Delivering dangerous cargoes

Ralph R. Isberg and Guillaume Duménil

The structures of four type III secretion chaperones provide insights into how they function in the translocation process.

© 2001 Nature Publishing Group http://structbio.nature.com

For much of the past century, microbiologists have been aware that bacteria are capable of secreting protein toxins that cause the death, dysfunction or aberrant regulation of host cells. It was assumed that most anti-host factors were secreted by the pathogens and then internalized by receptor-mediated endocytosis or some other pinocytotic vesicular uptake process. Recent advances have turned this model on its head, as it now appears that a wide variety of Gram-negative pathogens, including *Salmonella,* some pathogenic *Escherichia coli* species and plant pathogenic *Pseudomonas*, have the ability to directly inject proteins from the bacterium into the host eukaryotic cell cytoplasm[1]. The injection process is accomplished by the use of specialized protein secretion/translocation systems that transiently allow proteins to flow from the cytoplasm of the invader into the host cell.

Establishment of this protein flux is particularly problematic for Gram-negative bacteria[2]. The envelope of these microorganisms is complex, containing inner and outer membranes as well as a periplasmic space. This means that movement of proteins from the bacterial cytoplasm into the host cell involves transit across three membranes. Furthermore, there are distinct limitations to the housekeeping secretory systems that export signal sequence-containing proteins: they cannot readily promote secretion of proteins across the outer membrane. Bacteria have evidently overcome these problems by developing specialized export complexes, of which the most intensively studied are called type III secretion systems (TTSS)[3]. The proteins translocated by these systems, called TTSS effectors, have a variety of activities that damage host cells. These include hijacking of cytoskeletal functions and signal transduction cascades by virtue of having functions such as GTPase activation, guanosine exchange and tyrosine phosphatase activities (as seen in *Yersinia* and *Salmonella* species), or *via* insertion of unique receptors into the host cell membrane (as observed for enteropathogenic *E. coli*). Translocation of some of these effectors requires a set of chaperone proteins, but how they help deliver the effectors is not clearly understood. As reported in this issue[4] and in the November issue[5] of *Nature Structural Biology*, and in a recent issue of *Nature*[6], three papers described the crystal structures of four different TTSS chaperones. These studies provide insights into the functions of these chaperones in the translocation process.

Chaperoning effector translocation

TTSS, which may be encoded by as many as 20 or more genes, are marked by a number of common features. First, all have a core of at least nine genes of high sequence similarity to those found in bacterial flagella apparati[3]. In some cases, at least a portion of this apparatus has been isolated or visualized by electron microscopy and found to have a syringe-like appearance, which may represent the channel through which the exported proteins travel[7,8]. Second, two proteins are translocated *via* the TTSS into the host cell membrane[9,10]. These proteins, called translocator proteins, act as either pores or sites of fusion between the eukaryotic cell and the bacterial envelope[9–11]. Finally, the proteins translocated across the TTSS all lack classic hydrophobic N-terminal signal sequences, which would normally be required to direct secreted proteins into well-characterized export pathways. Instead, the information for export of these proteins lies in either an N-terminal signal of unclear nature, or is conferred by binding to soluble secretion chaperones that presumably maintain the export competency of the translocated protein[12].

The association of TTSS effectors with chaperones is reminiscent of bacterial signal sequence-dependent secretory systems, in which the general chaperone, SecB, maintains the exported proteins in a secretion-competent form[2,13]. For TTSS, however, there are several export chaperones of little obvious sequence similarity to one another, with each binding a single, or at most a few, exported proteins. Many of the chaperones — that is, those not involved in binding the translocator proteins — recognize a single contiguous region of the exported protein that appears to have no function other than chaperone binding[14–17].

The role of the *Yersinia* TTSS chaperone SycE in promoting translocation of the YopE RhoGAP protein[18,19] has been closely investigated[16,17]. The N-terminal 77 amino acids of YopE seem to be solely involved in promoting translocation to animal cells[16,20]. As is true of most TTSS effectors, the region covering the most N-terminal 15 amino acids is sufficient to confer fairly efficient translocation into mammalian cells in strains lacking most of the other TTSS substrates[16,20]. However, the nature of this short signal is very controversial. Evidence exists supporting conflicting ideas that the targeting information in this region is either due to the structure of the mRNA or conferred by the peptide sequence itself, which is proposed to have amphipathic helix properties[21,22]. Directly downstream from this signal is the region that binds the SycE chaperone[20]: the presence of this region obligate the requirement of SycE for YopE translocation[20].

In a number of cases, the TTSS chaperone has been shown to prevent aggregation or degradation of effector molecules that contain the intact chaperone binding region. One of the major questions is whether this is the only function of this interaction, or whether the chaperone–effector complex must exist to allow the effector to be efficiently recognized by the TTSS. There is now circumstantial evidence that the latter model is correct, at least for YopE[15].

Common features

The recently solved crystal structures of four TTSS chaperones — SycE from *Y. tuberculosis*[5], CesT from an enteropathogenic *E. coli* and SigE from *Salmonella*[4], and a complex between *Salmonella* SicP and the chaperone binding region of SptP[6] — allow direct investigation of some of the previously noted properties of these proteins. First, the presence of a predicted C-terminal amphipathic α-helix was con-

© 2001 Nature Publishing Group http://structbio.nature.com

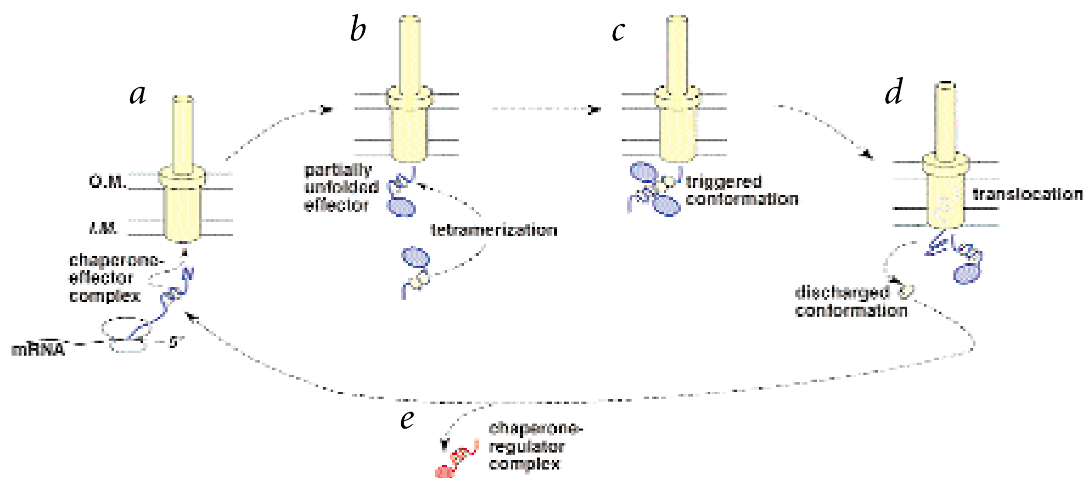

Fig. 1 Chaperone-effector cycle for translocation of proteins *via* TTSS. Model is described in text. ***a***, TTSS chaperone associates as a dimer with a nascent effector polypeptide during translation. Either the 5′ mRNA or an N-terminal signal allows association of the nascent chain with the translocation apparatus. ***b***, After translation is completed, the chaperone binding domain remains unfolded while the effector domain is folded, and the effector–chaperone complex is targeted to the TTSS, where it associates with an identical complex (tetramerization). ***c***, The 4:2 complex remains triggered in the TTSS, awaiting a signal from the host cell to be injected through the translocation system. ***d***, The chaperone undergoes a conformation change that releases the effector (discharged conformation), and regions of the effector that were previously folded proceed to unfold during translocation. ***e***, The discharged effector is now free to associate with transcriptional regulators (such as LcrQ), or be recharged by binding to a nascent effector during translation.

firmed in these structures[23], but the proposed role of hydrophobic residues in binding substrate cannot be correct. The hydrophobic face of the C-terminal helix is not surface exposed in any structure and not involved in effector binding in the SptP–SicP cocrystal[4–6]. Secondly, the dimeric nature of the chaperones appears to be universal[24]. This is true for each of the solved structures, although the nature of the dimer interface varies among the chaperones. The simplest interface is SycE, which has a helix and loop from one monomer plunged into a sheet of the partner subunit[5]. In the case of the enteropathogenic *E. coli* CesT (the chaperone for the bacterial intimin receptor Tir[4] which allows the bacterium to adhere to host cells), the solved dimer interface is huge and involves a domain swap between the individual monomers. Finally, the SptP–SicP cocrystal reveals the unexpected structure of a dimer of chaperone dimers, which had not been foreseen by most previous biochemical studies[6].

Other previously noted properties of the TTSS chaperones were that they were generally quite acidic, but otherwise exhibited very low primary sequence similarity between each other[12]. As expected, each of the solved structures showed a large negatively charged surface, but in addition each showed extensive hydrophobic patches on the surface. The cocrystal of SicP–SptP demonstrates that these hydrophobic patches are directly involved in binding the SptP effector[6], and their importance in CesT was demonstrated by site-directed mutagenesis[4]. The low sequence similarity, on the other hand, may be more apparent than real. Birtalan and Ghosh[5] point out that there is strong sequence conservation in regions of the dimer interface, the hydrophobic faces of amphipathic helices and in internal packing residues.

TTSS chaperones and the folding of effectors

The structure of the N-terminal fragment of SptP in complex with the SicP chaperone comes out strongly in support of the idea that chaperones are involved in presenting a secretion-competent conformation of the effector to the TTSS[6]. The partially unfolded SptP peptide is presented across the surface of SicP, in a fashion that is grossly reminiscent of DNA wrapped around a histone. SptP has considerable secondary structure in this complex, but its individual structural elements are extended about the SicP surface, maintained by contacts with four largely hydrophobic surface patches on SicP. The most controversial aspect of this structure is the 4:2 SicP:SptP stoichiometry, in which two SicP homodimers bind two SptP monomers. Amazingly, the SicP homodimers are held together by an SptP ‘helix exchange’, as helices at the carboxyl terminal ends of each of the chaperone binding domains of the SptP monomers cross over to link the two homodimers together.

In contrast to this cocrystal, the intact biochemical activities of the Tir and SigD effectors bound to chaperones indicate that the unfolding observed in the SicP–SptP structure is probably limited to the chaperone binding domains of the effectors[23]. As pointed out by Strynadka and coworkers[4], since chaperone binding and the effector activities are in two different regions of the protein, interaction with the chaperone would not necessarily be expected to disrupt these biochemical activities. It does emphasize, however, that a proposed secretion-competent conformation may be limited to a particular domain of the translocated protein. These results also do not rule out the possibility that the effector domain may unfold during translocation to the mammalian cell, as had been observed previously with anthrax toxin[25].

Domain exchange: a reality?

As mentioned above, two of the four chaperone structures have domain or helix exchanges between protein subunits that could be interpreted as crystallographic artifacts. These unusual structural elements, however, could have important biological functions. For instance, there must be some explanation for how the high affinity effector–chaperone interaction[15] can be reversed to allow translocation to occur. In the case of CesT[4], the crystal structure may represent a conformation that facilitates discharge of the effector during translocation, or that allows binding to proteins other than

© 2001 Nature Publishing Group http://structbio.nature.com

© 2001 Nature Publishing Group http://structbio.nature.com

the TTSS effector. For the *Yersinia* SycH and the *Salmonella* SicA chaperones, discharge of the effector results in chaperone binding of the transcriptional regulatory factors LcrQ and InvF, respectively[26,27]. Similarly, the helix exchange leading to the 4:2 SicP:SptP stoichiometry may be explained by postulating that this is the complex found in association with the membrane translocator. Most previous work had indicated a 2:1 chaperone:effector stoichiometry, but these studies were all performed on soluble proteins[14]. Furthermore, data consistent with the binding of tetrameric SycE to YopE has been presented[15], and dimer-tetramer transitions have previously been observed for the SecB secretion chaperone[13,28].

Assuming that the domain exchange structures represent species of the chaperone at different stages of the translocation process, a model can be constructed for the different conformations (Fig. 1). In the first step of the cycle, the dimeric chaperone interacts with the nascent effector polypeptide during translation (Fig. 1*a*). The chaperone–effector complex then interacts directly with the TTSS. Once associated with the TTSS, a second chaperone–effector complex is directed to this site (Fig. 1*b*), helix exchange occurs, and a dimer of 2:1 complexes is placed in a triggered position in the TTSS (Fig. 1*c*). After contact with mammalian cells, immediate release of the effector is signaled. During the translocation process, the mature folded activity domain of the effector may partially unfold (Fig. 1*b*) (similar to the translocation of the anthrax toxin lethal factor[25]), facilitating transfer to the mammalian cell. After release of the effector, the chaperone assumes the 'discharged conformation' (Fig. 1*d*) seen in the CesT structure, which directs the chaperone away from the TTSS, and may allow interaction with other factors in the bacterial cell, such as transcriptional regulators (Fig. 1*e*).

Clearly the big tests for the implications from the described structures will be to determine if there is further structural support for the surprising results obtained thus far, and to determine if the unusual aspects of these structures have biological relevance. Most interesting would be a comparison of SycH binding to its two partners, the YopH tyrosine phosphatase and the LcrQ regulatory molecule. Based on the recent successes described here, further structural information on this fascinating biological system should be coming on the scene soon.

Ralph R. Isberg is an investigator of the Howard Hughes Medical Institute. Ralph R. Isberg and Guillaume Duménil are in the Department of Molecular Biology and Microbiology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, Massachusetts 02111, USA. Correspondence should be addressed to R.R.I. email: ralph.isberg@tufts.edu

1. Finlay, B.B. & Falkow, S. *Microbiol. Mol .Biol. Rev.* **61**, 136–169 (1997).
2. Duong, F., Eichler, J., Price, A., Leonard,M. R. & Wickner, W. *Cell* **91**, 567–573 (1997).
3. Hueck, C.J. *Microbiol. Mol .Biol. Rev.* **62**, 379–433 (1998).
4. Luo, Y. *et al. Nature Struct. Biol.* **8**, 1042–1047 (2001).
5. Birtalan, S. & Ghosh, P. *Nature Struct. Biol.* **8**, 974–978 (2001).
6. Stebbins, C.E. & Galán, J.E. *Nature* **414**, 77–81 (2001).
7. Kubori, T., Sukhan, A., Aizawa, S.I. & Galan, J.E. *Proc. Natl. Acad. Sci. USA* **97**, 10225–10230 (2000).
8. Tamano, K. *et al. EMBO J.* **19**, 3876–3887 (2000).
9. Blocker, A. *et al. J.Cell.Biol.* **147**, 683–693 (1999).
10. Tardy, F. *et al. EMBO J.* **18**, 6793–6799 (1999).
11. Hayward, R.D., McGhie, E.J. & Koronakis, V. *Mol. Microbiol.* **37**, 727–739 (2000).
12. Cornelis, G.R. & Van Gijsegem, F. *Annu. Rev. Microbiol.* **54**, 735–774 (2000).
13. Muren, E.M., Suciu, D., Topping, T.B., Kumamoto, C.A. & Randall, L.L. *J. Biol. Chem.* **274**, 19397–19402 (1999).
14. Abe, A. *et al. Mol. Microbiol.* .**33**, 1162–1175 (1999).
15. Cheng, L.W. & Schneewind, O. *J. Biol. Chem.* **274**, 22102–22108 (1999).
16. Schesser, K., Frithz-Lindsten, E. & Wolf-Watz, H. *J. Bacteriol.* **178**, 7227–7233 (1996).
17. Woestyn, S., Sory, M.P., Boland, A., Lequenne, O. & Cornelis, G.R. *Mol. Microbiol.* **20**, 1261–1271 (1996).
18. Black, D.S. & Bliska, J.B. *Mol. Microbiol.* **37**, 515–527 (2000).
19. von Pawel-Rammingen, U. *et al. Mol. Microbiol.* **36**, 737–748 (2000).
20. Boyd, A.P., Lambermont, I. & Cornelis, G.R. *J. Bacteriol* .**182**, 4811–4821 (2000).
21. Anderson, D.M. & Schneewind, O. *Science* **278**, 1140–1143 (1997).
22. Lloyd, S.A., Norman, M., Rosqvist, R. & Wolf-Watz, H. *Mol. Microbiol*. **39**, 520–531 (2001).
23. Wattiau, P., Bernier, B., Deslee, P., Michiels, T. & Cornelis, G.R. *Proc. Natl. Acad. Sci. USA* **91**, 10493–10497 (1994).
24. Lloyd, S.A., Forsberg, A., Wolf-Watz, H. & Francis, M.S. *Trends Microbiol*. **9**, 367–371 (2001).
25. Wesche, J., Elliott, J.L., Falnes, P.O., Olsnes, S. & Collier, R.J. *Biochemistry* **37**, 15737–15746 (1998).
26. Cambronne, E.D., Cheng, L.W. & Schneewind, O. *Mol. Microbiol*. **37**, 263–273 (2000).
27. Darwin, K.H. & Miller, V.L. *EMBO J.* **20**, 1850–1862 (2001).
28. Topping, T.B., Woodbury, R.L., Diamond, D.L., Hardy, S.J. & Randall, L.L. *J. Biol. Chem.* **276**, 7437–7441 (2001).

Opening the door to mitochondrial protein import

Robert E. Jensen and Arthur E. Johnson

After reconstitution into liposomes, Tim23p, a mitochondrial inner membrane protein required for protein import, forms an aqueous pore that is activated by a transmembrane potential and mitochondrial targeting peptides. A report in this issue suggests that proteins are translocated into the mitochondrial matrix through a channel formed by Tim23p. These data also suggest a mechanism by which protein import can occur without disrupting the permeablility barrier of the inner membrane.

More than 90% of mitochondrial proteins are synthesized on cytoplasmic ribosomes and then imported into mitochondria. Previous research on mitochondrial protein import has identified many components of the import machinery, including members of the TOM complex in the mitochondrial outer membrane (OM), and the TIM23 and TIM22 complexes in the inner membrane (IM)[1–4]. However, questions remain about the mechanisms of protein import into the mitochondrion. For example, do imported proteins pass directly through the nonpolar interior of the mitochondrial OM and IM, or do they pass through aqueous pores in the membranes during translocation? In addition, since a proton gradient across

© 2001 Nature Publishing Group http://structbio.nature.com