Published in final edited form as:

Curr Opin Microbiol. 2010 February ; 13(1): 47–52. doi:10.1016/j.mib.2009.11.001.

Type 3 Secretion Systems Shape Up as They Ship Out

Thomas C. Marlovits^{1,2} and C. Erec Stebbins³

¹ Research Institute of Molecular Pathology, Dr. Bohr Gasse 7, A-1030 Vienna, Austria, Europe

² Institute of Molecular Biotechnology, Austrian Academy of Sciences, Dr. Bohr-Gasse 3-5, A-1030 Vienna, Europe

³ Laboratory of Structural Microbiology, RU Box 52, The Rockefeller University, New York, NY 10065

Summary

Virulence associated protein Type III Secretion Systems (T3SS) are intricately structured organic nanosyringes that achieve the translocation of bacterial proteins from the prokaryotic cytoplasm across three membranes into the host cytosol. The substrates for these systems number in the hundreds, with remarkably diverse biological activities, modulating host cell biology for the benefit of the pathogen. Although there has been tremendous progress on the structure and function of the T3SS substrates, there has been comparatively little progress on the much more highly conserved secretion apparatus itself. This review summarizes recent advances in the field of structural microbiology that have begun to address this shortcoming, finally bringing to bear the power of structural biology to this central virulence system of Gram negative bacterial pathogens.

Introduction

In the long and dynamic interaction between hosts and pathogens, virulence proteins have played a key role in rendering the host organism useful for the replication of the pathogen. The evolution of molecular machineries designed to translocate bacterial proteins into host cells marked an important transition in this host-pathogen relationship, providing a means to introduce directly into eukaryotic cells, proteins that could precisely modulate the biology of the targeted tissues.

Bacterial Type III Secretion Systems (T3SS) are one of the poster-children in such translocation systems. Aptly considered “molecular nanosyringes”, they are composed of numerous proteins, spanning multiple membranes with supramolecular organelle sized structures, harboring a narrow channel through which the substrates travel in energy (ATP) dependent manners [1, 2]. The T3SSs also have homology to systems used to translocate macromolecules in non-pathogenic contexts – the flagella export system and in symbiotic contexts [3,4].

Enormous progress has been made in the last two decades since these systems were first identified. However, there has been, until recently, very limited structural information, particularly at high resolution in a fashion that could provide mechanistic details. In this review, we consider recent advances in the structure and function of T3SS, examining where structural biology has taken the field, and where it still needs to go to achieve the level of understanding required to effectively model these machineries.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The T3SS

Nearly one billion years ago, according to bioinformatic reconstructions, bacteria were in possession of genes highly homologous to the modern virulence associated T3SS [5]. The genes for this protein secretion system have found their way, through pathogenicity islands and large plasmids, to a wide variety of Gram-negative pathogens of animals and plants, and also function in phytosymbiotic relationships [1,2,4]. The known virulence substrates for these systems currently number in the several hundred, modulating host biochemical functions such as cytoskeletal structure, programmed cell death, cell cycle progression, endocytic trafficking, and gene expression [6–10].

The secretion machinery itself is highly conserved, consisting of more than twenty proteins, many showing homology to flagellar export genes. A conceptual division can be made between the so-called *basal body* of the apparatus, which differs between the pathogenic and flagellar systems, and the core transmembrane proteins that have a much higher level of conservation [1–4,11].

The basal body may be thought of as the “syringe” body, the shell or tunnel that provides a solid structural framework for the secretion machinery to use for translocation, through which substrates travel (Figure 1). Remarkably, membrane preparations from *Salmonella*, *Shigella*, and *E. coli* show that the minimal, stable core of the basal body consists of only of three proteins (plus a filamentous needle, [1,11–13]). Since these three proteins assemble into a supramolecular structure spanning several hundred angstroms in width and height, it is clear that the individual proteins, none larger than 70kDa, must form oligomers of high symmetry. Indeed, electron microscopy has revealed that the basal body possesses radial symmetry (ring-like) of a very high order (at least 12-fold, although different image-processing approaches in EM have led to different orders of radial symmetry, [14,15*,16*]).

The three proteins of the basal body are (1) an outer-membrane spanning member of the secretin family (*Salmonella* InvG), and (2) an inner membrane complex consisting of two proteins without known parallel ((*Salmonella* PrgH and PrgK, Figure 1) [11]. The secretin has in most cases been associated with a “piloting” protein that is required for it to assemble within the outer membrane, although this protein appears to be lost in standard membrane preparations of the basal body [11,17–20].

Protruding from the outer membrane at the “top” of the basal body is a long filamentous structure composed of a single protein (Figure 1). This “needle” is in some pathogens surrounded also by a polymerized sheath [21,22]. The needle is anchored about halfway through the basal body in the inner ring structure, and forms part of the channel from bacterial cytoplasm to host cytosol [14,15*]. At the tip of the needle, is a complex of several proteins, also visualized by EM, which docks with a pore in the host cell formed of T3SS substrates (Figure 1) [23].

Believed to assemble underneath the basal body within the inner membrane, are a set of proteins that are the most highly conserved in all of T3SS, strongly so even between the pathogenic and flagellar systems. These proteins include an ATPase, and a set of more than five integral membrane proteins that are absolutely critical to the functioning of the system[1,11,24–29]. Outside of a few soluble domains of two of these proteins, structure-function information is extremely scarce [30]. For the integral membrane domains, there is very little biochemical, and no structural information at all.

Recent Advances in Electron Microscopy

More than a decade ago, structural work of the type III secretion system was launched by the first visual recognition of the *Salmonella* needle complex, the core structure of the type III secretion system, using conventional electron microscopy [12]. Since then, three dimensional electron microscopy of fully hydrated *Salmonella* [14] and negatively stained *Shigella* [16*, 31] needle complexes and subsequent single particle analysis provided a detailed view about the overall architecture of this core structure from various species. In both systems, the membrane embedded base is organized in a series of inner and outer rings, documenting on the one hand the close evolutionary relationship of this supramolecular system and on the other hand determining their role as a scaffold for a safe translocation of bacterial effectors thorough a number of hostile environments into a host cell.

The recent (scanning) transmission electron microscopy of negatively stained complexes from *Shigella* provided additional detail on the composition of the apparatus [16*]. It was seen, for example, that the outer membrane ring (secretin portion) is resolved into three separate rings. The two near the outer membrane are highly reminiscent of images obtained by EM of other outer membrane secretin proteins, such as PulD [16*,32]. The most periplasmic ring is linked to the inner membrane rings through what has been termed the “connector” ring (a thick structure with a height slightly larger than the outer membrane sub-rings). Underneath the connector ring, is the much larger (in diameter) inner membrane ring (Figure 1 and 2A). This ring may be connected to the socket/cup through what appear to be a series of “spokes” (Figure 2B). Finally, the inner membrane ring is connected through its lower side (cytoplasmic proximal) through two thin linkers to the “legs” of the basal body (which form a final, smaller ring at the very base of the apparatus).

The investigation of assembly states of the *Salmonella* needle complex [14] revealed remarkable results both compositionally and conformationally: the central part of the base is a chamber, closed by a septum at its apical and by a plate at its basal side. The ‘socket’ a newly discovered substructure reaches into the chamber, whereas the ‘cup’ is oriented towards the cytoplasmic space. The ‘socket’ creates a docking platform of the ‘inner rod’ – another newly identified substructure - which is only present in needle complexes and connects to the extracellularly located needle filament. Surprisingly, comparing the base and the needle complex as two subsequent assembly states uncovered that this supramolecular complex must undergo large conformational changes during the assembly process. This is highlighted at various positions throughout the complex. Such changes are observed for example by an increase of the base diameter especially around the neck region, but are also evident at the cytoplasmically oriented IR2 ring, which together with the ‘cup’ redefines this area. Moreover, the socket moves outwards and subsequently allows the docking of the inner rod structure to finally establish a ‘secretion tunnel’ that connects from the inner membrane to the needle filament.

Whether these conformational changes are a series of subsequent movements or whether they occur as a single concerted step is an open question; they establish however that the underlying protein building blocks must have the capability to maintain a stable, yet dynamic complex. The potential functional implication may be dramatic: the structural changes observed correlate with the reprogramming phase of the complex, during which the complex changes its substrate specificity from secreting the inner rod and needle filament proteins to the ones secreted at a later stage (translocon and effector proteins) [15*,33].

Recent Advances in X-ray Crystallography

Along with new EM data on the T3SS, there has been interesting and important progress at higher resolution with X-ray crystallographic studies of components of the system. The

Strynadka group published a series of structures of soluble subdomains of the three key proteins in the basal body [34,35*]. Beginning in 2005 with the smaller, lipidated inner-membrane component from *E. coli*, they published the crystal structure of EscJ(21–190) [34]. This subdomain packed as a tetramer in the asymmetric unit of the crystals. Intriguingly, studying the 6-fold symmetry axis in the crystals, they noticed that this tetramer formed a superhelix, that when constrained in two-dimensions, formed a 24-fold symmetric ring with dimensions very similar to the inner membrane ring from EM [11,34].

More recent work revealed a surprising set of conserved domains in the crystal structures of soluble, periplasmic subdomains of the T2SS secretin GspD [36], the T3SS secretin EscC (the *E. coli* outer-membrane secretin), and the T3SS protein PrgH (the larger of the two inner-membrane proteins, this one from *Salmonella*) [35*]. Figure 3 summarizes the conserved domain relationships.

The overall structures of these domains superficially appear somewhat similar: each has a three-stranded β -sheet core with two anti-parallel and interacting helices on one face of the sheet that run parallel to the strands. In fact, the overall folds can be roughly superimposed. However, the folds are distinct in their topology (Figure 3). One domain is a $\beta\alpha\beta\beta\alpha$ fold (strand-helix-strand-strand-helix), whereas the other domain is an $\alpha\beta\beta\alpha\beta$ fold (helix-strand-strand-helix-strand). This distinction is important in classifying the domains that reoccur as building blocks of the basal body.

For example, EscJ possesses each of these two domains: a $\beta\alpha\beta\beta\alpha$ fold (henceforth D1) is followed by an $\alpha\beta\beta\alpha\beta$ fold (henceforth D2). Despite a complete lack of detectable sequence similarity, the other inner-membrane protein, PrgH from *Salmonella*, was recently shown to possess two domains homologous to the D2 subdomain of EscJ. Completing the surprise, the recent crystal structure of the outer membrane secretins EscC and GspD show that they each possess a subdomain homologous to D1. These crystal structures reveal a completely unexpected relationship between the three individual proteins of the basal body (and indicated a high degree of conservation in the secretin family of outer membrane channels). Essentially, it appears that a large portion of the T3SS is composed of a highly similar set of primary building blocks.

The potential implications, however, may be even broader. As described, EscJ was modeled to form a 24-fold symmetric ring structure in the inner membrane. The domain homology between PrgH and EscJ (the two inner membrane proteins), as well as the relative orientation between the two domains, suggested that, like EscJ, PrgH could oligomerize into a highly symmetric ring structure [35*]. Furthermore, as the secretin family is known to generally form rings of 12–14 fold symmetry [16*,32,37–39], the presence of D1-like domains in EscC and GspD was suggestive of a ring-association. Therefore, the presence of the same fold in these ring-forming proteins, which in the case of EscJ had been shown to mediate a tetramer in the crystal structure that could be modeled as a ring structure, led to the hypothesis that these domains function generally, at least in T3SSs, as “ring-forming” motifs [35*]. Subsequent docking of the modeled rings into the EM maps led to several differing topological models [16*,35*].

The D1 subdomain is homologous to the eukaryotic RNA and DNA binding KH-domain [36,40]. It also shares structural similarity (but no sequence conservation) to bacterial and phage systems that function to bind peptidoglycan [41]. Therefore, in addition to a possible ring-forming function, the D1 domains may function to anchor the rings in the peptidoglycan space between the inner and outer membranes. However, the widespread appearance of the fold likely implies that it is a common structural core to many proteins of unrelated function. Whether these subdomains that continue to reappear in the T2SS and T3SS are a generalized

scaffold that has been co-opted for many purposes, or whether it mediates ring formation in other systems, is unclear. The fact that, to date, none of the fragments crystallized form rings by themselves in solution, has made it difficult to establish the ring-forming motif hypothesis directly.

Conclusions and Future directions

The progress in the structure and function of the T3SS gives great confidence that this complicated virulence molecular machine is amenable to detailed mechanistic studies. Future work will undoubtedly be centered on deriving higher resolution electron microscopic reconstructions of the apparatus, and in particular clarifying and applying the true symmetry of the system to improve resolution. Such maps in conjunction with further progress in crystallography and molecular modeling will allow for unprecedented accuracy in the building of models for this secretion machine. The ultimate goal will be to construct high-resolution models of the T3SS that reveal the mechanism of its function as a molecular syringe.

Acknowledgments

This work was supported by NIH grant AI052182 (to C.E.S.) and by ZIT (Center for Innovation and Technology Vienna) grant CMCN (Center for Molecular and Cellular Nanostructure) Vienna (to T.C.M.)

Reference sand recommended reading

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

1. Galan JE, Wolf-Watz H. Protein delivery into eukaryotic cells by type III secretion machines. *Nature* 2006;444:567–573. [PubMed: 17136086]
2. Cornelis GR. The type III secretion injectisome. *Nat Rev Microbiol* 2006;4:811–825. [PubMed: 17041629]
3. Macnab RM. How bacteria assemble flagella. *Annu Rev Microbiol* 2003;57:77–100. [PubMed: 12730325]
4. Tampakaki AP, Fadouloglou VE, Gazi AD, Panopoulos NJ, Kokkinidis M. Conserved features of type III secretion. *Cell Microbiol* 2004;6:805–816. [PubMed: 15272862]
5. Horn M, Collingro A, Schmitz-Esser S, Beier CL, Purkhold U, Fartmann B, Brandt P, Nyakatura GJ, Droege M, Frishman D, et al. Illuminating the evolutionary history of chlamydiae. *Science* 2004;304:728–730. [PubMed: 15073324]
6. Cunnac S, Lindeberg M, Collmer A. *Pseudomonas syringae* type III secretion system effectors: repertoires in search of functions. *Curr Opin Microbiol* 2009;12:53–60. [PubMed: 19168384]
7. Parsot C. *Shigella* type III secretion effectors: how, where, when, for what purposes? *Curr Opin Microbiol* 2009;12:110–116. [PubMed: 19157960]
8. McGhie EJ, Brawn LC, Hume PJ, Humphreys D, Koronakis V. *Salmonella* takes control: effector-driven manipulation of the host. *Curr Opin Microbiol* 2009;12:117–124. [PubMed: 19157959]
9. Poueymiro M, Genin S. Secreted proteins from *Ralstonia solanacearum*: a hundred tricks to kill a plant. *Curr Opin Microbiol* 2009;12:44–52. [PubMed: 19144559]
10. Galan JE. Common themes in the design and function of bacterial effectors. *Cell Host Microbe* 2009;5:571–579. [PubMed: 19527884]
11. Moraes TF, Spreter T, Strynadka NC. Piecing together the type III injectisome of bacterial pathogens. *Curr Opin Struct Biol* 2008;18:258–266. [PubMed: 18258424]
12. Kubori T, Matsushima Y, Nakamura D, Uralil J, Lara-Tejero M, Sukhan A, Galan JE, Aizawa SI. Supramolecular structure of the *Salmonella typhimurium* type III protein secretion system. *Science* 1998;280:602–605. [PubMed: 9554854]
13. Kimbrough TG, Miller SI. Contribution of *Salmonella typhimurium* type III secretion components to needle complex formation. *Proc Natl Acad Sci U S A* 2000;97:11008–11013. [PubMed: 10984518]

14. Marlovits TC, Kubori T, Sukhan A, Thomas DR, Galan JE, Unger VM. Structural insights into the assembly of the type III secretion needle complex. *Science* 2004;306:1040–1042. [PubMed: 15528446]
- 15*. Marlovits TC, Kubori T, Lara-Tejero M, Thomas D, Unger VM, Galan JE. Assembly of the inner rod determines needle length in the type III secretion injectisome. *Nature* 2006;441:637–640. [PubMed: 16738660]
- 16*. Hodgkinson JL, Horsley A, Stabat D, Simon M, Johnson S, da Fonseca PC, Morris EP, Wall JS, Lea SM, Blocker AJ. Three-dimensional reconstruction of the *Shigella* T3SS transmembrane regions reveals 12-fold symmetry and novel features throughout. *Nat Struct Mol Biol* 2009;16:477–485. [PubMed: 19396171]
17. Crago AM, Koronakis V. Salmonella InvG forms a ring-like multimer that requires the InvH lipoprotein for outer membrane localization. *Mol Microbiol* 1998;30:47–56. [PubMed: 9786184]
18. Daefler S, Russel M. The Salmonella typhimurium InvH protein is an outer membrane lipoprotein required for the proper localization of InvG. *Mol Microbiol* 1998;28:1367–1380. [PubMed: 9680224]
19. Lario PI, Pfuertner RA, Frey EA, Creagh L, Haynes C, Maurelli AT, Strynadka NC. Structure and biochemical analysis of a secretin pilot protein. *Embo J* 2005;24:1111–1121. [PubMed: 15775974]
20. Zenk SF, Stabat D, Hodgkinson JL, Veenendaal AK, Johnson S, Blocker AJ. Identification of minor inner-membrane components of the *Shigella* type III secretion system ‘needle complex’. *Microbiology* 2007;153:2405–2415. [PubMed: 17660405]
21. Sekiya K, Ohishi M, Ogino T, Tamano K, Sasakawa C, Abe A. 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 U S A* 2001;98:11638–11643. [PubMed: 11562461]
22. Ogino T, Ohno R, Sekiya K, Kuwae A, Matsuzawa T, Nonaka T, Fukuda H, Imajoh-Ohmi S, Abe A. Assembly of the type III secretion apparatus of enteropathogenic *Escherichia coli*. *J Bacteriol* 2006;188:2801–2811. [PubMed: 16585741]
23. Mueller CA, Broz P, Cornelis GR. The type III secretion system tip complex and translocon. *Mol Microbiol* 2008;68:1085–1095. [PubMed: 18430138]
24. Akeda Y, Galan JE. Chaperone release and unfolding of substrates in type III secretion. *Nature* 2005;437:911–915. [PubMed: 16208377]
25. Hensel M, Shea JE, Gleeson C, Jones MD, Dalton E, Holden DW. Simultaneous identification of bacterial virulence genes by negative selection. *Science* 1995;269:400–403. [PubMed: 7618105]
26. Li J, Ochman H, Groisman EA, Boyd EF, Solomon F, Nelson K, Selander RK. Relationship between evolutionary rate and cellular location among the Inv/Spa invasion proteins of *Salmonella enterica*. *Proc Natl Acad Sci U S A* 1995;92:7252–7256. [PubMed: 7638176]
27. Macnab RM. Type III flagellar protein export and flagellar assembly. *Biochim Biophys Acta* 2004;1694:207–217. [PubMed: 15546667]
28. Muller SA, Pozidis C, Stone R, Meesters C, Chami M, Engel A, Economou A, Stahlberg H. Double hexameric ring assembly of the type III protein translocase ATPase HrcN. *Mol Microbiol* 2006;61:119–125. [PubMed: 16824099]
29. Zarivach R, Vuckovic M, Deng W, Finlay BB, Strynadka NC. Structural analysis of a prototypical ATPase from the type III secretion system. *Nat Struct Mol Biol* 2007;14:131–137. [PubMed: 17237797]
30. Zarivach R, Deng W, Vuckovic M, Felise HB, Nguyen HV, Miller SI, Finlay BB, Strynadka NC. Structural analysis of the essential self-cleaving type III secretion proteins EscU and SpaS. *Nature* 2008;453:124–127. [PubMed: 18451864]
31. Blocker A, Jouihri N, Larquet E, Gounon P, Ebel F, Parsot C, Sansonetti P, Allaoui A. Structure and composition of the *Shigella flexneri* “needle complex”, a part of its type III secretion. *Mol Microbiol* 2001;39:652–663. [PubMed: 11169106]
32. Chami M, Guilvout I, Gregorini M, Remigy HW, Muller SA, Valerio M, Engel A, Pugsley AP, Bayan N. Structural insights into the secretin PulD and its trypsin-resistant core. *J Biol Chem* 2005;280:37732–37741. [PubMed: 16129681]
33. Wood SE, Jin J, Lloyd SA. YscP and YscU switch the substrate specificity of the *Yersinia* type III secretion system by regulating export of the inner rod protein YscI. *J Bacteriol* 2008;190:4252–4262. [PubMed: 18424518]

34. Yip CK, Kimbrough TG, Felise HB, Vuckovic M, Thomas NA, Pfuetzner RA, Frey EA, Finlay BB, Miller SI, Strynadka NC. Structural characterization of the molecular platform for type III secretion system assembly. *Nature* 2005;435:702–707. [PubMed: 15931226]
- 35*. Spreter T, Yip CK, Sanowar S, Andre I, Kimbrough TG, Vuckovic M, Pfuetzner RA, Deng W, Yu AC, Finlay BB, et al. A conserved structural motif mediates formation of the periplasmic rings in the type III secretion system. *Nat Struct Mol Biol* 2009;16:468–476. [PubMed: 19396170]
36. Korotkov KV, Pardon E, Steyaert J, Hol WG. Crystal structure of the N-terminal domain of the secretin GspD from ETEC determined with the assistance of a nanobody. *Structure* 2009;17:255–265. [PubMed: 19217396]
37. Opalka N, Beckmann R, Boisset N, Simon MN, Russel M, Darst SA. Structure of the filamentous phage pIV multimer by cryo-electron microscopy. *J Mol Biol* 2003;325:461–470. [PubMed: 12498796]
38. Collins RF, Frye SA, Kitmitto A, Ford RC, Tonjum T, Derrick JP. Structure of the *Neisseria meningitidis* outer membrane PilQ secretin complex at 12 Å resolution. *J Biol Chem* 2004;279:39750–39756. [PubMed: 15254043]
39. Burghout P, van Boxtel R, Van Gelder P, Ringler P, Muller SA, Tommassen J, Koster M. Structure and electrophysiological properties of the YscC secretin from the type III secretion system of *Yersinia enterocolitica*. *J Bacteriol* 2004;186:4645–4654. [PubMed: 15231798]
40. Valverde R, Edwards L, Regan L. Structure and function of KH domains. *FEBS J* 2008;275:2712–2726. [PubMed: 18422648]
41. Mishima M, Shida T, Yabuki K, Kato K, Sekiguchi J, Kojima C. Solution structure of the peptidoglycan binding domain of *Bacillus subtilis* cell wall lytic enzyme CwlC: characterization of the sporulation-related repeats by NMR. *Biochemistry* 2005;44:10153–10163. [PubMed: 16042392]

Figure 1. An Organic Nanosyringe

A schematic representation of the protein Type III Secretion System (T3SS) of *Salmonella* focusing on the basal body of the nanomachine. The inner and outer membranes of the bacterium are shown along with the peptidoglycan layer. The three proteins of the basal body (PrgH, PrgK, and InvG from the *Salmonella* SPI-1 pathogenicity island) are labeled, and drawn to roughly correspond the EM reconstructions and biochemical experiments localizing the polypeptides. The needle filament (PrgI) is also depicted, as are the tip protein (SipD) and the translocon pore (SipB/SipC) in the eukaryotic plasma membrane. Approximate dimensions for the apparatus are also shown.

Figure 2. Architecture of needle complexes from Shigella and Salmonella

Cut-away view of side views of the base and the needle complexes from Shigella (EmDep accession number EMD-1617) and Salmonella obtained from cryo-electron microscopy images and single particle reconstructions (EmDep accession numbers 1100, 1224).

Nomenclature given for the Shigella and Salmonella structure, respectively: OMR (outer membrane ring), (inner membrane ring), Conn. (connector), Le (leg), OM (outer membrane ring), (IM (inner membrane ring)). For the Salmonella density, the distribution of protein density along the central longitudinal section is shown as contour lines. Basal view of the Shigella needle complex base showing twelve spokes connecting the socket/cup with the IMR.

Figure 3. A structural building-block for the T3SS

Shown are the related structural domains of GspD, EscJ, EscC, and PrgH divided into two classes based on protein fold (PDB accession codes 3EZJ, 1YJ7, 3GR5, and 3GR0, respectively). A topology diagram is shown on the far right for each class to further illustrate the connectivity differences that are present in these folds despite the very similar three-dimensional appearance of the three-stranded β -sheet capped on one face by two interacting, anti-parallel helices. The location of these proteins in the basal body is roughly indicated on the left.