Type III machines of Gram-negative pathogens: injecting virulence factors into host cells and more

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Many Gram-negative bacteria that cause disease in either mammals or plants share a strategy of delivering toxic proteins into the cytoplasm of host cells known as type III secretion. Recent advances have provided a glimpse at the molecular nature of these lethal injection machines. Several groups have reported fibrous structures on bacterial surfaces that appear to be extensions of type III machines and necessary for toxin injection into host cells. Other research revealed complex mechanisms of secretion substrate recognition that presumably function to direct toxins to different locations during infection.

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Abbreviations

EPEC	enteropathogenic *E. coli*
ER	endoplasmic reticulum
SRP	signal recognition particle
Yop	*Yersinia* outer protein

Introduction

Upon infection, pathogenic bacteria need to evade the host's immune defense in order to multiply. For several Gram-negative pathogens this goal is achieved via a specialized protein secretion machine, known as type III secretion, whereby antihost factors are injected into the cytoplasm of immune cells [1,2]. Other pathogens use type III injection machines to damage epithelial tissues or to invade specific host cells [3–5]. Given the versatility of type III systems, it is not surprising that both mammalian and plant pathogens employ similar elements for the establishment of disease [6]. Genes required for the synthesis and assembly of type III machines are typically clustered. Transfer of such a gene cluster is thought to transform otherwise nonpathogenic species into virulent microbes [7].

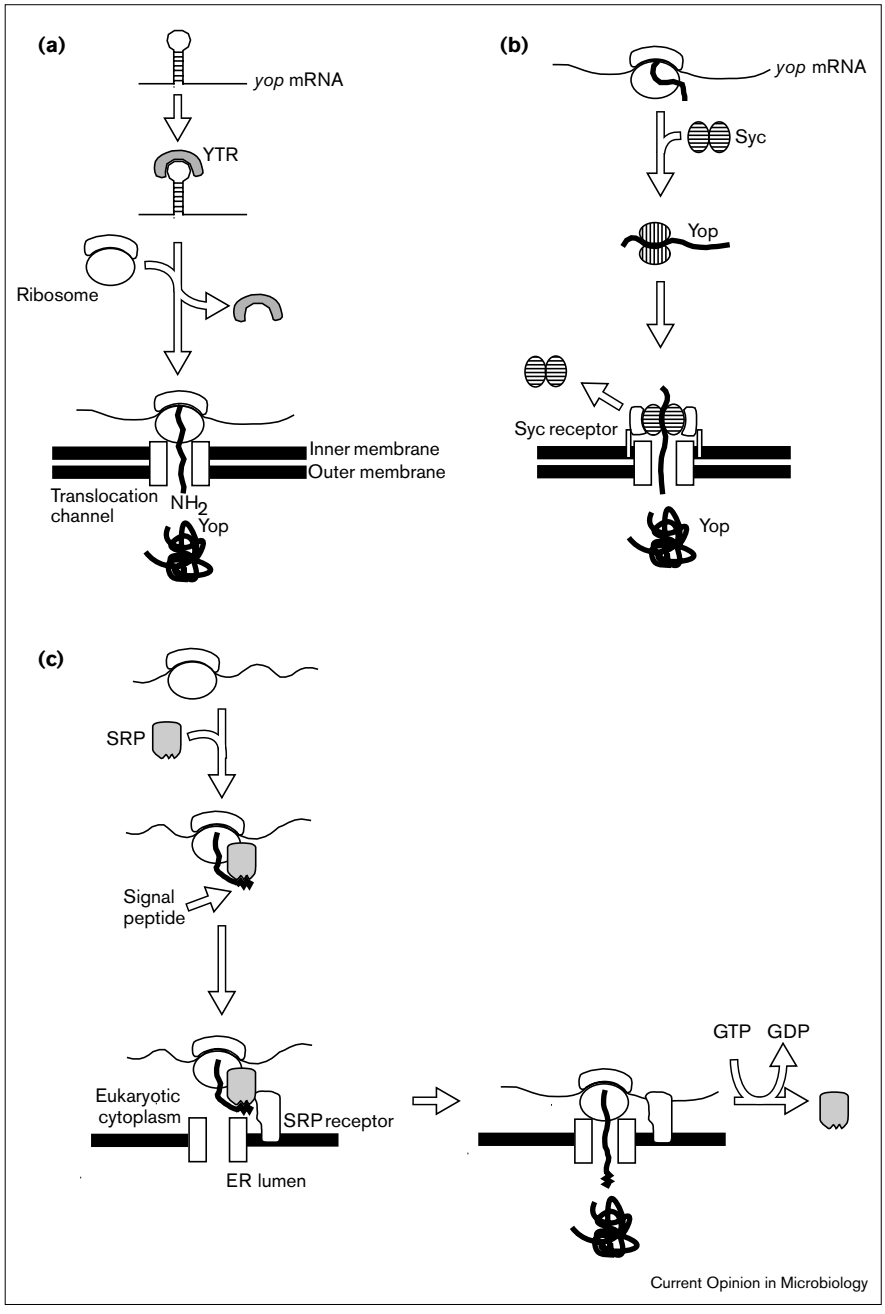
Structural components of the type III secretion machinery are highly conserved between different pathogenic species. Bacteria employing this pathway share at least eight genes, and many have over twenty components that are essential for the proper functioning of their secretion machines [6]. Although the subunits of type III machines are conserved between pathogenic species, their secretion substrates are not. At first glance, this appears odd if one assumes that all of these polypeptides need to be recognized by the same machine. In this review, we describe recent advances in understanding substrate recognition by type III machines. We highlight how these mechanisms may allow bacteria to direct secreted proteins to specific locations during infection and how the expression of genes appears to be intimately linked to the ability to secrete certain proteins. In addition, we discuss advances made in defining and visualizing elements that allow bacteria to inject proteins into eukaryotic cells.

Substrate recognition

Most of the work on recognition of type III secretion substrates has been described for *Yersinia enterocolitica*. Pathogenic *Yersinia* species, *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica*, can be artificially induced for type III secretion if grown at 37°C in the absence of calcium, thereby avoiding the otherwise necessary host cell contact. When induced by low calcium, *Y. enterocolitica* secretes fourteen proteins (Yops, for *Yersinia* outer proteins) abundantly into the culture medium: YopB, YopD, YopE, YopH, YopM, YopN, YopO, YopP, YopQ, YopR, YopT, YscM1, YscM2 and LcrV [8]. Mapping of the signal for type III secretion followed an experimental scheme by which *yop* gene sequences were fused to an open reading frame specifying a cytoplasmic reporter protein [9,10]. Export of the resulting hybrid polypeptide demonstrates recognition of the type III secretion signal. All Yops tested thus far contain a signal located in the first fifteen codons of the respective open reading frame [11,12]. Because these encoded amino acids can be extensively mutagenized, even by frame shift mutation, and still be secreted, it appears that *yop* mRNA signals export of the polypeptide [13•].

One way by which mRNA could signal type III export is to couple its translation with the secretion of the encoded polypeptide [14] (Figure 1a). If so, *yop* transcripts should not be translated unless ribosomes charged with these mRNAs are properly tethered to the type III secretion machinery. Translational repression might be a property of *yop* mRNA itself, as the RNAs are predicted to form folded structures that bury translational initiation signals, or it may depend on repressor molecules that recognize specific transcripts [13•]. Whatever the mechanism, translational repression of *yop* mRNAs must eventually be relieved for a productive interaction between charged ribosomes and type III secretion machines to occur. Yop polypeptides could thus be secreted across the bacterial envelope in a co-translational manner. A well known example of co-translational secretion is protein translocation into the lumen of the eukaryotic endoplasmic reticulum (ER) [15]. According to the signal peptide hypothesis for eukoryotic protein co-transitional secretion into the ER, a sequence of hydrophobic amino acids within nascent polypeptides

Figure 1



A comparison of mechanisms for protein translocation across membranes. **(a)** RNA signal hypothesis of type III secretion. Translation of the mRNA specifying secreted proteins (Yops) is repressed in the bacterial cytoplasm. A hypothetical element, the Yop translational repressor (YTR), is proposed to bind *yop* transcripts. Repression is relieved and YTR displaced once a ribosome charged with *yop* mRNA has docked on the type III secretion channel. Secretion occurs as the polypeptide is synthesized into the secretory pathway (co-translational secretion) **(b)** Syc-mediated recognition of type III secretion substrates. Homodimeric Syc binds newly synthesized Yop polypeptide in the bacterial cytoplasm. The Syc complex is recognized by the type III machine (Syc receptor) and unfolded Yop is initiated into the secretory pathway. The Syc protein is displaced and remains in the cytoplasm. **(c)** Signal hypothesis for eukaryotic proteins entering the secretory pathway. The signal recognition particle (SRP) binds the signal peptide of the nascent polypeptide chain. SRP binding stalls translation until the SRP complex contacts the SRP receptor on the ER membrane. Once the ribosome docks on the translocation channel, translation resumes, polypeptide is synthesized into the lumen of the channel. The signal peptide is cleaved on the lumenal side of the ER membrane and GTP hydrolysis of the SRP receptor releases SRP into the cytoplasm.

provides a signal that is recognized by the signal recognition particle (SRP) [16]. SRP binding to the nascent polypeptide arrests translation while it tethers the ribosome to a receptor at the ER membrane [17,18]. The SRP is displaced from the ribosome in a manner dependent on GTP hydrolysis by the SRP receptor and translation resumes with the nascent polypeptide being initiated into the translocation channel [19] (Figure 1c).

One prediction of the RNA signal hypothesis is that polypeptides secreted by such a mechanism should be located almost exclusively outside of the bacterial cell. Although this is clearly not true for all Yop proteins, at least one of them, YopQ, is found only in the culture medium but not in bacterial cells [20]. Recent work reveals that some Yops contain a second signal for secretion, as fusion of residues 15–220 of YopE to the reporter also lead to export of the hybrid polypeptide [20]. This second signal maps to the amino acid sequence for a domain of YopE that binds to a small cytosolic chaperone SycE [21]. Indeed, in the absence of SycE, the second secretion signal does not function and all YopE export of *sycE*$^-$ mutant cells is driven by the mRNA signal [20]. Several other Yops are known to bind to a cognate Syc protein and at least for YopE and

Table 1

Mutant phenotypes of *Yersinia* type III secretion genes during infection of tissue culture cells.

Phenotype	*Yersinia* genes	References
No secretion, no targeting	*yscC–L, yscN–U, lcrD*	[64–69]
Secretion, no targeting	*yopD*	[31,33,52]
Loss of targeting specificity	*yopN*, *lcrG*	[22,31,39–41]
Loss of some Yop targeting	*sycE*, *sycH*, *tyeA*	[12,21,22, 34,45••]
Loss of regulation of *yop* expression	*yopD*, *lcrQ, yscM1, yscM2, yopN, lcrG lcrV, lcrH, virF, virG*	[39,45••,47, 49,50•,51••, 70–75]

YopH this interaction seems absolutely necessary for their injection into the eukaryotic cytosol [12,22]. SycE is a small homodimeric protein and when complexed with YopE maintains this polypeptide in a soluble state within the cytoplasm [23,24]. Somehow, SycE is displaced from YopE to permit YopE export by the type III machine, thereby releasing the chaperone to bind another secretion substrate (Figure 1b). Both models of substrate recognition, RNA signal (Figure 1a and c) and chaperone mediated (Figure 1b), do not involve the direct recognition of secreted polypeptides by the type III machinery. Secretion chaperones have also been found in other Gram-negative pathogens [25–27]. Together with the observation that the expression of export substrates in heterologous species leads to their type III secretion [11,28••], this suggests that the RNA signal and chaperone-mediated export pathways may be common to all type III machines.

It seems clear that plant pathogens must also inject their type III substrates into the host, however, this has never been observed *in vivo*. To measure secretion in culture, Collmer and co-workers [28••] cloned the *Erwinia caratovora* type III genes on a cosmid in *Escherichia coli*. Expression of type III secretion substrates from *Pseudomonas syringae*, AvrPto and AvrB, in the recombinant *E. coli* strain led to the secretion of these polypeptides by the *E. caratovora* type III system into the culture media [28••]. This observation further corroborates our notion that the secretion signals described above may be universally employed by type III machines. Furthermore, the type III apparatus of *Erwinia* consists of only nine genes, a number that is significantly smaller than the 24 Yop secretion genes (*ysc*) genes of *Yersiniae*. Apparently, secretion across the double membrane envelope of Gram-negative bacteria can be made a lot simpler.

Location of type III secretion substrates during infection

By employing immunofluorescent detection of infected HeLa cells, Wolf-Watz and co-workers reported that YopE, YopH and YpkA (YopO) were injected into the cytosol of eukaryotic cells [29–31]. Sory and Cornelis [32] developed an experimental scheme that measured the injection of hybrid reporter proteins into macrophages and other host cells. A recently developed technique measures injection of Yops by employing digitonin, a detergent which selectively solubilizes the cholesterol containing eukaryotic plasma membrane. This assay permits localization of Yops by immunoblotting in either the eukaryotic cystol, the culture media or associated with the bacteria [22]. Together with protease protection experiments and immunofluorescence microscopy, Yops can be assigned to at least three distinct compartments: seven proteins of *Yersinia* species, also called effector Yops, are injected into the eukaryotic cytosol (i.e. YopE, YopH, YopM, YopN, YopO, YopP and YopT), whereas YopB, YopD and YopR are secreted into the extracellular milieu [22,33–36]. YopQ and LcrV remain associated with the bacteria, however, their precise location during infection is still unknown [37].

There are several ways by which type III machines could accomplish the task of directing proteins to different locations. Here we name two of our favorite models but many others are, of course, possible. Assuming that *Yersiniae* export all polypeptides through the same type III machine, it follows that the secretion of Yop proteins may be timed. Some polypeptides may be secreted at a time when type III export leads to their release into the extracellular milieu, whereas at another time a similar pathway may direct polypeptides into the eukaryotic cytosol. Alternatively, *Yersiniae* might modify their type III machines such that some direct the secretion of specific Yops, whereas others cause injection (targeting) into eukaryotic cells. Although we cannot yet distinguish between these possibilities, it seems obvious that both models require distinct substrate recognition events of Yop proteins that result in either secretion or injection.

Recent work suggests that the injection of YopE into host cells absolutely requires binding to SycE in the bacterial cytoplasm [22]. Does all Syc-mediated export lead to the targeting of proteins into eukaryotic cells? Although YopE, YopH and YopT appear to require a cognate Syc for targeting, YopO, YopP and YopM are also injected into eukaryotic cells but specific chaperones have not yet been identified for these Yops [33]. YopB and YopD, on the other hand, are secreted into the extracellular milieu and bind to SycD (LcrH) [38]; however, this protein has not been demonstrated to play a role as a secretion chaperone. Thus, a clear picture of the substrate requirements for all Yops has not yet emerged.

Genetic analysis of the *Yersinia yop* virulon has turned to identify components that are required for proper delivery of Yops. Mutations in *yopN* and *lcrG* abolish specificity of effector Yop targeting such that these proteins are found in all compartments during infection [22,31,39–41] (Table 1). Hence, LcrG and YopN seem to regulate the type III

machinery in a manner that ensures proper delivery. Boyd and colleagues [42] report that LcrG is positioned on the bacterial surface such that it can interact with glucosaminoglycans (heparan sulfate) on the eukaryotic cell surface. This interaction can be prevented by adding excess heparan sulfate to the medium. Others have viewed LcrG as part of an intra-cytoplasmic gate. LcrG requires binding to LcrV for proper function and the amount of free versus complexed LcrG could determine various degrees of gate opening (titration model) [43,44]. A mutation located in a gene immediately downstream of *yopN*, *tyeA*, is reported to display another remarkable phenotype. *tyeA* mutant *Yersiniae* cannot inject YopE and YopH, whereas their ability to target YopO and YopP into the eukaryotic cytosol is unaffected [45••]. TyeA is thought to be located on the bacterial surface, however, it is not yet clear how this protein regulates the injection of specific effector Yops.

Gene expression during type III secretion

In *Yersinia*, it has long been known that there is feedback from the secretion channel to the expression of type III substrates [46]. That is, when secretion is compromised by a mutation in an essential component of the machinery, expression of Yops is diminished. Previously, *Y. pseudotuberculosis* LcrQ (YscM1 and YscM2 in *Y. enterocolitica*) has been implicated in this feedback regulation [47–49]. Overexpression of LcrQ constitutively represses Yop expression even when secretion is fully induced. It was hypothesized that LcrQ, which is itself a substrate for type III secretion, represses transcription of *yop* genes when it is located in the bacterial cell [47]. When secretion is induced, LcrQ may be rapidly secreted, allowing for *yop* transcriptional activation. Whether LcrQ is essential for the targeting of Yops during infection has not yet been tested. Recent work has demonstrated that another secretion substrate YopD is also involved in feedback control of *yop* expression [50•,51••]. Mutations in YopD do not abrogate secretion of Yops in the low calcium model, however, they do abrogate repression of gene expression when secretion is compromised. Furthermore, YopD appears to be required for the proper function of LcrQ. When LcrQ is overexpressed in a YopD mutant, Yop synthesis remained induced suggesting that YopD acts prior to LcrQ in the cascade leading to repression of Yop expression when secretion is compromised.

YopD is essential for the efficient targeting of Yops into the eukaryotic cytosol and has been thought to be a component of the translocation pore into the host cell [31]. Recently it was shown, however, that a GST–YopD fusion could complement the targeting defect of a *yopD* null mutant (VT Lee, O Schneewind, unpublished data). This fusion cannot be exported, demonstrating that YopD must function in the bacterial cytoplasm. YopD thus cannot be a component of a proposed translocation pore (see below), as it would require an extracellular location for this function. This experiment also suggests that the regulation of Yop expression is essential for proper function of type III secretion during infection. The exact mechanism that YopD uses to control gene expression, as well as how it senses defects in secretion, remains to be defined.

Injection devices

Injection of Yop proteins into eukaryotic cells requires a mechanism of protein translocation across three membranes: the bacterial inner and outer membranes, as well as the eukaryotic plasma membrane. Although it is clear that the type III machine transports Yop proteins across the bacterial envelope, translocation across the eukaryotic membrane may require another element, here called the injection device. Its existence was suggested when Wolf-Watz's and Cornelis' laboratories reported *Yersinia* strains with mutations in *yopB* and *yopD* that were defective in the injection of effector Yops into eukaryotic cells but unaffected for low calcium induced type III secretion [31,33,52]. Both YopB and YopD contain segments of hydrophobic amino acids and it seemed probable that these polypeptides might insert into membranes. Indeed, purified YopB forms pores when incubated with eukaryotic membranes [52]. The role of YopD in the translocation of effector Yops is less obvious; however, Wolf-Watz's laboratory reports that this polypeptide is at least in part injected into the eukaryotic cytosol [50•]. The picture that emerges from this research suggests that YopB/YopD might function as an import pore through which previously secreted effector Yops travel into the eukaryotic cytosol. While YopB makes up the pore, YopD may shuttle effector molecules into the cytosol. At odds with this hypothesis is that secreted effector Yops do not harbor a conserved sequence element that would permit their recognition. Furthermore, effector Yops are not found soluble in the extracellular milieu or positioned on bacterial or eukaryotic surfaces, suggesting that a transport intermediate may not exist. The view that YopB/YopD may serve as a translocation pore has also been challenged by the observation of a *yopB* mutant that abrogates YopB synthesis but does not affect effector Yop injection into HeLa cells (VT Lee, O Schneewind, unpublished data).

Another mode of injecting proteins into eukaryotic cells has been reported for the plant pathogen *P. syringae*. This organism forms a pilus like structure on its surface that is composed of HrpA, a polypeptide that is secreted in a type III dependent manner [53]. It has been proposed that the HrpA pilus forms an extension of the type III machine that permits injection of other Hrp proteins directly into plant tissues. Consistent with this model is the observation that *hrpA* mutants of *P. syringae* are defective in causing plant disease. Thus, the HrpA filament could represent the functional equivalent of an injection needle for eukaryotic cells. What is attractive about this model is that it explains how type III machines might inject proteins directly into eukaryotic cells. If true, it would be surprising if such ingenious injection devices existed only in plant pathogens but not in related microbes that colonize animals. A search to identify filamentous structures on the surfaces of animal pathogens is under way and enteropathogenic *E. coli*

(EPEC) as well as *Salmonella typhimurium* display such structures [54,55]. *espA*– mutant EPEC were unable to form surface appendages and were also unable to inject other type III export substrates (Tir and EspB) into eukaryotic cells and this abolished pathogenicity [56••]. EspA surface appendages were also found to be required for intimate adherence of EPEC to the host cell and were only present in the early stages of infection. A future challenge will be to determine whether such filaments indeed represent a protein injection device and how this structure might assemble within the type III machinery.

Assembly of type IV pili and filamentous phage has been well studied and researchers studying type III secretion have already gained from common concepts. Morphogenesis of pili and filamentous phage occurs as a simultaneous export and assembly of protein subunits across the outer membrane, a process requiring a proteinaceous channel made up of a single polypeptide (f1 pIV and pIV homologues) [57,58]. pIV forms a multimeric gated channel in the outer membrane and details of its structure were recently revealed by electron microscopy [59]. Both phage and pili are thought to be assembled within the pIV outer membrane pore. All known type III secretion systems contain a pIV homologue and when purified from *Salmonella* and *Yersinia* spp. similar donut-shaped architectures were observed [60,61].

Several subunits of type III machines display homology to components of the flagellar secretion apparatus, named the basal body/hook complex [6]. This complex has been viewed by electron microscopy and because of its rotational symmetry is amenable to detailed structural analysis [62]. Aizawa and colleagues [63••] searched for *Salmonella* type III machines reasoning that its structure must be similar to the basal bodies and may be viewed in preparations otherwise designed to isolate this apparatus. Bacterial detergent lysates were subjected to ultra centrifugation and alkali extraction, yielding a complex structure composed of a needle with four attached perpendicular rings. This complex is inserted in the *Salmonella* envelope such that the needle protrudes onto the cell surface while the rings make contact with the inner or outer membrane. Purified needle complexes contain InvG, the *Salmonella* pIV homologue, and at least two other components. Future work will need to unveil the identity of the peptides that make up these ring and needle structures and test whether such complexes can be found in all bacteria expressing type III secretion systems.

Conclusions

Type III machines allow Gram-negative pathogens to establish disease in animals and plants by directing several different toxins either into the extracellular milieu or into the cytosol of host cells. To accomplish all this, type III machines appear to require two distinct subunits, a secretion machine that translocates proteins across the bacterial envelope and an injection device that directs a subset of polypeptides into host cells. Identification of the elements required for each of the two functions is pursued by genetic analysis and by searching for supramolecular structures that can accomplish these tasks. Mapping and mutational analysis of secretion signals suggest at least two if not several different modes by which type III machines recognize export substrates. Presumably, each mode of substrate recognition determines the final destination of type III exported polypeptides. Eventually these lines of research should converge to generate a clear picture of how Gram-negative bacteria deliver their toxins while attacking the eukaryotic host. Given the complexity of the tasks that type III machines accomplish, it seems safe to assume that research on this topic will yield astonishing solutions to fundamental biological problems.

Note added in proof

The study referred to in the text as (Lee VT, Schneewind O, unpublished data) has now been accepted for publication [76•].

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Cornelis GR, Wolf-Watz H: **The *Yersinia* Yop virulon: a bacterial system for subverting eukaryotic cells.** *Mol Microbiol* 1997, **23**:861-867.
2. Straley SC, Skrzypek E, Plano GV, Bliska JB: **Yops of *Yersinia spp.* pathogenic for humans.** *Infect Immun* 1993, **61**:3105-3110.
3. Collazo CM, Galan JE: **The invasion-associated type III protein secretion system in *Salmonella* — a review.** *Gene* 1997, **192**:51-59.
4. Frank DW: **The exoenzyme S regulon of *Pseudomonas aeruginosa*.** *Mol Microbiol* 1997, **26**:621-629.
5. Kaper JB: **EPEC delivers the goods.** *Trends Microbiol* 1998, **6**:169-172.
6. Hueck CJ: **Type III protein secretion systems in bacterial pathogens of animals and plants.** *Microbiol Mol Biol Rev* 1998, **62**:379-433.
7. Groisman E, Ochman H: **Cognate gene clusters govern invasion of host epithelial cells by *Salmonella typhimurium* and *Shigella flexneri*.** *EMBO J* 1993, **12**:3779-3787.
8. Michiels T, Wattiau P, Brasseur R, Ruysschaert J-M, Cornelis G: **Secretion of Yop proteins by *Yersiniae*.** *Infect Immun* 1990, **58**:2840-2849.
9. Michiels T, Cornelis GR: **Secretion of hybrid proteins by the *Yersinia* Yop export system.** *J Bacteriol* 1991, **173**:1677-1685.
10. Schesser K, Fritzh-Lindsten E, Wolf-Watz H: **Delineation and mutational analysis of the *Yersinia pseudotuberculosis* YopE domains which mediate translocation across bacterial and eukaryotic cellular membranes.** *J Bacteriol* 1996, **178**:7227-7233.
11. Rosqvist R, Hakansson S, Forsberg A, Wolf-Watz H: **Functional conservation of the secretion and translocation machinery for virulence proteins of *Yersinia*, *Salmonellae* and *Shigellae*.** *EMBO J* 1995, **14**:4187-4195.

12. Sory M-P, Boland A, Lambermont I, Cornelis GR: **Identification of the YopE and YopH domains required for secretion and internalization into the cytosol of macrophages, using the cyaA gene fusion approach.** *Proc Natl Acad Sci USA* 1995, **92**:11998-12002.

13. • Anderson DM, Schneewind O: **A mRNA signal for the type III secretion of Yop proteins by *Yersinia enterocolitica*.** *Science* 1997, **278**:1140-1143.
Extensive analysis of the type III secretion signal for Yops demonstrated that this signal is probably not encoded by amino acid sequence or protein secondary structure, but rather is encoded by the mRNA of the secretion substrate at the 5′ end of the coding sequence.

14. Silhavy TJ: **Death by lethal injection.** *Science* 1997, **278**:1085-1086.

15. Blobel G: **Intracellular protein topogenesis.** *Proc Natl Acad Sci USA* 1980, **77**:1496-1500.

16. Walter P, Gilmore R, Blobel G: **Protein translocation across the endoplasmic reticulum.** *Cell* 1984, **38**:5-8.

17. Gilmore R, Blobel G, Walter P: **Protein translocation across the endoplasmic reticulum. 1. Detection in the microsomal membrane of a receptor for the signal recognition particle.** *J Cell Biol* 1982, **95**:463-469.

18. Meyer DI, Dobberstein B: **Secretory protein translocation across membranes - the role of the 'docking protein'.** *Nature* 1982, **297**:647-650.

19. Ng DTW, Walter P: **Protein translocation across the endoplasmic reticulum.** *Curr Opin Cell Biol* 1994, **6**:510-516.

20. Cheng LW, Anderson DM, Schneewind O: **Two independent type III mechanisms for YopE in *Yersinia enterolitica*.** *Mol Microbiol* 1997, **24**:757-765.

21. Woestyn S, Sory M-P, Boland A, Lequenne O, Cornelis GR: **The cytosolic SycE and SycH chaperones of *Yersinia* protect the region of YopE and YopH involved in translocation across eukaryotic cell membranes.** *Mol Microbiol* 1996, **20**:1261-1271.

22. Lee VT, Anderson DM, Schneewind O: **Targeting of *Yersinia* Yop proteins into the cytosol of HeLa cells: one-step translocation of YopE across bacterial and eukaryotic membranes is dependent on SycE chaperone.** *Mol Microbiol* 1998, **28**:593-601.

23. Frithz-Lindsten E, Rosqvist R, Johansson L, Forsberg A: **The chaperone-like protein YerA of *Yersinia pseudotuberculosis* stabilizes YopE in the cytoplasm but is dispensible for targeting to the secretion loci.** *Mol Microbiol* 1995, **16**:635-647.

24. Wattiau P, Cornelis GR: **SycE, a chaperone-like protein of *Yersinia enterocolitica* involved in the secretion of YopE.** *Mol Microbiol* 1993, **8**:123-131.

25. Fu Y, Galan JE: **Identification of a specific chaperone for SptP, a substrate of the centisome 63 type III secretion system of *Salmonella typhimurium*.** *J Bacteriol* 1998, **180**:3393-3399.

26. Wainwright LA, Kaper JB: **EspB and EspD require a specific chaperone for proper secretion from enteropathogenic *Escherichia coli*.** *Mol Microbiol* 1998, **27**:1247-1260.

27. Wattiau P, Woestyn S, Cornelis GR: **Customized secretion chaperones in pathogenic bacteria.** *Mol Microbiol* 1996, **20**:255-262.

28. •• Ham JH, Bauer DW, Fouts DE, Collmer A: **A cloned *Erwinia chrysanthemi* Hrp (type III protein secretion) system functions in *Escherichia coli* to deliver *Pseudomonas syringae* Avr signals to plant cells and to secrete Avr proteins in culture.** *Proc Natl Acad Sci USA* 1998, **95**:10206-10211.
The first observation of secretion by *Erwinia* type III in bacterial culture. Not only can this system secrete heterologous type III substrates, but it also contains only nine type III genes thus defining a minimum type III secretion machine.

29. Hakansson S, Gaylov E, Rosqvist R, Wolf-Watz H: **The *Yersinia* YpkA Ser/Thr kinase is translocated and subsequently targeted to the inner surface of the HeLa cell plasma membrane.** *Mol Microbiol* 1996, **20**:593-603.

30. Persson C, Nordfelth R, Holmstrom A, Hakansson S, Rosqvist R, Wolf-Watz H: **Cell-surface-bound *Yersinia* translocate the protein tyrosine phosphatase YopH by a polarized mechanism into the target cell.** *Mol Microbiol* 1995, **18**:135-150.

31. Rosqvist R, Magnusson K-E, Wolf-Watz H: **Target cell contact triggers expression and polarized transfer of *Yersinia* YopE cytotoxin into mammalian cells.** *EMBO J* 1994, **13**:964-972.

32. Sory M, Cornelis G: **Translocation of a hybrid YopE-adenylate cyclase from *Yersinia enterocolitica* into HeLa cells.** *Mol Microbiol* 1994, **14**:583-594.

33. Boland A, Sory M-P, Iriarte M, Kerbourch C, Wattiau P, Cornelis GR: **Status of YopM and YopN in the *Yersinia* yop virulon: YopM of *Y. enterocolitica* is internalized inside the cytosol of PU5-1.8 macrophages by the YopB, D, N delivery apparatus.** *EMBO J* 1996, **15**:5191-5201.

34. Iriarte M, Cornelis GR: **YopT, a new *Yersinia* Yop effector protein, affects the cytoskeleton of host cells.** *Mol Microbiol* 1998, **29**:915-929.

35. Mills SD, Boland A, Sory MP, van der Smissen P, Kerbourch C, Finlay B, Cornelis G: ***Yersinia enterocolitica* induces apoptosis in macrophages by a process requiring functional type III secretion and translocation mechanisms and involving YopP, presumably acting as an effector protein.** *Proc Natl Acad Sci USA* 1997, **94**:12638-12643.

36. Monack DM, Mecsas J, Ghori N, Falkow S: ***Yersinia* signals macrophages to undergo apoptosis and YopJ is necessary for this cell death.** *Proc Natl Acad Sci USA* 1997, **94**:10385-10390.

37. Holmstrom A, Petterson J, Rosqvist R, Hakansson S, Tafazoli F, Fallman M, Magnusson K-E, Wolf-Watz H, Forsberg A: **YopK of *Yersinia pseudotuberculosis* controls translocation of Yop effectors across the eukaryotic cell membrane.** *Mol Microbiol* 1997, **24**:73-91.

38. Wattiau P, Bernier B, Deslee P, Michiels T, Cornelis GR: **Individual chaperones required for Yop secretion by *Yersinia*.** *Proc Natl Acad Sci USA* 1994, **91**:10493-10497.

39. Forsberg A, Viitanen A-M, Skunik M, Wolf-Watz H: **The surface-located YopN protein is involved in calcium signal transduction in *Yersinia pseudotuberculosis*.** *Mol Microbiol* 1991, **5**:977-986.

40. Sarker MR, Sory MP, Boyd AP, Iriarte M, Cornelis GR: **LcrG is required for efficient translocation of *Yersinia* Yop effector proteins into eukaryotic cells.** *Infect Immun* 1998, **66**:2976-2979.

41. Yother J, Goguen JD: **Isolation and characterization of Ca^{2+}-blind mutants of *Yersinia pestis*.** *J Bacteriol* 1985, **164**:704-711.

42. Boyd AP, Sory MP, Iriarte M, Cornelis GR: **Heparin interferes with translocation of Yop proteins into HeLa cells and binds to LcrG, a regulatory component of the *Yersinia* Yop apparatus.** *Mol Microbiol* 1998, **27**:425-436.

43. Nilles ML, Fields KA, Straley SC: **The V antigen of *Yersinia pestis* regulates Yop vectorial targeting as well as Yop secretion through effects on YopB and LcrG.** *J Bacteriol* 1998, **180**:3410-3420.

44. Nilles ML, Williams AW, Skrzypek E, Straley SC: ***Yersinia pestis* LcrV forms a stable complex with LcrG and may have a secretion-related regulatory role in the low-Ca^{2+} response.** *J Bacteriol* 1997, **179**:1307-1316.

45. •• Iriarte M, Sory MP, Boland A, Boyd AP, Mills SD, Lambermont I, Cornelis GR: **TyeA, a protein involved in control of Yop release and in translocation of *Yersinia* Yop effectors.** *EMBO J* 1998, **17**:1907-1918.
This paper describes a new protein involved in regulating Yop secretion. The remarkable phenotype is that YopE and YopH are not injected in the *tyeA* mutant, but the other Yop effectors are. This suggests that perhaps different modes of recognition are required for injected substrates.

46. Straley SC, Plano GV, Skrzypek E, Haddix PL, Fields KA: **Regulation by Ca^{2+} in the *Yersinia* low-Ca^{2+} response.** *Mol Microbiol* 1993, **8**:1005-1010.

47. Petterson J, Nordfelth R, Dubinina E, Bergman T, Gustafsson M, Magnusson KE, Wolf-Watz H: **Modulation of virulence factor expression by pathogen target cell contact.** *Science* 1996, **273**:1231-1233.

48. Rimpilainen M, Forsberg A, Wolf-Watz H: **A novel protein, LcrQ, involved in the low-calcium response of *Yersinia pseudotuberculosis* shows extensive homology to YopH.** *J Bacteriol* 1992, **174**:3355-3363.

49. Stainier I, Iriarte M, Cornelis GR: **YscM1 and YscM2, two *Yersinia enterocolitica* proteins causing down regulation of yop transcription.** *Mol Microbiol* 1997, **26**:833-843.

50. Francis MS, Wolf-Watz H: **YopD of *Yersinia pseudotuberculosis* is**
• **translocated into the cytosol of HeLa epithelial cells: evidence of a structural domain necessary for translocation.** *Mol Microbiol* 1998, **29**:799-815.
The authors find that YopD may be located in the eukaryotic cytosol. This suggests multiple roles for YopD in the pathogenesis of *Yersinia* infection.

51. Williams AW, Straley SC: **YopD of *Yersinia pestis* plays a role in**
•• **negative regulation of the low-calcium response in addition to its role in translocation of Yops.** *J Bacteriol* 1998, **180**:350-358.
Using genetic analysis, the authors report that YopD plays a role in negative regulation of Yop expression. By construction of a *yopD/lcrQ* double mutant, the authors show that YopD's regulatory function is required for LcrQ function.

52. Hakansson S, Schesser K, Persson C, Galyov EE, Rosqvist R, Homble F, Wolf-Watz H: **The YopB protein of *Yersinia pseudotuberculosis* is essential for the translocation of Yop effector proteins across the target cell plasma membrane and displays a contact-dependent membrane disrupting activity.** *EMBO J* 1996, **15**:5812-5823.

53. Roine E, Wei W, Yuan J, Nurmiaho-Lassila EL, Kalkkinen N, Romantschuk M, He SY: **Hrp pilus: An *hrp*-dependent bacterial surface appendage produced by *Pseudomonas syringae* pv. *tomato* DC3000.** *Proc Natl Acad Sci USA* 1997, **94**:3459-3464.

54. Ginocchio CC, Olmsted SB, Wells CL, Galan JE: **Contact with epithelial cells induces the formation of surface appendages on *Salmonella typhimurium*.** *Cell* 1994, **76**:717-724.

55. Reed KA, Clark MA, Booth TA, Hueck CJ, Miller SI, Hirst BH, Jepson MA: **Cell-contact stimulated formation of filamentous appendages by *Salmonella typhimurium* does not depend on the type III secretion system encoded by *Salmonella* pathogenicity island 1.** *Infect Immun* 1998, **66**:2007-2017.

56. Knutton S, Rosenshine I, Pallen MJ, Nisan I, Neves BC, Bain C,
•• Wolff C, Dougan G, Frankel G: **A novel EspA-associated surface organelle of enteropathogenic *Escherichia coli* involved in protein translocation into epithelial cells.** *EMBO J* 1998, **17**:2166-2176.
This paper identifies a surface structure present in the early stages of enteropathogenic *E. coli* (EPEC) infection that is absent in *espA* mutant cells. This structure appears to be essential to inject proteins into the eukaryotic cytosol and so may represent a type III injection device.

57. Hardie KR, Lory S, Pugsley AP: **Insertion of an outer membrane protein in *E. coli* requires a chaperone-like protein.** *EMBO J* 1996, **15**:978-988.

58. Russel M: **Phage assembly: a paradigm for bacterial virulence factor export?** *Science* 1994, **265**:612-614.

59. Linderoth NA, Simon MN, Russel M: **The filamentous phage pIV multimer visualized by scanning transmission electron microscopy.** *Science* 1997, **278**:1635-1638.

60. Koronakis V, Li J, Koronakis E, Stauffer K: **Structure of TolC, the outer membrane component of the bacterial type I efflux system, derived from two-dimensional crystals.** *Mol Microbiol* 1997, **23**:617-626.

61. Koster M, Bitter W, deCock H, Allaoui A, Cornelis GR, Tomassen J: **The outer membrane component, YscC, of the Yop secretion machinery of *Yersinia enterocolitica* forms a ring-shaped multimeric complex.** *Mol Microbiol* 1997, **26**:789-797.

62. DeRosier DJ: **The turn of the screw: the bacterial flagellar motor.** *Cell* 1998, **93**:17-20.

63. Kubori T, Matsushima Y, Nakamura D, Uralil J, Lara-Tejero M,
•• Sukhan A, Galan JE, Aizawa SI: **Supramolecular structure of the *Salmonella typhimurium* type III protein secretion system.** *Science* 1998, **280**:602-605.
The first purification of intact type III secretion machines. Needle-like structures containing known type III machine components were purified using a technique borrowed from the purification of flagellar basal body complexes.

64. Allaoui A, Schulte R, Cornelis GR: **Mutational analysis of the *Yersinia enterocolitica virC* operon: characterization of *yscE, F, G, I, J, K* required for Yop secretion and *yscH* encoding YopR.** *Mol Microbiol* 1995, **18**:343-355.

65. Allaoui A, Woestyn S, Sluiters C, Cornelis G: **YscU, a *Yersinia enterocolitica* inner membrane protein involved in Yop secretion.** *J Bacteriol* 1994, **176**:4534-4542.

66. Bergmann T, Erickson K, Galyov E, Persson C, Wolf-Watz H: **The *lcrB* (*yscN/U*) gene cluster of *Yersinia pseudotuberculosis* is involved in Yop secretion and shows high homology to the spa gene clusters of *Shigella flexneri* and *Salmonella typhimurium*.** *J Bacteriol* 1994, **176**:2619-2626.

67. Plano GV, Straley SC: **Multiple effects of *lcrD* mutations in *Yersinia pestis*.** *J Bacteriol* 1993, **175**:3536-3545.

68. Plano GV, Straley SC: **Mutations in *yscC*, *yscD*, and *yscG* prevent high level expression and secretion of V antigen and Yops in *Yersinia pestis*.** *J Bacteriol* 1995, **177**:3843-3854.

69. Woestyn S, Allaoui A, Wattiau P, Cornelis G: **YscN, the putative energizer of the *Yersinia* Yop secretion machinery.** *J Bacteriol* 1994, **176**:1561-1569.

70. Allaoui A, Scheen R, Lambert de Rouvroit C, Cornelis GR: **VirG, a *Yersinia enterocolitica* lipoprotein involved in Ca^{2+} dependency, is related to ExsB of *Pseudomonas aeruginosa*.** *J Bacteriol* 1995, **177**:4230-4237.

71. Cornelis G, Sluiters C, Lambert de Rouvroit C, Michiels T: **Homolgy between VirF, the transcriptional activator of the *Yersinia* virulence regulon, and AraC, the *Escherichia coli* arabinose operon regulator.** *J Bacteriol* 1989, **171**:254-262.

72. Goguen JD, Yother J, Straley SC: **Genetic analysis of the low calcium response in *Yersinia pestis* Mud1(Ap *lac*) insertion mutants.** *J Bacteriol* 1984, **160**:842-848.

73. Skrzypek E, Straley SC: **LcrG, a secreted protein involved in negative regulation of the low-calcium response in *Yersinia pestis*.** *J Bacteriol* 1993, **175**:3520-3528.

74. Skrzypek E, Straley SC: **Differential effects of deletions in *lcrV* on secretion of V antigen, regulation of the low-calcium response, and virulence of *Yersinia pestis*.** *J Bacteriol* 1995, **177**:2530-2542.

75. Viitanen A-M, Toivanen P, Skurnik M: **The *lcrE* gene is part of an operon in the *lcr* region of *Yersinia enterocolitica O:3*.** *J Bacteriol* 1990, **172**:3152-3162.

76. Lee VT, Schneewind O: **Type III machines of pathogenic *Yersiniae***
• **secrete virulence factors into the extracellular milieu.** *Mol Microbiol* in press.
Characterization of *Yersinia enterocolitica yopB, yopD* and *yopR* mutant strains with the digitonin fractionation technique suggests that YopB and YopR are not required for type III targeting. Although YopD is required, *yopD* mutants can be complemented with nonsecretable Gst–YopD fusion, suggesting that intrabacterial YopD, but not secreted YopD, appears to be necessary for type III targeting.