulic, M., Stewart, E.J., and Radman, M. (2008) Direct visualization of horizontal gene transfer. *Science* **319:** 1533-1536.

Backert, S., Fronzes, R., and Waksman, G. (2008) VirB2 and VirB5 proteins: specialized adhesins in bacterial type-IV secretion systems? *Trends Microbiol* **16:** 409-413.

Backert, S., and Meyer, T.F. (2006) Type IV secretion systems and their effectors in bacterial pathogenesis. *Curr Opin Microbiol* **9:** 207-217.

Backert, S., Tegtmeyer, N., and Fischer, W. (2015) Composition, structure and function of the *Helicobacter pylori cag* pathogenicity island encoded type IV secretion system. *Future Microbiol* **10:** 955–965.

Banta, L.M., Kerr, J.E., Cascales, E., Giuliano, M.E., Bailey, M.E., McKay, C., *et al.* (2011) An *Agrobacterium* VirB10 mutation conferring a type IV secretion system gating defect. *J Bacteriol* **193:** 2566-2574.

Bantwal, R., Bannam, T.L., Porter, C.J., Quinsey, N.S., Lyras, D., Adams, V., *et al.* (2012) The peptidoglycan hydrolase TcpG is required for efficient conjugative transfer of pCW3 in *Clostridium perfringens*. *Plasmid* **67:**139-147.

Barden, S., Lange, S., Tegtmeyer, N., Conradi, J., Sewald, N., Backert, S., *et al.* (2013) A helical RGD motif promoting cell adhesion: crystal structures of the *Helicobacter pylori* type IV secretion system pilus protein CagL. Structure **21:** 1931-1941.

Beare, P.A., Gilk, S.D., Larson, C.L., Hill, J., Stead, C.M., Omsland, A., *et al.* (2011) Dot/Icm type IVB secretion system requirements for *Coxiella burnetii* growth in human macrophages. *MBio* **2:** e00175–11.

Berger, B.R., and Christie, P.J. (1993) The *Agrobacterium tumefaciens virB4* gene product is an essential virulence protein requiring an intact nucleoside triphosphate-binding domain. *J Bacteriol* **175:** 1723-1734.

Berger, B.R., and Christie, P.J. (1994) Genetic complementation analysis of the *Agrobacterium tumefaciens virB* operon: *virB2* through *virB11* are essential virulence genes. *J Bacteriol* **176:** 3646-3660.

Bhatty, M., Camacho, M.I., Gonzalez-Rivera,C., Frank, K.L, Dale, J.L., Manias, D.A., *et al.* (2017) PrgU: a suppressor of sex pheromone toxicity in *Enterococcus faecalis*. *Mol Microbiol.* **103:** 398-412.

Bhatty, M., Cruz, M.R., Frank, K.L, Gomez, J.A., Andrade, F., Garsin, D.A., *et al.* (2015) *Enterococcus faecalis* pCF10-encoded surface proteins PrgA, PrgB (aggregation substance) and PrgC contribute to plasmid transfer, biofilm formation and virulence. *Mol Microbiol* **95:** 660-677.

Bhatty, M., Laverde Gomez, J.A., and Christie, P.J. (2013) The expanding bacterial type IV secretion lexicon. *Res Microbiol* **164**: 620-639.

Boschiroli, M.L., Ouahrani-Bettache, S., Foulongne, V., Michaux-Charachon, S., Bourg, G., Allardet-Servent, A., *et al.* (2002) Type IV secretion and *Brucella* virulence. *Vet Microbiol* **90:** 341-348.

Brombacher, E., Urwyler, S., Ragaz, C., Weber, S.S., Kami, K., Overduin, M., *et al.* (2009) Rab1 guanine nucleotide exchange factor SidM is a major phosphatidylinositol 4-phosphate-binding effector protein of *Legionella pneumophila. J Biol Chem* **284**: 4846–4856.

Cabezón, E., Ripoll-Rozada, J., Peña, A., de la Cruz, F., and Arechaga, I. (2015) Towards an integrated model of bacterial conjugation. *FEMS Microbiol Rev* **39:** 81-95.

Carey, K.L., Newton, H.J., Lührmann, A., and Roy, C.R. (2011) The *C. burnetii* Dot/Icm system delivers a unique repertoire of type IV effectors into host cells and is required for intracellular replication. *PLoS Pathog* **7:** e1002056.

Carraro, N., and Burrus, V. (2014) Biology of three ICE Families: SXT/R391, ICEB*s1*, and ICE*St1*/ICE*St3*. *Microbiol Spectr* **2** doi: 10.1128/microbiolspec.MDNA3-0008-2014.

Cascales, E., and Christie, P.J. (2004) Definition of a bacterial type IV secretion pathway for a DNA substrate. *Science* **304:** 1170-1173.

Casu, B., Smart, J., Hancock, M.A., Smith, M., Sygusch, J., and Baron, C. (2016) Structural analysis and inhibition of TraE from the pKM101 type IV secretion system. *J Biol Chem* **291:** 23817-23829.

Celli, J. (2015) The changing nature of the *Brucella*-containing vacuole. *Cell Microbiol* **17:** 951-958.

Celli, J., de Chastellier, C., Franchini, D.M., Pizarro-Cerda, J., Moreno, E., and Gorvel, J.P. (2003) *Brucella* evades macrophage killing via VirB-dependent sustained interactions with the endoplasmic reticulum. *J Exp Med* **198:** 545-556.

Chandran, V., Fronzes, R., Duquerroy, S., Cronin, N., Navaza, J., and Waksman, G. (2009) Structure of the outer membrane complex of a type IV secretion system. *Nature* **462:** 1011-1015.

Chandran Darbari, V., and Waksman, G. (2015) Structural biology of bacterial type IV secretion systems. *Annu Rev Biochem* **84:** 603-629.

Chen, C., Banga, S., Mertens, K., Weber, M.M., Gorbaslieva, I., Tan, Y., *et al.* (2010) Large-scale identification and translocation of type IV secretion substrates by *C. burnetii*. *Proc Natl Acad Sci USA* **107:** 21755-21760.

Choy, A., Dancourt, J., Mugo, B., O'Connor, T.J., Isberg, R.R., Melia, T.J., *et al.* (2012) The *Legionella* effector RavZ inhibits host autophagy through irreversible Atg8 deconjugation. *Science* **338:** 1072–1076.

Christie, E., Guzmán-Verri, C., Méresse, S., Desjardins, M., Pizarro-Cerdá, J., Badilla, J., *et al.* (2002) Activation of Rho and Rab GTPases dissociates *Brucella abortus* internalization from intracellular trafficking. *Cell Microbiol* **4:** 663-676.

Christie, P.J. (2016) The Mosaic Type IV Secretion Systems. *EcoSalPlus* **7** doi:10.1128/ecosalplus.ESP-0020-2015.

Clewell, D.B., Weaver, K.E., Dunny, G.M., Coque, T.M., Francia, M.V., and Hayes, F. (2014) Extrachromosomal and Mobile Elements in Enterococci: Transmission, Maintenance, and Epidemiology. In: Gilmore, M.S., Clewell, D.B., Ike, Y., Shankar, N., editors. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection. Boston: Massachusetts *Eye and Ear Infirmary*.

Colonne, P.M., Winchell, C.G., Graham, J.G., Onyilagha, F.I., MacDonald, L.J., Doeppler, H.R., *et al.* (2016) Vasodilator-stimulated phosphoprotein activity is required for *Coxiella burnetii* growth in human macrophages. *PLoS Pathog* **12:** e1005915.

Costa, T.R., Ilangovan, A., Ukleja, M., Redzej, A., Santini, J.M., Smith, T.K., Egelman, E.H., and Waksman, G. (2016) Structure of the bacterial sex F pilus reveals an assembly of a stoichiometric protein-phospholipid complex. *Cell* **166:** 1436-1444.e10.

Cunha, L.D., Ribeiro, J.M., Fernandes, T.D., Massis, L.M., Khoo, C.A., Moffatt, J.H., *et al.* (2015) Inhibition of inflammasome activation by *C. burnetii* type IV secretion system effector IcaA. *Nat Commun* **6:** 10205.

Dehio, C. (2005) *Bartonella*-host-cell interactions and vascular tumour formation. *Nat Rev Microbiol* **3:** 621-631.

De Jong, M.F., Sun, Y.H., den Hartigh, A.B., van Dijl, J.M., and Tsolis, R.M. (2008) Identification of VceA and VceC, two members of the VjbR regulon that are translocated into macrophages by the *Brucella* type IV secretion system. *Mol Microbiol* **70:** 1378-1396.

de la Cruz, F., Frost, L.S., Meyer, R.J., and Zechner, E.L. (2010) Conjugative DNA metabolism in Gram-negative bacteria. *FEMS Microbiol Rev* **34:** 18-40.

DeWitt, T., and Grossman, A.D. (2014) The bifunctional cell wall hydrolase CwlT is needed for conjugation of the integrative and conjugative element ICE*Bs1* in *Bacillus subtilis* and *B. anthracis*. *J Bacteriol* **196:** 1588-1596.

Dürrenberger, M.B., Villiger, W., and Bächi, T. (1991) Conjugational junctions: morphology of specific contacts in conjugating *Escherichia coli* bacteria. *J Struct Biol* **107:** 146-156.

Eicher, S.C., and Dehio, C. (2012) *Bartonella* entry mechanisms into mammalian host cells. *Cell Microbiol* **14:** 1166-1173.

Eisenbrandt, R., Kalkum, M., Lai, E.M., Lurz, R., Kado, C.I., and Lanka, E. (1999) Conjugative pili of IncP plasmids, and the Ti plasmid T pilus are composed of cyclic subunits. *J Biol Chem* **274:** 22548-22555.

Fercher, C., Probst, I., Goessweiner-Mohr, N., Arends, K., Grohmann, E., Meyer, N.-H., *et al.* (2016) VirB8-like protein TraH is crucial for DNA transfer in *Enterococcus faecalis*. *Sci Rep* **6:** 24643.

Fernández-González, E., de Paz, H.D., Alperi, A., Agúndez, L., Faustmann, M., Sangari, F.J., *et al.* (2011) Transfer of R388 derivatives by a pathogenesis-associated type IV secretion system into both bacteria and human cells. *J Bacteriol* **193:** 6257-6265.

Fernández-González, E., and Backert, S. (2014) DNA transfer in the gastric pathogen *Helicobacter pylori. J Gastroenterol* **49:**594–604.

Finsel, I., Ragaz, C., Hoffmann, C., Harrison, C.F., Weber, S., van Rahden, V.A., *et al.* (2013) The *Legionella* effector RidL inhibits retro-grade trafficking to promote intracellular replication. *Cell Host Microbe* **14:** 38–50.

Fischer, W., Püls, J., Buhrdorf, R., Gebert, B., Odenbreit, S., and Haas, R. (2001) Systematic mutagenesis of the *H. pylori* cag pathogenicity island: essential genes for CagA translocation in host cells and induction of interleukin-8. *Mol Microbiol* **42:** 1337-1348.

Franco, A.T., Johnston, E., Krishna, U., Yamaoka, Y., Israel, D.A., Nagy, T.A., *et al.* (2008) Regulation of gastric carcinogenesis by *H. pylori* virulence factors. *Cancer Res* **68:** 379-387.

Frick-Cheng, A.E., Pyburn, T.M., Voss, B.J., McDonald, W.H., Ohi, M.D., and Cover, T.L. (2016) Molecular and structural analysis of the *H. pylori* cag type IV secretion system core complex. *MBio* **12:** e02001-15.

Fronzes, R., Schafer, E., Wang, L., Saibil, H.R., Orlova, E.V., and Waksman, G. (2009) Structure of a type IV secretion system core complex. *Science* **323:** 266-268.

Frost, L.S., and Koraimann, G. (2010) Regulation of bacterial conjugation: balancing opportunity with adversity. *Future Microbiol* **5:** 1057-1071.

Gall, A., Gaudet, R.G., Gray-Owen, S.D., and Salama, N.R. (2017) TIFA signaling in gastric epithelial cells initiates the cag type 4 secretion system-dependent innate immune response to *Helicobacter pylori* infection. *MBio* **8:** pii: e01168-17.

Garcillan-Barcia, M.P., Francia, M.V., and de la Cruz, F. (2009) The diversity of conjugative relaxases and its application in plasmid classification. *FEMS Microbiol Rev* **33:** 657-687.

Ghosal, D., Chang, Y.W., Jeong, K.C., Vogel, J.P., and Jensen, G.J. (2017) In situ structure of the *Legionella* Dot/Icm type IV secretion system by electron cryotomography. *EMBO Rep* **18:** 726-732.

Gillespie, J.J., Ammerman, N.C., Dreher-Lesnick, S.M., Rahman, M.S., Worley, M.J., Setubal, J.C., *et al*. (2009) An anomalous type IV secretion system in *Rickettsia* is evolutionarily conserved. PLoS One **4**: e4833.

Gillespie, J.J., Brayton, K.A., Williams, K.P., Diaz, M.A., Brown, W.C., Azad, A.F., *et al*. (2010) Phylogenomics reveals a diverse *Rickettsiales* type IV secretion system. *Infect Immun* **78:** 1809-1823.

Gillespie, J.J., Phan, I.Q., Driscoll, T.P., Guillotte, M.L., Lehman, S.S., Rennoll-Bankert, K.E., *et al*. (2016) The *Rickettsia* type IV secretion system: unrealized complexity mired by gene family expansion. *Pathog Dis* 2016 **74**.

Goessweiner-Mohr, N., Grumet L., Arends, K., Pavkov-Keller, T., Gruber, C.C., Gruber, K., *et al*. (2013a) The 2.5 A structure of the *Enterococcus* conjugation protein TraM resembles VirB8 type IV secretion proteins. *J Biol Chem* **288**: 2018-2028.

Goessweiner-Mohr, N., Arends, K., Keller W., and Grohmann, E. (2013b) Conjugative type IV secretion in Gram-positive bacteria. *Plasmid* **70:** 289-302.

Goessweiner-Mohr, N., Arends, K., Keller, W., and Grohmann, E. (2014a) Conjugation in Gram-positive bacteria. *Microbiol Spectrum* **2:** PLAS-0004-2013.

Goessweiner-Mohr, N., Eder, M., Hofer, G., Fercher, C., Arends, K., Birner-Gruenberger, R., *et al*. (2014b) Structure of the double-stranded DNA-binding type IV secretion protein TraN from *Enterococcus. Acta Crystallogr D Biol Crystallogr* **70:** 2376-2389.

Gomis-Rüth, F.X., Moncalián, G., Pérez-Luque, R., González, A., Cabezón, E., de la Cruz, F., *et al.* (2001) The bacterial conjugation protein TrwB resembles ring helicases and F1-ATPase. *Nature* **409:** 637-641.

González-Prieto, C., Gabriel, R., Dehio, C., Schmidt, M., and Llosa, M. (2017) The conjugative relaxase TrwC promotes integration of foreign DNA in the human genome. *Appl Environ Microbiol* **83.** pii: e00207-17.

Gordon, J.E., Costa, T.R.D., Patel, R.S., Gonzalez-Rivera, C., Sarkar, M.K., Orlova, E.V., *et al.* (2017) Use of chimeric type IV secretion systems to define contributions of outer membrane subassemblies for contact-dependent translocation. *Mol Microbiol* **105:** 273-293.

Grohmann, E., Goessweiner-Mohr, N., and Brantl, S. (2016) DNA-binding proteins regulating pIP501 transfer and replication. Review. *Front Mol Biosci* 11.8.2016.

Gruber, C.J, Lang, S., Rajendra, V.K.H., Nuk, M., Raffl, S., Schildbach, J.F., *et al*. (2016) Conjugative DNA transfer is enhanced by plasmid R1 partitioning proteins. *Front Mol Biosci* **3**. doi: 10.3389/fmolb.2016.00032.

Guglielmini, J., de la Cruz, F., and Rocha, E.P. (2013) Evolution of conjugation and type IV secretion systems. *Mol Biol Evol* **30:** 315-331.

Guglielmini, J., Neron, B., Abby S.S., Garcillan-Barcia, M.P., de la Cruz F., and Rocha, E.P. (2014) Key components of the eight classes of type IV secretion systems involved in bacterial conjugation or protein secretion. *Nucleic Acids Res* **42:** 5715-5727.

Hare, S., Bayliss, R., Baron, C., and Waksman, G. (2006) A large domain swap in the VirB11 ATPase of *Brucella suis* leaves the hexameric assembly intact. *J Mol Biol* **360:** 56-66.

Hayashi, T., Senda, M., Morohashi, H., Higashi, H., Horio, M., Kashiba, Y., *et al.* (2012) Tertiary structure-function analysis reveals the pathogenic signaling potentiation mechanism of *H. pylori* oncogenic effector CagA. *Cell Host Microbe* **12:** 20-33.

Higashi, H., Tsutsumi, R., Muto, S., Sugiyama, T., Azuma, T., Asaka, M., *et al.* (2002) SHP-2 tyrosine phosphatase as an intracellular target of *H. pylori* CagA protein. *Science* **295:** 683-686.

Hilbi, H., Weber, S., and Finsel, I. (2011) Anchors for effectors: subversion of phospho-inositide lipids by *Legionella*. *Front Microbiol* **2:** 91.

Hofreuter, D., Odenbreit, S., Püls, J., Schwan, D., and Haas, R. (2000) Genetic competence in *Helicobacter pylori*: mechanisms and biological implications. *Res Microbiol.* **151:** 487-491.

Hofreuter, D., Odenbreit, S., Henke, G., and Haas, R. (1998) Natural competence for DNA transformation in *Helicobacter pylori*: identification and genetic characterization of the *comB* locus. *Mol Microbiol* **28:** 1027–1038.

Hofreuter, D., Karnholz, A., and Haas, R. (2003) Topology and membrane interaction of *Helicobacter pylori* ComB proteins involved in natural transformation competence. *Int J Med Microbiol* **293:** 153–165.

Horwitz, M.A. (1983) Formation of a novel phagosome by the Legionnaires' disease bacterium (*Legionella pneumophila*) in human monocytes. *J Exp Med* **158:** 1319–1331.

Hospenthal, M.K., Costa, T.R.D., and Waksman, G. (2017) A comprehensive guide to pilus biogenesis in Gram-negative bacteria. *Nat Rev Microbiol* **15:** 365-379.

Howe, D., and Heinzen, R.A. (2006) *Coxiella burnetii* inhabits a cholesterol-rich vacuole and influences cellular cholesterol metabolism. *Cell Microbiol* **8:** 496–507.

Hsu, F., Zhu, W., Brennan, L., Tao, L., Luo, Z.Q., and Mao, Y. (2012) Structural basis for substrate recognition by a unique *Legionella* phosphoinositide phosphatase. *Proc Natl Acad Sci USA* **109:** 13567–13572.

Ilangovan, A., Kay, C.W.M., Roier, S., El Mkami, H., Salvadori, E., Zechner, E.L., *et al.* (2017) Cryo-EM structure of a relaxase reveals the molecular basis of DNA unwinding during bacterial conjugation. *Cell* **169:** 708-721.

Isberg, R.R., O'Connor, T.J., and Heidtman, M. (2009) The *Legionella pneumophila* replication vacuole: making a cozy niche inside host cells. *Nat Rev Microbiol* **7:** 13–24.

Itzen, A. and Goody, R.S. (2011) Covalent coercion by *Legionella pneumophila*. *Cell Host Microbe* **10:** 89–91.

Jakubowski, S.J., Kerr, J.E., Garza, I., Krishnamoorthy, V., Bayliss, R., Waksman, G., *et al.* (2009) *Agrobacterium* VirB10 domain requirements for type IV secretion and T pilus biogenesis. *Mol Microbiol* **71:** 779-794.

Javaheri, A., Kruse, T., Moonens, K., Mejías-Luque, R., Debraekeleer, A., Asche, C.I., *et al.* (2016) *Helicobacter pylori* adhesin HopQ engages in a virulence-enhancing interaction with human CEACAMs. *Nat Microbiol* **2:** 16189.

Jeong, K.C., Ghosal, D., Chang, Y.W., Jensen, G.J., and Vogel, J.P. (2017) Polar delivery of *Legionella* type IV secretion system substrates is essential for virulence. *Proc Natl Acad Sci* USA **107:** 18997-9001.

Jiang, X., Yang, Y., Zhou, J., Zhu, L., Gu, Y., Zhang, X., *et al.* (2016) Roles of the putative type IV-like secretion system key component VirD4 and PrsA in pathogenesis of *Streptococcus suis* type 2. *Front Cell Infect Microbiol* **6:** 172.

Johnson, C.M., and Grossman, A.D. (2015) Integrative and Conjugative Elements (ICEs): What they do and how they work. *Annu Rev Genet* **49:** 577-601.

Kaplan-Türköz, B., Jiménez-Soto, L.F., Dian, C., Ertl, C., Remaut, H., Louche, A., *et al.* (2012) Structural insights into *H. pylori* oncoprotein CagA interaction with β_1 integrin. *Proc Natl Acad Sci* **109:** 14640-14645.

Ke, Y., Wang, Y., Li, W., Chen, Z. (2015) Type IV secretion system of *Brucella spp*. and its effectors. *Front Cell Infect Microbiol* **5:** 72.

Keestra-Gounder, A.M., Byndloss, M.X., Seyffert, N., Young, B.M., Chávez-Arroyo, A., Tsai, A.Y., *et al.* (2016) NOD1 and NOD2 signaling links ER stress with inflammation. *Nature* **532:** 394-397.

Kohler, P.L., Chan, Y.A., Hackett, K.T., Turner, N., Hamilton, H.L., Cloud-Hansen, K.A., *et al.* (2013) Mating pair formation homologue TraG is a variable membrane protein essential for contact-independent type IV secretion of chromosomal DNA by *Neisseria gonorrhoeae*. *J Bacteriol* **195:** 1666-1679.

Kohler, L.J., and Roy, C.R. (2015) Biogenesis of the lysosome-derived vacuole containing *Coxiella burnetii.* *Microbes Infect* **17:** 766-771.

Kohler, L.J., Reed, S.C., Sarraf, S.A., Arteaga, D.D., Newton, H.J., and Roy, C.R. (2016) Effector protein Cig2 decreases host tolerance of infection by directing constitutive fusion of autophagosomes with the *Coxiella*-containing vacuole. *MBio* **7:** pii: e01127-16.

Kohler, V., Probst, I., Aufschnaiter, A., Büttner, S., Schaden, L., Rechberger, G.N., *et al.* (2017) Conjugative type IV secretion in Gram-positive pathogens: TraG, a lytic transglycosylase and endopeptidase, interacts with translocation channel protein TraM. *Plasmid* **91:**9-18.

Königer, V., Holsten, L., Harrison, U., Busch, B., Loell, E., Zhao, Q., *et al.* (2016) *Helicobacter pylori* exploits human CEACAMs via HopQ for adherence and translocation of CagA. *Nat Microbiol* **2:** 16188.

Koraimann, G., and Wagner, M.A. (2014) Social behavior and decision making in bacterial conjugation. *Front Cell Infect Microbiol* **4:** 54.

Krüger, N.-J., and Stingl, K. (2011) Two steps away from novelty – principles of bacterial DNA uptake. *Mol Microbiol* **80:** 860–867.

Kubori, T., Koike, M., Bui, X.T., Higaki, S., Aizawa, S., and Nagai, H. (2014) Native structure of a type IV secretion system core complex essential for *Legionella* pathogenesis. *Proc Natl Acad Sci USA* **111:** 11804-11809.

Kwak, M.J., Kim, J.D., Kim, H., Kim, C., Bowman, J.W., Kim, S., *et al.* (2017) Architecture of the type IV coupling protein complex of *Legionella pneumophila*. *Nat Microbiol* **2**: 17114.

Kwok, T., Zabler, D., Urman, S., Rohde, M., Hartig, R., Wessler, S., *et al.* (2007) *Helicobacter* exploits integrin for type IV secretion and kinase activation. *Nature* **449:** 862-866.

Lang, S., Gruber, C.J., Raffl, S., Reisner, A., Zechner, E.L. (2014) Common requirement for the relaxosome of plasmid R1 in multiple activities of the conjugative type IV secretion system. *J Bacteriol* **196**: 2108-2121.

Latomanski, E.A., Newton, P., Khoo, C.A., and Newton, H.J. (2016) The effector Cig57 hijacks FCHO-mediated vesicular trafficking to facilitate intracellular replication of *Coxiella burnetii*. *PLoS Pathog* **12:** e1006101.

Laverde Gomez, J.A., Bhatty, M., and Christie, P.J. (2014) PrgK, a multidomain peptidoglycan hydrolase, is essential for conjugative transfer of the pheromone-responsive plasmid pCF10. *J Bacteriol* **196:** 527-539.

Laverde, D., Probst, I., Romero-Saavedra, F., Kropec, A., Wobser, D., Keller, W., *et al.* (2017) Targeting type IV secretion system proteins to combat multiresistant Gram-Positive pathogens. *J Infect Dis* **215:** 1836-1845.

Lawley, T.D., Klimke, W.A., Gubbins, M.J., and Frost, L.S. (2003) F factor conjugation is a true type IV secretion system. *FEMS Microbiol Lett.* **224:** 1-15.

Lee, J.J., Kim, D.G., Kim, D.H., Simborio, H.L., Min, W., Lee, H.J., *et al.* (2013) Interplay between clathrin and Rab5 controls the early phagocytic trafficking and intracellular survival of *Brucella abortus* within HeLa cells. *J Biol Chem* **288:** 28049-28057.

Leonard, T.A., Moller-Jensen, J., and Lowe, J. (2005). Towards understanding the molecular basis of bacterial DNA segregation. *Philos Trans R Soc Lond B Biol Sci* **360:** 523–535.

Leonetti, C.T., Hamada, M.A., Laurer, S.J., Broulidakis, M.P., Swerdlow, K.J., Lee, C.A., *et al.* (2015) Critical components of the conjugation machinery of the Integrative and Conjugative Element ICE*Bs1* of *Bacillus subtilis*. *J Bacteriol* **197:** 2558-2567.

Li, M., Shen, X., Yan, J., Han, H., Zheng, B., Liu, D., *et al.* (2011) GI-type T4SS-mediated horizontal transfer of the 89K pathogenicity island in epidemic *Streptococcus suis* serotype 2. *Mol Microbiol* **79:** 1670-1683.

Li, F., Alvarez-Martinez, C., Chen, Y., Choi, K.J., Yeo, H.J., and Christie, P.J. (2012) *Enterococcus faecalis* PrgJ, a VirB4-like ATPase, mediates pCF10 conjugative transfer through substrate binding. *J Bacteriol* **194:** 4041-4051.

Lifshitz Z, Burstein D, Peeri M, Zusman T, Schwartz K, Shuman HA, *et al.* (2013) Computational modeling and experimental validation of the *Legionella* and *Coxiella* virulence-related type-IVB secretion signal. *Proc Natl Acad Sci USA* **110:** E707-15.

Lin, M., den Dulk-Ras, A., Hooykaas, P.J. and Rikihisa, Y. (2007) *Anaplasma phagocytophilum* AnkA secreted by type IV secretion system is tyrosine phosphorylated by Abl-1 to facilitate infection. *Cell Microbiol* **9:** 2644-2657.

Lipscomb, G.L., Stirrett, K., Schut, G.J., Yang, F., Jenney, F.E. Jr., Scott, R.A. *et al.* (2011) Natural competence in the hyperthermophilic archaeon *Pyrococcus furiosus* facilitates genetic manipulation: construction of markerless deletions of genes encoding the two cytoplasmic hydrogenases. *Appl Env Microbiol* **77:** 2232–2238.

Llosa, M., and de la Cruz, F. (2005) Bacterial conjugation: a potential tool for genomic engineering. *Res Microbiol* **156:** 1-6.

Llosa, M., Schroder, G., and Dehio, C. (2012) New perspectives into bacterial DNA transfer to human cells. *Trends Microbiol* **20:** 355-359.

Lockwood, S., Voth, D.E., Brayton, K.A., Beare, P.A., Brown, W.C., Heinzen, R.A., *et al.* (2011) Identification of *Anaplasma marginale* type IV secretion system effector proteins. *PLoS One* **6:** e27724.

Low, H.H., Gubellini, F., Rivera-Calzada, A.F., Braun, N., Connery, S., Dujeancourt, A., *et al.* (2014) Structure of a type IV secretion system. *Nature* **508:** 550-553.

Lu, H., and Clarke, M. (2005) Dynamic properties of *Legionella*-containing phagosomes in *Dictyostelium* amoebae. *Cell Microbiol* **7:** 995–1007.

Lührmann, A., Nogueira, C.V., Carey, K.L., and Roy, C.R. (2010) Inhibition of pathogen-induced apoptosis by a *Coxiella burnetii* type IV effector protein. *Proc Natl Acad Sci USA* **107:** 8997-9001.

Machner, M.P., and Isberg, R.R. (2006) Targeting of host Rab GTPase function by the intravacuolar pathogen *Legionella pneumophila*. *Dev Cell* **11:** 47–56.

Marchesini, M.I., Herrmann, C.K., Salcedo, S.P., Gorvel, J.P., and Comerci, D.J. (2011) In search of *Brucella abortus* type IV secretion substrates: screening and identification of four proteins translocated into host cells through VirB system. *Cell Microbiol* **13:** 1261-1274.

McDonough, J.A., Newton, H.J., Klum, S., Swiss R, Agaisse H, and Roy CR. (2013) Host pathways important for *Coxiella burnetii* infection revealed by genome-wide RNA interference screening. *MBio* **4:** e00606–12.

Mimuro, H., Suzuki, T., Tanaka, J., Asahi,M., Haas, R., and Sasakawa, C. (2002) Grb2 is a key mediator of *H. pylori* CagA protein activities. *Mol Cell* **10:** 745-755.

Moffatt, J.H., Newton, P., and Newton, H.J. (2015) *Coxiella burnetii*: turning hostility into a home. *Cell Microbiol* **17:** 621-631.

Morgan, J.K., Luedtke, B.E., and Shaw, E.I. (2010) Polar localization of the *Coxiella burnetii* type IVB secretion system. *FEMS Microbiol Lett* **305:** 177-183.

Mueller, D., Tegtmeyer, N., Brandt, S., Yamaoka, Y., De Poire, E., Sgouras, D., *et al.* (2012) c-Src and c-Abl kinases control hierarchic phosphorylation and function of the CagA effector protein in Western and East Asian *Helicobacter pylori* strains. *J Clin Invest* **122:** 1553-1566.

Murata, T., Delprato, A., Ingmundson, A., Toomre, D.K., Lambright, D.G., and Roy, C.R. (2006) The *Legionella pneumophila* effector protein DrrA is a Rab1 guanine nucleotide-exchange factor. *Nat Cell Biol* **8:** 971–977.

Myeni, S., Child, R., Ng, T.W., Kupko, J.J. 3rd, Wehrly, T.D., Porcella, S.F., *et al.* (2013) *Brucella* modulates secretory trafficking via multiple type IV secretion effector proteins. *PLoS Pathog* **9:** e1003556.

Nagai, H., Kagan, J.C., Zhu, X., Kahn, R.A., and Roy, C.R. (2002) A bacterial guanine nucleotide exchange factor activates ARF on *Legionella* phagosomes. *Science* **295:** 679–682.

Nagai, H., and Kubori, T. (2011) Type IVB secretion systems of *Legionella* and other Gram-negative bacteria. *Front Microbiol* **2:** 136.

Nešić, D., Buti, L., Lu, X., and Stebbins, C.E. (2014) Structure of the *Helicobacter pylori* CagA oncoprotein bound to the human tumor suppressor ASPP2. *Proc Natl Acad Sci USA* **111:** 1562-1567.

Niu, H., Kozjak-Pavlovic, V., Rudel T., and Rikihisa, Y. (2010) *Anaplasma phagocytophilum* Ats-1 is imported into host cell mitochondria and interferes with apoptosis induction. *PLoS Pathog* **6**: e1000774.

Niu, H., and Rikihisa, Y. (2013) Ats-1: a novel bacterial molecule that links autophagy to bacterial nutrition. *Autophagy* **9**: 787-788.

Niu, H., and Rikihisa, Y. (2014) Investigating interference with apoptosis induction by bacterial proteins. *Methods Mol Biol* **1197:** 169-184.

Obergfell, K.P., and Seifert, H.S. (2015) Mobile DNA in the pathogenic *Neisseria*. *Microbiol Spectr* **3:** MDNA3-0015-2014.

Ohashi, N., Zhi, N., Lin, Q., and Rikihisa, Y. (2002) Characterization and transcriptional analysis of gene clusters for a type IV secretion machinery in human granulocytic and monocytic ehrlichiosis agents. *Infect Immun* **70:** 2128-2138.

Ohnishi, N., Yuasa, H., Tanaka, S., Sawa, H., Miura, M., Matsui, A., *et al.* (2008) Transgenic expression of *Helicobacter pylori* CagA induces gastrointestinal and hematopoietic neoplasms in mouse. *Proc Natl Acad Sci USA* **105:** 1003-1008.

Pachulec, E., Siewering, K., Bender, T., Heller, E.M., Salgado-Pabon, W., Schmoller, S.K., *et al.* (2014) Functional analysis of the gonococcal genetic island of *Neisseria gonorrhoeae. PLOs One* **9:** e109613.

Palanivelu, D.V., Goepfert, A., Meury, M., Guye, P., Dehio, C., and Schirmer, T. (2011) Fic domain-catalyzed adenylylation: insight provided by the structural analysis of the type IV secretion system effector BepA. *Protein Sci* **20:** 492-499.

Pan, X., Lührmann, A., Satoh, A., Laskowski-Arce, M.A., and Roy, C.R. (2008) Ankyrin repeat proteins comprise a diverse family of bacterial type IV effectors. *Science* **320:** 1651–1654.

Paschos, A., den Hartigh, A., Smith, M.A., Atluri, V.L., Sivanesan, D., Tsolis, R.M., *et al.* (2011) An in vivo high-throughput screening approach targeting the type IV secretion system component VirB8 identified inhibitors of *Brucella abortus* 2308 proliferation. *Infect Immun* **79:** 1033-1043.

Personnic, N., Bärlocher, K., Finsel, I., and Hilbi, H. (2016) Subversion of retrograde trafficking by translocated pathogen effectors. *Trends Microbiol* **24:** 450-462.

Pizarro-Cerdá, J., Moreno, E., Sanguedolce, V., Mege, J.L., and Gorvel, J.P. (1998) Virulent *Brucella abortus* prevents lysosome fusion and is distributed within autophagosome-like compartments. *Infect Immun* **66:** 2387-2392.

Porter, C.J., Bantwal, R., Bannam, T.L., Rosado, C.J., Pearce, M.C., Adams, V., *et al.* (2012) The conjugation protein TcpC from *Clostridium perfringens* is structurally related to the type IV secretion system protein VirB8 from Gram-negative bacteria. *Mol Microbiol* **83:** 275-288.

Ragaz, C., Pietsch, H., Urwyler, S., Tiaden, A., Weber, S.S., and Hilbi, H. (2008) The *Legionella pneumophila* phosphatidylinositol-4 phosphate-binding type IV substrate SidC recruits endoplasmic reticulum vesicles to a replication-permissive vacuole. *Cell Microbiol* **10:** 2416–2433.

Ramírez-Puebla, S.T., Ormeño-Orrillo, E., Vera-Ponce de León, A., Lozano, L., Sanchez-Flores, A., Rosenblueth, M., *et al.* (2016) Genomes of candidatus *Wolbachia bourtzisii* wDacA and

candidatus *Wolbachia pipientis* wDacB from the cochineal insect *Dactylopius coccus* (Hemiptera: Dactylopiidae). *G3 (Bethesda)* **6:** 3343-3349.

Redzej, A., Ilangovan, A., Lang, S., Gruber, C.J., Topf, M., Zangger, K., *et al*. (2013) Structure of a translocation signal domain mediating conjugative transfer by type IV secretion systems. *Mol Microbiol* **89:** 324–333.

Redzej, A., Ukleja, M., Connery, S., Trokter, M., Felisberto-Rodrigues, C., Cryar, A., *et al*. (2017) Structure of a VirD4 coupling protein bound to a VirB type IV secretion machinery. *EMBO J* pii: e201796629.

Regier, Y., O Rourke, F., and Kempf, V.A. (2016) *Bartonella spp.* - a chance to establish One Health concepts in veterinary and human medicine. *Parasit Vectors* **9:** 261.

Rikihisa, Y. (2015) Molecular pathogenesis of *Ehrlichia chaffeensis* infection. *Annu Rev Microbiol* **69:** 283-304.

Rikihisa, Y. (2017) Subversion of RAB5-regulated autophagy by the intracellular pathogen *Ehrlichia chaffeensis*. *Small GTPases* **26:** 1-7.

Rivera-Calzada, A., Fronzes, R., Savva, C.G., Chandran, V., Lian, P.W., Laeremans, T., *et al.* (2013) Structure of a bacterial type IV secretion core complex at subnanometre resolution. *EMBO J* **32:** 1195-1204.

Robinson, C.G., and Roy, C.R. (2006) Attachment and fusion of endoplasmic reticulum with vacuoles containing *Legionella pneumophila*. *Cell Microbiol* **8:** 793–805.

Rohrer, S., Holsten, L., Weiss, E., Benghezal, M., Fischer, W., and Haas, R. (2012) Multiple pathways of plasmid DNA transfer in *Helicobacter pylori*. *PLOS ONE* **7:** e45623.

Rothmeier, E., Pfaffinger, G., Hoffmann, C., Harrison, C.F., Grabmayr, H., Repnik, U., *et al.* (2013) Activation of Ran GTPase by a *Legionella* effector promotes microtubule polymerization, pathogen vacuole motility and infection. *PLoS Pathog* **9:** e1003598.

Saadat, I., Higashi, H., Obuse, C., Umeda, M., Murata-Kamiya, N., Saito, Y., *et al.* (2007) *Helicobacter pylori* CagA targets PAR1/MARK kinase to disrupt epithelial cell polarity. *Nature* **447:** 330-333.

Salama, N.R., Hartung, M.L., and Mueller, A. (2013) Life in the human stomach: persistence strategies of the bacterial pathogen *Helicobacter pylori*. *Nat Rev Microbiol* **11:** 385–399.

Samuels, A.L., Lanka, E., and Davies, J.E. (2000) Conjugative junctions in RP4- mediated mating of *Escherichia coli*. *J Bacteriol* **182:** 2709-2715.

Savvides, S.N., Yeo, H.J., Beck, M.R., Blaesing, F., Lurz, R., Lanka, E., *et al.* (2003) VirB11 ATPases are dynamic hexameric assemblies: new insights into bacterial type IV secretion. *EMBO J* **22:** 1969-1980.

Schmidt, T.P., Perna, A.M., Fugmann, T., Böhm, M., Hiss, J., Haller, S., *et al*. (2016) Identification of E-cadherin signature motifs functioning as cleavage sites for *Helicobacter pylori* HtrA. *Sci Rep* **6:** 23264.

Schoebel, S., Blankenfeldt, W., Goody, R.S., and Itzen A. (2010) High-affinity binding of phosphatidyl-linositol 4-phosphate by *Legionella pneumophila* DrrA. *EMBO Rep* **11:** 598–604.

Schröder, G., Schuelein, R., Quebatte, M., and Dehio, C. (2011) Conjugative DNA transfer into human cells by the VirB/VirD4 type IV secretion system of the bacterial pathogen *Bartonella henselae*. *Proc Natl Acad Sci USA* **108:** 14643-14648.

Schulein, R., Guye, P., Rhomberg, T.A., Schmid, M.C., Schröder, G., Vergunst, A.C., *et al.* (2005) A bipartite signal mediates the transfer of type IV secretion substrates of *Bartonella henselae* into human cells. *Proc Natl Acad Sci USA* **102:** 856-861.

Segal, G., Feldman, M., and Zusman, T. (2005) The Icm/Dot type-IV secretion systems of *Legionella pneumophila* and *Coxiella burnetii*. *FEMS Microbiol Rev* **29:** 65-81.

Selbach, M., Paul, F.E., Brandt, S., Guye, P., Daumke, O., Backert, S., *et al.* (2009) Host cell interactome of tyrosine-phosphorylated bacterial proteins. *Cell Host Microbe* **5:** 397-403.

Shaffer, C.L., Good J.A., Kumar, S., Krishnan, K.S., Gaddy, J.A., Loh, J.T., *et al*. (2016) Peptidomimetic small molecules disrupt type IV secretion system activity in diverse bacterial pathogens. *MBio* **7:** e00221-00216.

Sharma, P., Teymournejad, O., and Rikihisa, Y. (2017) Peptide Nucleic Acid knockdown and intra-host cell complementation of *Ehrlichia* type IV secretion system effector. *Front Cell Infect Microbiol.* **7:** 228.

Sherwood, R.K., and Roy, C.R. (2016) Autophagy Evasion and Endoplasmic Reticulum Subversion: The Yin and Yang of *Legionella* Intracellular Infection. *Annu Rev Microbiol* **70:** 413-433.

Siamer, S., and Dehio, C. (2015) New insights into the role of *Bartonella* effector proteins in pathogenesis. *Curr Opin Microbiol* **23:** 80–85.

Simon, S., Wagner, M.A., Rothmeier, E., Müller-Taubenberger, A., and Hilbi, H. (2014) Icm/Dot-dependent inhibition of phagocyte migration by *Legionella* is antagonized by a translocated Ran GTPase activator. *Cell Microbiol* **16:** 977–992.

Smeets, L.C., Bijlsma, J.J., Kuipers, E.J., Vandenbroucke-Grauls, C.M., and Kusters, J.G. (2000a) The *dprA* gene is required for natural transformation of *Helicobacter pylori*. *FEMS Immunol Med Microbiol* **27:** 99–102.

Smeets, L.C., Bijlsma, J.J., Boomkens, S.Y., Vandenbroucke-Grauls, C.M., and Kusters, J.G. (2000b) *comH*, a novel gene essential for natural transformation of *Helicobacter pylori*. *J Bacteriol* **182:** 3948–3954.

Smith, E.P., Miller, C.N., Child, R., Cundiff, J.A., and Celli, J. (2016) Postreplication roles of the *Brucella* VirB Type IV secretion system uncovered via conditional expression of the VirB11 ATPase. *MBio* **7:** pii: e01730-16.

Sonthayanon, P., Peacock, S.J., Chierakul, W., Wuthiekanun, V., Blacksell, S.D., Holden, M.T., *et al*. (2010) High rates of homologous recombination in the mite endosymbiont and opportunistic human pathogen *Orientia tsutsugamushi*. *PLoS Negl Trop Dis* **4**: e752.

Souza, D.P., Oka, G.U., Alvarez-Martinez, C.E., Bisson-Filho, A.W., Dunger, G., Hobeika, L., *et al.* (2015) Bacterial killing via a type IV secretion system. *Nat Commun* **6:** 6453.

Souza, G., Oka, G., Sgro, L., Coutinho de Oliveira, N., Bueno, W., Cenens, E., *et al*. (2016) Bacterial Wars: a new role for Type IV Secretion Systems. www.t4ss-conference.de

Starr, T., Ng, T.W., Wehrly, T.D., Knodler, L.A., and Celli, J. (2008) *Brucella* intracellular replication requires trafficking through the late endosomal/lysosomal compartment. *Traffic* **9:** 678-694.

Stein, S.C., Faber, E., Bats, S.H., Murillo, T., Speidel, Y., Coombs, N., *et al.* (2017) *Helicobacter pylori* modulates host cell responses by CagT4SS-dependent translocation of an intermediate metabolite of LPS inner core heptose biosynthesis. *PLoS Pathog* **13:** e1006514.

Stingl, K., Müller, S., Scheidgen-Kleyboldt, G., Clausen, M., and Maier, B. (2010) Composite system mediates two-step DNA uptake into *Helicobacter pylori*. *Proc Natl Acad Sci USA* **107:** 1184–1189.

Tegtmeyer, N., Wessler, S., Necchi, V., Rohde, M., Harrer, A., Rau, T.T., *et al*. (2017a) A unique basolateral type IV secretion model for the CagA oncoprotein of *Helicobacter pylori. Cell Host Microbe* **22**: 552-560.e5.

Tegtmeyer, N., Neddermann, M., Asche, C.I., and Backert, S. (2017b) Subversion of host kinases: a key network in cellular signaling hijacked by *Helicobacter pylori* CagA. *Mol Microbiol* **105:** 358-372.

Toulabi, L., Wu, X., Cheng, Y., and Mao, Y. (2013) Identification and structural characterization of a *Legionella* phosphoinositide phosphatase. *J Biol Chem* **288:** 24518–24527.

Trokter, M., Felisberto-Rodrigues, C., Christie, P.J., and Waksman, G. (2014) Recent advances in the structural and molecular biology of type IV secretion systems. *Curr Opin Struct Biol* **27:** 16-23.

Urwyler, S. (2009) Proteome analysis of *Legionella* vacuoles purified by magnetic immunoseparation reveals secretory and endosomal GTPases. *Traffic* **10:** 76–87.

Varga, M.G., Shaffer, C.L., Sierra, J.C., Suarez, G., Piazuelo, M.B., Whitaker, M.E., *et al*. (2016) Pathogenic *Helicobacter pylori* strains translocate DNA and activate TLR9 via the cancer-associated cag type IV secretion system. *Oncogene* **35:** 6262-6269.

Vayssier-Taussat, M., Le Rhun, D., Deng, H.K., Biville, F., Cescau, S., Danchin, A., *et al.* (2010) The Trw type IV secretion system of *Bartonella* mediates host-specific adhesion to erythrocytes. *PLoS Pathog* **6:** e1000946.

Viala, J., Chaput, C., Boneca, I.G., Cardona, A., Girardin, S.E., Moran, A.P., *et al.* (2004) Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori* cag pathogenicity island. *Nat Immunol* **5:** 1166-1174.

Vogel, J.P., and Isberg, R.R. (1999) Cell biology of *Legionella pneumophila*. *Curr Opin Microbiol* **2:** 30-34.

Voth, D.E., and Heinzen, R.A. (2007) Lounging in a lysosome: the intracellular lifestyle of *Coxiella burnetii. Cell Microbiol* **9:** 829–840.

Voth, D.E., and Heinzen, R.A. (2009) *Coxiella* type IV secretion and cellular microbiology. *Curr Opin Microbiol* **12:** 74-80.

Wagner, A., Whitaker, R.J., Krause, D.J., Heilers, J.H., van Wolferen, M., van der Does, C., *et al*. (2017) Mechanisms of gene flow in archaea. *Nat Rev Microbiol* **15:** 492-501.

Wallden, K., Williams, R., Yan, J., Lian, P.W., Wang, L., Thalassinos, K., *et al.* (2012) Structure of the VirB4 ATPase, alone and bound to the core complex of a type IV secretion system. *Proc Natl Acad Sci U S A* **109:** 11348-11353.

Wang, Y.A., Yu, X., Silverman, P.M., Harris, R.L., and Egelman, E.H. (2009) The structure of F-pili. *J Mol Biol* **385:** 22-29.

Wang, J., Feng, Y., Wang, C., Srinivas, S., Chen, C., Liao, H., *et al*. (2017) Pathogenic *Streptococcus* strains employ novel escape strategy to inhibit bacteriostatic effect mediated by mammalian peptidoglycan recognition protein. *Cell Microbiol* **19** doi: 10.1111/cmi.12724.

Weber, S., Wagner, M., and Hilbi, H. (2014) Live-cell imaging of phosphoinositide dynamics and membrane architecture during *Legionella* infection. *MBio* **5:** e00839–13.

Weber, S.S., Ragaz, C., and Hilbi, H. (2009) The inositol polyphosphate 5-phosphatase OCRL1 restricts intracellular growth of *Legionella*, localizes to the replicative vacuole and binds to the bacterial effector LpnE. *Cell Microbiol* **11:** 442–460.

Weber, S.S., Ragaz, C., Reus, K., Nyfeler, Y., and Hilbi, H. (2006) *Legionella pneumophila* exploits PI(4)P to anchor secreted effector proteins to the replicative vacuole. *PLoS Pathog* **2:** e46.

Whitaker, N., Chen, Y., Jakubowski, S.J., Sarkar, M.K., Li, F., and Christie, P.J. (2015) The all-alpha domains of coupling proteins from the *Agrobacterium tumefaciens* VirB/VirD4 and *Enterococcus faecalis* pCF10-encoded type IV secretion systems confer specificity to binding of cognate DNA substrates. *J Bacteriol.* **197:** 2335-2349.

Wisniewski, J.A., Traore, D.A., Bannam, T.L., Lyras, D., Whisstock, J.C., and Rood, J.I. (2016) TcpM: a novel relaxase that mediates transfer of large conjugative plasmids from *Clostridium perfringens*. *Mol Microbiol* **99:** 884–896.

Wisniewski, J.A., and Rood, J.I. (2017) The Tcp conjugation system of *Clostridium perfringens. Plasmid* **91:**28-36.

Wisniewski, J.A., Teng, W.L., Bannam, T.L., and Rood, J.I. (2015) Two novel membrane proteins, TcpD and TcpE, are essential for conjugative transfer of pCW3 in *Clostridium perfringens*. *J Bacteriol* **197:** 774-781.

Wong, J.J.W., Lu, J., and Glover, J.N.M. (2012) Relaxosome function and conjugation regulation in F-like plasmids - a structural biology perspective. *Mol Microbiol* **85:** 602-617.

Xu, L., Shen, X., Bryan, A., Banga, S., Swanson, M.S., and Luo, Z.Q. (2010) Inhibition of host vacuolar H^+-ATPase activity by a *Legionella pneumophila* effector. *PLoS Pathog* **6:** e1000822.

Yeh, Y.C., Lin, T.L., Chang, K.C., and Wang, J.T. (2003) Characterization of a ComE3 homologue essential for DNA transformation in *Helicobacter pylori. Infect Immun* **71:** 5427–5431.

Yeo, H.J., Savvides, S.N., Herr, A.B., Lanka, E., and Waksman, G. (2000) Crystal structure of the hexameric traffic ATPase of the *Helicobacter pylori* type IV secretion system. *Mol Cell* **6:** 1461-1472.

Yin, S., Li, M., Rao, X., Yao, X., Zhong, Q., Wang, M., *et al.* (2016) Subtilisin-like protease-1 secreted through type IV secretion system contributes to high virulence of *Streptococcus suis* 2. *Sci Rep* **6:** 27369.

Zamboni, D.S., McGrath, S., Rabinovitch M., and Roy, C.R. (2003) *Coxiella burnetii* express type IV secretion system proteins that function similarly to components of the *Legionella pneumophila* Dot/Icm system. *Mol Microbiol* **49:** 965–976.

Zechner, E.L., Lang, S., and Schildbach, J.F. (2012) Assembly and mechanisms of bacterial type IV secretion machines. *Philos Trans R Soc Lond B Biol Sci.* **367:** 1073-1087.

Zhang, X.S., Tegtmeyer, N., Traube, L., Jindal, S., Perez-Perez, G., Sticht, H., *et al.* (2015) A specific A/T polymorphism in Western tyrosine phosphorylation B-motifs regulates *Helicobacter pylori* CagA epithelial cell interactions. *PLoS Pathog* **11:** e1004621.

Zhao, Y., Liu, G., Li, S., Wang, M., Song, J., Wang, J., *et al.* (2011) Role of a type IV-like secretion system of *Streptococcus suis* 2 in the development of streptococcal toxic shock syndrome. *J Infect Dis* **204:** 274-281.

Zimmermann, S., Pfannkuch, L., Al-Zeer, M., Bartfeld, S., Koch, M., Liu, J., *et al*. (2017) ALPK1 and TIFA dependent innate immune response triggered by the *H. pylori* type IV secretion system. *Cell Rep* **20:** 2384-2395.

Zweig, M.A., Schork, S., Koerdt, A., Siewering, K., Sternberg, C., Thormann, K., *et al.* (2013). Secreted single-stranded DNA is involved in the initial phase of biofilm formation by *Neisseria gonorrhoeae*. *Environ Microbiol* **3:** 1462–2920.

Zusman, T., Yerushalmi, G., and Segal, G. (2003) Functional similarities between the *icm/dot* pathogenesis systems of *Coxiella burnetii* and *Legionella pneumophila*. *Infect. Immun* **71:** 3714–3723.

Figure legend

Figure 1. Schematic representation of type IV secretion architecture and functions in bacteria.

A. Conjugative T4SSs translocate DNA from the donor bacterium into various recipients, including other bacteria or eukaryotic cells.

B. DNA release systems facilitate an exchange of DNA with the extracellular space as well as biofilm formation.

C. DNA uptake from the environment proceeds by the ComB T4SS.

D. The *Xanthomonas citri* T4SS can deliver a protein toxin to kill neighboring Gram$^-$ bacterial competitors.

E. Various pathogenic bacteria and symbionts have evolved T4SSs to deliver effector proteins or DNA–protein complexes into their host (either eukaryotic target cells or protozoan hosts). The T4SSs can either inject their effectors directly into the host cell or secrete them into the medium, thereby exerting remarkably different effects on host cell functions during infection.

F. EM reconstructions showing the structure of the plasmid R388 T4SS complex and the core complex. Front view (left) and cut-away front view (right) of the T4SS complex (EMD-2567) comprising the core/outer membrane complex (core/OMC, green), the stalk (grey) and the inner membrane complex (IMC, blue). U-tier, M-tier and L-tier stand for upper, middle and lower tier, respectively. The inner (IM) and outer (OM) membranes are indicated.

G. pKM101 core complex (EMD-2232) (top) and truncated core complex lacking the N-terminal part of VirB10 (EMD-2233) (bottom): side view (left) and cut-away side view (right). The bottom right panel shows the superposition of the difference map (between the full-length and the truncated core complex cryo-EM maps) in green, and the cryo-EM structure of the truncated core complex in orange (as in bottom left). The VirB10 N-terminus forms the inner wall of the I-layer and the base.

H. Cryo-EM structure of the TraI relaxase-ssDNA complex revealed the molecular basis of DNA unwinding during bacterial conjugation.

I. To achieve genetic exchange during bacterial conjugation, two relaxase monomers collaborate, adopting distinct structural conformations to provide the two necessary enzymatic activities for processing the DNA.

J. Individual steps are indicated: (1) TraI opens to bind ssDNA and closes to surround DNA entirely during unwinding. (2) DNA binding to transesterase in closed TraI inhibits nicking. (3) DNA splitting by vestigial helicase. This figure was extensively updated from Backert and Meyer (2006), Trokter *et al.* (2014) and Ilangovan *et al.* (2017) with permission from CELL Press.

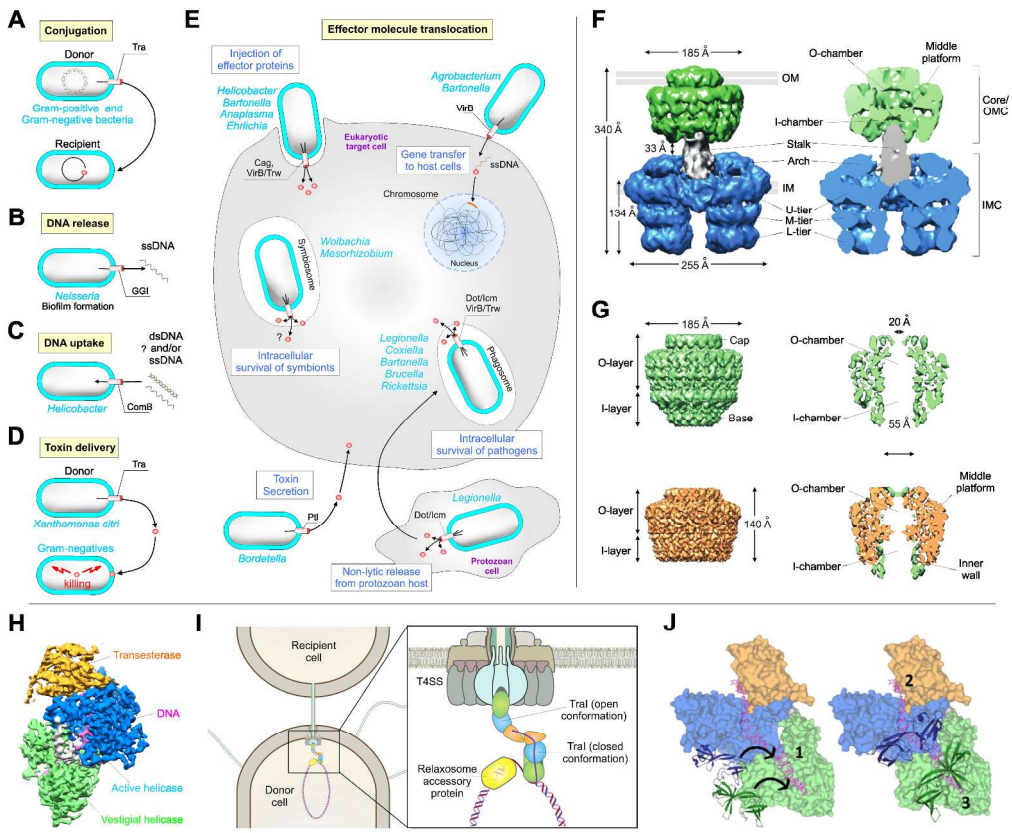
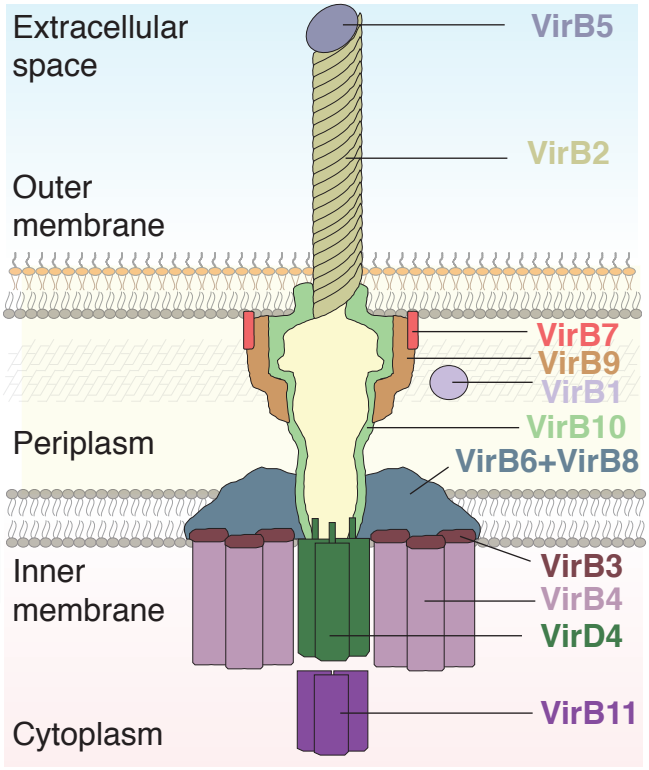


Figure 1. Schematic representation of type IV secretion architecture and functions in bacteria.
A. Conjugative T4SSs translocate DNA from the donor bacterium into various recipients, including other bacteria or eukaryotic cells.
B. DNA release systems facilitate an exchange of DNA with the extracellular space as well as biofilm formation.
C. DNA uptake from the environment proceeds by the ComB T4SS.
D. The Xanthomonas citri T4SS can deliver a protein toxin to kill neighboring Gram- bacterial competitors.
E. Various pathogenic bacteria and symbionts have evolved T4SSs to deliver effector proteins or DNA–protein complexes into their host (either eukaryotic target cells or protozoan hosts). The T4SSs can either inject their effectors directly into the host cell or secrete them into the medium, thereby exerting remarkably different effects on host cell functions during infection.
F. EM reconstructions showing the structure of the plasmid R388 T4SS complex and the core complex. Front view (left) and cut-away front view (right) of the T4SS complex (EMD-2567) comprising the core/outer membrane complex (core/OMC, green), the stalk (grey) and the inner membrane complex (IMC, blue). U-tier, M-tier and L-tier stand for upper, middle and lower tier, respectively. The inner (IM) and outer (OM) membranes are indicated.
G. pKM101 core complex (EMD-2232) (top) and truncated core complex lacking the N-terminal part of VirB10 (EMD-2233) (bottom): side view (left) and cut-away side view (right). The bottom right panel shows the superposition of the difference map (between the full-length and the truncated core complex cryo-EM maps) in green, and the cryo-EM structure of the truncated core complex in orange (as in bottom left). The VirB10 N-terminus forms the inner wall of the I-layer and the base.
H. Cryo-EM structure of the TraI relaxase-ssDNA complex revealed the molecular basis of DNA unwinding during bacterial conjugation.
I. To achieve genetic exchange during bacterial conjugation, two relaxase monomers collaborate, adopting distinct structural conformations to provide the two necessary enzymatic activities for processing the DNA.
J. Individual steps are indicated: (1) TraI opens to bind ssDNA and closes to surround DNA entirely during

unwinding. (2) DNA binding to transesterase in closed TraI inhibits nicking. (3) DNA splitting by vestigial helicase. This figure was extensively updated from Backert and Meyer (2006), Trokter et al. (2014) and Ilangovan et al. (2017) with permission from CELL Press.



Abbreviated Summary

Type IV secretion systems (T4SSs) are highly sophisticated nanomachines in the cell envelope of many bacteria. They exhibit crucial roles during infection of humans by the secretion of effector proteins, conjugative transfer of DNA and exchange of DNA with the extracellular environment. In this MicroReview, we summarize recent progress on T4SS composition, assembly and structure, and highlight how basic understanding of their functions is aiding in the design of novel strategies for antimicrobial therapies.