Int J Clin Exp Pathol 2014;7(9):5404-5414
www.ijcep.com /ISSN:1936-2625/IJCEP0001642

Review Article

Conserved type III secretion system exerts important roles in Chlamydia trachomatis

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Received July 31, 2014; Accepted August 23, 2014; Epub August 15, 2014; Published September 1, 2014

Abstract: Upon infection, *Chlamydiae* alter host cellular functions in a variety of ways. *Chlamydial* infection prevents host cell apoptosis, induces re-organization of the actin cytoskeleton and alters host cellular signaling mechanisms. *Chlamydia* is among the many pathogenic Gram-negative bacteria that employ the type III secretion system (T3SS) to overcome host defenses and exploit available resources. T3SS are used by many Gram-negative bacterial pathogens to manipulate eukaryotic host cells through the delivery of effector proteins into their cytosol and membranes. T3SS is an evolutionarily refined, virulence determinant of Gram-negative bacteria where more than 20 proteins form an apparatus, generally termed injectisome, to achieve the vectorial secretion and translocation of anti-host effector proteins. This review describes challenges and recent advances that have revealed how *Chlamydia trachomatis* utilizes diversification to produce a conserved T3SS that exerts an important role in *Chlamydia trachomatis*.

Keywords: *Chlamydia trachomatis*, type III secretion system, effectors, chaperones, injectisomes, regulators

Introduction

The obligate intracellular pathogen *Chlamydia trachomatis* (*C. trachomatis*) infects epithelial cells of conjunctiva and genital tract, causing conjunctivitis and the sexually transmitted diseases. Inflammatory damage resulted from chronic *C. trachomatis* infections can lead to severe sequelae, including infectious blindness, pelvic inflammatory disease, and infertility. *C. trachomatis* is a type of obligate intracellular bacterium that develops in parasitophorous vesicles termed inclusions. This requires lifestyle necessitates well arranged when *C. trachomatis* enters into non-phagocytic cells, creates a privileged intracellular niche, and subverts potent host defenses. *C. trachomatis* contains the coding ability for T3SS, and this mechanism has emerged to essentially contribute in its virulence.

Preliminary studies

C. trachomatis possess a bi-phasic developmental cycle. The infections are initiated when infectious particles, elementary bodies (EBs), invade host cells. EBs are the invasive, metabolically inactive form of *C. trachomatis*. After entry, EBs differentiate into metabolically active reticulate bodies (RBs) and replicate within a membrane-bound vacuole (inclusion). RBs accumulate within inclusions from mid-to late-cycle during which a subset of RBs differentiate back into EBs. At the end of the infectious cycle, EBs are released by lysis of the host cells or extrusion of the inclusions and then infect neighboring cells. Due to the limited metabolic activity of EBs [1], type III secretion apparatus (T3SA) and effectors required for the invasion of epithelial cells must be pre-packaged during late-cycle conversion from RBs to EBs. Intuitively, T3SS is activated when EB contacts a host cell, after which actively secreting injectisomes are present on RBs. Secretion activity are turned off after differentiation of RBs back to EBs, followed by the detachment of EBs from the inclusion membrane.

It is clear that EBs contain functional T3SS, and activation of effector secretion occurs rapidly upon contact with a host cell [2]. These injectisomes must mediate the secretion of some early-cycle effectors because de novo synthesis of T3SA genes does not begin until mid-cycle

when *C. trachomatis* first start to divide [3]. Moreover, Dumoux et al. [4] detected CdsF localization at synapse points where *Chlamydiae* contacted inclusion membranes. Interestingly, a quantitative proteomics approach indicated that EBs were pre-loaded with great levels of effectors and chaperones while late-cycle levels of chaperones and key apparatus components were decreased in RBs [5]. The molecular mechanisms responsible for secretion activity remain unknown. It is possible that Chlamydial T3SS is regulated partly by disulfide bonding within T3SA components. EB envelopes are highly cross-linked by disulfide bonds which are decreased when intracellular *Chlamydiae* differentiates into RBs. *C. trachomatis* needle protein, CdsF, contains cysteine residues [6] that are unique among T3SS needle proteins. Disulfide bonding in polymerized needle is related to oxidation state of the Chlamydial envelope and developmental stages [7]. Therefore, the disulfide bonding found in EB-localized CdsF could play a role in manipulating secretion activity.

T3SS effectors

T3SS-expressing pathogens express many kinds of effector proteins that function by direct association, enzymatic modification, or mimicry of target host factors to promote molecular requirements of virulence in the system [8]. The obligate intracellular existence would promote the secretion of a large and intricate set of effectors. Detection of Chlamydial effector proteins has been blocked by the fact that T3SS substrates lack a predictable secretion signal. To date, the ectopic expression and secretion of Chlamydial proteins in surrogate T3SS [9] have been proven to be an effective approach to identify candidate substrates. Here, we mainly discuss two categories of effectors: invasion-related translocated actin recruiting phosphoprotein (Tarp) and inclusion membrane protein (Inc)-class effectors. Tarp is also referred to as CT456, and is the only well-characterized T3SS effector in *Chlamydia* [10]. CT875, a new Slc1 client protein and T3SS effector, is renamed TepP [11]. Additional secreted effectors such as the putative histone methyltransferase NUE [12] or the GCIP-interacting protein CT847 [13] have already been described, but their respective roles in *C. trachomatis* infection remain unknown. The in vitro deubiquitinase activity of ChlaDUB1/CT868 and ChlaDUB2/CT867 [14], and the capability of ChlaDUB1/CT868 in suppressing the nuclear factor (NF)-κB pathway in transfected cells, indicate that these two proteins should be effectors [15]. Finally, there is an additional group of proteins that we regard as possible T3SS substrates, including putative deubiquitinases ChlaDub1/2, the class I accessible protein Cap1, and hypothetical proteins CT082 [16], CopN/CT089 [12], Cap1/CT529 [17], CT695 [16], CT620 [18], CT621 [19], CT622 [20], CT711 [18], a novel T3SS dependent substrate CT694 [21], lipid droplet-associated (Lda) proteins Lda1/CT156, Lda2/CT163, and Lda3/CT473 [22], and Nue/CT737 [12].

Cunha et al. [23] reported 10 *C. trachomatis* proteins (CT053, CT105, CT142, CT143, CT144, CT161, CT338, CT429, CT656, and CT849) that were likely T3SS substrates and possible effectors. CT105 and CT142 have been previously identified as possible modulators of host cell functions. In addition, genes encoding CT142, CT143, and CT144 have been shown to be transcriptionally regulated by a protein (Pgp4) encoded by the *Chlamydia* virulence plasmid. In *C. trachomatis*, besides CT849, a DUF720 domain is found in CT847, a T3SS effector that interacts with human Grap2 cyclin D-interacting protein (GCIP) [13], and in CT848, which has been indicated as a T3SS substrate using *S. flexneri* as a heterologous system [24].

Invasion-related Tarp and CT694

Currently, Tarp and CT694 represent the most thoroughly characterized invasion-related effector proteins, but we think there are additional factors to be discovered. Both Tarp and CT694 are secreted within minutes after contact with a host cell [2, 21]. *C. trachomatis* Tarp recruits actin [21] and is able to nucleate actin polymerization independently [25] or in cooperation with Arp2/3 [26]. *C. trachomatis* Tarp is tyrosine-phosphorylated at entry sites by multiple host kinases [27]. Tyrosine phosphorylation of host and/or bacterial proteins has been implicated in signaling pathways that trigger the entry of many intracellular pathogens. Tarp is tyrosine-phosphorylated and functions in the nucleation of actin required for entry. A functional T3SS may be required for Tarp to integrate into EBs, and the conversion from RB to EB may depend on Tarp secretion. Nevertheless, the findings that Tarp is synthesized at the very

late stage of Chlamydial growth cycle and rapidly packaged into EBs are consistent with its primary function, which is to facilitate Chlamydial EB invasion into new target cells. The conserved C-terminal domain of Tarp may be responsible for initiating actin recruitment regardless of tyrosine phosphorylation [28].

Previous studies showed that Tarp was dominantly recognized by antisera from patients with *C. trachomatis* infection in the urogenital tract or ocular tissues. Interestingly, immunization of mice with Tarp induced Th1-dominant cellular immunity and significantly attenuated inflammatory pathologies in oviduct tissues [29]. Rac1 is essential for *C. trachomatis* invasion [30], and Rac1 activating factors Sos1 and Vav2 have been shown to interact with phospho-Tarp [31]. Tarp facilitates invasion by mediating actin re-arrangements through the direct nucleation of F-actin polymerization and the recruitment of Rac-specific guanine nucleotide exchange factors. Actin remodeling is critical for *Chlamydia* invasion, so timely T3SS-dependent Tarp translocation is key to the survival of the obligate intracellular bacteria.

C. trachomatis CT694 does not interact directly with actin, but is found to interact with human Ahnak to interfere with Ahnak-mediated actin mobilization [21]. CT694 also contains a membrane localization domain analogous to that found in Yersinia YopE, Pseudomonas ExoS, and Salmonella SptP [32]. It is possible that CT694 also influences actin dynamics by manipulating the activity of Rho GTPases. Based on these findings, we speculate that CT694 may invert *Chlamydia*-mediated actin reorganization after the invasion process. Therefore, CT694 may antagonize Tarp function.

Inc-class effectors

Following invasion, the early inclusion remains isolated from endosomal compartments, trafficking to the peri-nuclear region and intercepts material from multivesicular bodies, lipid droplets, endoplasmic reticulum (ER), and Golgi apparatus. Therefore, *C. trachomatis* is similar to other intracellular pathogens that reside in parasitophorous vacuoles and manipulate host membrane trafficking. In the Chlamydial system, many of these processes are orchestrated by a large group of effectors termed Inc proteins. The family of Inc proteins containing a bilobed hydrophobic domain is translocated by T3SS mechanism into the inclusion membrane, and serves to anchor proteins in the inclusion membrane. Inc proteins share a feature that becomes a hallmark of the family: a large hydrophobic domain of 40 to 60 residues with hydrophilic residues in its middle. The bilobed hydrophobic domain of Inc proteins is predicted to enable its insertion into the inclusion membrane. Furthermore, it is assumed that at least one segment of the protein faces the cytosol of the host. Type III secretion signals have been found in the amino terminal domain of several Inc proteins, indicating that this is the secretion mechanism used to transit the bacterial outer membranes. From their localization at the interface between the bacteria and the host, Inc proteins are expected to be involved in various processes.

The Inc proteins, characterized by a bilobed hydrophobic motif, are thought to mediate their insertion into the inclusion membrane. *C. trachomatis* Inc proteins CT119/IncA, CT115/IncD, CT147, CT228, CT229, and CT813 have been suggested to subvert host cell vesicular and nonvesicular transport [33, 34].

IncA is involved in the homotypic fusion of the *C. trachomatis* inclusions [35]. IncB, CT101, CT222 and CT850 co-localize with activated Fyn and Src kinases in inclusion membrane microdomains. These microdomains also interact with centrosomes. It has been proposed that these four inclusion proteins are involved in the interaction of the inclusion with the microtubule network [36]. CT229 and CT813 have been assigned putative functions in intercepting the host vesicular trafficking, based on their respective interaction with the host proteins Rab4 [34] and VAMP7-8. Derre et al. [37] suggested a model in which the *C. trachomatis* effector protein IncD specifically interacted with the non-vesicular ceramide transfer protein (CERT), at membrane contact sites between *C. trachomatis* inclusion membrane and ER tubules harboring the VAPA/B proteins. The IncD-CERT-VAPA/B interaction may be involved in the non-vesicular transfer of ceramide from the ER to the inclusion. The depletion of either CERT or VAP proteins impaired bacterial development. Therefore, the presence of IncD, CERT, VAPA/B, and potential-

ly additional host and/or bacterial factors, at points of contact between the ER and the inclusion membrane provides a specialized metabolic and/or signaling micro-environment favorable to bacterial development. Previous study showed that IncG/CT118 specifically interacted with the mammalian signal transducer protein 14-3-3β at inclusion membrane [38].

C. trachomatis replicates in a parasitophorous membrane-bound compartment called inclusion. The inclusions corrupt host vesicle trafficking networks to avoid the degradative endolysosomal pathway, but promote fusion with each other in order to sustain higher bacterial loads in a process known as homotypic fusion. The *Chlamydia* protein IncA appears to play central roles in both of these processes as it participates in homotypic fusion and inhibits endocytic SNARE-mediated membrane fusion. Ronzone et al. [39] found that inhibition of SNARE-mediated fusion required IncA to be on the same membrane as the endocytic SNARE proteins. IncA displays two coiled-coil domains showing high homology with SNARE proteins. Domain swap and deletion experiments revealed that although both of these domains were capable of independently inhibiting SNARE-mediated fusion, the two coiled-coil domains cooperated in mediating IncA multimerization and homotypic membrane interaction. In total, there are two functional SNARE-like domains (SLDs) in IncA. The N-terminal SNARE-like domain SLD1 and the C-terminal SNARE-like domain SLD2 work independently to inhibit membrane fusion. However, they are both required to promote homotypic fusion. It has been previously shown that the N-terminal domain functions in the targeting of the protein to the inclusion membrane through T3SS. Therefore, it seems that the function of the N-terminus of IncA is restricted to protein translocation to the inclusion surface. Both SLDs are required to efficiently interfere with homotypic fusion in a cellular assay, suggesting that both domains are involved in the formation of a fusogenic coiled-coil bundle. The C-terminal cytoplasmic domain is necessary to promote IncA oligomerization and fusion of multiple inclusions in the same cell. Either domain is capable of inhibiting late endosome/lysosome fusion to protect the inclusion from degradation, but both domains appear to be required to promote homotypic interaction between inclusions.

With the Inc interfere with endocytic membrane fusion events to avoid destruction, Rab-interacting or alternative SNARE-interacting Incs may promote vesicle fusion with nutrient-rich compartments. Therefore, Inc proteins play an important role in creating and maintaining a *Chlamydia*-specific privileged intracellular niche.

TepP

TepP (Ct875) is tyrosine-phosphorylated upon translocation into host cells [11]. TepP is translocated early during bacterial entry into epithelial cells and is phosphorylated at tyrosine residues by host kinases. Phosphorylated TepP associates with host scaffolding proteins Crk-I and Crk-II. The recruitment of Crk proteins to nascent inclusions is absent in cells infected with *Chlamydia* mutant harboring TepP null allele and is restored in complemented strain. TepP is phosphorylated at both tyrosine and serine residues, with the phosphotyrosine residue mapping to the peptide ASDYDLPR, which is repeated in tandem between amino acids 496-504. This phosphorylation site matches the pYxxP consensus binding site for the host adaptor proteins Crk-I and Crk-II. Crk-I and Crk-II are well-characterized scaffolding proteins that organize cytoskeletal rearrangement and signal transduction events [40]. The SH2 domain of Crk interacts with tyrosine-phosphorylated proteins. Crk-binding partners Sos1 and Dock180 activate Rac1, which is partially required for *C. trachomatis* invasion. Therefore, it is possible that TepP, in addition to Tarp, contributes to Rac1 activation at bacteria entry sites through its recruitment of Crk. Many immune-related genes showed TepP-dependent activation. TepP acts as a scaffolding protein that upon tyrosine phosphorylation, recruits additional scaffolding proteins like Crk, which in turn recruit more proteins to nascent inclusions, presumably to help establish an early niche for replication within the host. In addition, the transcriptional response of host cells to infection with TepP mutants suggest that there is a distinct gene expression program that is dependent on TepP-mediated signaling events.

CT621

CT621 is a member of a family of proteins containing a domain of unknown function (DUF582) that is only found within the genus *Chlamydia*

[41]. Within the DUF582 domain, CT621 and CT620 have a leucine zipper with four leucine residues at a fixed spacing of seven amino acids. Leucine zippers form protein dimers, resulting in the formation of helical structures wrapped around each other in a coiled coil, which is often associated with DNA binding.

CT621 is secreted late in the Chlamydial developmental cycle. It is the first Chlamydial protein found to be localized within both cytoplasm and nucleus of the host cells. The inclusions contain CT621 at all time points after secretion is first observed. It is therefore possible that secretion of CT621 continues after the initial secretion. CT621 secretion through T3SS takes place from RB that is located close to the inclusion membrane. CT621 is the first Chlamydial protein shown to be translocated to the nucleus of infected cells, even though the nuclear localization, in theory, could be explained by lateral diffusion. The fact that CT621 is secreted late in the Chlamydial developmental cycle indicates that the protein is functional in the late stage of infection.

T3SS chaperones

Type III secretion chaperones (T3SCs) have three types: bind effectors (class I), pore-forming translocators (class II), and subunits of injectisome or flagellar substructures (class III). Class I chaperones are the best studied. They share low sequence similarity between each other but display a conserved three-dimensional (3D) structure. T3SCs have been classified based on their substrate specificity. Class I T3SCs bind single (class IA) or multiple (class IB) effector substrates, class II T3SCs bind translocon components and class III chaperones bind needle subunit proteins.

Depending on the number of effectors with which they can associate, class I chaperones are further divided into classes IA and IB [42]. Class IA chaperones are specific for single effectors and the genes encoding the chaperone effector pair are often co-transcribed or adjacent to each other on the bacterial genome. Class IB chaperones associate with more than one effector and the genes encoding these chaperones are often unlinked from that of its cognate cargo. According to amino acid sequence analysis, *C. trachomatis* encodes at least six putative T3SCs: Slc1 (CT043), Scc1 (CT088), Scc2 (CT576), Scc3 (CT862), CT274 and Scc4 (CT663). Several studies have validated their function as chaperones and identified the substrates they engaged. For instance, Slc1 from *C. trachomatis* interacts with Tarp and enhances its translocation/secretion in a heterologous *Yersinia* T3SS [43, 44]. Scc1 and Scc4 from *C. pneumoniae* enhance the secretion of CopN whereas Scc3 inhibits CopN secretion. Additional T3SCs have been defined functionally. For example, Multiple cargo secretion chaperone (Mcsc) that does not share any obvious sequence homology with known chaperones, was identified based on its ability to bind and stabilize the Inc proteins Cap1 and CT618 [45]. Slc1 engaged at least four new substrates of T3SS: CT365, CT694, CT695 and CT875/TepP (a protein not previously thought to harbor a type III secretion signal based on early prediction algorithms) [46]. CT584 [47], a potential T3SC, interacts with at least six T3SS substrates, including CT082. CT584 secretion becomes possible since this protein has been indicated to serve as a tip-complex protein that is able to bind host lipids *in vitro*.

T3SCs directly bind T3SS substrates and facilitate their secretion. In general, these small (15-20 kDa), acidic [isoelectric point (pI) = 6], and dimeric, cytosol-localized proteins function by (i) protecting their substrates from degradation, (ii) keeping their substrates in a partially unfolded secretion competent state, (iii) preventing premature and/or unproductive protein interactions with their substrates, and (iv) directly promoting the secretion of their substrates via direct interaction with select components of T3SA. In addition to their traditional role in escorting cargo for secretion, T3SCs can regulate additional cellular functions. In *Chlamydia*, the chaperone Scc4 (CT663) negatively regulates σ66-dependent transcription by directly interacting with both σ66 and β subunits of RNA polymerase. T3SCs enhance the secretion of their bound cargo by stabilizing effectors in the bacterial cytoplasm and maintaining them in a secretion competent state. In addition, T3SCs can also prioritize the secretion of effectors. The most abundant T3SC in EBs is Slc1, which, like CesT, engages multiple effectors. Tarp, one of Slc1's cargos [10], is secreted within 5 min upon EB attachment to epithelial cells. Interestingly, the majority of Tarp in EBs is found pre-complexed with Slc1,

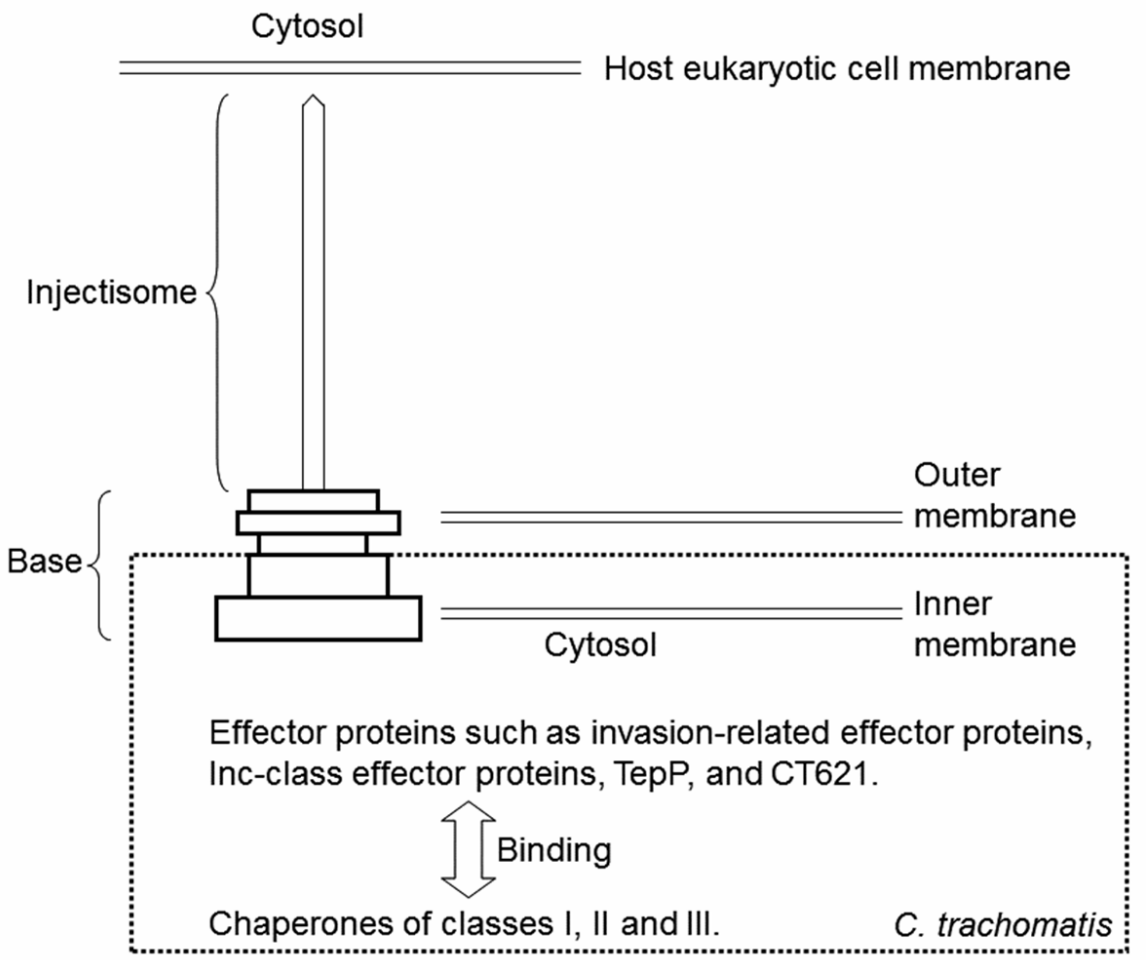

Figure 1. Diagram of type III secretion system of Chlamydia trachomatis.

implying that this pre-engagement with its chaperone could prime Tarp for rapid secretion. Similarly, a significant proportion of CT694 within EBs is pre-complexed with Slc1, suggesting that CT694 may also be secreted very early during infection, possibly at the same time as Tarp. Chen et al. considered a model wherein T3SS substrates pre-bound by Slc1 in EBs, such as Tarp and CT694, were delivered first, followed by the delivery of TepP and potentially other effectors which did not exist as preformed effector-chaperone complexes in EBs.

Additionally, a class II T3SC, Scc3, binds to the C-terminal region of CopN, resulting in *Chlamydia* T3SS-specific regulatory interaction that functions to reduce CopN secretion. *C. trachomatis* T3SS also employs two translocator-specific class II T3SCs termed Scc2 and Scc3 [48]. These tetratricopeptide repeat-containing chaperones are homologous to the SycD T3SC of *Yersinia*, and their coding genes are in close proximity to the CopB and CopB2 coding genes. Both chaperones have been shown to interact with *Yersinia* YopD translocator protein. Scc2 co-precipitates with *C. trachomatis* CopB and interacts with CopB in two-hybrid studies [49]. Although the regulatory interaction of Scc3 with CopN seems to be unique to *Chlamydiae*, class II chaperones in other T3SS also participate in regulatory interactions that coordinate the expression and/or delivery of T3SS substrates. *C. trachomatis* also has a heterodimeric (CdsE and CdsG) class III T3SC that is specific for the CdsF needle subunit protein [50]. In conclusion, *C. trachomatis* T3SCs are multifunctional proteins that may play important roles in T3SA assembly, translocon secretion and assembly, establishment of a secretion hierarchy, and the efficient translocation of effector proteins into the host cells. From a more practical perspective, studies on these chaperones also provid-

ed an additional way for the identification of novel effector proteins.

T3SS injectisomes

In one model, a hierarchy of effector secretion is established by the timing of its synthesis. The presence of early, mid and late effectors competing to engage a common injectisome argues that additional components are involved to ensure an orderly translocation of effectors. Most effector proteins contain a T3SS-targeting signal at their extreme amino terminus that is broadly recognized by divergent injectisomes. Additional targeting information that is contained within approximately the first 200 amino acid residues of the effector provides binding sites for the secretion of chaperones. These chaperones are multi-functional: they target effectors to the proper injectisome, stabilize preformed effector proteins, mask membrane targeting domains that are prone to aggregation, and possibly impart a translocation hierarchy.

T3SS contains macromolecular structures composed of 20-35 proteins that are often referred to as "injectisomes" due to their resemblance to an injection needle (**Figure 1**). The injectisome is a nanomachine that is evolved for the delivery of bacterial proteins, by type III secretion, across eukaryotic cell membranes. In the present stage of knowledge, injectisome consists of a basal structure, which resembles the basal structure of the flagellum, surmounted by either a needle or a long pilus. CdsF is concentrated in the outer membrane of EBs and is surface-exposed as a component of an extracellular needle-like projection. CdsF is able to polymerize into multi-subunit complexes. CdsF represents at least one component of the extended *Chlamydia* T3SS injectisome.

Chlamydia T3SS is a non-flagellar T3SS. *Chlamydia* injectisome components are present at all stages of infection. In addition, needle-like structures have been observed on the surface of EBs and at the sites of RB attachment to inclusion membranes, suggesting that this secretion system is functional. These T3SS substrates include more than 25 soluble proteins and a large family of 40-50 integral membrane proteins of unknown function that localize to the inclusion membrane (Incs). T3SS substrates are likely to translocate in a hierarchical fashion to regulate specific cellular functions at distinct stages of infection. Injectisomes belong to at least seven distinct families, in which three (Ysc, SPI-1 and SPI-2) are predominantly found in free-living pathogens of animals and two are more common in plant pathogens (Hrp1 and Hrp2). The remaining injectisome families are limited to the *Chlamydiae* phylum and the Rhizobiale order [51]. CdsQ, a conserved structural component predicted to be at the base of the injectisome, interacts with multiple proteins, including a new chaperone that binds to and stabilizes secretory cargo destined for the membrane of the pathogenic vacuole. The base of the secretion apparatus serves as a docking site for a chaperone and a subset of chaperone-cargo complexes.

Spaeth et al. [45] identified networks of *Chlamydia*-specific proteins that interacted with the basal structure of the injectisome, including two hubs of protein-protein interactions that linked known secreted effector proteins to CdsQ, the putative cytoplasmic C-ring component of the secretion apparatus. One of these protein-interaction hubs is defined by CT260/Mcsc. Mcsc binds to and stabilizes at least two secreted hydrophobic proteins, Cap1 and CT618, which localize to the membrane of the pathogenic vacuole. The resulting complexes bind to CdsQ, suggesting that in *Chlamydia*, the C-ring of the injectisome mediates the recognition of a subset of Incs in complexes with their chaperones. The selective recognition of Incs by chaperones may provide a mechanism to coordinate the translocation of subsets of Incs at different stages in infection. Two novel hubs of protein-protein interactions (CT260 and CT700) link multiple Incs to the secretion apparatus. CT260/Mcsc is of particular interest because, in addition to its direct interaction with CdsQ and many secretory cargo proteins (Cap1, CT225 and CT618), it has predicted secondary structural similarity to other T3SCs. Mcsc is expressed in both developmental forms of *Chlamydia* and forms stable dimers and complexes with at least two Incs. One of the functions of Mcsc is to stabilize these Incs. Mcsc acts as a real Class I T3SC. By analogy to these findings, CdsQ recruits Mcsc effector complexes directly to the base of the injectisome. However, because Mcsc can bind to the C-ring component CdsQ in the absence of its bound substrate, it is also possible that Mcsc is pre-docked on the C-ring of the injectisome. Because the stability of effectors requires bind-

ing by Mcsc, either Mcsc dimers that detach from the injectisome and bind to newly synthesized effectors at distal sites or the translating ribosome itself is recruited to the C-ring. Furthermore, one of the CdsQ-interacting proteins identified by Y2H is CT677, a putative ribosome recycling factor. These findings raise the possibility that the injectisome may interact with the bacterial translational machinery. The prominence of CdsQ as a hub of protein-protein interactions, including secretion chaperones, suggests a central role in regulating the recognition of effector proteins.

T3SS regulators

T3SS is utilized by numerous Gram-negative bacteria to efficiently interact with a host. Appropriate expression of type III genes is achieved through the integration of several regulatory pathways that ultimately coordinate the activity of a central transcriptional activator usually belonging to the AraC family [52]. The complex regulatory cascades allow this virulence strategy to be utilized by different bacteria even if they occupy diverse niches that define a unique set of environmental cues. It is not surprising that multifaceted regulatory systems are required to impart spatial and temporal control of type III gene expression. It is apparent that regulation occurs in at least two distinct steps: expression of genes required for assembly of the secretion apparatus followed by expression of genes whose products are substrates for T3SS. There are several environmental cues, such as nucleoid-associated proteins, phosphorelay two-component systems, AraC-like transcriptional activators and global regulators cross-talk to modulate the expression of TTSS genes. However, it is worth noting that recent developments indicate that cell contact, assembly switching and specific chaperone molecules play a unique role in the regulation of T3SS. By contrast, researches on *C. trachomatis* T3SS regulators are rare. By now, it's unclear what regulatory factors are functional in *C. trachomatis* T3SS. Therefore, it's important to find the regulatory systems that mediate *C. trachomatis* T3SS.

Future prospects

Type III secretion is an important virulence determinant of many Gram-negative bacteria including *C. trachomatis*, and an attractive target for the interruption of pathogen development. Small molecule inhibitors of T3SS have been identified to inhibit T3SS and *C. trachomatis* development [53]. An improved understanding of the signals mediating *C. trachomatis* T3SS and the secreted complement of effectors and chaperones should provide additional targets to inhibit *C. trachomatis* pathogenesis.

Recently, the advances in *Chlamydial* genetics may have the potential to make significant progress. Many kinds of newly published methods laid on the foundations for the genetic manipulation of *Chlamydia*. Transformation of *Chlamydia* with exogenous DNA has also been achieved by a variety of techniques, such as electroporation, $CaCl_2$ treatment [54], and the use of dendrimers. Because of the limited size of *C. trachomatis* genomes, the final identification and confirmation of all effectors and chaperones is possible. With a complete list of effectors and corresponding gene sequences, the inferred contributions of effector polymorphisms to virulence may eventually be confirmed genetically. A more thorough characterization of *C. trachomatis* T3SS, and the effects of T3SS to virulence would provide significant insights into the prevention and treatment of *C. trachomatis* infection.

Acknowledgements

This work was supported by grants from National Natural Science Foundation of China (Nos. 30970165 and 81102230), the Innovation Platform of Open Fund Project for Universities in Hunan Province (No. 13K081), Construct Program of the Key Discipline in Hunan Province (No. 2011-76) and Hunan Provincial Key Laboratory for Special Pathogens Prevention and Control (No. 2014-5).

Disclosure of conflict of interest

None.

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