PHILOSOPHICAL TRANSACTIONS B

rstb.royalsocietypublishing.org

Review

Cite this article: Diepold A, Armitage JP. 2015 Type III secretion systems: the bacterial flagellum and the injectisome. *Phil. Trans. R. Soc. B* **370**: 20150020.
http://dx.doi.org/10.1098/rstb.2015.0020

Accepted: 23 July 2015

One contribution of 12 to a theme issue 'The bacterial cell envelope'.

Subject Areas:
microbiology

Keywords:
regulation of virulence, pathogenicity, bacterial movement, translocation, transmembrane complexes, chemotaxis

Author for correspondence:
Judith P. Armitage
e-mail: judith.armitage@bioch.ox.ac.uk

Electronic supplementary material is available at http://dx.doi.org/10.1098/rstb.2015.0020 or via http://rstb.royalsocietypublishing.org.

THE ROYAL SOCIETY PUBLISHING

Type III secretion systems: the bacterial flagellum and the injectisome

Andreas Diepold and Judith P. Armitage

Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK

AD, 0000-0002-4475-3923; JPA, 0000-0003-4983-9731

The flagellum and the injectisome are two of the most complex and fascinating bacterial nanomachines. At their core, they share a type III secretion system (T3SS), a transmembrane export complex that forms the extracellular appendages, the flagellar filament and the injectisome needle. Recent advances, combining structural biology, cryo-electron tomography, molecular genetics, *in vivo* imaging, bioinformatics and biophysics, have greatly increased our understanding of the T3SS, especially the structure of its transmembrane and cytosolic components, the transcriptional, post-transcriptional and functional regulation and the remarkable adaptivity of the system. This review aims to integrate these new findings into our current knowledge of the evolution, function, regulation and dynamics of the T3SS, and to highlight commonalities and differences between the two systems, as well as their potential applications.

1. The flagellum and the injectisome share a type III secretion system

Far from being 'bags of enzymes', bacteria have evolved to employ remarkably complex structures and pathways to ensure survival and spreading. Two of the most impressive examples of large macromolecular complexes span both membranes of Gram-negative bacteria: the bacterial flagellum, a rotating filament used in chemotaxis, and the injectisome, an injection device used for interkingdom transfer of proteins.

Although these two machineries clearly differ both in overall structure and function, at their core, they both consist of a conserved machinery for protein export, the type III secretion system (T3SS). In the flagellum, the T3SS is used to export the distal flagellar components and build the extracellular filament. Within the injectisome, the T3SS is at the centre of the export machinery and enables both the formation of the extracellular needle and the direct transfer of substrates from the bacterial cytosol into the host cells. While the term 'type III secretion system' is often applied to the whole injectisome, we will use it for the export machinery within both systems (and, accordingly, for statements valid in both cases) and use 'flagellum' or 'injectisome' to specify the respective complete system.

The flagellum is a rotating semi-rigid helical filament that is anchored within the bacterial membranes and driven by the influx of protons or Na^+ ions. It allows bacteria to move within fluid environments, including through liquid films on surfaces. Usually, the rotational direction of the flagellum is coupled to chemosensory receptors, allowing taxis, the movement of bacteria within gradients of chemicals, oxygen and even temperature [1,2]. As most bacteria are too small to sense spatial gradients, they use temporal sensing, comparing conditions at a given time with those a few seconds ago and bias their overall swimming in a favourable direction. The flagellum is a target of the chemotaxis pathway. In the well-studied case of *Escherichia coli*, the flagella usually rotate in a counterclockwise direction, forming a bundle of flagella which leads to forward movement of the bacterium. Movement away from favourable conditions leads to fewer effector molecules being bound to the cluster of membrane spanning chemoreceptors, and this increases the activity

of a kinase, CheA, which phosphorylates a small 14 kDa response regulator, CheY. CheY ~ P binds to the switch complex of the flagellar motor causing the rotor to switch its rotation direction. This leads to dispersal of the flagellar bundle and tumbling, which randomly reorients the bacterium, causing it to swim in a new direction when the bundle reforms. If this is an improved direction the cell will swim for longer before tumbling, leading to a biased random walk with an overall movement towards favourable conditions. Although the detailed mechanism and integration into the chemotaxis network differs between species, similar mechanisms are found in most flagellated bacteria [3]. While biasing the overall swimming pattern towards an improving environment appears to be the main function of the flagellum, there is evidence that in some species the flagellum can have additional roles. It is known to remodel in response to changes in external load or ion concentration [4,5] and there is increasing evidence of motors being slowed or stabilized by proteins activated in response to the growth state [6–9]. The flagellum may also directly act as a sensor, for example to detect surface proximity by higher load or slowed rotation, which can then lead to a switch in the bacterial lifestyle towards a sessile, biofilm-like mode [10,11].

Injectisomes are transmembrane complexes used by Gram-negative bacteria to translocate so-called effector proteins into eukaryotic host cells, where the effectors influence host behaviour in favour of the bacterium [12,13]. In many species, this process is essential for pathogenesis; however, it is also implicated in symbiosis [14–16]. The pool of effector proteins greatly varies between different bacterial species, reflecting the needs of the respective bacterium. In most studied cases, protein translocation[1] is a tightly regulated process that is only initiated upon contact with the host cell.

In some cases, bacteria harbour more than one type of injectisome (reviewed in [17]). The most prominent example is *Salmonella*, which uses its different injectisomes, encoded in two different *Salmonella* pathogenicity islands (SPI), for entry into host cells (in the case of SPI-1) and the subsequent survival, replication and dissemination therein (in the case of SPI-2). While a number of bacterial species have the genetic information for more than one set of flagella, there are only very few cases, most prominently some *Vibrio* species, in which more than one type is expressed at any given time [18–20].[2] However, quite often, flagella and injectisomes are present at the same time and both systems have been shown to heavily modulate each other, suggesting crosstalk between systems.

The aim of this review is to compare the two types of T3SS and to show commonalities and differences between them. There are excellent earlier reviews on the T3SS [12,13,17,22,23] and we will particularly focus on new aspects of T3SS that have emerged in the last few years and key open questions.

[1]In this review, we define 'translocation' as the process of transporting a protein from the bacterial cytosol into a host cell, while we use 'secretion' to describe the mere export of a protein from the bacterium into the extracellular milieu.

[2]A recent study reported the formation of a single polar flagellum formed by flagellins from two different gene clusters in a *Sphingomonas* strain [21].

2. Structural and functional homologies among the T3SS and other complexes

The common core of all T3SS resides in the transmembrane and cytosolic parts (table 1 and figure 1) and includes five proteins forming the export apparatus[3] and the surrounding MS ring in the inner membrane (IM), as well as the cytosolic ATPase, its negative regulator, the proposed stalk at the cytosolic interface and the cytosolic C-ring.

Notably, several of these core T3SS components are structurally similar to components of F/V-type ATPases; most significantly, the ATPase FliI/SctN, the central stalk FliJ/SctO and the negative regulator FliH/SctL resemble the α/β subunits, the γ subunit and the b/δ subunits of the F_0F_1-ATP synthase, respectively [33,35–37] (figure 1). Assuming that the T3SS ATPase itself does not rotate, this suggests that the FliJ/SctO stalk might rotate and be linked to substrate export [29,33].

The outer membrane (OM) ring structures are not related between the two systems. While flagella include a P-ring spanning the peptidoglycan layer and an L-ring transversing the OM, injectisomes contain a secretin-type ring, probably acquired from various sources later in evolution [38], that spans both of these layers. The reason for this divergence is unclear; however, the flagellar P- and L-rings need to function as bushings for the rotating flagellum, whereas secretins are very stable structures, shown to seal the OM until their conformation is changed to an open state [39,40], possibly by the growing needle.

The extracellular filaments of T3SS clearly vary in structure. The needles in injectisomes of animal pathogens and the flagellar hook differ in size and structure, but have a very similar helical arrangement [41]. In plant pathogens, the needle is replaced by a pilus, which can be up to several micrometres long, and is thought to be able to penetrate the thick plant cell wall. Beyond the injectisome needle the structures vary. In most animal pathogens there is a small tip structure built by a hydrophilic translocator protein [42] thought to allow pore formation in the host cell by two interacting hydrophobic translocators, but the injectisomes in enteropathogenic *E. coli* (EPEC) have a flexible sheath of up to 0.6 µm length and are able to span the mucous layer in the intestine [43]. The flagellar hook is extended by a long (up to 10 µm) semi-rigid helical filament made of flagellin; hook and filament are joined by two junction proteins. The C- and N-terminal domains of flagellins are conserved and allow the folding and polymerization into an 11 start helical tube, while the middle domain, exposed on the outer surface of the filament, varies between species [44].

With the possibility of imaging T3SS *in situ* by cryo-electrotomography, the interest in structural studies of the T3SS has been revived. A study of flagellar motors from different bacteria revealed that while a structural core of the conserved proteins was visible in all cases, the overall appearance of the motors as well as peripheral structural elements showed striking differences, highlighting the adaptability of the flagellum [45]. Recent studies have visualized the injectisome *in situ* under similar conditions [27,46,47]. They showed

[3]The sixth protein of the flagellar export apparatus, FliO, which has no homolog in the injectisome, has recently been found to regulate FliP during assembly of the flagellum [34].

Table 1. Structural components of the flagellar and injectisome T3SS. As the nomenclature for injectisome components is currently species-specific, we will use the unified Sct nomenclature [24] in this review. Where no Sct name exists, we will use the Ysc nomenclature (denoted by *). Refer to electronic supplementary material, table S1 for a list of names of injectisome components in specific organisms. Grey shading denotes homologous proteins, italics denotes that the protein can be exported by the T3SS. Extra, extracellular location; OM, outer membrane; PP, periplasm; IM, inner membrane; cyto, cytosolic.

cellular location	flagellar component	injectisome component	degree of similarity[a]	function
extra	**FliD**			**Filament-capping protein** required for proper folding of the flagellin
extra	**FliC**			**Flagellin** forming the filament
extra	**FlgL**			**Hook-filament junction** proteins connecting the filament and the hook
extra	**FlgK**			
extra		**YopD***		**Pore**-forming hydrophobic translocators
extra		**YopB***		
extra		**LcrV***		**Tip** protein (hydrophilic translocator)
extra	**FlgD**			**Hook-capping protein**, scaffold protein required for hook assembly
extra	**FlgE**	**SctF**	low	**Hook/needle** subunit, helical arrangement with 5–6 subunits per turn
OM		**SctC**		**Secretin ring** in the OM, requires assistance of pilotin lipoprotein for integration in OM
OM	**FlgH**			**L-ring**, lipoprotein, part of bushing
PP	**FlgI**			**P-ring**, part of bushing
PP		**SctI**		**Inner rod** in the injectisome. Exported T3SS substrate in some systems. Involved in needle anchoring and/or length determination, not required for connection of the membrane rings
PP	**FlgJ**			**Rod-capping protein** with muramidase function
PP	**FlgB**			**Flagellar rod**/transmission shaft. Requires functional T3SS export for assembly
PP	**FlgC**			
PP	**FlgD**			
PP	**FlgF**			
PP	**FliE**			
IM		**SctD**		Bitopic outer **MS ring** protein, connecting the secretin (SctC) in the OM and the inner MS ring (SctJ) in the IM
IM	**FliF**	**SctJ**	medium	**MS ring** protein, thought to surround the export apparatus. Large bitopic protein in the flagellum, significantly smaller and predominantly or completely periplasmic in the injectisome
IM[b]	**FliO**			Flagellum-specific **IM export apparatus** protein regulating FliP during assembly of the flagellum
IM[b]	**FliP**	**SctR**	high	**IM export apparatus** proteins, mainly transmembrane helices and periplasmic domains, possibly forming the pore in the IM
IM[b]	**FliQ**	**SctS**	high	
IM[b]	**FliR**	**SctT**	high	
IM[b]	**FlhB**	**SctU**	high	**IM export apparatus** protein with C-terminal cytosolic domain, involved in **substrate specificity switch** upon hook/needle completion
IM[b]	**FlhA**	**SctV**	high	**IM export apparatus** protein with large C-terminal cytosolic domain. Present as a multimer, probably forming a nonameric ring
cyto	**FliJ**	**SctO**	low	**Stalk** protein with homology to γ subunit of F_oF_1-ATP synthase. Also shown to bind empty chaperones ('chaperone escort' function). T3SS substrate in some systems

(*Continued*.)

Table 1. (*Continued.*)

cellular location	flagellar component	injectisome component	degree of similarity[a]	function
cyto	**FliI**	**SctN**	high	**Hexameric ATPase**, probably required for chaperone release and substrate unfolding
cyto	**FliH**	**SctL**	medium	**Negative regulator of ATPase**, also shown to interact with stalk and large export apparatus component, part of injectisome sorting platform
cyto	**FliG**			Part of the flagellar **switch complex** controlling the rotation direction (together with FliM and FliN)
cyto	**FliM**	**SctQ**	low	Forming the **cytosolic C-ring**, part of the switch complex in the flagellum and the injectisome sorting platform
cyto	**FliN**	**SctQ$_C$**	high	Part of the **cytosolic C-ring**. SctQ$_C$ is expressed from an internal translation initiation site in SctQ [25,26]. FliM + FliN and SctQ + SctQ$_C$ form stable complexes (with a 1 : 4 or 1 : 2 ratio, respectively)
cyto		**SctK**		**Accessory protein** interacting with the C-ring, part of injectisome sorting platform.

[a]Degree of similarity between flagellar and injectisome components in *Y. enterocolitica* (see electronic supplementary material, table S2 for details): high, $E < 10^{-5}$; medium, $10^{-5} < E < 0.01$; low, $E > 0.01$.
[b]Located within MS ring, most likely in a membrane-like environment.

a certain flexibility within the structure and differences to structures based on purified injectisomes [46], and allowed the comparison of the structures of the T3SS in the flagellum and the injectisome [27,47]. A particularly interesting structural detail is the C-ring, which is clearly visible as part of the switch complex in the flagellum. While there are homologous proteins in the injectisome, no C-ring structure was detected in the first injectisome cryo-ET studies [27,46]. However, six cytosolic 'pods', that were absent in strains lacking the C-ring component, were visible in a recent cryo-ET structure [47], suggesting that the architecture of the corresponding structure is significantly different in the injectisome and the flagellum.

Flagellar motors spin against a ring of stators, which are not present in hook-basal body isolations or visible in cryo-ET of species with extracellular filaments. However, they are visible in spirochaetes with periplasmic filaments [48], which rotate at high load. No equivalent proteins have been detected in injectisomes, and although there is a report of rotation of the injectisome needle tip [49], this observation has so far not been repeated or functionally linked to any T3SS component.

Further details on the structure of injectisomes and flagella can be obtained from dedicated recent reviews [30,50–53].

3. Injectisome-T3SS derived from flagellar ancestors

The evolutionary relationship between the flagellum and the injectisome has been the subject of considerable debate. Flagella exist in both Gram-positive and Gram-negative bacteria, while injectisomes have so far only been found in Gram-negative species, suggesting a flagellar T3SS as ancestor [24,54]. However, early phylogenetic studies suggested that both systems share a common ancestor, and have since evolved differently from each other [55]. With the ever-growing number of genome sequences [56,57], it became clear that flagellar components are almost always encoded on the bacterial chromosome and co-evolved with the rest of the genome to a certain degree [58,59], while the genes coding for the injectisome are often encoded on virulence plasmids or pathogenicity islands, and are more closely related to each other than their flagellar counterparts. They are distributed independently of the phylogeny of the respective species, and have probably been frequently transferred between species [38,55,60]. Latest analyses suggest that the ancestral T3SS was used for locomotion and was similar to current-day flagella, while modern injectisomes were derived through a series of gene losses and subsequent acquisitions via a non-flagellar ancestor that did not translocate proteins, leading to a protein translocation machinery [38] (figure 2).

Within the injectisomes, eight subfamilies can be distinguished (table 2). While, as mentioned above, there is no clear correlation between the phylogenetic tree of bacteria and their injectisome, injectisome subfamilies do confer host specificity. While the Ysc, SPI-1, SPI-2 and *Chlamydia* subfamilies target animal cells, Hrp1, Hrp2 and *Rhizobia* have plant host cells, and the more distantly related *Myxococcus* subfamily is present in free-living, non-pathogenic or symbiotic bacteria [12,38]. The *Myxococcus* subgroup lacks the secretin ring in the OM and any detectable needle subunit [38,56,61]. While it might form a fully functional T3SS by associating with a secretin of one of the type II secretion systems present in *Myxococcus*, it was also proposed to belong to an intermediate ancestral form of T3SS (figure 2) specialized in transport across the IM [38,61]. Interestingly, many pathogens harbour two different injectisomes, which almost always belong to two different subfamilies [17,38]. As an example, many *Yersinia*

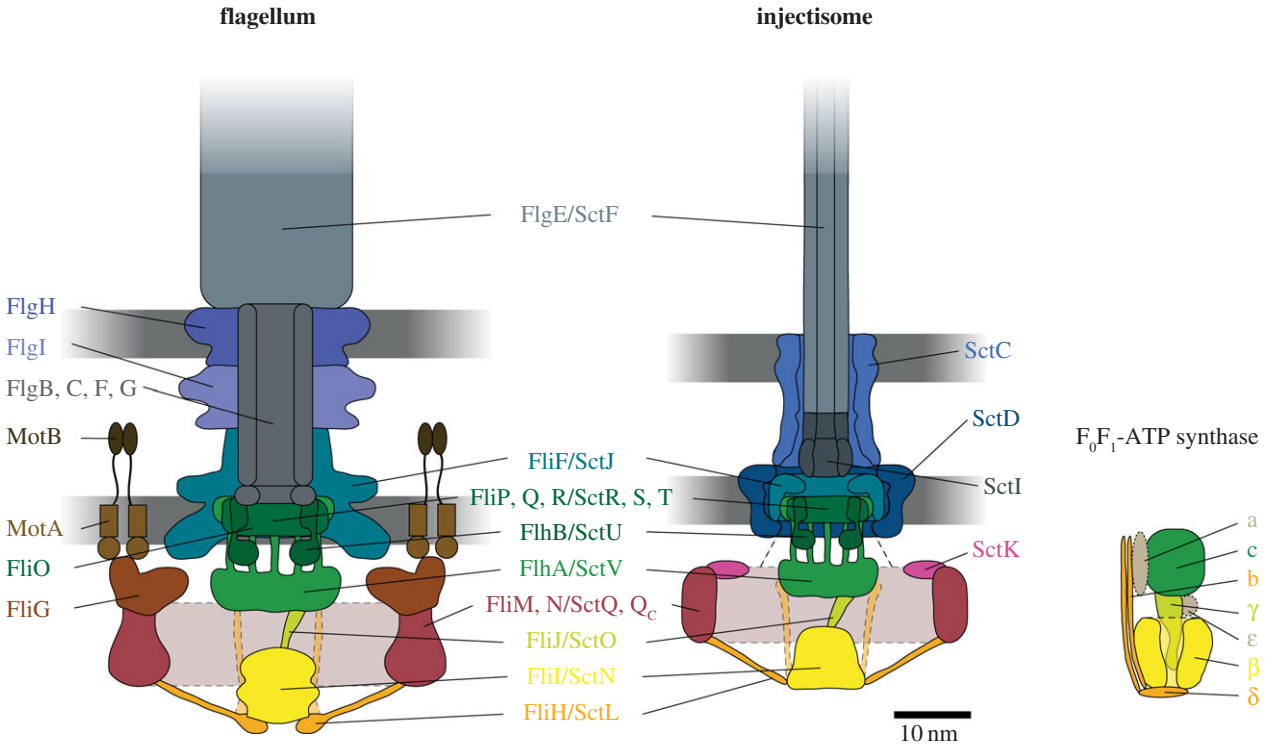

Figure 1. Structural model of the flagellar and injectisome T3SS, and comparison with structurally related parts of the F_0F_1-ATP synthase. Components present in both T3SS are listed in the centre, flagellum-specific and injectisome components on the left and right side, respectively. An alternative location for FliH/SctL [27,28] is indicated by dashed lines. Subunits of the F_0F_1-ATP synthase are listed on the far right side, components that are structurally related to T3SS components are coloured correspondingly. The c-ring of the F_0F_1-ATP synthase spans the IM, but has been drawn as corresponding to the FlhA/SctV ring in this image [28,29]. Flagellar model based on the study of Minamino & Imada [30]; injectisome model modified from [31]; based on cryo-electromicroscopic data [32]; F_0F_1-ATP synthase and structural homologies based on Ibuki *et al.* [33]. Scale bar and relative size of flagellum/injectisome based on Kawamoto *et al.* [27].

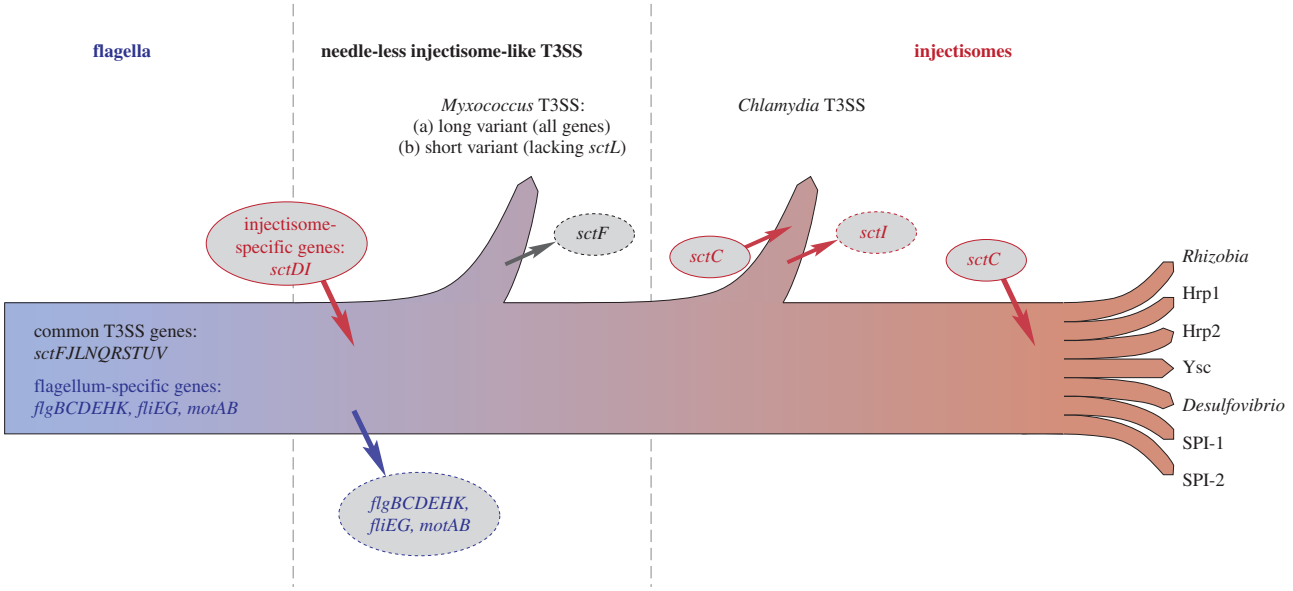

Figure 2. Proposed scenario for the evolution of T3SS. Adapted from [38]. Loss of flagellum-specific genes (blue) and acquisition of the injectisome-specific (red) genes *sctD* and *sctI* lead to a needle-less injectisome-like T3SS that is still present in *Myxococcus*. Acquisition of *sctC* secretins from different cellular machineries allowed the formation of contact-dependent injectisome-type T3SS. In addition to the depicted gene acquisitions and losses, family-specific changes occurred, leading to the eight injectisome subfamilies indicated—the more ancestral *Chlamydia* T3SS and the *Rhizobia*, Hrp1, Hrp2, Ysc, *Desulfovibrio*, SPI-1 and SPI-2 injectisomes. In the rhizobial family, a Tad-like secretin has been acquired independently. Gene acquisition is depicted by incoming arrows and solid lines, gene loss is depicted by outgoing arrows and dashed lines. See Abby & Rocha [38] for details.

strains have the genes for the Ysc T3SS expressed in animal cells at 37°C and the Ysa T3SS which is not expressed under these conditions. Recently, the Ysa system was shown to be involved in intracellular survival and to be required for the infection of *Drosophila* S2 cells at lower temperatures, suggesting a role in an alternative insect host [62,63].

4. Transcriptional and post-transcriptional regulation of the T3SS

It appears obvious that both the expression and the function of T3SS must be regulated. Environmental inputs control not only whether a bacterium needs a flagellum, but also how

Table 2. Different subfamilies of injectisome T3SS. The names of the subfamilies, selected species containing T3SS of this subfamily, the distribution throughout bacteria and particularities of the subfamily are listed. The SPI-1 subfamily is sometimes referred to as Inv-Mxi-Spa, the SPI-2 subfamily is also called Ssa-Esc. Data compiled from [12,17,38].

name	selected species	distribution/comments
Ysc	*Yersinia* spp. (Ysc)	present in β-, γ- and δ-proteobacteria, predominantly in animal pathogens
	Pseudomonas aeruginosa	
	Vibrio parahaemolyticus	
	Bordetella pertussis	
SPI-1	*Salmonella enterica* (SPI-1)	present in β- and γ-proteobacteria, predominantly in animal pathogens
	Shigella flexneri	
	Burkholderia pseudomallei	
	Yersina enterocolitica (Ysa)	
SPI-2	*Salmonella enterica* (SPI-2)	present in β- and γ-proteobacteria, predominantly in animal pathogens
	Escherichia coli EHEC/EPEC	
	Citrobacter rodentium	
	Yersinia pestis (chromosome-encoded)	
Chlamydia	*Chlamydia* spp.	present in Chlamydiales, obligate intracellular pathogens
Hrp1	*Pseudomonas syringae*	present in γ-proteobacteria, predominantly in plant pathogens
	Erwinia amylovora	
Hrp2	*Xanthomonas campestris*	present in β- and γ-proteobacteria, predominantly in plant pathogens
	Ralstonia solanacearum	
	Burkholderia pseudomallei	
Rhizobium	*Rhizobium* spp.	present in plant symbiotic α-proteobacteria, involved in nitrogen fixation
	Mesorhizobium loti	
Myxococcus	*Myxococcus xanthus*	present in two different variants in these δ-proteobacteria, incomplete and presumably needle-less T3SS

many, while injectisomes are generally only useful within the host organism.

Expression of flagellar genes is tightly regulated, ensuring sequential production of proteins for ordered assembly. In *E. coli*, a class 1 master operon, *flhDC*, regulated by metabolic state and cell cycle, controls the expression of class 2 operons, which encode for the proximal structural components of the flagellum including its T3SS core. After completion of the T3SS, the anti-σ^{28} factor FlgM, a T3SS substrate, is exported, which allows the expression of class 3 genes, including the filament subunit, by the alternative sigma factor σ^{28} [64]. In addition, the expression levels of flagellar components are often regulated as part of a switch between a free swimming lifestyle and a sessile or biofilm-associated lifestyle. A key player in this switch is the small cytoplasmic signal molecule cyclic-di-GMP (c-di-GMP) [65–67]), which itself may be influenced by the cell cycle, linking flagellar biosynthesis to cell division. In general, high c-di-GMP levels lead to downregulation of flagellar transcription [68,69].

For the injectisome, a related transcription hierarchy exists. However, in this case, all structural components belong to class 2 operons, with the effectors corresponding to class 3. Effector transcription is strongly upregulated upon contact to host cells. While the underlying mechanisms seem to differ between organisms [17], this generally involves the export of a regulatory protein, leading to release of its cytosolic binding partner, which then directly or indirectly induces transcription.

Interestingly, transcription of the two types of T3SS often influences each other, and in the majority of the cases flagella and injectisomes are counter-regulated. In species with both systems, flagellar genes are often activated only at temperatures below 30°C, while injectisome genes are transcribed within the host organism, which for animal pathogens is sensed by a temperature of 37°C [70–74]. In addition, there is direct counter-regulation involving the main transcription factors FlhDC and VirF [75–79], in agreement with a general switch between motility and virulence. As the export signals for flagellar and injectisome substrates are non-exclusive (see below), such a strategy might help to prevent the export of flagellum substrates through the injectisome (which in the case of flagellin might induce host cell defence) and vice versa.

It has become obvious that the carbon storage regulator (Csr) system [80] is a key factor in the post-transcriptional regulation of T3SS components. Csr is a regulatory complex in which non-coding Csr RNAs titrate the binding of CsrA to mRNA targets, leading to degradation of these targets. The Csr system has pleiotropic effects on the expression levels of injectisome components in *Salmonella* [81,82], EPEC [83] and *Yersinia* (reviewed in [84]), and was recently shown to downregulate the injectisome in persistent *Yersinia* infections [85]. CsrA

also governs the co-regulation of the injectisome with other virulence mechanisms. In *Pseudomonas aeruginosa*, the RetS sensor protein, another system involved in regulating Csr, switches between expression of the T3SS and the type VI secretion system by regulating c-di-GMP levels [86].

CsrA also controls motility, with CsrA upregulating the expression of flagella by stabilizing the transcript of the flagellar master operon *flhDC* [87,88]. Interestingly, CsrA not only regulates motility in this post-transcriptional manner, but is also directly involved in flagellum biosynthesis by suppressing flagellin biosynthesis prior to completion of the flagellar T3SS [89]. Based on the fact that the CsrA gene is mainly present in genomes that encode flagellin, this has been postulated to be the primordial function of the Csr system [89]. In addition, CsrA directly regulates the expression of several main regulators of c-di-GMP levels, connecting the two main pathways influencing flagellar biosynthesis [90,91].

Both T3SS also can sense and react to the proximity of host cells or other surfaces. Flagella can directly sense the increased load on the motor, once a flagellum is stuck because of contact to a surface, which can lead to a 'swim-or-stick switch', resulting in the downregulation of expression of proteins needed for motility and induction of biofilm formation (reviewed in [92]). This effect has been clearly demonstrated in *Caulobacter crescentus*, where production of holdfasts, adhesive polysaccharides at the attached cell pole, could be visualized within minutes after attachment and flagellar arrest [10,93], and in *Bacillus subtilis*, where inhibition of flagellar rotation leads to biofilm formation by triggering the DegS–DegU two-component signal transduction system and other pathways [11,94,95].

Injectisomes also react to approaching cells, often upregulating expression in preparation for effector translocation upon cell contact. To detect this proximity, both changes in local oxygenation [96,97] and local pH [98] can be sensed. Similarly, intracellular pathogens can determine the correct time for expression of the injectisome, as shown for *Salmonella* SPI-2, which is upregulated and activated by the decreased pH in the vacuole [98], sensed by acidification of the bacterial cytosol [99]. Interestingly, some species have also been shown to display bimodal expression of injectisome subunits during infection, leading to injectisome-positive and -negative subpopulations of the same strain, as was shown for *Salmonella* SPI-1 [100], EPEC [101] and *Shigella* [102], which might allow a subset of bacteria to benefit from the effects of the T3SS while circumventing the associated energy expense. The regulation of the T3SS is summarized in figure 4.

5. What determines the number and cellular distribution of T3SS?

The production and assembly of injectisomes and especially flagella, with around 20 000 flagellin subunits in the filament [103], come at a high energetic cost. Perhaps even more importantly, the number and localization of T3SS is crucial for their physiological function: although a recent study has shown remarkable robustness of *E. coli* swimming to variations in the number of flagella [104], both the number and the relative positioning of the flagella will directly influence the movement of the bacterium. Likewise, the chance of a bacterium establishing contact to a host cell with an injectisome directly depends on their number and distribution.

Depending on the species, flagella can be distributed peritrichous (distributed across the bacterium), medial or polar, either as a single flagellum or in tufts. While peritrichous flagella are usually evenly spread out across the bacterium (with the possible exception of the tips) and their number largely correlates with the cell size within a species, the insertion of new polar flagella is controlled by the cell cycle and often requires a polar marker [105]. Examples for such markers and their relation to the cell cycle are the GTPase FlhF and its negative regulator, the MinD-like ATPase FlhG (reviewed by [106]), found in many species, and the c-di-GMP receptor TipF and its interactor TipN in *C. crescentus* [107,108]. Interestingly, in both cases, the localization of flagella involves an interaction with components of the flagellar switch complex [108,109], suggesting that the final localization of the flagellum is at least influenced by the cytosolic components. Notably, c-di-GMP is involved not only in flagellar positioning but also in direct regulation of motility (see below, section 'Functional regulation and dynamics').

Injectisomes are more difficult to visualize than flagella. However, based on electron micrographs, the number of injectisomes strongly varies between different systems. The *Salmonella* SPI-2 system was found to be present in very low numbers (one to a few [110]), whereas 10–100 SPI-1 needle complexes [111] and 50–100 *Shigella* needle complexes [112] were observed per bacterium. The distribution of injectisomes was determined by fluorescence microscopy of functional fluorescently labelled components in *Yersinia enterocolitica* [113,114]. All analysed components were distributed in relatively evenly spaced foci around the bacterium, which is also the case in *Shigella* [115]. Recently, it was shown that these foci in *Y. enterocolitica* may correspond to clusters of injectisomes, which themselves tend to maximize the distance to their next neighbours, and that the number of injectisomes per cluster, rather than the number of clusters, increased upon activation of the T3SS [116]. The average number of injectisomes per *Y. enterocolitica* cell ranged from 10 to 30 in this study.

Although these numbers may generally represent the lower limits of possible ranges, due to the possible loss, non-visualization, or clustering of injectisomes, these data indicate that number and distribution of injectisomes strongly vary between different systems, based on their requirement. As an example, the difference between the number of SPI-1 and SPI-2 T3SS in *Salmonella* may reflect the different roles of these systems in the infection process [110]. SPI-1 injectisomes are formed by extracellular *Salmonella* and a large number conceivably increases the chance of establishing host cell contact, while SPI-2 is activated by bacteria tightly surrounded by the vacuolar membrane, where a single injectisome seems to be sufficient to establish contact and translocate the corresponding effectors.

The distribution of T3SS is probably linked to where they are integrated into the membranes and, importantly, the peptidoglycan (PG) layer; therefore, PG-modifying enzymes, such as PG lyases, play an important role in the number and positioning of T3SS. Interestingly, many such PG lyases or muramidases are encoded within T3SS gene clusters [117–121] and a recent report even indicated an interaction between a lyase and the injectisome inner rod [121]. Similarly, in the flagellum, the PG lyase FlgJ has been shown to assist in the formation of the inner rod with its N-terminus, while the C-terminus exhibits a muramidase function [122,123]. However, PG lyases appear to be functionally redundant as not all T3SS have a dedicated PG lyase, and while the absence of

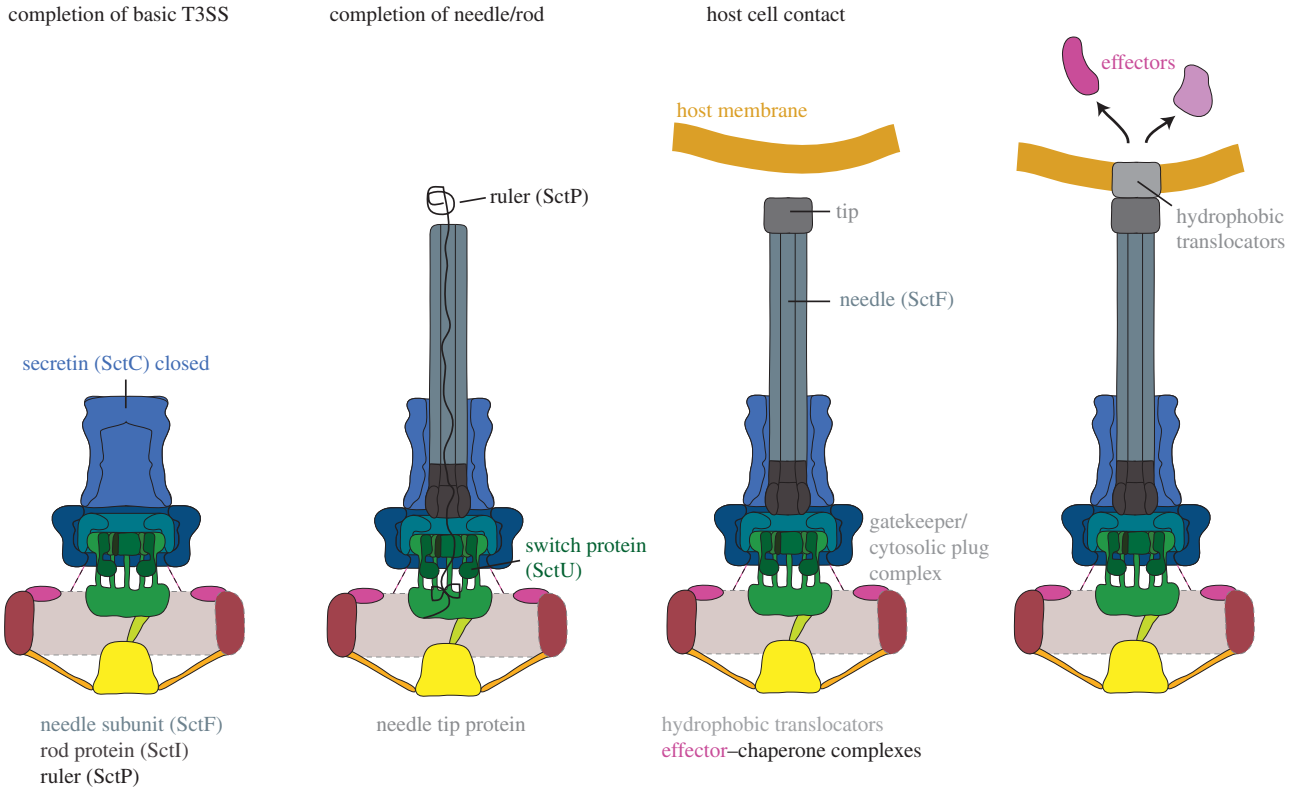

Figure 3. Substrate switching events. This image depicts the switching events at the injectisome; the first two switching events occur in all T3SS, whereas the switch upon host cell contact is injectisome-specific. Proteins involved in the respective switching events are denoted; proteins that are exported upon switching are listed on the cytosolic side. Scheme adapted from [31]. Upon completion of the basic T3SS, consisting of the membrane rings, export apparatus and cytosolic components, the export of early T3SS substrates occurs, including the hook/needle, rod and ruler. In the injectisome, formation of the needle is thought to open the previously closed secretin ring. The length of the rod/needle is surveyed by the molecular ruler FliK/SctP and once the correct length is reached, the ruler and the switch protein FlhB/SctU in the export apparatus are involved in switching the substrate specificity. This allows export of the next substrate class, the tip protein in the injectisome and flagellin and its associated proteins in the flagellum. The injectisome is fully assembled at this point, but does not secrete effectors, until contact to a host cell has been established. This signal is thought to be sensed by the needle tip and transferred via the needle, releasing a cytosolic gatekeeper/plug complex with unknown position and allowing effector export. Refer to main text for details and references.

single PG lyases may influence the positioning of T3SS, it does not lead to strong overall T3SS phenotypes.

6. Transcription hierarchy, assembly and activation of the T3SS

In the flagellum, the assembly order roughly reflects the three-tiered expression hierarchy mentioned earlier. Comparing the structure of flagellar precursors in strains lacking single components, Kubori *et al.* [124] concluded that the actual assembly of the flagellum starts with the MS ring in the IM. Next, the C-ring/switch complex can form. The integration of the export apparatus could not be visualized in these experiments. The presence of the large export apparatus component FlhA has been shown to induce and stabilize MS ring formation, arguing for an early role of FlhA in the assembly of the flagellum [125], although it is not absolutely required for MS ring formation [126]. For more detailed reviews on the assembly of the flagellum refer to [127–129].

In the injectisome, all structural components belong to class 2 operons and the proteins that make up the injectisome are therefore likely to be expressed and present at the same time. The assembly of the machinery (reviewed in [31]) is therefore thought to be mainly governed by protein interactions. While studies in *Salmonella* SPI-1 suggest an inside-out assembly of the membrane rings [130–132], similar to the model for the flagellum, the secretin was shown to nucleate membrane ring assembly in *Y. enterocolitica* [113], similar to type IV pili [133]. Independently, the export apparatus can assemble within the IM [114,134]. After the concurrent assembly of the cytosolic complex, requiring all of its components SctN, SctL, SctQ and SctL (but not the stalk SctO), the T3SS is functional and the needle can be formed [113,135].

The membrane rings and IM export apparatus are exported via the Sec pathway. However, once the core T3SS is assembled, it starts to independently export early substrates, the flagellar hook FlgE and the hook-capping protein FlgD in the case of the flagellum and the needle subunit in the case of the injectisome. In both cases, the correct length of the filament or needle is measured, in a so-far incompletely understood way, by a ruler protein (FliK/SctP) [136–138]. In interaction with an export apparatus component, the type III secretion substrate specificity switch (T3S4) protein FlhB/SctU, it induces a change in substrate specificity, allowing secretion and assembly of the flagellin subunits and the needle tip proteins, respectively (figure 3).

While the injectisome is functional at this stage, the secretion of effectors is still suppressed, until contact to a host cell has been established (figure 3). At the moment, it is unclear which molecular events lead to effector export, but the large variety in experimentally determined activation signals in different species (apart from host membrane contact, effector secretion is controlled by extracellular Ca^{2+} levels in *Yersinia*, O_2 levels in *Shigella* [96] and the local pH in *Salmonella*

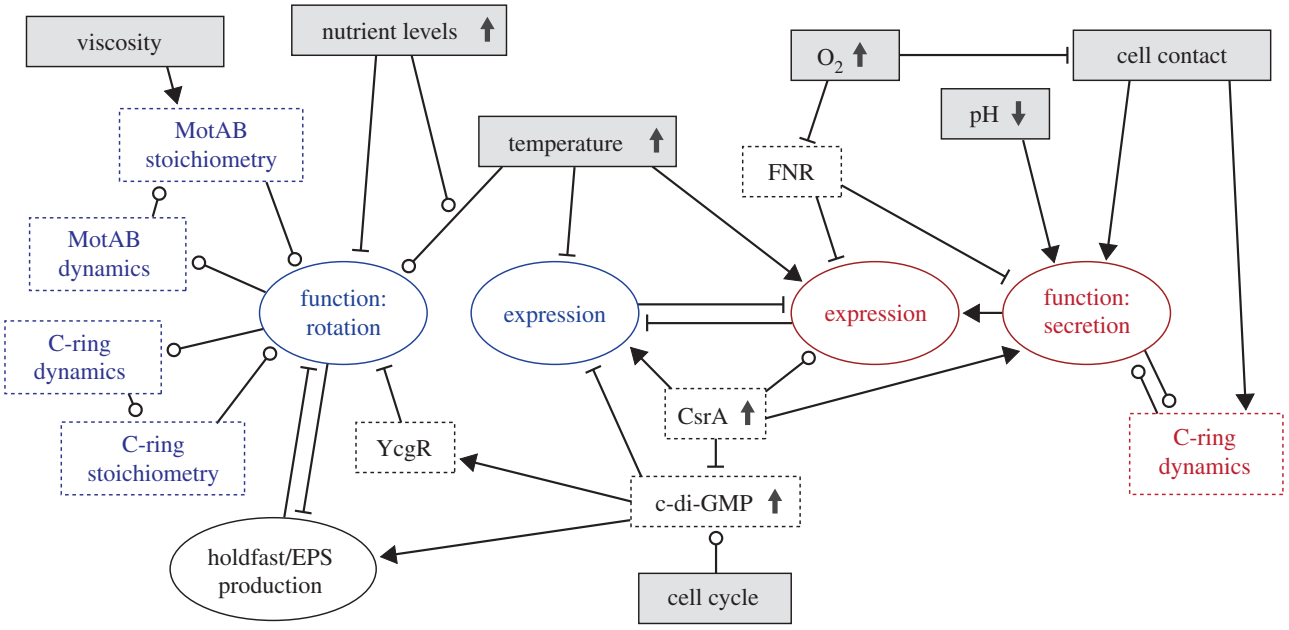

Figure 4. Inputs into the flagellum/the injectisome. Influence of several external cues (shaded boxes) on expression and function of the flagellum (blue, left side) and the injectisome (red, right side). Arrows: positive influence, lines with blocked end: negative influence, lollipops: pleiotropic/unknown influence. Note that this scheme contains data from various organisms under different conditions and not all mechanisms may be present in all species and at all times. FNR, fumarate and nitrate reductase. Refer to main text for references. (Online version in colour.)

SPI-2 [98,139]) suggest that this process might be triggered by species-specific signals. The existence of various signalling-deficient mutants in the tip and the needle [140–145] suggests that at least some of these signals are sensed at the tip, and transferred via the needle. Deletions of the tip protein in *P. aeruginosa* and *Shigella* lead to uncontrolled secretion [142,146], implicating a plug at the tip of the needle. However, this is not the case for all injectisomes and a cytosolic protein complex present in all injectisomes, termed the cytosolic plug or gatekeeper complex, is involved also in substrate control. Absence of either component of this complex of cytosolic proteins leads to uncontrolled secretion of effectors [147–151]. A similar role has been shown for a regulatory protein, LcrG, in *Yersinia*, which binds to the tip protein in the cytosol [152–154]. Constitutive secretion in the absence of any of these cytosolic components suggests an active prevention of secretion of late substrates prior to the second switch. Interestingly, it has been recently shown that the secretion control by the tip plug and cytosolic gatekeeper complex may be a part of the same mechanism, which possibly involves the inner rod protein [155,156].

Formation of the flagellar filament, which is the equivalent substrate class in the flagellum, does not seem to require an additional signal. Also, in contrast to the hook, which has a controlled length, the length control of the filament may vary from species to species, with some species appearing to have a defined length and others continuously growing filaments [157–161]. In all studied cases, the filament rapidly regrows if it is sheared despite the loss of the cap proteins catalysing filament growth.

7. Export signals

Export signals ensure that cargo is delivered to and exported by the correct secretion system. In contrast to other export pathways like Sec or Tat, the T3SS export signals are remarkably vaguely defined. While an uncleaved and generally unstructured N-terminal sequence seems to be common for all T3SS substrates, the length of the minimal signal greatly varies, additional signals seem to be required for some classes of substrates and, despite increasingly sophisticated algorithms [162–166], it is still difficult to precisely predict T3SS substrates.

While T3SS export signals appear to exclude export via unrelated export systems, there are numerous reports that the signals are interchangeable between different T3SS, and that flagellar substrates can be exported by injectisomes and vice versa [167–173]. Although flagella and injectisomes are rarely coexpressed *in vivo*, these findings highlight that the respective signal sequences are not exclusive.

The injectisome export signal has been studied in more detail. Most studied T3SS effectors have one N-terminal signal sequence located within the N-terminal 15–40 amino acids. While this sequence is sufficient to target even unrelated proteins for export through the injectisome [174], in some cases, the signal required for translocation into the host cells was found to be longer than the one sufficient for export via the T3SS [175,176]. Studies from various organisms show numerous silent mutations within the signal sequence have no or little effect on export, implying that the export signal is predominantly encoded in the amino acid sequence [177–179]. However, it has also been shown that frameshifted mRNA export sequences can still support the export of substrates and that the mRNA sequence around the start codon plays a role in export ([180,181], reviewed in [182]), indicating that the effects of mRNA and protein sequence on targeting may be additive.

While most effector proteins have one N-terminal secretion signal, other substrate classes appear to have additional export signals: the *Yersinia* ruler protein features a central stretch that is also required for export [183], and translocators as well as some effectors were shown to require both N- and C-terminal signals [184–186].

In the flagellum, the N-terminal parts of the flagellin or hook protein have been identified as secretion signals, albeit the very N-termini were not strictly required for secretion [187–189].

Box 1. T3SS chaperones.

T3SS chaperones are proteins assisting assembly and/or export of their cargo, effectors (class I chaperones), hydrophobic translocators (class II chaperones) or the components of the needle or flagellum (class III) [12]. Class I chaperones are separated into two subclasses. While class IB chaperones (also called general chaperones) bind a variety of effectors (similar to the class II chaperones that often can bind both hydrophobic translocators), the class IA chaperones, representing the majority of T3SS chaperones, have one specific target protein and are often encoded next to this protein. They are relatively small acidic proteins that form dimers. Different, non-exclusive roles have been assigned to T3SS chaperones: (i) they might keep effectors in a (partially) unfolded state, facilitating the unfolding prior to export; (ii) they have a role in signalling, indicating the export of their binding partner [192–199]; (iii) they can mask a localization domain for targeting in the host cell, preventing mislocalization within the bacterium [200]; and (iv) they may play a role in targeting of their cognate effector and introduction of a secretion hierarchy. Concerning the last role, chaperones have been found to specifically target effector proteins to the injectisome rather than the flagellum [168], to impose a secretion hierarchy for the injectisome through decreasing affinities to a cytosolic 'sorting platform' [201] and, in a similar way, but with another interaction partner, the export apparatus component FlhA, to coordinate the assembly of the flagellum [202].

Both the identified minimal export signal of the flagellin and full-length anti-σ^{28} factor FlgM have been successfully used as export signals for substrates which could subsequently cleaved by TEV protease [190,191]. As in the injectisome, both the peptide sequence and an mRNA signal seem to play a role in substrate recognition [189].

Export signals are intrinsically unstructured and unconserved, and it has not been possible to identify a common interaction partner at the T3SS. Rather, the export signal has been proposed to increase the local concentration of the target protein at the flagellum. While this process appears to be sufficient to allow the export of (usually overexpressed) non-native secretion substrates, it has been suggested that the targeting of native substrates is further enhanced or rendered more specific by the binding of their chaperones (box 1).

8. Type III secretion export is a multi-step process with many unknown features

Despite intense research, the export process through the T3SS is not well-understood. A myriad of interactions among the proteins involved at the cytosolic interface of the T3SS, and between these proteins and export cargo have been reported (reviewed in [17]). In addition, the interaction network of the cytosolic components might well change between different functional states of the T3SS. A candidate for such a switch is FliH/SctL, the negative regulator of the ATPase, which can additionally interact with both the major export apparatus component and the C-ring [27,28,203].

Even if not all of these interactions are necessarily relevant *in vivo*, their multitude suggests a multi-step binding and export process rather than one or two decisive interactions. Beside the low specificity of export signals, this notion is supported by the fact that loss of some conserved cytosolic components of the T3SS can be compensated for. As an example, the flagellar ATPase FliI is not absolutely required for the formation and rotation of flagella, especially in the absence of its negative regulator FliH and at increased proton-motive force (PMF) [204,205], an effect that was to some extent also observed for a catalytically inactive injectisome ATPase [204]. Similarly, absence of the flagellar C-ring component FliMN could be compensated by overexpression of the flagellar main transcription factor FlhDC [206] or even the ATPase FliI alone [207], although similar experiments did not lead to the same outcome for the injectisome (A Diepold 2009, unpublished data).

Although the path of cargo is thus unclear, the narrow diameter of both the injectisome needle[4] and the flagellar filament (20–25 Å, accommodating a maximum of one to two α-helices) [211,212] means that proteins travelling through these appendages will have to be at least partially unfolded prior to export [213]. In agreement with this assumption, very stably folded artificial T3SS substrates such as ubiquitin, GST or GFP were shown to be rejected by the export machinery [214,215] or even block the machinery in the cases of fusions to the ruler protein [216]. A recent study exploited this fact to visualize a trapped export substrate in the injectisome needle [208] and showed that presence of this substrate leads to distinct conformational changes within the basal body.

The main player in unfolding the substrate (and the concurrent removal of the chaperone) is the ATPase [215], which has been shown in many studies to engage with effectors and/or their chaperones. Recent structural data showed that some effector/chaperone complexes can form hexamers, which would fit the hexameric ATPase structure [217]. Interestingly, it was shown that targeting to the ATPase and secretion are largely independent events: while chaperone binding, rather than presence of the secretion signal, was required for association of a substrate with the ATPase, this was insufficient for secretion of the substrate [218].

Besides its role in the flagellar switch complex, the C-ring is also involved in the export process. Many reported interactions with both cargo proteins and machinery components indicate a multifaceted role in cargo export. More specifically, in the *Salmonella* injectisome, the C-ring protein participates in a cytosolic 'sorting platform' (together with SctL and SctK), proposed to ensure the secretion hierarchy through different binding affinities to T3SS chaperones, which bind cargo proteins [201]. In the *Yersinia* injectisome, the C-ring protein exchanges with a cytosolic pool [219] and since the exchange rate

[4]Effector export is generally believed to occur through the needle, as visualized for the trapped ruler protein in [208]. However, based on the presence of translocators and effectors on the surface of the bacterium prior to host cell contact and the observation that such extracellular effectors could be translocated into the host cell in a separate (but still T3SS-dependent) step, Wolf-Watz and co-workers proposed a binary AB toxin-like export mechanism translocation model [209,210]; the significance of this finding *in vivo* is so far unclear.

correlated with effector export, the C-ring might be involved in the functional regulation of the T3SS [219].

9. Energy transduction for export

Most transport processes in bacteria use energy provided by either the PMF across membranes or by ATPases. For the T3SS, both factors have been shown to be important—while mutations in the T3SS-associated ATPase FliI/SctN abolished type III secretion [220–222], the same was true for loss of the PMF [213,223,224] (reviewed in [23,225]). So far, the specific roles of both factors are not completely understood, although the finding that absence of the ATPase can be overcome to a certain degree by overexpression of other flagellar components, mutations in the export apparatus components FlhA and FlhB, or increased PMF [204,223] suggests that the PMF is more central to energizing export. The main role of the ATPase could therefore be to detach chaperones and unfold the cargo protein prior to export [215]. On the other hand, such unfolding may directly contribute to the energy balance of translocation, as the entropic force stored in the unfolded subunits could be used to direct transport through stepwise refolding after export. Two variants of this latter model have been proposed for flagellin export: cargo might enter the flagellum through single-file diffusion [226] or, more efficiently, by coupling subunits: the folding of the N-terminus of one filament subunit would generate a pulling force allowing the C-terminus of the same subunit to pull along the N-terminus of the next subunit, and so on [227]. Such a model is compatible with a recent publication showing a constant growth rate of the flagellum at the distal tip [161], in contrast to earlier work observing an exponential decrease in growth rate with length [158,228,229], which fits a model where flagellin subunits were pumped into the flagellum from the base and slowed down by protein compression and friction [160]. Which of the models best describes export, and whether the export process is energized similarly for all cargo classes, remains to be shown.

Several proteins have been shown to influence the energy transduction, most interestingly FliJ/SctO, which in *P. aeruginosa* has been recently proposed to harness the PMF together with another cytosolic regulator, PcrD [230]. However, as SctO also influences other processes in T3SS and has been shown to interact with and positively regulate the ATPase activity in EPEC [33,231] and to be an important component in the determination of substrate hierarchy through interactions with FlhA and other components [232,233], it is very difficult to distinguish between its direct and secondary effects in energy transduction. Energization of T3SS export is described in more detail in a recent review [225].

Type III secretion export is very efficient. Injectisomes can export hundreds to thousands of effector molecules per second [234–236], while flagella grow with a rate of about 10 flagellin subunits per second, corresponding to about 5000 amino acids [228]. Interestingly, the high rate of export in injectisomes correlates with strong inhibition of growth and division in different species [100,237]—in fact, the correlation of *Yersinia* growth and pathogenicity at 37°C (with the absence of Ca^{2+} inducing effector secretion and preventing growth under these conditions) was one of the earliest indications of type III secretion [238–240]. Despite this, it is still not known what exactly leads to this growth inhibition. It is unlikely to be the cost of producing injectisomes, as under non-secreting conditions, a considerable number of injectisomes (about half the number compared to secreting conditions [116]) are produced with little effect on growth. Similarly, effector production and export, or an adverse effect of effector secretion on the bacterium are unlikely to be the sole cause, as growth restriction under secreting conditions does not correlate well with presence or absence of effectors. Also, growth inhibition and secretion could be experimentally uncoupled under certain conditions [241]. This suggests either crosstalk between T3SS and bacterial metabolism [242] or a specific mechanism resulting in loss of division upon type III secretion activation [100]. In either case, the involved pathways remain to be identified.

10. Functional regulation and dynamics

The activity of T3SS must be under tight control—chemotaxis is only possible if flagellar rotation can be adjusted, and tightly regulating the translocation activity of injectisomes is crucial to ensure an optimal amount of protein is translocated into the host cell without eliciting host responses that are detrimental to the survival of the bacterium, especially in the case of persistent infections. However, in contrast to the transcriptional regulation of T3SS, the functional regulation is less well-understood. The best-studied case is the control of flagellar rotation by the chemotactic pathway, which is mediated by the response regulator CheY, which in its phosphorylated form can bind the C-ring protein FliM [3], leading to reversal or braking of the flagellum. The same pathway is used for bacterial thermotaxis [243]. Additionally, c-di-GMP plays an important role in the functional control of flagella, in addition to its effects on the transcriptional and post-transcriptional regulation of motility: the c-di-GMP-binding protein YcgR represses motility by binding to the MotAB stator [6,9], as well as to the C-ring [7,8]. In addition, high c-di-GMP levels lead to accumulation of extracellular cellulose in *Salmonella*, which sterically reduces motility [244]. Heterogeneity in the cellular levels of c-di-GMP, caused by the activity of a phosphodiesterase, which in turn is controlled and asymmetrically partitioned by binding to the chemotaxis histidine kinase CheA in *P. aeruginosa*, accordingly leads to different motility and chemotactic response between the two daughter cells after division [245,246].

In the case of the injectisome, activation of secretion can be triggered by an increase in local oxygen levels (occurring close to the gastrointestinal tract mucosa, the site of *Shigella* invasion) [96], a drop in pH (in the vacuole activating *Salmonella* SPI-2) [98] or extrabacterial Ca^{2+} depletion (which might mimic the low levels of free Ca^{2+} in the host cytosol for *Yersinia*). In addition, it is conceivably beneficial to regulate the amount of effectors that are translocated into a host cell as well as the long-term activity of the system, especially in persistent infections. Indeed, it was shown that previous secretion of certain effectors into a host cell suppresses the T3SS activity of bacteria that subsequently attach to the same cell [146,247], suggesting an active suppression of excess translocation.

The rapid development of fluorescence microscopy techniques has allowed the composition of T3SS to be monitored in real time, and revealed that T3SS are by no means static complexes, but can exchange subunits while functioning (reviewed in [248]). In the flagellum, various components of the cytosolic interface were shown to be dynamic: MotAB, the flagellar stator

complex, exchanges with an average dwell time of about 30 s [249], and indeed is only maintained in place by a conformational change caused by the ion flow [4,250]. In the *E.coli* flagellum, there can be up to 11 MotAB stator complexes and their number increases with the external force on the filament [4,251–254].[5] While the PMF is required for the association of stator complexes with the rotor, individual stators only stay associated for about 30 s before exchanging with stators in the membrane, even at full PMF. In the case of *Shewanella*, H^+-driven MotAB stator complexes exchange with their Na^+-driven PomAB counterparts if the extracellular concentrations of H^+ and Na^+ ions change [5]. Interestingly, the rotating flagellar C-ring components FliMN also exchange within the working flagellum [255–259]; in this case, turnover depends on the rotation direction of the motor, which *in vivo* is regulated by the presence of the activated response regulator CheY [255,258]. The number of FliM and FliN proteins in the C-ring of the counterclockwise rotating flagellum is 26–29% higher than in the clockwise rotating flagellum [258,259]. These results suggest that remodelling of the flagellar motor is involved in its adaptation, which has been shown to account for the extreme sensitivity of the motor to environmental changes [257]. FliI, the flagellar ATPase, was also proposed to dynamically bind to the C-ring and other components of the flagellar cytosolic interface in a complex with its regulator FliH, based on binding studies [128]; recently, this exchange has been shown directly [260].

In the injectisome, less is known about the behaviour of the cytosolic components. Recently, however, the C-ring component SctQ has been shown to exchange with a cytosolic pool in the functioning T3SS [219], and the SctQ exchange rate correlated with the export process.

In all of these cases, the T3SS appears to react differently to a variety of signals, suggesting that these dynamic components allow modulation of the function of the T3SS in response to internal and external cues, ensuring both efficiency and adaptivity of these important molecular machines.

Figure 4 summarizes the influence of external signals on the expression and function of the T3SS.

11. Applications of the T3SS in medicine and biotechnology

Both the injectisome and the bacterial flagellum are targets for the development of novel antimicrobial agents, preventing essential steps in colonization. On the other hand, they can also be exploited. The most obvious application of the T3SS is to export specific proteins. Proteins of choice can easily be targeted to the machinery by a short N-terminal signal (which may be cleaved off at a later stage), and are specifically exported into the supernatant, which leads to significant enrichment of soluble protein without additional purification steps. Both types of T3SS have been used for this purpose: the injectisome was used to export various proteins, including spider silk proteins [261], and has recently been shown to be a useful tool for the production of biopolymer-forming proteins, with increased export levels upon overexpression of the master transcriptional regulator [262]. Similarly, the flagellar T3SS was shown to be able to export a variety of different protein types [191] and subsequently optimized for protein secretion [263].

The injectisome, a system evolutionarily optimized for protein translocation, has also been used to directly transport proteins into host cells, especially to deliver vaccines [264–267], but also for immunomodulatory cytokines [268]. Traditionally, attenuated strains were used for this purpose, but recently, achromosomal, but T3SS-active minicells were also shown to be functional for translocation [269]. Besides such medical approaches, injectisomes might be useful carriers for the delivery of proteins into cultured eukaryotic cells [270,271].

Beyond the exploitation of the T3SS for protein transport, its application as a biosensor is conceivable. Possible future applications include the use of the flagellum as a sensor for biofilm formation, or, similarly, the injectisome to sense cell contact.

12. Open questions

Despite intense research, the molecular events that govern the export of substrates via the T3SS remain largely unknown. Many interactions between T3SS components and substrates and their chaperones have been recorded, but so far no clear picture emerges. Given the recent findings that several core parts of the machinery are not absolutely essential for export, it is possible that T3SS export relies on a series of local enrichment steps. Cross-linking studies and single-molecule fluorescence microscopy experiments for different classes of cargo under various conditions might help to uncover the path of cargo through the T3SS.

A closely linked question is the energy transfer for T3SS export. While it appears clear that the PMF plays an important role in export, no conclusive theory for how the PMF is linked to export has yet been provided.

An important player in the T3SS is the export apparatus in the IM. Owing to the high proportion of transmembrane helices in the export apparatus, both structural and functional knowledge of its components is limited, but with the application of cryotomography and selective cross-linkers, important insights into the structure of the export apparatus and possible homologies to other export systems are to be expected.

While the influence of many global regulatory networks on the expression and, in some cases, function of both types of T3SS has been revealed in the last years, it is largely unknown how these signals are processed and implemented at the T3SS. This is especially true for the injectisome, where very little is known about the functional regulation of the system.

Understanding the regulation and molecular mechanism of the T3SS will not only help us to appreciate the function of these complex nanomachines, but may also help to control and interfere with the T3SS, both in preventing the function of the system to specifically prevent pathogenicity, and to tailor the T3SS for biochemical applications from targeted translocation to locomotion.

Competing interests. We declare we have no competing interests.

Funding. We thank EMBO for a long-term fellowship (ALTF 170-2011) to A.D. BBSRC is thanked for continuous funding of the work in the Armitage laboratory. This review does not claim to be comprehensive. The authors tried to preferentially highlight recent studies that have not been mentioned in previous reviews and apologize for any omissions.

[5]In agreement with this observation, species swimming under high load like spirochaetes, as well as *Helicobacter*, have more than 11 stators, supporting the idea that a higher number allows increased torque generation [45,48].

References

1. Wadhams GH, Armitage JP. 2004 Making sense of it all: bacterial chemotaxis. *Nat. Rev. Mol. Cell Biol*. **5**, 1024–1037. (doi:10.1038/nrm1524)
2. Sourjik V, Wingreen NS. 2012 Responding to chemical gradients: bacterial chemotaxis. *Curr. Opin. Cell Biol*. **24**, 262–268. (doi:10.1016/j.ceb.2011.11.008)
3. Porter SL, Wadhams GH, Armitage JP. 2011 Signal processing in complex chemotaxis pathways. *Nat. Rev. Microbiol*. **9**, 153–165. (doi:10.1038/nrmicro2505)
4. Tipping MJ, Delalez NJ, Lim R, Berry RM, Armitage JP. 2013 Load-dependent assembly of the bacterial flagellar motor. *MBio* **4**, e00551-13. (doi:10.1128/mBio.00551-13)
5. Paulick A, Delalez NJ, Brenzinger S, Steel BC, Berry RM, Armitage JP, Thormann KM. 2015 Dual stator dynamics in the *Shewanella oneidensis* MR-1 flagellar motor. *Mol. Microbiol*. **96**, 993–1001. (doi:10.1111/mmi.12984)
6. Boehm A *et al.* 2010 Second messenger-mediated adjustment of bacterial swimming velocity. *Cell* **141**, 107–116. (doi:10.1016/j.cell.2010.01.018)
7. Fang X, Gomelsky M. 2010 A post-translational, c-di-GMP-dependent mechanism regulating flagellar motility. *Mol. Microbiol*. **76**, 1295–1305. (doi:10.1111/j.1365-2958.2010.07179.x)
8. Paul K, Nieto V, Carlquist WC, Blair DF, Harshey RM. 2010 The c-di-GMP binding protein YcgR controls flagellar motor direction and speed to affect chemotaxis by a 'backstop brake' mechanism. *Mol. Cell* **38**, 128–139. (doi:10.1016/j.molcel.2010.03.001)
9. Kuchma SL, Delalez NJ, Filkins LM, Snavely EA, Armitage JP, O'Toole GA. 2015 Cyclic di-GMP-mediated repression of swarming motility by *Pseudomonas aeruginosa* PA14 requires the MotAB stator. *J. Bacteriol*. **197**, 420–430. (doi:10.1128/JB.02130-14)
10. Kirkpatrick CL, Viollier PH. 2012 Reflections on a sticky situation: how surface contact pulls the trigger for bacterial adhesion. *Mol. Microbiol*. **83**, 7–9. (doi:10.1111/j.1365-2958.2011.07913.x)
11. Cairns LS, Marlow VL, Bissett E, Ostrowski A, Stanley-Wall NR. 2013 A mechanical signal transmitted by the flagellum controls signalling in *Bacillus subtilis*. *Mol. Microbiol*. **90**, 6–21. (doi:10.1111/mmi.12342)
12. Cornelis GR. 2006 The type III secretion injectisome. *Nat. Rev. Microbiol*. **4**, 811–825. (doi:10.1038/nrmicro1526)
13. Galán JE, Wolf-Watz H. 2006 Protein delivery into eukaryotic cells by type III secretion machines. *Nature* **444**, 567–573. (doi:10.1038/nature05272)
14. Dale C, Moran NA. 2006 Molecular interactions between bacterial symbionts and their hosts. *Cell* **126**, 453–465. (doi:10.1016/j.cell.2006.07.014)
15. Preston GM. 2007 Metropolitan microbes: type III secretion in multihost symbionts. *Cell Host Microbe* **2**, 291–294. (doi:10.1016/j.chom.2007.10.004)
16. Fauvart M, Michiels J. 2008 Rhizobial secreted proteins as determinants of host specificity in the rhizobium–legume symbiosis. *FEMS Microbiol. Lett*. **285**, 1–9. (doi:10.1111/j.1574-6968.2008.01254.x)
17. Büttner D. 2012 Protein export according to schedule: architecture, assembly, and regulation of type III secretion systems from plant- and animal-pathogenic bacteria. *Microbiol. Mol. Biol. Rev*. **76**, 262–310. (doi:10.1128/MMBR.05017-11)
18. Allen RD, Baumann P. 1971 Structure and arrangement of flagella in species of the genus *Beneckea* and *Photobacterium fischeri*. *J. Bacteriol*. **107**, 295–302.
19. Atsumi T, McCarter L, Imae Y. 1992 Polar and lateral flagellar motors of marine *Vibrio* are driven by different ion-motive forces. *Nature* **355**, 182–184. (doi:10.1038/355182a0)
20. Li N, Kojima S, Homma M. 2011 Sodium-driven motor of the polar flagellum in marine bacteria *Vibrio*. *Genes Cells* **16**, 985–999. (doi:10.1111/j.1365-2443.2011.01545.x)
21. Maruyama Y, Kobayashi M, Murata K, Hashimoto W. In press. Formation of a single polar flagellum by two distinct flagellar gene sets in *Sphingomonas* sp. strain A1. *Microbiology*. (doi:10.1099/mic.0.000119)
22. Erhardt M, Namba K, Hughes KT. 2010 Bacterial nanomachines: the flagellum and type III injectisome. *Cold Spring Harb. Perspect. Biol*. **2**, a000299. (doi:10.1101/cshperspect.a000299)
23. Minamino T. 2013 Protein export through the bacterial flagellar type III export pathway. *Biochim. Biophys. Acta Mol. Cell Res*. **1843**, 1642–1648. (doi:10.1016/j.bbamcr.2013.09.005)
24. Hueck CJ. 1998 Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiol. Mol. Biol. Rev*. **62**, 379–433.
25. Yu X-J, Liu M, Matthews S, Holden DW. 2011 Tandem translation generates a chaperone for the *Salmonella* type III secretion system protein SsaQ. *J. Biol. Chem*. **286**, 36 098–36 107. (doi:10.1074/jbc.M111.278663)
26. Bzymek KP, Hamaoka BY, Ghosh P. 2012 Two translation products of *Yersinia yscQ* assemble to form a complex essential to type III secretion. *Biochemistry* **51**, 1669–1677. (doi:10.1021/bi201792p)
27. Kawamoto A, Morimoto YV, Miyata T, Minamino T, Hughes KT, Kato T, Namba K. 2013 Common and distinct structural features of *Salmonella* injectisome and flagellar basal body. *Sci. Rep*. **3**, 3369. (doi:10.1038/srep03369)
28. Hara N, Morimoto YV, Kawamoto A, Namba K, Minamino T. 2012 Interaction of the extreme N-terminal region of FliH with FlhA is required for efficient bacterial flagellar protein export. *J. Bacteriol*. **194**, 5353–5360. (doi:10.1128/JB.01028-12)
29. Abrusci P *et al.* 2013 Architecture of the major component of the type III secretion system export apparatus. *Nat. Struct. Mol. Biol*. **20**, 99–104. (doi:10.1038/nsmb.2452)
30. Minamino T, Imada K. 2015 The bacterial flagellar motor and its structural diversity. *Trends Microbiol*. **23**, 267–274. (doi:10.1016/j.tim.2014.12.011)
31. Diepold A, Wagner S. 2014 Assembly of the bacterial type III secretion machinery. *FEMS Microbiol. Rev*. **38**, 802–822. (doi:10.1111/1574-6976.12061)
32. Schraidt O, Marlovits TC. 2011 Three-dimensional model of *Salmonella*'s needle complex at subnanometer resolution. *Science* **331**, 1192–1195. (doi:10.1126/science.1199358)
33. Ibuki T, Imada K, Minamino T, Kato T, Miyata T, Namba K. 2011 Common architecture of the flagellar type III protein export apparatus and F- and V-type ATPases. *Nat. Struct. Mol. Biol*. **18**, 277–282. (doi:10.1038/nsmb.1977)
34. Barker CS, Meshcheryakova IV, Inoue T, Samatey FA. 2014 Assembling flagella in *Salmonella* mutant strains producing a type III export apparatus without FliO. *J. Bacteriol*. **196**, 4001–4011. (doi:10.1128/JB.02184-14)
35. Pallen MJ, Bailey CM, Beatson SA. 2006 Evolutionary links between FliH/YscL-like proteins from bacterial type III secretion systems and second-stalk components of the FoF1 and vacuolar ATPases. *Protein Sci*. **15**, 935–941. (doi:10.1110/ps.051958806)
36. Imada K, Minamino T, Tahara A, Namba K. 2007 Structural similarity between the flagellar type III ATPase FliI and F1-ATPase subunits. *Proc. Natl Acad. Sci. USA* **104**, 485–490. (doi:10.1073/pnas.0608090104)
37. Worrall LJ, Vuckovic M, Strynadka NCJ. 2010 Crystal structure of the C-terminal domain of the *Salmonella* type III secretion system export apparatus protein InvA. *Protein Sci*. **19**, 1091–1096. (doi:10.1002/pro.382)
38. Abby SS, Rocha EPC. 2012 The non-flagellar type III secretion system evolved from the bacterial flagellum and diversified into host-cell adapted systems. *PLoS Genet*. **8**, e1002983. (doi:10.1371/journal.pgen.1002983)
39. Reichow SL, Korotkov KV, Hol WGJ, Gonen T. 2010 Structure of the cholera toxin secretion channel in its closed state. *Nat. Struct. Mol. Biol*. **17**, 1226–1232. (doi:10.1038/nsmb.1910)
40. Korotkov KV, Gonen T, Hol WGJ. 2011 Secretins: dynamic channels for protein transport across membranes. *Trends Biochem. Sci*. **36**, 433–443. (doi:10.1016/j.tibs.2011.04.002)
41. Cordes FS, Komoriya K, Larquet E, Yang S, Egelman E, Blocker AJ, Lea SM. 2003 Helical structure of the needle of the type III secretion system of *Shigella flexneri*. *J. Biol. Chem*. **278**, 17 103–17 107. (doi:10.1074/jbc.M300091200)
42. Mueller CA, Broz P, Müller SA, Ringler P, Erne-Brand F, Sorg I, Kuhn M, Engel A, Cornelis GR. 2005 The V-antigen of *Yersinia* forms a distinct structure at

the tip of injectisome needles. *Science* **310**, 674–676. (doi:10.1126/science.1118476)

43. Sekiya K, Ohishi M, Ogino T, Tamano K, Sasakawa C, Abe A. 2001 Supermolecular structure of the enteropathogenic *Escherichia coli* type III secretion system and its direct interaction with the EspA-sheath-like structure. *Proc. Natl Acad. Sci. USA* **98**, 11 638–11 643. (doi:10.1073/pnas.191378598)
44. Malapaka RRV, Adebayo LO, Tripp BC. 2007 A deletion variant study of the functional role of the *Salmonella* flagellin hypervariable domain region in motility. *J. Mol. Biol*. **365**, 1102–1116. (doi:10.1016/j.jmb.2006.10.054)
45. Chen S *et al.* 2011 Structural diversity of bacterial flagellar motors. *EMBO J*. **30**, 2972–2981. (doi:10.1038/emboj.2011.186)
46. Kudryashev M *et al.* 2013 *In situ* structural analysis of the *Yersinia enterocolitica* injectisome. *Elife* **2**, e00792. (doi:10.7554/eLife.00792)
47. Hu B, Morado DR, Margolin W, Rohde JR, Arizmendi O, Picking WL, Picking WD, Liu J. 2015 Visualization of the type III secretion sorting platform of *Shigella flexneri*. *Proc. Natl Acad. Sci. USA* **112**, 1047–1052. (doi:10.1073/pnas.1411610112)
48. Murphy GE, Leadbetter JR, Jensen GJ. 2006 *In situ* structure of the complete *Treponema primitia* flagellar motor. *Nature* **442**, 1062–1064. (doi:10.1038/nature05015)
49. Ohgita T, Hayashi N, Hama S, Tsuchiya H, Gotoh N, Kogure K. 2013 A novel effector secretion mechanism based on proton-motive force-dependent type III secretion apparatus rotation. *FASEB J*. **27**, 2862–2872. (doi:10.1096/fj.13-229054)
50. Chatterjee S, Chaudhury S, McShan AC, Kaur K, De Guzman RN. 2013 Structure and biophysics of type III secretion in bacteria. *Biochemistry* **52**, 2508–2517. (doi:10.1021/bi400160a)
51. Burkinshaw BJ, Strynadka NCJ. 2014 Assembly and structure of the T3SS. *Biochim. Biophys. Acta* **1843**, 1648–1693. (doi:10.1016/j.bbamcr.2014.01.035)
52. Abrusci P, McDowell MA, Lea SM, Johnson S. 2014 Building a secreting nanomachine: a structural overview of the T3SS. *Curr. Opin. Struct. Biol*. **25**, 111–117. (doi:10.1016/j.sbi.2013.11.001)
53. Mukherjee S, Kearns DB. 2014 The structure and regulation of flagella in *Bacillus subtilis*. *Annu. Rev. Genet*. **48**, 319–340. (doi:10.1146/annurev-genet-120213-092406)
54. Macnab RM. 2004 Type III flagellar protein export and flagellar assembly. *Biochim. Biophys. Acta* **1694**, 207–217. (doi:10.1016/j.bbamcr.2004.04.005)
55. Gophna U, Ron E, Graur D. 2003 Bacterial type III secretion systems are ancient and evolved by multiple horizontal-transfer events. *Gene* **312**, 151–163. (doi:10.1016/S0378-1119(03)00612-7)
56. Pallen MJ, Beatson SA, Bailey CM. 2005 Bioinformatics, genomics and evolution of non-flagellar type-III secretion systems: a Darwinian perspective. *FEMS Microbiol. Rev*. **29**, 201–229. (doi:10.1016/j.femsre.2005.01.001)
57. Pallen MJ, Gophna U. 2007 Bacterial flagella and type III secretion: case studies in the evolution of complexity. *Genome Dyn*. **3**, 30–47. (doi:10.1159/000107602)
58. Liu R, Ochman H. 2007 Stepwise formation of the bacterial flagellar system. *Proc. Natl Acad. Sci. USA* **104**, 7116–7121. (doi:10.1073/pnas.0700266104)
59. Snyder LAS, Loman NJ, Fütterer K, Pallen MJ. 2009 Bacterial flagellar diversity and evolution: seek simplicity and distrust it? *Trends Microbiol*. **17**, 1–5. (doi:10.1016/j.tim.2008.10.002)
60. Naum M, Brown EW, Mason-Gamer RJ. 2009 Phylogenetic evidence for extensive horizontal gene transfer of type III secretion system genes among enterobacterial plant pathogens. *Microbiology* **155**, 3187–3199. (doi:10.1099/mic.0.029892-0)
61. Konovalova A, Petters T, Søgaard-Andersen L. 2010 Extracellular biology of *Myxococcus xanthus*. *FEMS Microbiol. Rev*. **34**, 89–106. (doi:10.1111/j.1574-6976.2009.00194.x)
62. Walker KA, Maltez VI, Hall JD, Vitko NP, Miller VL. 2013 A phenotype at last: essential role for the *Yersinia enterocolitica* Ysa type III secretion system in a *Drosophila melanogaster* S2 cell model. *Infect. Immun*. **81**, 2478–2487. (doi:10.1128/IAI.01454-12)
63. Bent ZW *et al.* 2015 A transcriptomic analysis of *Yersinia enterocolitica* biovar 1B infecting murine macrophages reveals new mechanisms of extracellular and intracellular survival. *Infect. Immun.* **83**, 2672–2685. (doi:10.1128/IAI.02922-14)
64. Anderson JK, Smith TG, Hoover TR. 2010 Sense and sensibility: flagellum-mediated gene regulation. *Trends Microbiol*. **18**, 30–37. (doi:10.1016/j.tim.2009.11.001)
65. Römling U, Gomelsky M, Galperin MY. 2005 C-di-GMP: the dawning of a novel bacterial signalling system. *Mol. Microbiol*. **57**, 629–639. (doi:10.1111/j.1365-2958.2005.04697.x)
66. Hengge R. 2009 Principles of c-di-GMP signalling in bacteria. *Nat. Rev. Microbiol*. **7**, 263–273. (doi:10.1038/nrmicro2109)
67. Mills E, Pultz IS, Kulasekara HD, Miller SI. 2011 The bacterial second messenger c-di-GMP: mechanisms of signalling. *Cell. Microbiol*. **13**, 1122–1129. (doi:10.1111/j.1462-5822.2011.01619.x)
68. Wolfe AJ, Visick KL. 2008 Get the message out: cyclic-Di-GMP regulates multiple levels of flagellum-based motility. *J. Bacteriol*. **190**, 463–475. (doi:10.1128/JB.01418-07)
69. Guttenplan SB, Kearns DB. 2013 Regulation of flagellar motility during biofilm formation. *FEMS Microbiol. Rev*. **37**, 849–871. (doi:10.1111/1574-6976.12018)
70. Skurnik M, Bölin I, Heikkinen H, Piha S, Wolf-Watz H. 1984 Virulence plasmid-associated autoagglutination in *Yersinia* spp. *J. Bacteriol*. **158**, 1033–1036.
71. Michiels T, Vanooteghem J, Lambert de Rouvroit C, China B, Gustin A, Boudry P, Cornelis GR. 1991 Analysis of virC, an operon involved in the secretion of Yop proteins by *Yersinia enterocolitica*. *J. Bacteriol*. **173**, 4994–5009.
72. Lambert de Rouvroit C, Sluiters C, Cornelis GR. 1992 Role of the transcriptional activator, VirF, and temperature in the expression of the pYV plasmid genes of *Yersinia enterocolitica*. *Mol. Microbiol*. **6**, 395–409. (doi:10.1111/j.1365-2958.1992.tb01483.x)
73. Hoe N, Goguen J. 1993 Temperature sensing in *Yersinia pestis*: translation of the LcrF activator protein is thermally regulated. *J. Bacteriol*. **175**, 7901–7909.
74. Falconi M, Colonna B, Prosseda G, Micheli G, Gualerzi CO. 1998 Thermoregulation of *Shigella* and *Escherichia coli* EIEC pathogenicity. A temperature-dependent structural transition of DNA modulates accessibility of *virF* promoter to transcriptional repressor H-NS. *EMBO J*. **17**, 7033–7043. (doi:10.1093/emboj/17.23.7033)
75. Bleves S, Marenne M-N, Detry G, Cornelis GR. 2002 Up-regulation of the *Yersinia enterocolitica* yop regulon by deletion of the flagellum master operon flhDC. *J. Bacteriol*. **184**, 3214–3223. (doi:10.1128/JB.184.12.3214-3223.2002)
76. Horne SM, Prüß BM. 2006 Global gene regulation in *Yersinia enterocolitica*: effect of FliA on the expression levels of flagellar and plasmid-encoded virulence genes. *Arch. Microbiol*. **185**, 115–126. (doi:10.1007/s00203-005-0077-1)
77. Iyoda S, Koizumi N, Satou H, Lu Y, Saitoh T, Ohnishi M, Watanabe H. 2006 The GrlR-GrlA regulatory system coordinately controls the expression of flagellar and LEE-encoded type III protein secretion systems in enterohemorrhagic *Escherichia coli*. *J. Bacteriol*. **188**, 5682–5692. (doi:10.1128/JB.00352-06)
78. Soscia C, Hachani A, Bernadac A, Filloux A, Bleves S. 2007 Cross talk between type III secretion and flagellar assembly systems in *Pseudomonas aeruginosa*. *J. Bacteriol*. **189**, 3124–3132. (doi:10.1128/JB.01677-06)
79. Singer HM, Kühne C, Deditius JA, Hughes KT, Erhardt M. 2014 The *Salmonella* Spi1 virulence regulatory protein HilD directly activates transcription of the flagellar master operon *flhDC*. *J. Bacteriol*. **196**, 1448–1457. (doi:10.1128/JB.01438-13)
80. Romeo T. 1998 Global regulation by the small RNA-binding protein CsrA and the non-coding RNA molecule CsrB. *Mol. Microbiol*. **29**, 1321–1330. (doi:10.1046/j.1365-2958.1998.01021.x)
81. Johnston C, Pegues DA, Hueck CJ, Lee A, Miller SI. 1996 Transcriptional activation of *Salmonella typhimurium* invasion genes by a member of the phosphorylated response-regulator superfamily. *Mol. Microbiol*. **22**, 715–727. (doi:10.1046/j.1365-2958.1996.d01-1719.x)
82. Altier C, Suyemoto M, Lawhon SD. 2000 Regulation of *Salmonella enterica* serovar typhimurium invasion genes by *csrA*. *Infect. Immun*. **68**, 6790–6797. (doi:10.1128/IAI.68.12.6790-6797.2000)
83. Bhatt S, Edwards AN, Nguyen HTT, Merlin D, Romeo T, Kalman D. 2009 The RNA binding protein CsrA is a pleiotropic regulator of the locus of enterocyte effacement pathogenicity island of enteropathogenic *Escherichia coli*. *Infect. Immun*. **77**, 3552–3568. (doi:10.1128/IAI.00418-09)
84. Heroven AK, Böhme K, Dersch P. 2012 The Csr/Rsm system of *Yersinia* and related pathogens: a

post-transcriptional strategy for managing virulence. *RNA Biol*. **9**, 379–391. (doi:10.4161/rna.19333)

85. Avican K, Fahlgren A, Huss M, Heroven AK, Beckstette M, Dersch P, Fällman M. 2015 Reprogramming of *Yersinia* from virulent to persistent mode revealed by complex *in vivo* RNA-seq analysis. *PLoS Pathog.* **11**, e1004600. (doi:10.1371/journal.ppat.1004600)
86. Moscoso JA, Mikkelsen H, Heeb S, Williams P, Filloux A. 2011 The *Pseudomonas aeruginosa* sensor RetS switches type III and type VI secretion via c-di-GMP signalling. *Environ. Microbiol*. **13**, 3128–3138. (doi:10.1111/j.1462-2920.2011.02595.x)
87. Wei BL, Brun-Zinkernagel A-M, Simecka JW, Prüß BM, Babitzke P, Romeo T. 2001 Positive regulation of motility and *flhDC* expression by the RNA-binding protein CsrA of *Escherichia coli*. *Mol. Microbiol*. **40**, 245–256. (doi:10.1046/j.1365-2958.2001.02380.x)
88. Vakulskas CA, Potts AH, Babitzke P, Ahmer BMM, Romeo T. 2015 Regulation of bacterial virulence by Csr (Rsm) systems. *Microbiol. Mol. Biol. Rev*. **79**, 193–224. (doi:10.1128/MMBR.00052-14)
89. Mukherjee S, Yakhnin H, Kysela D, Sokoloski J, Babitzke P, Kearns DB. 2011 CsrA-FliW interaction governs flagellin homeostasis and a checkpoint on flagellar morphogenesis in *Bacillus subtilis*. *Mol. Microbiol*. **82**, 447–461. (doi:10.1111/j.1365-2958.2011.07822.x)
90. Jonas K, Tomenius H, Römling U, Georgellis D, Melefors O. 2006 Identification of YhdA as a regulator of the *Escherichia coli* carbon storage regulation system. *FEMS Microbiol. Lett*. **264**, 232–237. (doi:10.1111/j.1574-6968.2006.00457.x)
91. Jonas K, Edwards AN, Ahmad I, Romeo T, Römling U, Melefors O, Melefors Ö. 2010 Complex regulatory network encompassing the Csr, c-di-GMP and motility systems of *Salmonella typhimurium*. *Environ. Microbiol*. **12**, 524–540. (doi:10.1111/j.1462-2920.2009.02097.x)
92. Belas R. 2014 Biofilms, flagella, and mechanosensing of surfaces by bacteria. *Trends Microbiol*. **22**, 517–527. (doi:10.1016/j.tim.2014.05.002)
93. Li G, Brown PJB, Tang JX, Xu J, Quardokus EM, Fuqua C, Brun YV. 2012 Surface contact stimulates the just-in-time deployment of bacterial adhesins. *Mol. Microbiol.* **83**, 41–51. (doi:10.1111/j.1365-2958.2011.07909.x)
94. Chan JM, Guttenplan SB, Kearns DB. 2014 Defects in the flagellar motor increase synthesis of poly-γ-glutamate in *Bacillus subtilis*. *J. Bacteriol*. **196**, 740–753. (doi:10.1128/JB.01217-13)
95. Cairns LS, Hobley L, Stanley-Wall NR. 2014 Biofilm formation by *Bacillus subtilis*: new insights into regulatory strategies and assembly mechanisms. *Mol. Microbiol*. **93**, 587–598. (doi:10.1111/mmi.12697)
96. Marteyn BS *et al.* 2010 Modulation of *Shigella* virulence in response to available oxygen *in vivo*. *Nature* **465**, 355–358. (doi:10.1038/nature08970)
97. Schüller S, Phillips A. 2010 Microaerobic conditions enhance type III secretion and adherence of enterohaemorrhagic *Escherichia coli* to polarized human intestinal epithelial cells. *Environ. Microbiol*. **12**, 2426–2435. (doi:10.1111/j.1462-2920.2010.02216.x)
98. Yu X-J, McGourty K, Liu M, Unsworth KE, Holden DW. 2010 H sensing by intracellular *Salmonella* induces effector translocation. *Science* **328**, 1040–1043. (doi:10.1126/science.1189000)
99. Chakraborty S, Mizusaki H, Kenney LJ. 2015 A FRET-based DNA biosensor tracks OmpR-dependent acidification of *Salmonella* during macrophage infection. *PLoS Biol*. **13**, e1002116. (doi:10.1371/journal.pbio.1002116)
100. Sturm A, Heinemann M, Arnoldini M, Benecke A, Ackermann M, Benz M, Dormann J, Hardt W-D. 2011 The cost of virulence: retarded growth of *Salmonella typhimurium* cells expressing type III secretion system 1. *PLoS Pathog*. **7**, e1002143. (doi:10.1371/journal.ppat.1002143)
101. Mills E, Baruch K, Aviv G, Nitzan M, Rosenshine I. 2013 Dynamics of the type III secretion system activity of enteropathogenic *Escherichia coli*. *MBio* **4**, e00303-13. (doi:10.1128/mBio.00303-13)
102. Campbell-Valois F-X, Schnupf P, Nigro G, Sachse M, Sansonetti PJ, Parsot C. 2014 A fluorescent reporter reveals on/off regulation of the *Shigella* type III secretion apparatus during entry and cell-to-cell spread. *Cell Host Microbe* **15**, 177–189. (doi:10.1016/j.chom.2014.01.005)
103. Macnab RM. 2003 How bacteria assemble flagella. *Annu. Rev. Microbiol*. **57**, 77–100. (doi:10.1146/annurev.micro.57.030502.090832)
104. Mears PJ, Koirala S, Rao CV, Golding I, Chemla YR. 2014 *Escherichia coli* swimming is robust against variations in flagellar number. *Elife* **3**, e01916. (doi:10.7554/eLife.01916)
105. Laloux G, Jacobs-Wagner C. 2014 How do bacteria localize proteins to the cell pole? *J. Cell Sci*. **127**, 11–19. (doi:10.1242/jcs.138628)
106. Kazmierczak BI, Hendrixson DR. 2013 Spatial and numerical regulation of flagellar biosynthesis in polarly flagellated bacteria. *Mol. Microbiol*. **88**, 655–663. (doi:10.1111/mmi.12221)
107. Huitema E, Pritchard S, Matteson D, Radhakrishnan SK, Viollier PH. 2006 Bacterial birth scar proteins mark future flagellum assembly site. *Cell* **124**, 1025–1037. (doi:10.1016/j.cell.2006.01.019)
108. Davis NJ *et al.* 2013 De- and repolarization mechanism of flagellar morphogenesis during a bacterial cell cycle. *Genes Dev*. **27**, 2049–2062. (doi:10.1101/gad.222679.113)
109. Schuhmacher JS *et al.* 2015 MinD-like ATPase FlhG effects location and number of bacterial flagella during C-ring assembly. *Proc. Natl Acad. Sci. USA* **112**, 3092–3097. (doi:10.1073/pnas.1419388112)
110. Chakravortty D, Rohde M, Jäger L, Deiwick J, Hensel M. 2005 Formation of a novel surface structure encoded by *Salmonella* Pathogenicity Island 2. *EMBO J*. **24**, 2043–2052. (doi:10.1038/sj.emboj.7600676)
111. Kubori T, Matsushima Y, Nakamura D, Uralil J, Lara-Tejero M, Sukhan A, Galán JE, Aizawa S-I. 1998 Supramolecular structure of the *Salmonella typhimurium* type III protein secretion system. *Science* **280**, 602–605. (doi:10.1126/science.280.5363.602)
112. Blocker AJ, Gounon P, Larquet E, Niebuhr K, Cabiaux V, Parsot C, Sansonetti P. 1999 The tripartite type III secreton of *Shigella flexneri* inserts IpaB and IpaC into host membranes. *J. Cell Biol*. **147**, 683–693. (doi:10.1083/jcb.147.3.683)
113. Diepold A, Amstutz M, Abel S, Sorg I, Jenal U, Cornelis GR. 2010 Deciphering the assembly of the *Yersinia* type III secretion injectisome. *EMBO J*. **29**, 1928–1940. (doi:10.1038/emboj.2010.84)
114. Diepold A, Wiesand U, Cornelis GR. 2011 The assembly of the export apparatus (YscR,S,T,U,V) of the *Yersinia* type III secretion apparatus occurs independently of other structural components and involves the formation of an YscV oligomer. *Mol. Microbiol*. **82**, 502–514. (doi:10.1111/j.1365-2958.2011.07830.x)
115. Notti RQ, Bhattacharya S, Lilic M, Stebbins CE. 2015 A common assembly module in injectisome and flagellar type III secretion sorting platforms. *Nat. Commun*. **6**, 7125. (doi:10.1038/ncomms8125)
116. Kudryashev M, Diepold A, Amstutz M, Armitage JP, Stahlberg H, Cornelis GR. 2015 *Yersinia enterocolitica* type III secretion injectisomes form regularly spaced clusters, which incorporate new machines upon activation. *Mol. Microbiol*. **95**, 875–884. (doi:10.1111/mmi.12908)
117. Miras I, Hermant D, Arricau N, Popoff MY. 1995 Nucleotide sequence of i*agA* and *iagB* genes involved in invasion of HeLa cells by *Salmonella enterica* subsp. *enterica* ser. Typhi. *Res. Microbiol*. **146**, 17–20. (doi:10.1016/0923-2508(96)80267-1)
118. Koraimann G. 2003 Lytic transglycosylases in macromolecular transport systems of Gram-negative bacteria. *Cell. Mol. Life Sci*. **60**, 2371–2388. (doi:10.1007/s00018-003-3056-1)
119. Oh H-S, Kvitko BH, Morello JE, Collmer A. 2007 *Pseudomonas syringae* lytic transglycosylases coregulated with the type III secretion system contribute to the translocation of effector proteins into plant cells. *J. Bacteriol*. **189**, 8277–8289. (doi:10.1128/JB.00998-07)
120. Yu Y-C, Lin C-N, Wang S-H, Ng S-C, Hu WS, Syu W-J. 2010 A putative lytic transglycosylase tightly regulated and critical for the EHEC type three secretion. *J. Biomed. Sci*. **17**, 52. (doi:10.1186/1423-0127-17-52)
121. Burkinshaw BJ, Deng W, Lameignère E, Wasney GA, Zhu H, Worrall LJ, Finlay BB, Strynadka NCJ. 2015 Structural analysis of a specialized type III secretion system peptidoglycan-cleaving enzyme. *J. Biol. Chem*. **290**, 10 406–10 417. (doi:10.1074/jbc.M115.639013)
122. Hirano T, Minamino T, Macnab RM. 2001 The role in flagellar rod assembly of the N-terminal domain of *Salmonella* FlgJ, a flagellum-specific muramidase. *J. Mol. Biol*. **312**, 359–369. (doi:10.1006/jmbi.2001.4963)
123. Nambu T, Minamino T, Macnab RM, Kutsukake K. 1999 Peptidoglycan-hydrolyzing activity of the FlgJ protein, essential for flagellar rod formation in *Salmonella typhimurium*. *J. Bacteriol*. **181**, 1555–1561.

124. Kubori T, Yamaguchi S, Aizawa S-I. 1997 Assembly of the switch complex onto the MS ring complex of *Salmonella typhimurium* does not require any other flagellar proteins. *J. Bacteriol*. **179**, 813–817.
125. Li H, Sourjik V. 2011 Assembly and stability of flagellar motor in *Escherichia coli*. *Mol. Microbiol*. **80**, 886–899. (doi:10.1111/j.1365-2958.2011.07557.x)
126. Morimoto YV, Ito M, Hiraoka KD, Che YS, Bai F, Kami-ike N, Namba K, Minamino T. 2014 Assembly and stoichiometry of FliF and FlhA in *Salmonella* flagellar basal body. *Mol. Microbiol*. **91**, 1214–1226. (doi:10.1111/mmi.12529)
127. Chevance FFV, Hughes KT. 2008 Coordinating assembly of a bacterial macromolecular machine. *Nat. Rev. Microbiol*. **6**, 455–465. (doi:10.1038/nrmicro1887)
128. Minamino T, Imada K, Namba K. 2008 Mechanisms of type III protein export for bacterial flagellar assembly. *Mol. Biosyst*. **4**, 1105–1115. (doi:10.1039/b808065h)
129. Evans LDB, Hughes C, Fraser GM. 2014 Building a flagellum outside the bacterial cell. *Trends Microbiol*. **22**, 566–572. (doi:10.1016/j.tim.2014.05.009)
130. Kimbrough TG, Miller SI. 2000 Contribution of *Salmonella typhimurium* type III secretion components to needle complex formation. *Proc. Natl Acad. Sci. USA* **97**, 11 008–11 013. (doi:10.1073/pnas.200209497)
131. Kimbrough TG, Miller SI. 2002 Assembly of the type III secretion needle complex of *Salmonella typhimurium*. *Microbes Infect*. **4**, 75–82. (doi:10.1016/S1286-4579(01)01512-X)
132. Schraidt O, Lefebre MD, Brunner MJ, Schmied WH, Schmidt A, Radics J, Mechtler K, Galán JE, Marlovits TC. 2010 Topology and organization of the *Salmonella typhimurium* type III secretion needle complex components. *PLoS Pathog*. **6**, e1000824. (doi:10.1371/journal.ppat.1000824)
133. Friedrich C, Bulyha I, Søgaard-Andersen L. 2014 Outside-in assembly pathway of the type IV pilus system in *Myxococcus xanthus*. *J. Bacteriol*. **196**, 378–390. (doi:10.1128/JB.01094-13)
134. Wagner S, Königsmaier L, Lara-Tejero M, Lefebre MD, Marlovits TC, Galán JE. 2010 Organization and coordinated assembly of the type III secretion export apparatus. *Proc. Natl Acad. Sci. USA* **107**, 17 745–17 750. (doi:10.1073/pnas.1008053107)
135. Diepold A, Wiesand U, Amstutz M, Cornelis GR. 2012 Assembly of the *Yersinia* injectisome: the missing pieces. *Mol. Microbiol*. **85**, 878–892. (doi:10.1111/j.1365-2958.2012.08146.x)
136. Journet L, Agrain C, Broz P, Cornelis GR. 2003 The needle length of bacterial injectisomes is determined by a molecular ruler. *Science* **302**, 1757–1760. (doi:10.1126/science.1091422)
137. Shibata S, Takahashi N, Chevance FFV, Karlinsey JE, Hughes KT, Aizawa S-I. 2007 FliK regulates flagellar hook length as an internal ruler. *Mol. Microbiol*. **64**, 1404–1415. (doi:10.1111/j.1365-2958.2007.05750.x)
138. Wee DH, Hughes KT. 2015 Molecular ruler determines needle length for the *Salmonella* Spi-1 injectisome. *Proc. Natl Acad. Sci. USA* **112**, 4098–4103. (doi:10.1073/pnas.1423492112)
139. Rappl C, Deiwick J, Hensel M. 2003 Acidic pH is required for the functional assembly of the type III secretion system encoded by *Salmonella* pathogenicity island 2. *FEMS Microbiol. Lett*. **226**, 363–372. (doi:10.1016/S0378-1097(03)00638-4)
140. Picking WL, Nishioka H, Hearn P, Baxter M, Harrington A, Blocker AJ. 2005 IpaD of *Shigella flexneri* is independently required for regulation of Ipa protein secretion and efficient insertion of IpaB and IpaC into host membranes. *Infect. Immun*. **73**, 1432–1440. (doi:10.1128/IAI.73.3.1432-1440.2005)
141. Kenjale R, Wilson J, Zenk S, Saurya S, Picking WL, Blocker AJ. 2005 The needle component of the type III secreton of *Shigella* regulates the activity of the secretion apparatus. *J. Biol. Chem*. **280**, 42 929–42 937. (doi:10.1074/jbc.M508377200)
142. Veenendaal AKJ, Hodgkinson J, Schwarzer L, Stabat D, Zenk S, Blocker AJ. 2007 The type III secretion system needle tip complex mediates host cell sensing and translocon insertion. *Mol. Microbiol*. **63**, 1719–1730. (doi:10.1111/j.1365-2958.2007.05620.x)
143. Lee P-C, Stopford CM, Svenson AG, Rietsch A. 2010 Control of effector export by the *Pseudomonas aeruginosa* type III secretion proteins PcrG and PcrV. *Mol. Microbiol*. **75**, 924–941. (doi:10.1111/j.1365-2958.2009.07027.x)
144. Martinez-Argudo I, Blocker AJ. 2010 The *Shigella* T3SS needle transmits a signal for MxiC release, which controls secretion of effectors. *Mol. Microbiol*. **78**, 1365–1378. (doi:10.1111/j.1365-2958.2010.07413.x)
145. Shen D-K, Saurya S, Wagner C, Nishioka H, Blocker AJ. 2010 Domains of the *Shigella flexneri* type III secretion system IpaB protein involved in secretion regulation. *Infect. Immun*. **78**, 4999–5010. (doi:10.1128/IAI.00470-10)
146. Cisz M, Lee P-C, Rietsch A. 2008 ExoS controls the cell contact-mediated switch to effector secretion in *Pseudomonas aeruginosa*. *J. Bacteriol*. **190**, 2726–2738. (doi:10.1128/JB.01553-07)
147. Forsberg Å, Viitanen A, Skurnik M, Wolf-Watz H. 1991 The surface-located YopN protein is involved in calcium signal transduction in *Yersinia pseudotuberculosis*. *Mol. Microbiol*. **5**, 977–986. (doi:10.1111/j.1365-2958.1991.tb00773.x)
148. Day J, Plano GV. 1998 A complex composed of SycN and YscB functions as a specific chaperone for YopN in *Yersinia pestis*. *Mol. Microbiol*. **30**, 777–788. (doi:10.1046/j.1365-2958.1998.01110.x)
149. Cheng LW, Kay O, Schneewind O. 2001 Regulated secretion of YopN by the type III machinery of *Yersinia enterocolitica*. *J. Bacteriol*. **183**, 5293–5301. (doi:10.1128/JB.183.18.5293-5301.2001)
150. Deng W *et al.* 2005 Regulation of type III secretion hierarchy of translocators and effectors in attaching and effacing bacterial pathogens. *Infect. Immun*. **73**, 2135–2146. (doi:10.1128/IAI.73.4.2135-2146.2005)
151. Deane JE, Roversi P, King C, Johnson S, Lea SM. 2008 Structures of the *Shigella flexneri* type 3 secretion system protein MxiC reveal conformational variability amongst homologues. *J. Mol. Biol*. **377**, 985–992. (doi:10.1016/j.jmb.2008.01.072)
152. Sundin C, Thelaus J, Bröms JE, Forsberg Å. 2004 Polarisation of type III translocation by *Pseudomonas aeruginosa* requires PcrG, PcrV and PopN. *Microb. Pathog*. **37**, 313–322. (doi:10.1016/j.micpath.2004.10.005)
153. Torruellas J, Jackson M, Pennock J, Plano GV. 2005 The *Yersinia pestis* type III secretion needle plays a role in the regulation of Yop secretion. *Mol. Microbiol*. **57**, 1719–1733. (doi:10.1111/j.1365-2958.2005.04790.x)
154. Hamad MA, Nilles ML. 2007 Structure-function analysis of the C-terminal domain of LcrV from *Yersinia pestis*. *J. Bacteriol*. **189**, 6734–6739. (doi:10.1128/JB.00539-07)
155. Cherradi Y, Schiavolin L, Moussa S, Meghraoui A, Meksem A, Biskri L, Azarkan M, Allaoui A, Botteaux A. 2013 Interplay between predicted inner-rod and gatekeeper in controlling substrate specificity of the type III secretion system. *Mol. Microbiol*. **87**, 1183–1199. (doi:10.1111/mmi.12158)
156. Roehrich AD, Guillossou E, Blocker AJ, Martinez-Argudo I. 2013 *Shigella* IpaD has a dual role: signal transduction from the type III secretion system needle tip and intracellular secretion regulation. *Mol. Microbiol*. **87**, 690–706. (doi:10.1111/mmi.12124)
157. Ikeda T, Yamaguchi S, Hotani H. 1993 Flagellar growth in a filament-less *Salmonella* fliD mutant supplemented with purified hook-associated protein 2. *J. Biochem*. **114**, 39–44.
158. Keener JP. 2006 How *Salmonella typhimurium* measures the length of flagellar filaments. *Bull. Math. Biol*. **68**, 1761–1778. (doi:10.1007/s11538-005-9033-0)
159. Wösten MMSM, van Dijk L, Veenendaal AKJ, De Zoete MR, Bleumink-Pluijm NMC, van Putten JPM. 2010 Temperature-dependent FlgM/FliA complex formation regulates *Campylobacter jejuni* flagella length. *Mol. Microbiol*. **75**, 1577–1591. (doi:10.1111/j.1365-2958.2010.07079.x)
160. Tanner DE, Ma W, Chen Z, Schulten K. 2011 Theoretical and computational investigation of flagellin translocation and bacterial flagellum growth. *Biophys. J*. **100**, 2548–2556. (doi:10.1016/j.bpj.2011.04.036)
161. Turner L, Stern AS, Berg HC. 2012 Growth of flagellar filaments of *Escherichia coli* is independent of filament length. *J. Bacteriol*. **194**, 2437–2442. (doi:10.1128/JB.06735-11)
162. Arnold R *et al.* 2009 Sequence-based prediction of type III secreted proteins. *PLoS Pathog*. **5**, e1000376. (doi:10.1371/journal.ppat.1000376)
163. Samudrala R, Heffron F, McDermott JE. 2009 Accurate prediction of secreted substrates and identification of a conserved putative secretion signal for type III secretion systems. *PLoS Pathog*. **5**, e1000375. (doi:10.1371/journal.ppat.1000375)
164. McDermott JE *et al.* 2011 Computational prediction of type III and IV secreted effectors in Gram-negative bacteria. *Infect. Immun*. **79**, 23–32. (doi:10.1128/IAI.00537-10)

165. Yang X, Guo Y, Luo J, Pu X, Li M. 2013 Effective identification of Gram-negative bacterial type III secreted effectors using position-specific residue conservation profiles. *PLoS ONE* **8**, e84439. (doi:10.1371/journal.pone.0084439)
166. Wang Y, Sun M, Bao H, White AP. 2013 T3_MM: a Markov model effectively classifies bacterial type III secretion signals. *PLoS ONE* **8**, e58173. (doi:10.1371/journal.pone.0058173)
167. Young BM, Young GM. 2002 YplA is exported by the Ysc, Ysa, and flagellar type III secretion systems of *Yersinia enterocolitica*. *J. Bacteriol.* **184**, 1324–1334. (doi:10.1128/JB.184.5.1324-1334.2002)
168. Lee SH, Galán JE. 2004 *Salmonella* type III secretion-associated chaperones confer secretion-pathway specificity. *Mol. Microbiol.* **51**, 483–495. (doi:10.1046/j.1365-2958.2003.03840.x)
169. Warren SM, Young GM. 2005 An amino-terminal secretion signal is required for YplA export by the Ysa, Ysc, and flagellar type III secretion systems of *Yersinia enterocolitica* biovar 1B. *J. Bacteriol.* **187**, 6075–6083. (doi:10.1128/JB.187.17.6075-6083.2005)
170. Ehrbar K, Winnen B, Hardt W-D. 2006 The chaperone binding domain of SopE inhibits transport via flagellar and SPI-1 TTSS in the absence of InvB. *Mol. Microbiol.* **59**, 248–264. (doi:10.1111/j.1365-2958.2005.04931.x)
171. Lilic M, Vujanac M, Stebbins CE. 2006 A common structural motif in the binding of virulence factors to bacterial secretion chaperones. *Mol. Cell* **21**, 653–664. (doi:10.1016/j.molcel.2006.01.026)
172. Sun Y-H, Rolán HG, Tsolis RM. 2007 Injection of flagellin into the host cell cytosol by *Salmonella enterica* serotype Typhimurium. *J. Biol. Chem.* **282**, 33 897–33 901. (doi:10.1074/jbc.C700181200)
173. Ince D, Sutterwala FS, Yahr TL. 2015 Secretion of flagellar proteins by the *Pseudomonas aeruginosa* type III secretion-injectisome system. *J. Bacteriol.* **197**, 2003–2011. (doi:10.1128/JB.00030-15)
174. Michiels T, Cornelis GR. 1991 Secretion of hybrid proteins by the *Yersinia* Yop export system. *J. Bacteriol.* **173**, 1677–1685.
175. Sory M-PP, Boland A, Lambermont I, Cornelis GR. 1995 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* **92**, 11 998–12 002. (doi:10.1073/pnas.92.26.11998)
176. Schesser K, Frithz-Lindsten E, Wolf-Watz H. 1996 Delineation and mutational analysis of the *Yersinia* pseudotuberculosis YopE domains which mediate translocation across bacterial and eukaryotic cellular membranes. *J. Bacteriol.* **178**, 7227–7233.
177. Lloyd SA, Norman M, Rosqvist R, Wolf-Watz H. 2001 *Yersinia* YopE is targeted for type III secretion by N-terminal, not mRNA, signals. *Mol. Microbiol.* **39**, 520–531. (doi:10.1046/j.1365-2958.2001.02271.x)
178. Rüssmann H, Kubori T, Sauer J, Galán JE. 2002 Molecular and functional analysis of the type III secretion signal of the *Salmonella enterica* InvJ protein. *Mol. Microbiol.* **46**, 769–779. (doi:10.1046/j.1365-2958.2002.03196.x)
179. Karavolos MH, Roe AJ, Wilson M, Henderson J, Lee JJ, Gally DL, Khan CMA. 2005 Type III secretion of the *Salmonella* effector protein SopE is mediated via an N-terminal amino acid signal and not an mRNA sequence. *J. Bacteriol.* **187**, 1559–1567. (doi:10.1128/JB.187.5.1559-1567.2005)
180. Blaylock B, Sorg JA, Schneewind O. 2008 *Yersinia enterocolitica* type III secretion of YopR requires a structure in its mRNA. *Mol. Microbiol.* **70**, 1210–1222. (doi:10.1111/j.1365-2958.2008.06474.x)
181. Niemann GS *et al.* 2013 RNA type III secretion signals that require Hfq. *J. Bacteriol.* **195**, 2119–2125. (doi:10.1128/JB.00024-13)
182. Sorg JA, Miller NC, Schneewind O. 2005 Substrate recognition of type III secretion machines—testing the RNA signal hypothesis. *Cell. Microbiol.* **7**, 1217–1225. (doi:10.1111/j.1462-5822.2005.00563.x)
183. Agrain C, Sorg I, Paroz C, Cornelis GR. 2005 Secretion of YscP from *Yersinia enterocolitica* is essential to control the length of the injectisome needle but not to change the type III secretion substrate specificity. *Mol. Microbiol.* **57**, 1415–1427. (doi:10.1111/j.1365-2958.2005.04758.x)
184. Allen-Vercoe E, Toh MCW, Waddell B, Ho H, DeVinney R. 2005 A carboxy-terminal domain of Tir from enterohemorrhagic *Escherichia coli* O157:H7 (EHEC O157:H7) required for efficient type III secretion. *FEMS Microbiol. Lett.* **243**, 355–364. (doi:10.1016/j.femsle.2004.12.027)
185. Kim BH, Kim HG, Kim JS, Jang JI, Park YK. 2007 Analysis of functional domains present in the N-terminus of the SipB protein. *Microbiology* **153**, 2998–3008. (doi:10.1099/mic.0.2007/007872-0)
186. Tomalka AG, Stopford CM, Lee P-C, Rietsch A. 2012 A translocator-specific export signal establishes the translocator-effector secretion hierarchy that is important for type III secretion system function. *Mol. Microbiol.* **86**, 1464–1481. (doi:10.1111/mmi.12069)
187. Kornacker MG, Newton A. 1994 Information essential for cell-cycle-dependent secretion of the 591-residue *Caulobacter* hook protein is confined to a 21-amino-acid sequence near the N-terminus. *Mol. Microbiol.* **14**, 73–85. (doi:10.1111/j.1365-2958.1994.tb01268.x)
188. Végh BM, Gál P, Dobó J, Závodszky P, Vonderviszt F. 2006 Localization of the flagellum-specific secretion signal in *Salmonella* flagellin. *Biochem. Biophys. Res. Commun.* **345**, 93–98. (doi:10.1016/j.bbrc.2006.04.055)
189. Singer HM, Erhardt M, Hughes KT. 2014 Comparative analysis of the secretion capability of early and late flagellar type III secretion substrates. *Mol. Microbiol.* **93**, 505–520. (doi:10.1111/mmi.12675)
190. Dobó J, Varga J, Sajó R, Végh BM, Gál P, Závodszky P, Vonderviszt F. 2010 Application of a short, disordered N-terminal flagellin segment, a fully functional flagellar type III export signal, to expression of secreted proteins. *Appl. Environ. Microbiol.* **76**, 891–899. (doi:10.1128/AEM.00858-09)
191. Singer HM, Erhardt M, Steiner AM, Zhang MM, Yoshikami D, Bulaj G, Olivera BM, Hughes KT. 2012 Selective purification of recombinant neuroactive peptides using the flagellar type III secretion system. *MBio* **3**, pe00115-12. (doi:10.1128/mBio.00115-12)
192. Ménard R, Sansonetti P, Parsot C. 1994 The secretion of the *Shigella flexneri* Ipa invasins is activated by epithelial cells and controlled by IpaB and IpaD. *EMBO J.* **13**, 5293–5302.
193. Neyt C, Cornelis GR. 1999 Role of SycD, the chaperone of the *Yersinia* Yop translocators YopB and YopD. *Mol. Microbiol.* **31**, 143–156. (doi:10.1046/j.1365-2958.1999.01154.x)
194. Day J, Plano GV. 2000 The *Yersinia pestis* YscY protein directly binds YscX, a secreted component of the type III secretion machinery. *J. Bacteriol.* **182**, 1834–1843. (doi:10.1128/JB.182.7.1834-1843.2000)
195. Francis MS, Lloyd SA, Wolf-Watz H. 2001 The type III secretion chaperone LcrH co-operates with YopD to establish a negative, regulatory loop for control of Yop synthesis in *Yersinia pseudotuberculosis*. *Mol. Microbiol.* **42**, 1075–1093. (doi:10.1046/j.1365-2958.2001.02702.x)
196. Mavris M, Page A-L, Tournebize R, Demers B, Sansonetti P, Parsot C. 2002 Regulation of transcription by the activity of the *Shigella flexneri* type III secretion apparatus. *Mol. Microbiol.* **43**, 1543–1553. (doi:10.1046/j.1365-2958.2002.02836.x)
197. Cambronne ED, Sorg JA, Schneewind O. 2004 Binding of SycH chaperone to YscM1 and YscM2 activates effector yop expression in *Yersinia enterocolitica*. *J. Bacteriol.* **186**, 829–841. (doi:10.1128/JB.186.3.829-841.2004)
198. Pilonieta MC, Munson GP. 2008 The chaperone IpgC copurifies with the virulence regulator MxiE. *J. Bacteriol.* **190**, 2249–2251. (doi:10.1128/JB.01824-07)
199. Li Y, Li L, Huang L, Francis MS, Hu Y, Chen S. 2013 *Yersinia* Ysc-Yop type III secretion feedback inhibition is relieved through YscV-dependent recognition and secretion of LcrQ. *Mol. Microbiol.* **91**, 494–507. (doi:10.1111/mmi.12474)
200. Letzelter M, Sorg I, Mota LLJ, Meyer S, Stalder J, Feldman M, Kuhn M, Callebaut I, Cornelis GR. 2006 The discovery of SycO highlights a new function for type III secretion effector chaperones. *EMBO J.* **25**, 3223–3233. (doi:10.1038/sj.emboj.7601202)
201. Lara-Tejero M, Kato J, Wagner S, Liu X, Galán JE. 2011 A sorting platform determines the order of protein secretion in bacterial type III systems. *Science* **331**, 1188–1191. (doi:10.1126/science.1201476)
202. Kinoshita M, Hara N, Imada K, Namba K, Minamino T. 2013 Interactions of bacterial flagellar chaperone–substrate complexes with FlhA contribute to co-ordinating assembly of the flagellar filament. *Mol. Microbiol.* **90**, 1249–1261. (doi:10.1111/mmi.12430)
203. Minamino T, Yoshimura SDJ, Morimoto YV, González-Pedrajo B, Kami-Ike N, Namba K. 2009 Roles of the extreme N-terminal region of FliH for efficient localization of the FliH–FliI complex to the bacterial flagellar type III export apparatus. *Mol.*

Microbiol. **74**, 1471–1483. (doi:10.1111/j.1365-2958.2009.06946.x)

204. Erhardt M, Mertens ME, Fabiani FD, Hughes KT. 2014 ATPase-independent type-III protein secretion in *Salmonella enterica*. *PLoS Genet*. **10**, e1004800. (doi:10.1371/journal.pgen.1004800)
205. Sajó R, Liliom K, Muskotál A, Klein Á, Závodszky P, Vonderviszt F, Dobó J. 2014 Soluble components of the flagellar export apparatus, FliI, FliJ, and FliH, do not deliver flagellin, the major filament protein, from the cytosol to the export gate. *Biochim. Biophys. Acta* **1843**, 2414–2423. (doi:10.1016/j.bbamcr.2014.07.004)
206. Erhardt M, Hughes KT. 2009 C-ring requirement in flagellar type III secretion is bypassed by FlhDC upregulation. *Mol. Microbiol*. **75**, 376–393. (doi:10.1111/j.1365-2958.2009.06973.x)
207. Konishi M, Kanbe M, McMurry JL, Aizawa S-I. 2009 Flagellar formation in C-ring-defective mutants by overproduction of FliI, the ATPase specific for flagellar type III secretion. *J. Bacteriol*. **191**, 6186–6191. (doi:10.1128/JB.00601-09)
208. Radics J, Königsmaier L, Marlovits TC. 2014 Structure of a pathogenic type 3 secretion system in action. *Nat. Struct. Mol. Biol*. **21**, 82–87. (doi:10.1038/nsmb.2722)
209. Akopyan K, Edgren T, Wang-Edgren H, Rosqvist R, Fahlgren A, Wolf-Watz H, Fallman M. 2011 Translocation of surface-localized effectors in type III secretion. *Proc. Natl Acad. Sci. USA* **108**, 1639–1644. (doi:10.1073/pnas.1013888108)
210. Edgren T, Forsberg A, Rosqvist R, Wolf-Watz H. 2012 Type III secretion in *Yersinia*: injectisome or not? *PLoS Pathog*. **8**, e1002669. (doi:10.1371/journal.ppat.1002669)
211. Loquet A *et al*. 2012 Atomic model of the type III secretion system needle. *Nature* **486**, 276–279. (doi:10.1038/nature11079)
212. Yonekura K, Maki-Yonekura S, Namba K. 2003 Complete atomic model of the bacterial flagellar filament by electron cryomicroscopy. *Nature* **424**, 643–650. (doi:10.1038/nature01830)
213. Wilharm G, Lehmann V, Krauss K, Lehnert B, Richter S, Ruckdeschel K, Heesemann J, Trülzsch K. 2004 *Yersinia enterocolitica* type III secretion depends on the proton motive force but not on the flagellar motor components MotA and MotB. *Infect. Immun*. **72**, 4004–4009. (doi:10.1128/IAI.72.7.4004-4009.2004)
214. Lee VT, Schneewind O. 2002 Yop fusions to tightly folded protein domains and their effects on *Yersinia enterocolitica* type III secretion. *J. Bacteriol*. **184**, 3740–3745. (doi:10.1128/JB.184.13.3740-3745.2002)
215. Akeda Y, Galán JE. 2005 Chaperone release and unfolding of substrates in type III secretion. *Nature* **437**, 911–915. (doi:10.1038/nature03992)
216. Riordan KE, Sorg JA, Berube BJ, Schneewind O. 2008 Impassable YscP substrates and their impact on the *Yersinia enterocolitica* type III secretion pathway. *J. Bacteriol*. **190**, 6204–6216. (doi:10.1128/JB.00467-08)
217. Roblin P, Dewitte F, Villeret V, Biondi EG, Bompard C. 2015 A *Salmonella* type three secretion effector/chaperone complex adopts a hexameric ring-like structure. *J. Bacteriol*. **197**, 688–698. (doi:10.1128/JB.02294-14)
218. Chen L, Ai X, Portaliou AG, Minetti CASA, Remeta DP, Economou A, Kalodimos CG. 2013 Substrate-activated conformational switch on chaperones encodes a targeting signal in type III secretion. *Cell Rep*. **3**, 709–715. (doi:10.1016/j.celrep.2013.02.025)
219. Diepold A, Kudryashev M, Delalez NJ, Berry RM, Armitage JP. 2015 Composition, formation, and regulation of the cytosolic C-ring, a dynamic component of the type III secretion injectisome. *PLOS Biol*. **13**, e1002039. (doi:10.1371/journal.pbio.1002039)
220. Woestyn S, Allaoui A, Wattiau P, Cornelis GR. 1994 YscN, the putative energizer of the *Yersinia* Yop secretion machinery. *J. Bacteriol*. **176**, 1561–1569.
221. Eichelberg K, Ginocchio CC, Galán JE. 1994 Molecular and functional characterization of the *Salmonella typhimurium* invasion genes invB and invC: homology of InvC to the F0F1 ATPase family of proteins. *J. Bacteriol*. **176**, 4501–4510.
222. Fan F, Macnab RM. 1996 Enzymatic characterization of FliI. An ATPase involved in flagellar assembly in *Salmonella typhimurium*. *J. Biol. Chem*. **271**, 31 981–31 988. (doi:10.1074/jbc.271.50.31981)
223. Minamino T, Namba K. 2008 Distinct roles of the FliI ATPase and proton motive force in bacterial flagellar protein export. *Nature* **451**, 485–488. (doi:10.1038/nature06449)
224. Paul K, Erhardt M, Hirano T, Blair DF, Hughes KT. 2008 Energy source of flagellar type III secretion. *Nature* **451**, 489–492. (doi:10.1038/nature06497)
225. Lee P-C, Rietsch A. 2015 Fueling type III secretion. *Trends Microbiol*. **23**, 296–300. (doi:10.1016/j.tim.2015.01.012)
226. Stern AS, Berg HC. 2013 Single-file diffusion of flagellin in flagellar filaments. *Biophys. J*. **105**, 182–184. (doi:10.1016/j.bpj.2013.05.030)
227. Evans LDB, Poulter S, Terentjev EM, Hughes C, Fraser GM. 2013 A chain mechanism for flagellum growth. *Nature* **504**, 287–290. (doi:10.1038/nature12682)
228. Iino T. 1974 Assembly of *Salmonella* flagellin *in vitro* and *in vivo*. *J. Supramol. Struct*. **2**, 372–384. (doi:10.1002/jss.400020226)
229. Koroyasu S, Yamazato M, Hirano T, Aizawa S-I. 1998 Kinetic analysis of the growth rate of the flagellar hook in *Salmonella typhimurium* by the population balance method. *Biophys. J*. **74**, 436–443. (doi:10.1016/S0006-3495(98)77801-4)
230. Lee P-C, Zmina SE, Stopford CM, Toska J, Rietsch A. 2014 Control of type III secretion activity and substrate specificity by the cytoplasmic regulator PcrG. *Proc. Natl Acad. Sci. USA* **111**, E2027–E2036. (doi:10.1073/pnas.1402658111)
231. Romo-Castillo M, Andrade A, Espinosa N, Monjarás Feria J, Soto E, Díaz-Guerrero M, González-Pedrajo B. 2014 EscO, a functional and structural analog of the flagellar FliJ protein, is a positive regulator of EscN ATPase activity of the enteropathogenic *Escherichia coli* injectisome. *J. Bacteriol*. **196**, 2227–2241. (doi:10.1128/JB.01551-14)
232. Cherradi Y, Hachani A, Allaoui A. 2014 Spa13 of *Shigella flexneri* has a dual role: chaperone escort and export gate-activator switch of the type III secretion system. *Microbiology* **160**, 130–141. (doi:10.1099/mic.0.071712-0)
233. Bange G, Kümmerer N, Engel C, Bozkurt G, Wild K, Sinning I. 2010 FlhA provides the adaptor for coordinated delivery of late flagella building blocks to the type III secretion system. *Proc. Natl Acad. Sci. USA* **107**, 11 295–11 300. (doi:10.1073/pnas.1001383107)
234. Schlumberger MC, Müller A, Ehrbar K, Winnen B, Duss I, Stecher B, Hardt W-D, Mu AJ. 2005 Real-time imaging of type III secretion: *Salmonella* SipA injection into host cells. *Proc. Natl Acad. Sci. USA* **102**, 12 548–12 553. (doi:10.1073/pnas.0503407102)
235. Enninga J, Mounier J, Sansonetti P, Tran Van Nhieu G, Van Nhieu GT. 2005 Secretion of type III effectors into host cells in real time. *Nat. Methods* **2**, 959–965. (doi:10.1038/nmeth804)
236. Mills E, Baruch K, Charpentier X, Kobi S, Rosenshine I. 2008 Real-time analysis of effector translocation by the type III secretion system of enteropathogenic *Escherichia coli*. *Cell Host Microbe* **3**, 104–113. (doi:10.1016/j.chom.2007.11.007)
237. Rietsch A, Mekalanos JJ. 2006 Metabolic regulation of type III secretion gene expression in *Pseudomonas aeruginosa*. *Mol. Microbiol*. **59**, 807–820. (doi:10.1111/j.1365-2958.2005.04990.x)
238. Kupferberg LL, Higuchi K. 1958 Role of calcium ions in the stimulation of growth of virulent strains of *Pasteurella pestis*. *J. Bacteriol*. **76**, 120–121.
239. Brubaker RR, Surgella MJ. 1964 The effect of Ca^{++} and Mg^{++} on lysis, growth, and production of virulence antigens by *Pasteurella pestis*. *J. Infect. Dis*. **114**, 13–25. (doi:10.1093/infdis/114.1.13)
240. Gemski P, Lazere JR, Casey T. 1980 Plasmid associated with pathogenicity and calcium dependency of *Yersinia enterocolitica*. *Infect. Immun*. **27**, 682–685.
241. Brubaker RR. 2005 Influence of Na^{+}, dicarboxylic amino acids, and pH in modulating the low-calcium response of *Yersinia pestis*. *Infect. Immun*. **73**, 4743–4752. (doi:10.1128/IAI.73.8.4743-4752.2005)
242. Schmid A *et al*. 2009 Cross-talk between type three secretion system and metabolism in *Yersinia*. *J. Biol. Chem*. **284**, 12 165–12 177. (doi:10.1074/jbc.M900773200)
243. Salman H, Libchaber A. 2007 A concentration-dependent switch in the bacterial response to temperature. *Nat. Cell Biol*. **9**, 1098–1100. (doi:10.1038/ncb1632)
244. Zorraquino V, García B, Latasa C, Echeverz M, Toledo-Arana A, Valle J, Lasa I, Solano C. 2013 Coordinated cyclic-Di-GMP repression of *Salmonella* motility through ycgR and cellulose. *J. Bacteriol*. **195**, 417–428. (doi:10.1128/JB.01789-12)
245. Christen M, Kulasekara HD, Christen B, Kulasekara BR, Hoffman LR, Miller SI. 2010 Asymmetrical distribution of the second messenger c-di-GMP upon bacterial cell division. *Science* **328**, 1295–1297. (doi:10.1126/science.1188658)

246. Kulasekara BR, Kamischke C, Kulasekara HD, Christen M, Wiggins PA, Miller SI. 2013 c-di-GMP heterogeneity is generated by the chemotaxis machinery to regulate flagellar motility. *Elife* **2**, e01402. (doi:10.7554/eLife.01402)
247. Aili M, Isaksson EL, Carlsson SE, Wolf-Watz H, Rosqvist R, Francis MS. 2008 Regulation of *Yersinia* Yop-effector delivery by translocated YopE. *Int. J. Med. Microbiol.* **298**, 183–192. (doi:10.1016/j.ijmm.2007.04.007)
248. Brown MT, Delalez NJ, Armitage JP. 2011 Protein dynamics and mechanisms controlling the rotational behaviour of the bacterial flagellar motor. *Curr. Opin. Microbiol.* **14**, 734–740. (doi:10.1016/j.mib.2011.09.009)
249. Leake MC, Chandler J, Wadhams GH, Bai F, Berry RM, Armitage JP. 2006 Stoichiometry and turnover in single, functioning membrane protein complexes. *Nature* **443**, 355–358. (doi:10.1038/nature05135)
250. Fukuoka H, Wada T, Kojima S, Ishijima A, Homma M. 2009 Sodium-dependent dynamic assembly of membrane complexes in sodium-driven flagellar motors. *Mol. Microbiol.* **71**, 825–835. (doi:10.1111/j.1365-2958.2008.06569.x)
251. Reid S, Leake MC, Chandler J, Lo C-J, Armitage JP, Berry RM. 2006 The maximum number of torque-generating units in the flagellar motor of *Escherichia coli* is at least 11. *Proc. Natl Acad. Sci. USA* **103**, 8066–8071. (doi:10.1073/pnas.0509932103)
252. Yuan J, Berg HC. 2008 Resurrection of the flagellar rotary motor near zero load. *Proc. Natl Acad. Sci. USA* **105**, 1182–1185. (doi:10.1073/pnas.0711539105)
253. Lele PP, Hosu BG, Berg HC. 2013 Dynamics of mechanosensing in the bacterial flagellar motor. *Proc. Natl Acad. Sci. USA* **110**, 11 839–11 844. (doi:10.1073/pnas.1305885110)
254. Tipping MJ, Steel BC, Delalez NJ, Berry RM, Armitage JP. 2013 Quantification of flagellar motor stator dynamics through *in vivo* proton-motive force control. *Mol. Microbiol.* **87**, 338–347. (doi:10.1111/mmi.12098)
255. Delalez NJ, Wadhams GH, Rosser G, Xue Q, Brown M, Dobbie I, Berry RM, Leake MC, Armitage JP. 2010 Signal-dependent turnover of the bacterial flagellar switch protein FliM. *Proc. Natl Acad. Sci. USA* **107**, 11 347–11 351. (doi:10.1073/pnas.1000284107)
256. Fukuoka H, Inoue Y, Terasawa S, Takahashi H, Ishijima A. 2010 Exchange of rotor components in functioning bacterial flagellar motor. *Biochem. Biophys. Res. Commun.* **394**, 130–135. (doi:10.1016/j.bbrc.2010.02.129)
257. Yuan J, Branch RW, Hosu BG, Berg HC. 2012 Adaptation at the output of the chemotaxis signalling pathway. *Nature* **484**, 233–236. (doi:10.1038/nature10964)
258. Lele PP, Branch RW, Nathan VSJ, Berg HC. 2012 Mechanism for adaptive remodeling of the bacterial flagellar switch. *Proc. Natl Acad. Sci. USA* **109**, 20 018–20 022. (doi:10.1073/pnas.1212327109)
259. Delalez NJ, Berry RM, Armitage JP. 2014 Stoichiometry and turnover of the bacterial flagellar switch protein FliN. *MBio* **5**, e01216-14. (doi:10.1128/mBio.01216-14)
260. Bai F, Morimoto YV, Yoshimura SDJ, Hara N, Kami-Ike N, Namba K, Minamino T. 2014 Assembly dynamics and the roles of FliI ATPase of the bacterial flagellar export apparatus. *Sci. Rep.* **4**, 6528. (doi:10.1038/srep06528)
261. Widmaier DM, Tullman-Ercek D, Mirsky EA, Hill R, Govindarajan S, Minshull J, Voigt CA. 2009 Engineering the *Salmonella* type III secretion system to export spider silk monomers. *Mol. Syst. Biol.* **5**, 309. (doi:10.1038/msb.2009.62)
262. Azam A, Li C, Metcalf KJ, Tullman-Ercek D. In press. Type III secretion as a generalizable strategy for the production of full-length biopolymer-forming proteins. *Biotechnol. Bioeng.* (doi:10.1002/bit.25656)
263. Guo S, Alshamy I, Hughes KT, Chevance FFV. 2014 Analysis of factors that affect FlgM-dependent type III secretion for protein purification with *Salmonella enterica* serovar typhimurium. *J. Bacteriol.* **196**, 2333–2347. (doi:10.1128/JB.01572-14)
264. Rüssmann H, Shams H, Poblete F, Fu Y, Galán JE, Donis RO. 1998 Delivery of epitopes by the *Salmonella* type III secretion system for vaccine development. *Science* **281**, 565–568. (doi:10.1126/science.281.5376.565)
265. Rüssmann H, Igwe EI, Sauer J, Hardt WD, Bubert A, Geginat G. 2001 Protection against murine listeriosis by oral vaccination with recombinant *Salmonella* expressing hybrid *Yersinia* type III proteins. *J. Immunol.* **167**, 357–365. (doi:10.4049/jimmunol.167.1.357)
266. Chamekh M. 2010 Immunomodulation using genetically engineered bacteria for type III-mediated delivery of heterologous antigens and cytokines: potential application in vaccine and therapeutical developments. *Immunopharmacol. Immunotoxicol.* **32**, 1–4. (doi:10.3109/08923970902817890)
267. Wieser A, Magistro G, Nörenberg D, Hoffmann C, Schubert S. 2012 First multi-epitope subunit vaccine against extraintestinal pathogenic *Escherichia coli* delivered by a bacterial type-3 secretion system (T3SS). *Int. J. Med. Microbiol.* **302**, 10–18. (doi:10.1016/j.ijmm.2011.09.012)
268. Chamekh M, Phalipon A, Quertainmont R, Salmon I, Sansonetti P, Allaoui A. 2008 Delivery of biologically active anti-inflammatory cytokines IL-10 and IL-1ra *in vivo* by the *Shigella* type III secretion apparatus. *J. Immunol.* **180**, 4292–4298. (doi:10.4049/jimmunol.180.6.4292)
269. Carleton HA, Lara-Tejero M, Liu X, Galán JE. 2013 Engineering the type III secretion system in non-replicating bacterial minicells for antigen delivery. *Nat. Commun.* **4**, 1590. (doi:10.1038/ncomms2594)
270. iGEM Hokkaido University. 2010 Dr. *E. coli*: The smallest protein injector in the world. See http://2010.igem.org/Team:HokkaidoU_Japan/Projects.
271. iGEM Hokkaido University. 2011 Dr. *E. coli*. See http://2011.igem.org/Team:HokkaidoU_Japan.