Microreview

Structural microbiology at the pathogen–host interface

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Summary

Bacterial pathogens achieve the internalization of a multitude of virulence factors into eukaryotic cells. Some secrete extracellular toxins which bring about their own entry, usually by hijacking cell surface receptors and endocytic pathways. Others possess specialized secretion and translocation systems to directly inject bacterial proteins into the host cytosol. Recent advances in the structural biology of these virulence factors has begun to reveal at the molecular level how these bacterial proteins are delivered and modulate host activities ranging from cytoskeletal structure to cell cycle progression.

Introduction

The pathogen–host interaction is a catch-all phrase that harbours beneath its umbrella a myriad of interactions between microbial pathogens and their hosts. Most research in this area tends to stem from one of two perspectives: (i) the immunological response of the host to infection and (ii) the microbial products or virulence factors that target the host in the disease process. While it is clear that nothing is so discrete and easily divided in such a complicated interaction between organisms, both for historical reasons and technical challenges, this division has persisted and has offered both advantages and disadvantages.

The last 10 years have witnessed an explosion in our knowledge of bacterial virulence factors. Long thought to be the realm of a few specialized toxins and adhesions, the marriage of cell biology and microbiology has revealed a hidden landscape of sophisticated and diverse virulence systems in bacteria. Not only is it now clear that bacteria can precisely target specific host cell activities — such as cytoskeletal organization, cell cycle progression, vesicular trafficking and apoptosis — but the pathogenic factors responsible for these effects have begun to be characterized genetically, biochemically and, most recently, at the structural level. Here we examine some of the contributions of structural microbiology to our understanding of bacterial virulence systems.

Secretion and translocation

Key to the successful colonization and persistence in many plant and animal hosts, as well as for the formation of many productive symbiotic relationships, are the contact-dependent, or type III secretion systems (TTSS) of bacteria (Galan and Collmer, 1999). These are protein export and delivery systems that are evolutionarily related to the flagellar secretion system and which have been specialized for the translocation of bacterial proteins directly into the host cell. More than 20 proteins are known to be components of TTSS, although only a small number physically comprise the supermolecular, organelle-sized ‘molecular syringe’ that serves as the channel through which virulence factor substrates travel. Oligomeric ring-shaped structures span the inner and outer membranes of the Gram-negative bacteria that possess TTSS, and these structures are capped extracellularly with a filamentous ‘needle’ with a diameter of approximately 25 Å (Kim-brough and Miller, 2002; Marlovits et al., 2004).

On the bacterial side, there is a known requirement for a highly conserved $F_0F_1$ family ATPase that has been hypothesized to use the energy of ATP hydrolysis to drive translocation through the needle complex (Galan and Collmer, 1999). It has also been postulated that this energy dependence is coupled to an ‘unfoldase’ to render the virulence factors non-globular for transport through the system. This stems from the narrow channel diameter of the TTSS as observed from electron microscopy (EM) studies as well as the fact that significant indirect evidence exists that the substrates of the TTSS are maintained in a partially non-globular, partially folded ‘secretion competent’ state that is primed for movement through this system. This latter observation in fact stems from interesting structural work discussed next.

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Bacterial secretion chaperones

The majority of pathogenic TTSS substrates are found bound to a so-called ‘secretion chaperone’ in the bacterium before delivery into the host (Page and Parsot, 2002; Stebbins and Galan, 2003). These proteins are small, acidic dimer-forming molecules that bind to N-terminal domains in one or more virulence factors, but which show little detectable sequence similarity. Absence of a secretion chaperone for a given virulence factor often results in premature degradation or massive overaccumulation in the bacterium with a concurrent dramatic decrease in translocation into the host. Recently, it was shown in *Salmonella* that one role for these molecules is in the targeting of the virulence factor to the pathogenic TTSS, and not to the flagellar TTSS (Lee and Galan, 2004). Other roles proposed for these molecules have included a function in maintaining the virulence factor substrates of the TTSS in a ‘secretion-competent’ state, and also a function in preventing inappropriate interactions, such as might lead to aggregation, in the bacterium. These different proposed functions are not mutually exclusive.

Structure characterization of these chaperones and their complexes with virulence factors has revealed two important concepts (Stebbins and Galan, 2003; Ghosh, 2004): (i) that the secretion chaperones share a very similar fold and general mode of binding virulence factors, and therefore constitute a diversified but highly related family, and (ii) that the secretion chaperones bind their cognate virulence factors by interacting with an extended, non-globular N-terminal peptide that wraps around the chaperone dimer (Fig. 1A). The details of this interaction include the burying of large hydrophobic surfaces on the chaperones by the virulence factor-extended peptides as well as the interesting observation that the three-dimensional path of the non-globular peptide is generally similar (Fig. 1B) (Stebbins and Galan, 2001a; Birtalan et al., 2002; Phan et al., 2004; Schubot et al., 2005).

These structures have provided support for three models of chaperone function. The first is that of secretion competence, as the non-globular state of the chaperone molecule is very consistent with priming the virulence factors for secretion through the narrow channel of the TTSS (Stebbins and Galan, 2001a). The second is a signal to target the virulence factors to the TTSS. That such a signal, at least for certain virulence factors in *Salmonella*, is required to prevent secretion through the flagellar TTSS is now established (Lee and Galan, 2004).

![Fig. 1. Chaperones in type III secretion.](image)

A. Three different classes of chaperones associated with type III secretion systems (TTSS). The SicP–SptP complex represents an example in the pathogenic secretion chaperones that bind to translocated virulence factors and aid in their proper secretion. CesA–EspA represents another category, in which the CesA chaperone prevents polymerization of the filamentous EspA within the bacterium. Similarly, but in the context of flagellar type III secretion, the FliS chaperone serves as an antipolymerization factor for flagellin (FliC).

B. The mode of interaction of translocated virulence factors of TTSS with their cognate secretion chaperones. The chaperone dimers are shown as molecular surfaces coloured orange and grey for regions with hydrophobic and polar character respectively. Coloured circles represent disordered regions in the chaperone-binding domains.
It has also been proposed that the conserved three-dimensional path of the polypeptide in the SicP–SptP and SycE–YopE complexes may be a universal conformational signal recognized by the TTSS (Birtalan et al., 2002). The third functional role for TTSS chaperones that garner support is that of preventing inappropriate interactions. While there are two known examples of virulence factors with chaperone-binding domains that fold into stable, soluble domains in the absence of the chaperone, YopH (Evdokimov et al., 2001; Smith et al., 2001; Khandelwal et al., 2002) and SipA (M. Lilic and C.E. Stebbins, submitted), there are several examples of chaperone-binding domains that massively aggregate or are proteolysed without their cognate chaperone. Because of the large hydrophobic protein–protein interactions found in all the known chaperone–virulence factor complexes to date, if the peptide itself cannot fold into a globular molecule and bury its hydrophobic residues, the absence of the chaperone can lead to the formation of protein aggregates. Although the C-terminal domains have been shown in vitro to fold even when the N-terminal domain is bound by the chaperone in a non-globular state, the high solubility of these domains is not enough in many cases to salvage the protein in the absence of the chaperone.

The secretion chaperones have been divided into two classes based on their properties in binding a single or multiple virulence factors (Page et al., 2002; Stebbins and Galan, 2003; Ghosh, 2004; M. Lilic and C.E. Stebbins, submitted). Structural studies have supported this subdivision (van Eerde et al., 2004). Currently, the two examined secretion chaperones that are known to bind multiple substrates, Spa15 of Shigella and InvB of Salmonella, possess the same non-globular interaction, but assemble into a different dimer that is characterized by a relative rotation between the two proteins (van Eerde et al., 2004; M. Lilic and C.E. Stebbins, submitted). More strikingly, the complex of InvB with one of its associated virulence factors, SipA, shows that the protein–protein interface between the chaperone and substrate differs in this subclass as well (M. Lilic and C.E. Stebbins, submitted). The non-globular interaction is preserved, but it does not extend around the chaperone dimer (Fig. 1B). Instead, the non-globular peptide of SipA binds to only one InvB protein in the chaperone homodimer, leaving the other hydrophobic surfaces exposed. Modelling analysis indicates that the large globular region of the N-terminal domain of SipA, which makes significant protein–protein interactions with the InvB, precludes the binding of a second SipA molecule due to steric clash.

Finally, the structures of a heterodimeric secretion chaperone complex, that of SycN–YscB with the translocated Yersinia factor YopN (Schubot et al., 2005), as well as the complex of the negative regulator YscM2 with SycH (Phan et al., 2004), have been recently determined. Because of divergences in the conserved fold between SycN and YscB, this heterodimeric complex displays an asymmetry in its molecular surface, which creates several novel aspects to its interaction with YopN. In common with other TTSS chaperone complexes, it is an extended, non-globular region of YopN that interacts with the heterodimer (Fig. 1B). Large hydrophobic tracks also serve as a significant component of the interaction, although there are more polar contacts than have been observed in the other complexes. Unlike InvB–SipA, however, and like the remaining chaperones, YopN interacts with both chaperones, wrapping around the dimer interface (Fig. 1B). The complex of YscM2 and SycH also confirms that the non-globular interaction is a characteristic feature of this interaction (Phan et al., 2004).

Other proteins that interact with molecules that are substrates for TTSS have been termed chaperones, and until recently it was unclear from the sequence analysis whether they belonged to the families described. One example is CesA of Escherichia coli, which binds to EspA and prevents its polymerization before secretion through the TTSS (Creasey et al., 2003). Once extracellular, EspA polymerizes into a large filamentous appendage that associates with the E. coli needle complex (Sekiya et al., 2001; Daniell et al., 2003). EspA is therefore not a translocated protein that functions within the host cell, but is part of the secretion system itself (while also a substrate for it). The crystal structure of a CesA–EspA complex reveals that CesA is clearly of a different family of chaperone (Fig. 1A). It is an elongated helical structure that forms extensive coiled-coil interactions with EspA (Yip et al., 2005a), likely burying those regions of EspA that associate to form the polymer. Similarly, the flagellar TTSS secretes flagellin (FliC) to form the flagella. FliC is also prevented from polymerization within the bacterium by a chaperone, FliS (Auvray et al., 2001). The structure of this complex reveals yet another family of TTSS secretion chaperone that, like CesA, likely functions primarily to prevent premature polymerization, although intriguingly the FliC peptide bound to FliS is in a non-globular conformation reminiscent to those of the pathogenic TTSS chaperone-binding domains (Fig. 1A) (Evdokimov et al., 2003). FliS itself, however, possesses a very different fold from these chaperones, and both FliS and CesA prevent polymerization of their respective partners by binding as monomers.

**TTSS apparatus**

Understanding the structural and functional underpinnings of the molecular syringe itself is at the cutting edge of EM and X-ray crystallography. The sheer size of the needle complex, as well as its clear heterogeneity in preparations from bacteria, seems to place it into the realm of the
former technique. Indeed, impressive work using cryo-EM has imaged this organelle-sized secretion machine at 17-angstrom resolution, revealing aspects of the internal structure never-before glimpsed (Marlovits et al., 2004).

Recently, a substantial step forward was taken in the high-resolution characterization of this complex. Two groups have determined the structure of the EscJ protein from the E. coli TTSS – one by nuclear magnetic resonance (NMR) and one by X-ray crystallography (Crepin et al., 2005; Yip et al., 2005b). This ~20 kDa protein is the major component of the basal body of the needle complex. Strynadka and colleagues were able to crystallize EscJ in a very intriguing form (Yip et al., 2005b). The unique portion of the crystal structure (the asymmetric unit) was found to contain a tetramer of this protein (Fig. 2A), but the truly fascinating aspect of these crystals is the crystallographic packing of this tetramer. With great fortune, it was found that the tetramer formed in a crystal lattice in which it wound with sixfold symmetry around an axis of translation. Looking down this super-helical axis, and thereby ‘compressing’ the helical winding into a single plane, produces a ring-shaped structure with nearly the same number of molecules and dimensions as is observed in the basal ring structures by EM and other techniques (Fig. 2B). The authors hypothesized that the extensive interactions observed in this crystal packing argue that this projection of their crystals can serve as a model for the actual ring, providing for the first time a molecular image of a portion of the secretion apparatus. The central channel of this structure narrows like a cone from 120 Å to 73 Å in going from the inner membrane to the periplasmic face. The inner surface of the ring is lined with acidic residues, creating a very negatively charged interior. The overall appearance of the structure is reminiscent of the FliF ring from the flagellar TTSS as imaged by cryo-EM. These features altogether led to the hypothesis that the basal ring does not form a pore within the inner membrane, but that it is instead perched atop the periplasmic face of the membrane. The shape and charge distribution of the ring suggest that these are key elements for the assembly of the transmembrane elements and central cylindrical components of the needle complex.

**TTSS substrates**

The virulence factor substrates of TTSS possess a very wide variety of biochemical activities, and include proteases, kinases, protein and lipid phosphatases, nucleotide exchange factors and GTPase-activating proteins for the Rho family, actin-polymerizing and -bundling factors, and tubulin-binding proteins, to name a few. Structural studies have revealed at the molecular level how many of these factors function, shining light on horizontal and convergent evolution of host mimicry as well as completely novel methods to manipulate the host.

One of the earliest insights from structural biology came with the determination of the crystal structure of the Yersinia protein tyrosine phosphatase catalytic domain, YopH (Stuckey et al., 1994). Determined around the same time as the crystal structure of the eukaryotic PTP1B, these two structures, which proved to be homologous, and subsequent structural and biochemical work, elucidated the mechanisms of phosphatase removal for this family of enzymes. Later, structural work with the Salmonella tyrosine phosphatase, SptP, showed that the active site and fold of these bacterial phosphatases is highly conserved.
but that there have been considerable divergence in surface features, consistent with the very different targets and functions to which these enzymes are put by different pathogens (Fu and Galan, 1998; Galan and Zhou, 2000; Stebbins and Galan, 2000). In addition, complexes with phosphopeptides and small molecule inhibitors have both enhanced the biochemical knowledge of these enzymes, and have also opened the door for the rational design of inhibitors of virulence (Liang et al., 2003; Sun et al., 2003; Hu et al., 2004; Hu and Stebbins, 2005; Tautz et al., 2005). Such compounds have the potential to be used as therapeutic agents (Fig. 3A). Interestingly, the N-terminal domain of YopH also possesses a host cell function in addition to its chaperone binding function within the bacterium (Montagna et al., 2001). This domain is able to bind to phosphotyrosine substrates of the catalytic C-terminal domain. The crystal structure of the YopH N-terminal domain, and NMR structures of this domain bound to a phosphopeptide (Evdokimov et al., 2001; Smith et al., 2001; Khandelwal et al., 2002), has revealed the molecular basis of this interaction and confirmed and extended a wealth of genetic data (Fig. 3B). Finally, two recent structures have shown that the YopH catalytic domain possesses a second phosphotyrosine binding site on the face of the structure nearly opposite to that of the catalytic site, and that this site cooperates with the N-terminal domain of YopH to promote efficient recognition of its substrate p130Cas in epithelial cells (Phan et al., 2003; Ivanov et al., 2005).

Several bacterial virulence factors target the Rho family GTPases (Galan and Zhou, 2000; Barbieri et al., 2002). Structures of the Salmonella-translocated proteins SptP and SopE in complex with their host cell targets Rac1 and Cdc42 have been particularly informative in explaining the Yin and Yang of this pathogen’s effect of the host cell cytoskeleton (Stebbins and Galan, 2000; Buchwald et al., 2002). Salmonella uses SopE, a guanine nucleotide exchange factor, to activate signalling from Rac1 and Cdc42 (Galan and Zhou, 2000), contributing to membrane ruffles that internalize the bacterium. After targeted degradation by the host proteasome (Kubori and Galan, 2003), the GAP domain of SptP functions as a GTPase-activating protein to stimulate GTP hydrolysis, which downregulates signalling from these molecules. The co-crystal structures of SopE–Cdc42 and SptP–Rac1 have revealed fascinating mechanisms for the activity of these proteins. SptP is shown to recapitulate through a novel structural scaffold most of the contacts observed of host GAP enzymes, in effect mimicking their activity (Fig. 3C) (Stebbins and Galan, 2001b). In particular, contacts to the nucleotide and the insertion of a catalytic arginine from an alpha helix of the four helix bundle GAP domain have many parallels in host factors, as do the contacts to the GTPase catalytic residue Gin-61. Structures of homologous GAP domains in the proteins YopE of Yersinia spp. and ExoS of Pseudomonas aeruginosa reveal a highly conserved fold and mode of modulation for the Rho-GTPases (Wurtele et al., 2001a,b; Evdokimov et al., 2002). SopE, on the other hand, possesses a unique method for disrupting the active site of Cdc42, inserting a conserved loop that splays apart the key Switch I and Switch II regulatory and nucleotide binding regions of the GAP domain (Fig. 3D). These conformational changes in the active site make GDP binding unfavourable, but they leave the nucleotide binding pocket open for the binding of GTP, thereby enhancing nucleotide exchange.

Another common target in host cells are the cytoskeletal proteins themselves. Salmonella invasion protein A, or SipA, is important for full virulence and rapid entry of bacteria into host cells (Zhou et al., 1999). The C-terminal domain of this virulence factor has actin binding and polymerization activity (Zhou et al., 1999). A combination of X-ray crystallography and EM has shown that this C-terminal domain likely functions as a ‘molecular staple’, in which a core globular domain of SipA serves as a centre for two, non-globular ‘arms’ to reach out in opposite directions and tether actin protomers, thereby stabilizing the filament (Fig. 3E) (Liilic et al., 2003). Deletions of the putative arms lead to an impairment of polymerization of G-actin by SipA and a decrease in the measured elongation of the molecule in solution, while have no impact on the solubility of the molecule or its ability to bind to pre-formed F-actin.

A fascinating protein from the pathogen Yersinia is YpkA. YpkA (or YopO) possesses an N-terminal domain with homology to host ser/threonine kinases (Aepfelbacher, 2004), and possesses kinase activity in vitro that is dependent on the presence of actin and a putative actin-binding domain at the C-terminus of the protein (Juris et al., 2000). Between these two domains is a Rho family GTPase-binding domain (Aepfelbacher, 2004). Bioinformatic studies have drawn comparisons between this region and the ACC-finger domains of host proteins that bind to Rho GTPases (Aepfelbacher, 2004). The structure of a C-terminal domain of YpkA, containing the Rho-binding and actin-activation subdomains, reveals an unusual fold (G. Prehna, X. Hu and C.E. Stebbins, submitted). Two small subdomains are separated by a very long helix, giving the molecule an unusual, dumbbell shape. The uniquely folded C-terminal subdomain contains a 30-amino-acid peptide previously shown to be critical to actin-dependent activity of the kinase domain. This peptide corresponds to several elements of secondary structure whose removal would likely destabilize the entire C-terminal domain, as supported by biochemical experiments (G. Prehna, X. Hu and C.E. Stebbins, submitted). The N-terminal subdomain appears to differ from host cell ACC-finger domains, suggesting that YpkA uses a different mechanism to bind small GTPases.
Fig. 3. Virulence factors of type III secretion systems.

A. Molecular surface of the *Yersinia* virulence factor YopH, coloured blue, red, cyan and yellow, for basic, acidic, polar and non-polar character respectively. The phosphatase inhibitor, pNCS, is shown bound in the active site as a ball-and-stick model.

B. The N-terminal domain of YopH, used in the bacterium in binding a secretion chaperone, is used in the host cell to bind phosphotyrosine substrates of the C-terminal phosphatase domain. The structure of the N-terminal domain is shown as a grey transparent molecular surface over a cyan ribbon diagram, and the phosphotyrosine peptide is coloured yellow and red, and the tyrosine residues are in red.

C. The exchange factor SopE (violet) of *Salmonella* disrupts the nucleotide binding site of Cdc42 (yellow) facilitating binding of GTP. The GAGA loop is noted that inserts into the GTPase active site.

D. The GAP enzyme of *Salmonella* (violet) is shown inserting an arginine residue into Rac1 (yellow) to stimulate GTP hydrolysis.

E. Membrane ruffles (background image) of intestinal cells contain actin filaments (red ribbon diagram) 'stapled' together with the SipA protein of *Salmonella* (green). The EM density of SipA–actin complexes is shown in blue. A close-up of SipA with modelled 'arms' that extend to tether actin protomers together.

F. Crystal structure of the *Yersinia* kinase YpkA coloured in a spectrum from blue (N-terminus) to red (C-terminus). The genetically and biochemically functional regions are labelled.
**Toxins and receptors**

Type III secretion systems of Gram-negative bacteria have been a recent area of intense study. However, the understanding of toxins and extracellular adhesins and so-called 'invasins' have also benefited recently from structural biology. A recent example is a toxin called 'cytolethal distending toxin', or CDT, which over the last decade was shown to be a tripartite AB type holotoxin, the active subunit of which enters the nucleus and damages DNA (Pickett and Whitehouse, 1999; Lara-Tejero and Galan, 2000). This damage leads to permanent cell cycle arrest (or apoptosis in lymphocytes) and massive cellular distension over a period of several days (Fig. 4A). The crystal structure of the CDT holotoxin – composed of the active subunit CdtB bound to CdtA and CdtC – reveals that it has a great resemblance to the ricin holotoxin (Nesic et al., 2004). As in ricin, the active subunit, CdtB, binds two closely spaced repeats of a β-trefoil lectin fold. The active subunits have very different host cell targets (the ribosome for ricin, host DNA for CdtB), but the putative receptor-binding subunits of CDT occupy a similar position to elements with such function in ricin. Lending support to the idea that lectin folds of CdtA and CdtC are the cell surface-binding elements that achieve the internalization of the toxin, the structure reveals that these two proteins present a remarkable surface at one end of the holotoxin. This surface consists of a deep and extended groove 20 × 10 Å long (and nearly 8 Å at its deepest), and a large aromatic patch (Fig. 4B). Structure-based mutations in residues in the patch render the toxin inactive and unable to bind to the surface of cells (Nesic et al., 2004; D. Nesic and C.E. Stebbins, submitted). CdtB is shown to be a close structural homologue of proteins in the DNase

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**Fig. 4.** Recent structures of secreted toxins and internalization factors.

A. Comparison of CDT with ricin, in which the lectin domains are coloured blue and cyan, and the active enzymatic subunit is coloured yellow in both holotoxins. The lectin domains are demarked by a red line.

B. Surface of the CDT holotoxin coloured by amino acid type – yellow for non-polar, cyan for polar, except for acidic and basic residues which are in red and blue respectively.

C. The complex of InlA with the host target domain of E-cadherin shown as a ribbon diagram.
I family, and several residues with DNA binding function in DNase I occupy identical positions in the active site of CdtB, and mutations of these residues abolishes toxicity (Nesic et al., 2004).

Extracellularly, bacteria have evolved many specialized molecules to bind to host cell surface proteins. One interesting class of such molecules are the ‘internalins’ of the Gram-positive pathogen *Listeria monocytogenes*, specifically, InlA and InlB, that are sufficient when attached even to latex beads to induce their entry into cells (Cossart and Sansonetti, 2004). The target of InlA is the host protein E-cadherin, and the recent crystal structure of the complex of the E-cadherin-binding domain of InlA with the domain of E-cadherin that it recognizes was determined (Schubert et al., 2002). This structure reveals that the leucinerich repeat of InlA wraps around the E-cadherin domain (Fig. 4C). Pro16, a residue that is responsible for a species tropism in *Listeria* infection, is found to be nestled into a tight binding pocket in InlA, explaining why the mutation to glutamate (as found in the mouse) causes a steric clash and prevents binding. This is an amazing example of a macroscopic interaction between pathogen and host – namely, resistance to a pathogen for some species – finding explanation at the level of individual atoms through structural studies.

Structures of InlB itself have revealed that it is a modular protein that centres on an LRR domain at its N-terminus (with an N-terminal ‘cap’ and C-terminal Ig-like domain) (Marino et al., 1999; 2002; Schubert et al., 2001). This is connected to a B-repeat domain and three C-terminal ‘GW’ repeats, with the GW repeats 40 Å away from the N-terminal domain. The structure is thus highly elongated with dimensions of 60 Å × 165 Å in a plane in which it forms an ‘L’ shape. The GW repeat domain is found to possess an extremely basic molecular surface consistent with its known function in binding to the bacterial cell membrane (Marino et al., 2002).

**Conclusion**

This is a golden age in microbial pathogenesis, and at an ever-increasing rate the mechanisms that bacteria use to engage their hosts are being identified and characterized. Providing an essential foundation to understanding these pathogens is a structural knowledge of the bacterial virulence systems, and in particular, how they interact with and modulate host cell factors. This knowledge provides a molecular explanation for activity, and a blueprint for possible inactivation of key virulence systems by targeted therapeutics. As the diversity of characterized virulence systems increases, new themes in the bacterial manipulation of host cell biology are emerging, revealing both novel methods to achieve biochemical modulation of the host and exquisite mechanisms to mimic the activity of host factors for the benefit of the pathogen. In all, structural microbiology is proving to be an important compliment to more traditional studies, and providing a rich harvest of exciting biology at the pathogen–host interface.

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**References**


