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SecA: a tale of two protomers

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Summary

Bacteria, Archaea and Eukaryotes have evolved a plethora of mechanisms to translocate proteins across their various membranes. The bacterial Sec pathway is ubiquitous and essential for cell viability and is used by most proteins destined for the inner membrane, the periplasm or beyond. In bacteria, Sec system components include the heterotrimers SecY/SecE/SecG and SecD/SecF/YajC and the peripherally associated ATPase motor SecA. SecA in solution is mainly dimeric. Unexpectedly, structures of SecA dimers from different or even the same bacterium do not have a consistent dimerization interface. Analysis of the functional assembled translocase complexes blurs the picture even further as the functional quaternary state of the SecYEG channel is also disputed. Several experimental approaches tried to define the oligomeric state of SecA during preprotein 'pushing' through SecYEG. One high-resolution SecA–SecYEG complex has been visualized. This snapshot might be a step closer to the actual translocating machinery. Nevertheless, because of the use of detergent, the true quartenary state of the translocase might have been disturbed. Hence, even after this and other studies, several issues remain puzzling. New approaches must be combined with current tools to gain insight into the functionally relevant quartenary states of SecA and SecYEG during preprotein translocation.

Introduction

More than a third of the total cellular proteome becomes incorporated into biological membranes or is transported across them. Bacteria have invented more than 16 systems that traffic proteins to various extracytoplasmic locations (Papanikou *et al.*, 2007). Most proteins destined to exit the cytoplasm are synthesized as 'preproteins' bearing amino-terminal cleavable signal peptides (Blobel and Dobberstein, 1975) and utilize the Sec pathway (Fig. 1). Export can take place co-translationally (Fig. 1, I) (as is common for most inner membrane proteins and some lipoproteins) or post-translationally, i.e. after most or all of the chain has been synthesized (stage II; as is common for most periplasmic and outer membrane proteins and lipoproteins). The two pathways engage different piloting molecules to usher the exiting chains: the signal recognition particle is the co-translational pilot (Froderberg *et al.*, 2004; Halic and Beckmann, 2005), while SecB (Ullers *et al.*, 2004; Zhou and Xu, 2005), cytoplasmic SecA (Vrontou and Economou, 2004; Gouridis *et al.*, 2009) or possibly even other chaperones (Maier *et al.*, 2005) are at play in the post-translational mode. Both pathways eventually converge on the protein-conducting channel formed by a single or twin version of the membrane-embedded SecYEG heterotrimer (Osborne *et al.*, 2005; Papanikou *et al.*, 2007; Gouridis *et al.*, 2009). The ATPase motor SecA is a peripheral subunit of the channel (Papanikou *et al.*, 2007) and is called upon whenever large polar preprotein sequences are to be transported (Hegde and Bernstein, 2006). SecA interacts with preproteins in the cytoplasm or, with higher affinity, when it is bound to SecYEG (Gouridis *et al.*, 2009). It is this post-translational, SecA-mediated leg of the translocation pathway that will be discussed here. Other chaperones such as SecB (Zhou and Xu, 2005), trigger factor (Maier *et al.*, 2005) and possibly even DnaK (Agashe *et al.*, 2004; Genevaux *et al.*, 2007) might participate in the ribosome to membrane targeting process of secretory proteins. After preprotein binding to the translocase, SecA converts the chemical energy of ATP to mechanical work that drives the threading and moves the preprotein through the SecYEG channel (Papanikou *et al.*, 2007). This SecA-mediated secretion process involves a complex series of precise molecular events that have been discussed in detail elsewhere (Papanikou *et al.*, 2007; Rapoport, 2007). Briefly, some of these in temporal order are: the binding of signal peptides to SecA

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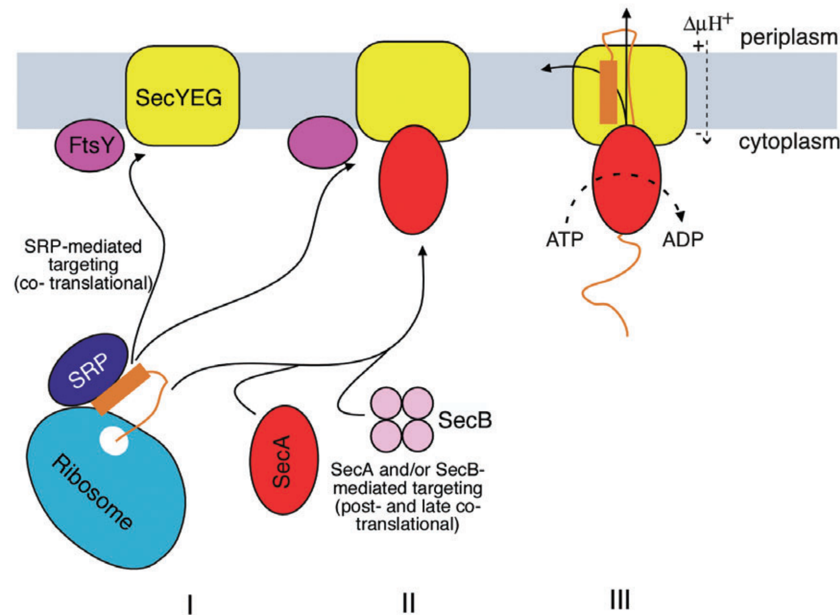

Fig. 1. Schematic model of preprotein secretion pathways in bacteria. The translocase complex consists of the membrane-embedded heterotrimeric complex SecYEG (yellow) and the peripherally associated ATPase motor SecA (red). Proteins destined for the inner membrane and beyond are synthesized as preproteins bearing a cleavable signal peptide (orange). I, membrane proteins and proteins with extremely hydrophobic signal peptides exit the cytoplasm co-translationally. During this process the ribosome-bound signal recognition particle (SRP) recognizes these signal sequences and targets the nascent preprotein to SecYEG, with the help of FtsY (pink), which acts as a SRP docking receptor in the cytoplasm and at the membrane. Additional proteins such as YidC, the bacterial homologue of the mitochondrial Oxa1 (Xie and Dalbey, 2008) (not shown), might participate in this process. II, SecB and/or SecA interact with preproteins when their synthesis is almost complete and transfers them to SecYEG. This largely post-translational mode of translocation occurs mainly with secretory preproteins. Another heterotrimeric membrane-embedded complex, SecD/SecF/YajC (not shown) binds transiently to SecYEG and optimizes secretion. III, the mechanical 'pushing' of preproteins through SecYEG is energized by ATP hydrolysis via the SecA motor (Tomkiewicz *et al*., 2006) and by the proton motive force (PMF or $\Delta\mu H^+$) when the chain is not bound to SecA (Schiebel *et al*., 1991). Signal peptides are cleaved off at the periplasmic face of the membrane by signal peptidase I or, II (for those on lipoproteins) (not shown).

(Gelis *et al*., 2007; Gouridis *et al*., 2009) and to the lateral gate of SecY (Osborne and Rapoport, 2007), the allosteric triggering of the translocase by signal peptides and the trapping of mature domains in the export channel (Gouridis *et al*., 2009), the activation of the helicase motor ATPase by opening of an electrostatic gate (Karamanou *et al*., 2007), the melting and dissociation of the SecA motor domains (Keramisanou *et al*., 2006) that cause ADP release (Fak *et al*., 2004; Karamanou *et al*., 2007; Robson *et al*., 2009), the release of a periplasmic 'plug' element in SecY that reduces rigidity of the channel (Li *et al*., 2007) and prepares it for opening and the interaction and possible pushing of the trapped mature domain by the IRA1 helix-loop-helix element in SecA known to act as an intramolecular regulator of its ATPase (Karamanou *et al*., 1999; Vrontou *et al*., 2004; Erlandson *et al*., 2008; Zimmer *et al*., 2008). Throughout the secretion process the preprotein is thought to be maintained in a non-native, possibly extended state.

Most proteins function as oligomeric assemblies. This can have various functional advantages, such as advanced allosteric control, regulatory flexibility and economic construction of large structures necessary for function (Marianayagam *et al*., 2004; Ali and Imperiali, 2005). Similarly, the quaternary oligomeric state of the functional translocase channel and its motor is central to understanding their function as it inpinges on important mechanistic questions. These include how secretory substrates are moved onto downstream subunits, if preproteins are maintained unfolded by more than one SecA molecule and whether large SecYEG/A assemblies can accommodate large membrane proteins prior to lateral release. As a result, the issue of oligomerization has attracted a lot of attention. Several lines of evidence indicate that both the channel (Manting *et al*., 2000; Bessonneau *et al*., 2002; Tziatzios *et al*., 2004; Scheuring *et al*., 2005) and the motor (Bessonneau *et al*., 2002; Or *et al*., 2002; Woodbury *et al*., 2002) undergo dynamic monomer–dimer equilibria and might even acquire higher-order states. While these transitions are now better documented, they are not yet comprehensively resolved or even directly linked to mechanical events of the secretion reaction. Data from

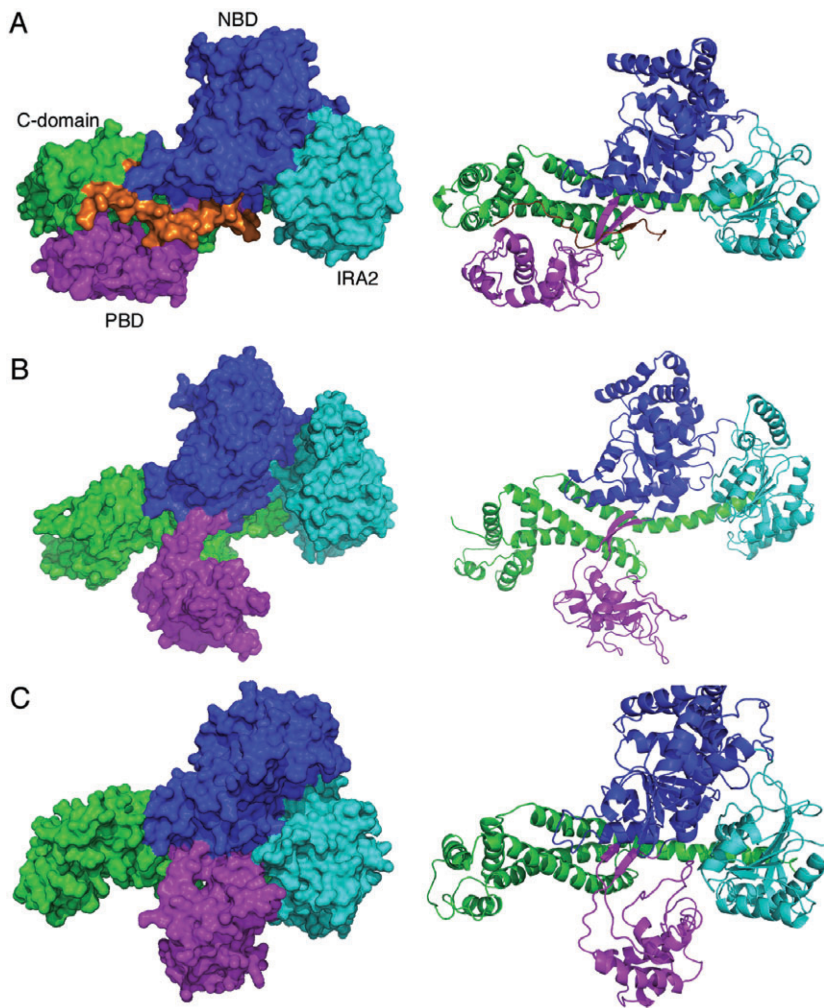

Fig. 2. Space filling (left) and ribbon models (right) of SecA protomers from three organisms: (A) (1M6N; *Bacillus subtilis*) (Hunt *et al*., 2002), (B) (2FSF; *Escherichia coli*) (Papanikolau *et al*., 2007) and (C) (3DIN; *Thermotoga maritima*) (Zimmer *et al*., 2008). SecA consists of four domains: the nucleotide binding domain (NBD; dark blue), the intramolecular regulator of ATPase 2 (IRA2; cyan), the preprotein binding domain (PBD; magenta) and the C-domain (green). Domain boundaries are as in Table 1. The three proteins were chosen to illustrate the major domain motions of the helicase motor sub-domains and the swivelling of the PBD. The extreme carboxyterminal residues of SecA (831–901 in *ec*SecA) termed C-tail are very flexible and control signal peptide accessibility to SecA (Gelis *et al*., 2007). In one structure (A; orange) they fold up against the body of SecA and interact with the stem of the PBD. SecYEG is bound as a vertical tube at the back of the SecA structures shown (see also the corresponding structure in Fig. 4C).

these studies, alternative interpretations and suggestions for future experiments will be presented below.

Form dictates function: the SecA protomer

SecA belongs to the superfamily 2 (SF2) 'DExH/D' proteins (Koonin and Gorbalenya, 1992; Vrontou and Economou, 2004; Papanikou *et al*., 2007). SF2 proteins include nucleic acid modifying enzymes (Solem *et al*., 2006; Yang and Jankowsky, 2006) and helicases (Cavanaugh *et al*., 2006; Cordin *et al*., 2006). In SecA, the helicase motor is formed by two domains: the discontinuous nucleotide binding domain (NBD; Fig. 2, dark blue; Table 1) and the intramolecular regulator of ATP hydrolysis (IRA2; light blue) and contains the nine conserved motifs of the DEAD helicase subfamily (Vrontou and Economou, 2004; Cordin *et al*., 2006; Papanikou *et al*., 2007). Nucleotides bind and get hydrolysed when sandwiched between the two helicase motor sub-domains. Nucleotide binding and release drive tightening and loosening of the motor respectively

Table 1. Organization and residue boundaries of SecA domains.

Organism	NBD (NBFI, NBD1)	PBD (PPXD)	IRA2 (NBFII, NBD2)	C-domain
E. coli	1–221, 376–416	222–375	417–621	622–836
M. tuberculosis	1–220, 356–397	221–355	398–608	608–835
B. subtilis	1–219, 356–396	219–355	397–567	568–780
T. thermophilus	1–221, 370–410	222–370	411–723	724–939
T. maritima	1–264, 401–441	265–400	442–613	614–816

Aminoacyl residues that demarkate SecA domains are indicated.
Names in parentheses indicate alternative names.

(Sianidis *et al*., 2001; Hunt *et al*., 2002; Keramisanou *et al*., 2006). These nucleotide-driven effects appear to be transferred to two 'specificity domains' (Sianidis *et al*., 2001; Gelis *et al*., 2007) that allow SecA to operate on polypeptides rather than nucleic acids. These two domains are bolted onto the helicase motor: the preprotein binding domain (PBD; magenta) that connects to NBD through a slender 'Stem' and the C-domain (green) (Table 1). The PBD is the site where preproteins bind on SecA (Papanikou *et al*., 2005; Gelis *et al*., 2007; Musial-Siwek *et al*., 2007) and from there they control conformation and ATP catalysis in the helicase motor (Karamanou *et al*., 2007). The C-domain binds like a latch to both helicase domains and suppresses the ATPase activity (Karamanou *et al*., 1999; Vrontou *et al*., 2004; Keramisanou *et al*., 2006; Mori and Ito, 2006). Cycles of ATP-controlled PBD (Gelis *et al*., 2007; Karamanou *et al*., 2007; Economou, 2008; Zimmer *et al*., 2008; Gouridis *et al*., 2009) and C-domain (Economou and Wickner, 1994; Karamanou *et al*., 1999; Vrontou *et al*., 2004; Mori and Ito, 2006; Zimmer *et al*., 2008) motions are expected to be responsible for somehow 'pushing' preprotein chains forward in the channel (Economou and Wickner, 1994; Papanikou *et al*., 2007; Economou, 2008; Zimmer *et al*., 2008). Swivelling of the PBD around its stem is a particularly prominent structural change (Fig. 2). Nevertheless, up to now there is no evidence that distinct PBD and C-domain motions are coupled to and driven by the presence of nucleotide or preprotein ligands.

O second protomer, where art thou?

SecA folds via a dimeric intermediate (Doyle *et al*., 2000), exists in a monomer to dimer equilibrium in solution with a dissociation constant of 0.25–0.5 μM (Woodbury *et al*., 2002) and is seen as a dimer by cryo-electron microscopy (cryo-EM) (Chen *et al*., 2008). At a cellular concentration estimated to be up to 8 μM (Akita *et al*., 1991) and split equally between the cytoplasm and a peripheral membrane state, the majority of cellular SecA is expected to be dimeric (Woodbury *et al*., 2002; Ding *et al*., 2003). Dimer formation is favoured by high temperature and concentration and disfavoured by high ionic strength (Woodbury *et al*., 2002), which leads to monomerization (Woodbury *et al*., 2002; Jilaveanu *et al*., 2005). These results indicate that electrostatic and possibly hydrophobic interactions are important to stabilize the dimer (Woodbury *et al*., 2002; Chen *et al*., 2008).

Eight high-resolution SecA structures have been solved by X-ray crystallography (Table 2) and one by nuclear magnetic reasonance (NMR) (Gelis *et al*., 2007). Low-resolution structures have been obtained by cryo-EM (Wang *et al*., 2003; Chen *et al*., 2007; 2008), atomic force microscopy (Wang *et al*., 2003), small angle X-ray scattering (Shilton *et al*., 1998) and small angle neutron scattering (SANS) (Bu *et al*., 2003). A structure of the isolated helicase motor has also been visualized with (Dempsey *et al*., 2002) or without its PBD domain (Nithianantham and Shilton, 2008). Most of the structures (Shilton *et al*., 1998; Bu *et al*., 2003; Sharma *et al*., 2003; Wang *et al*., 2003; Vassylyev *et al*., 2006; Zimmer *et al*., 2006; Chen *et al*., 2007; 2008; Papanikolau *et al*., 2007) contain SecA molecules packed as dimers (Fig. 3A–E). One structure contains a single protomer in the asymmetric unit but a crystallographic dimer can be discerned (Fig. 3F) (Hunt *et al*., 2002).

Surprisingly, the positioning of each protomer relative to one another is unique in every dimeric structure solved (Fig. 3) and the dimeric particles vary in diameter from ~10 to 15 nm (Table 2) (Shilton *et al*., 1998; Chen *et al*., 2008). Most dimers have an anti-parallel orientation (Fig. 3A–C, E and F) and their C-domains face towards opposite directions, whereas one (Fig. 3D) has its protomers oriented in a parallel fashion. Protomers are held together via electrostatic and hydrophobic interactions (Sharma *et al*., 2003; Zimmer *et al*., 2006; Chen *et al*., 2008). In the cryo-EM structure (Fig. 3B, *ec*SecA 2), the anti-parallel protomers lock onto each other with their helicase motors arranged back to back (Chen *et al*., 2008).

What is the reason for this remarkable variety in SecA dimers? First of all it can not be excluded that at least some of the crystallized dimers might not be physiologically relevant. Lest we forget, the SecA dimer is sensitive to high ionic strength and several of the crystallography studies made use of high concentrations of salts (Table 2). The highly charged and flexible SecA protomer might have first dissociated and then reassembled in some of the structures by making use of new interfaces dictated by crystal packing restraints. The hydrodynamic properties of SecA in the same solutions used for crystallization would help discriminate between various dimeric states but have not been determined in any crystallography study to date. Hydrodynamic approaches (e.g. size exclusion chromatography coupled with multi-angle laser light scattering, analytical ultracentrifugation) could provide information about the oligomerization equilibria and the hydrodynamic diameters under dynamic conditions, where protein flexibility and mobility are unrestrained.

Several strategies have been applied to probe the orientation and oligomerization state of the SecA protomers under more physiological buffer conditions. Förster resonance energy transfer (FRET) measurements support the anti-parallel orientation of the *bs*SecA 2 dimer (Fig. 3F) (Ding *et al*., 2003). Furthermore, cysteine cross-linking revealed intermolecular disulfide bonds (Or *et al*., 2005; Zimmer *et al*., 2006) that are compatible with the

Table 2. List of SecA structures that have been resolved by X-ray crystallography.

	Organism	Structure (accession code)	Crystallization solution	pH	*T* (°C)	Oligomeric state	Ligand	Additional structures	Diameter (nm)	References
1	*M. tuberculosis*	1NL3	10 mM Tris-HCl, 3.5–3.7 M sodium formate and 5–10% glycerol	7.5	16	Dimer		1NKT (ADP, Mg^{2+})	14.6	Sharma *et al.* (2003)
2	*B. subtilis*	2IBM	1.8 M malonate	6.8	28	Dimer	ADP		13	Zimmer *et al.* (2006)
3	*T. thermophilus*	2IPC	87.5 mM Tris-HCl, 9% PEG4000, 60 mM lithium sulphate, 0.2 M NaCl	8.5	20	Dimer			13.7	Vassylyev *et al.* (2006)
4	*E. coli*	2FSF	50 mM sodium citrate, 6–9% (w/v) polyethylene glycol 35000, 6–10% glycerol and 50 mM ammonium sulphate	5.8	18	Dimer		2FSG (ATP), 2FSH (AMP-PNP), 2FSI(ADP)	15.8	Papanikolau *et al.* (2007)
5	*B. subtilis*	1M6N	2 M ammonium sulphate, 30% glycerol, 1 mM DTT, 100 mM BES	7.0	25.8	Dimer (crystallographic)		1M74 (ADP, Mg^{2+}, SO_4)	12	Hunt *et al.* (2002)
6	*B. subtilis*	3JV2	0.1 M sodium citrate, 8% PEG 4000, 0.1 M NaCl,	5.6	25	Monomer	ADP, Mg^{2+}, peptide		10.6	Zimmer and Rapoport (2009)
7	*B. subtilis*	3DL8	26–28% (w/v) PEG 3350 and 250 mM lithium sulphate	6.5	25	Monomer (bound to *Aquifex aeolicus* SecYEG)			11.3	Zimmer *et al.* (2008)
8	*B. subtilis*	1TF5	0.1 M HEPES, 250 mM magnesium acetate, 7–12% PEG 8000	7.0	25	Monomer		1TF2 (ADP, Mg^{2+})	10.3	Osborne *et al.* (2004)
9	*T. maritima*	3JUX	0.1 M HEPES pH 7.5, 60% MPD	7.5	25	Monomer	ADP, Mg^{2+}		10.2	Zimmer and Rapoport (2009)
10	*T. maritima*	3DIN	20% PEG 3350, 200 mM (NH4)2SO4	7.5	25	Monomer (bound to *tm*SecYEG)	ADP, Mg^{2+}, BEF		10.6	Zimmer *et al.* (2008)
11	*E. coli*	3BXZ	25% ethylene glycol, 0.025 M spermidine	7.5	18	Monomer (helicase motor only)	ADP, Mg^{2+}, spermidine		11	Nithianantham and Shilton (2008)

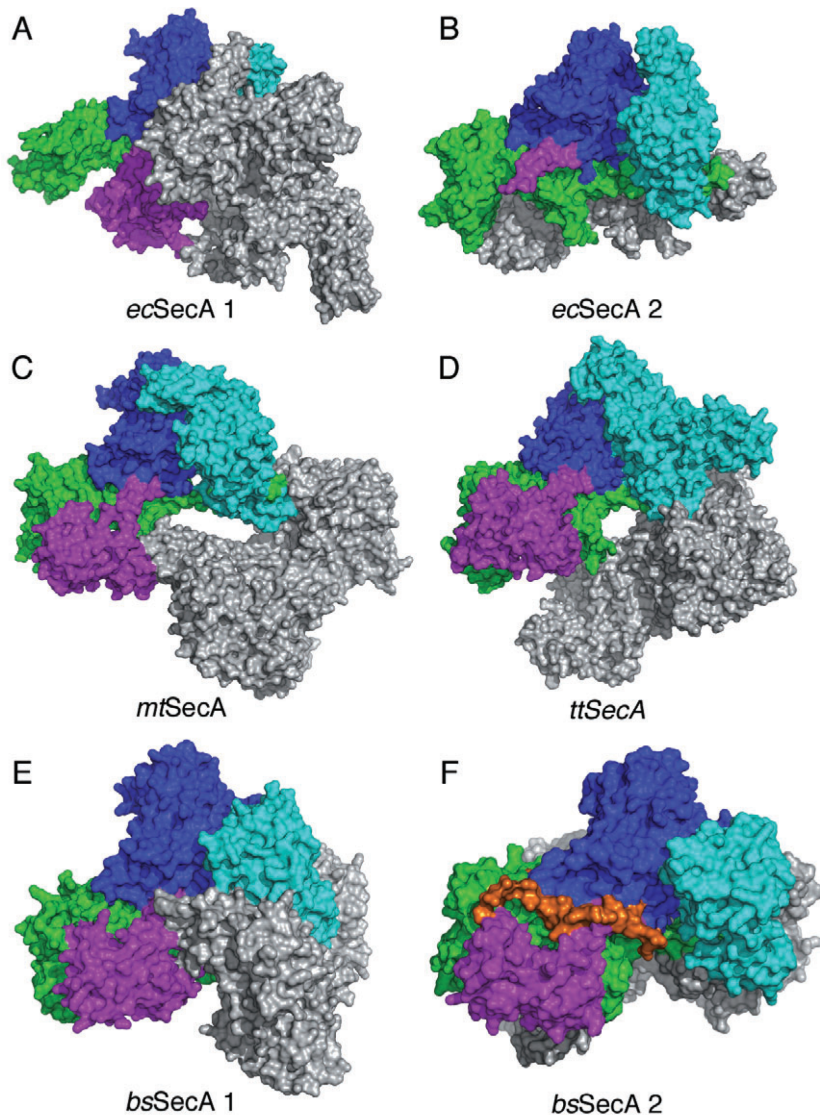

Fig. 3. Space filling models of SecA dimers. Dimeric SecA proteins were structurally aligned on one of their protomers (coloured) so as to demonstrate the variable position that the second (grey) protomer occupies. The structures used (with PDB codes where available) are: *Escherichia coli* (*ec*SecA 1; 2FSF) (Papanikolau *et al.*, 2007), *E. coli* (*ec*SecA 2; missing parts of the PBD) (Chen *et al.*, 2008), *Mycobacterium tuberculosis* (*mt*SecA; 1NL3) (Sharma *et al.*, 2003), *Thermus thermophilus* (*tt*SecA; 2IPC) (Vassylyev *et al.*, 2006), *Bacillus subtilis* (*bs*SecA *1*; 2IBM) (Zimmer *et al.*, 2006) and *B. subtilis* (*bs*SecA *2*; 1M6N) (Hunt *et al.*, 2002).

organization of three of the dimers seen by crystallography (Fig. 3D–F). Boundary sedimentation velocity centrifugation experiments (Woodbury *et al.*, 2002) support the idea that SecA dimers might populate different conformational states that could be affected by ligands such as Mg^{2+} (Gold *et al.*, 2007). One important recent development was the solution structure of SecA by NMR (Gelis *et al.*, 2007). This method has a unique power to dissect the structure of SecA dimers in solution in the presence of any combination of ligands. In addition, NMR is unique in its ability to provide information on the dynamics of the SecA protomer/dimer.

Taken together these data lead to a number of hypotheses that can be tested in future studies:

i. SecA can indeed form multiple different dimeric assemblies in the cytoplasm in the same organism or across bacteria.
ii. Some crystal structures may have frozen dimers that reflect the membrane-bound rather than the soluble, cytoplasmic state of SecA.
iii. The astonishing variability seen in SecA dimers is the result of crystallization and other artefacts and only one or a few of the identified structures is physiologically relevant and observed in the cell.
iv. There is no strong selective pressure for a fixed dimeric structure in the cytoplasm since, when bound to SecYEG, only one dimeric organization is stabilized and functional.
v. A defined cytoplasmic dimeric state might be irrelevant, if it is assumed all of the different dimers observed are capable of satisfying the potential ‘chaperone or pilot-like’ roles of cytoplasmic SecA and if SecA monomerizes upon binding to SecYEG and subsequently catalyses secretion strictly as a monomer.

Nucleotides and preproteins modulate SecA dimerization

The oligomeric status of SecA is affected by ligands and other factors it encounters during its catalytic cycle and by its association with SecYEG (see next section). FRET experiments indicated that signal peptides bind to monomeric and dimeric SecA with the same affinity (Auclair *et al*., 2010). Acidic phospholipids, detergents and synthetic signal peptides cause dissociation of dimers, as determined by FRET and cross-linking experiments (Or *et al*., 2002; Benach *et al*., 2003; Musial-Siwek *et al*., 2005; 2007). In other studies signal peptides caused either re-dimerization of phospholipid-monomerized SecA (Benach *et al*., 2003) or had no effect (Gelis *et al*., 2007). When SecA interacts with bilayers containing negatively charged phospholipids, it forms dumbbell and ring-shaped structures that correspond to dimeric species (Wang *et al*., 2003; Chen *et al*., 2007). The dumbbell structures seem to resemble the organization of the *mt*SecA dimer (Fig. 3C), whereas the ring structures are reminiscent of the *tt*SecA dimer organization (Fig. 3D). SecB-proOmpA, ATP, ATP-γ-S, ADP and liposomes did not seem to cause dimer dissociation in two studies (Driessen, 1993; Shilton *et al*., 1998). In agreement with these results, SecB interacts with the dimeric form of SecA (Fekkes *et al*., 1997; Mao *et al*., 2009) near the C-terminus of each protomer (Fekkes *et al*., 1997; Randall *et al*., 2004). Furthermore, SecA achieves maximal coupling of ATP hydrolysis to preprotein translocation when it interacts with SecB as a dimer, and only one preprotein interacts with a SecA dimer (Mao *et al*., 2009). In contrast to results with soluble SecA, SANS measurements of SecA bound on lipid bilayers suggest that ADP-Mg^{2+} and a non-hydrolysable ATP-Mg^{2+} analogue cause dissociation of the dimer (Bu *et al*., 2003).

Although it is clear from these disparate observations that the conformation and monomer–dimer equilibrium of SecA is modulated by various ligands, a future challenge will to present a coherent model that would integrate all of these observations.

Monomer or dimer during translocation catalysis?

What is the quartenary state of SecA in the assembled translocase and during catalysis? ‘Monomerists’ and ‘dimerists’ in the field have addressed this question by agreeing to disagree. Early FRET data suggested that SecA remains dimeric during preprotein translocation (Driessen, 1993). Nevertheless, FRET is sensitive at distances as great as 8 nm (Jares-Erijman and Jovin, 2003); hence, judicious interpretation of FRET results that either favour dimeric (Driessen, 1993) or monomeric (Or *et al*., 2002; Alami *et al*., 2007) states leaves a lot of room for alternative possibilities such as partial or complete protomer dissociation or conformational effects that do not move the fluorophores apart far enough. Tethering the two SecA protomers by the native cysteinyl residues located in the C-tail (de Keyzer *et al*., 2005) or by engineered cysteinyl residues in the *bs*SecA 2 dimer interface (Fig. 3F) (Jilaveanu and Oliver, 2006) or by fusion in tandem (Wang *et al*., 2008) did not prevent SecYEG interaction and translocation *in vitro*, suggesting that complete dissociation of the two protomers is not necessary for function. SecY monomers and dimers appear to form higher-order oligomers upon interaction with SecA, SecB, ATP and preprotein (Manting *et al*., 2000; Scheuring *et al*., 2005).

Other studies offer the even more dynamic possibility of SecA monomer to dimer transitions being stabilized depending on ligand occupancy. Thus a SecY dimer stabilized with an antibody formed complexes with monomeric SecA (Tziatzios *et al*., 2004). However, the same SecY dimer bound dimeric SecA preferentially when AMP-PNP was included in the reaction. A tandemly fused (SecY-SecY)EG dimer was able to associate with SecA monomers and dimers (Duong, 2003). In this study SecA dimers dissociated from $SecY_2EG$-containing IMVs upon addition of ATP, but only in the absence of Mg^{2+}. Preprotein was found associated with one SecY–SecA complex, while the second SecY remained bound because of the covalent linkage.

Yet other studies have proposed that SecA monomerizes upon binding to SecYEG and actually might function as a monomer. This possibility could render the issue of the precise dimeric form and state purely academic. A single SecYEG trapped in an artificial acidic phospholipid nanodisc was shown to bind to monomeric SecA (Alami *et al*., 2007), although the ability to bind preproteins or to catalyse translocation was not demonstrated for this 1:1 stoichiometric complex. Addition of SecYEG-containing proteoliposomes to dimeric SecA caused dissociation to monomers, as judged from cross-linking experiments and loss of FRET signals (Or *et al*., 2002; 2005). A SecA dimer with intermolecularly cross-linked protomers became non-functional (Or and Rapoport, 2007). However, it is not clear from such negative results whether the protein needs to monomerize to function or whether loss of function stems from the obstruction of essential conformational motions. Removal of the 11 N-terminal amino acids (SecAΔ11/N95) from SecA shifted the equilibrium of SecA towards the monomer in solution (Jilaveanu *et al*., 2005; Or *et al*., 2005; Das *et al*., 2008) [for an alternative view see (Karamanou *et al*., 2005; Gold *et al*., 2007; Wang *et al*., 2008)] without affecting translocation ATPase activity *in vitro* and function *in vivo*. An important point that is difficult to address in such studies is whether the quartenary state of SecA determined in solution remained unchanged after binding to SecYEG/lipids.

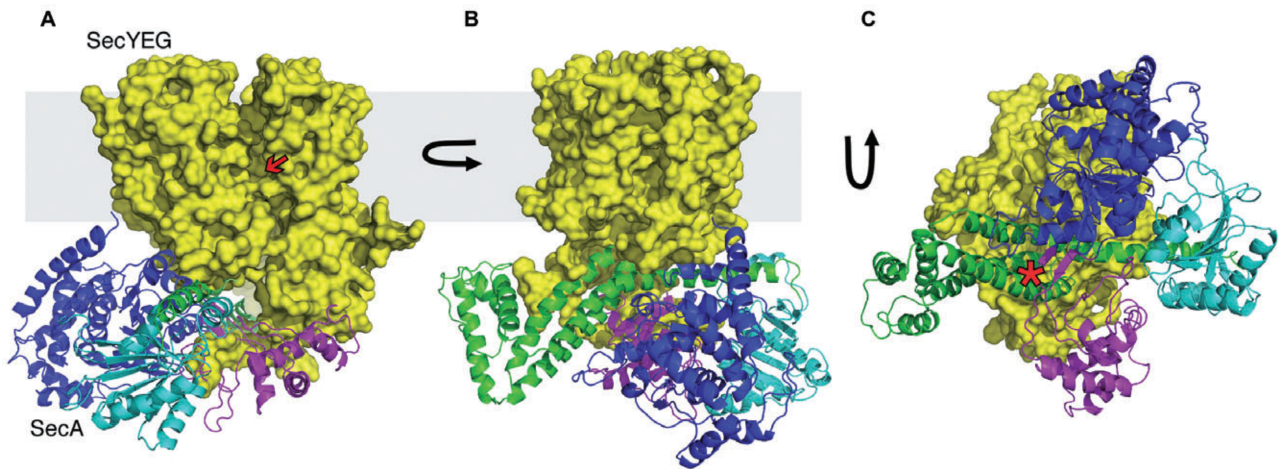

Fig. 4. Model of the SecYEG/SecA translocase complex determined by X-ray crystallography (Zimmer *et al*., 2008). (A) Front view and (B) side view (after 90° rotation) of the complex. The approximate position of the lipid bilayer in which SecYEG (yellow; space filling) is embedded is indicated as a grey band. SecA (ribbon) lies flat on SecYEG and faces the cytoplasm. The red arrow in (A) indicates the lateral gate in SecYEG believed to allow preproteins lateral access to the lipid bilayer. (C) The complex in (B) rotated 90° so that the SecYEG tube is vertical to the plane of the figure. The red asterisk denotes the cytoplasmic entrance of the SecY protein-conducting channel.

A recent landmark study presented the crystal structure of one SecA molecule bound to one SecYEG heterotrimer (Fig. 4) (Economou, 2008; Zimmer *et al*., 2008). SecA is positioned such that a continuous groove can be imagined formed from SecA to the SecY channel (Fig. 4, right, red asterisk). This groove would be formed between the PBD and IRA2 domains such that they would ‘embrace’ the chain during its transit. The unavoidable use of detergent in this study is likely responsible for the loss of the second copy of the presumed dimeric SecYEG but also possibly of a second SecA protomer (Economou, 2008; Zimmer *et al*., 2008). Nevertheless, based on this structure one SecA protomer should be considered as the minimal unit that interacts with a single SecYEG trimer. A single SecYEG trimer may also be necessary and sufficient for ribosome-mediated co-translational docking and secretion (Becker *et al*., 2009). In SecA-mediated secretion one current working hypothesis posits that one protomer of SecYEG may act as the docking site for SecA, whereas the other, probably the one crystallized by Zimmer *et al*. provides the secretory conduit for one preprotein molecule (Osborne and Rapoport, 2007). SecA could interact with the non-translocating copy of SecYEG with its helicase motor region (Karamanou *et al*., 2008; Tsukazaki *et al*., 2008).

In an attempt to visualize the proposed assemblies, we constructed models of a single SecA monomer (Zimmer *et al*., 2008) bound to either the ‘back-to-back’ (Fig. 5, left) (Breyton *et al*., 2002) or the ‘front-to-front’ (Mitra *et al*., 2005) (right) dimer. In both cases the SecYEG complex presumed missing from the crystal structure is greyed. A main difference between the two models is that in the ‘front-to-front’ model the lateral gates of SecYEG, through which membrane proteins are thought to slip out, face

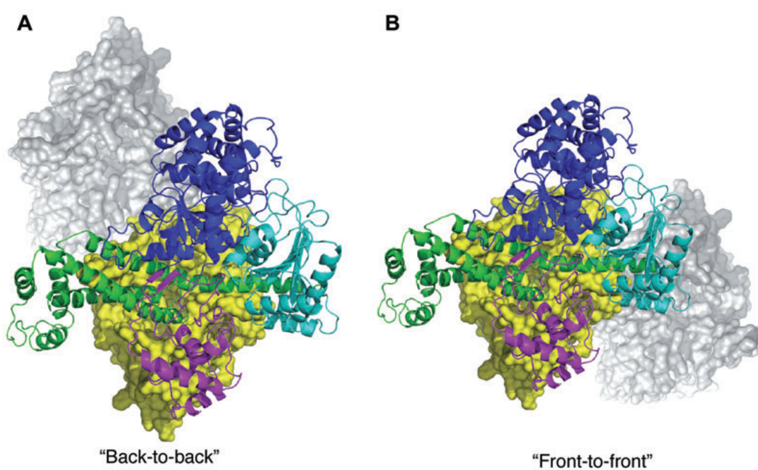

Fig. 5. Schematic representation of two of the proposed dimeric models of the SecYEG channel. (A) ‘Back-to-back’ (Breyton *et al*., 2002) and (B) ‘front-to-front’ dimer (Mitra *et al*., 2005), viewed from the cytoplasmic side of the membrane. The structures have been aligned on the yellow-coloured SecYEG trimer to better visualize the differences of the two models. The protein-conducting pore is located at the centre of each SecYEG trimer. The yellow SecYEG with the bound SecA corresponds to that depicted in Fig. 4C. SecYEG dimeric structures have been assembled manually and serve only for visualization purposes.

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each other while in the 'back-to-back' dimer they face away from one another. Two main conclusions become obvious from this analysis. First, only small regions of the SecA NBD domain and of the C-domain protrude out of the body of SecYEG and would be proximal to the membrane plane, available for additional interactions with a second SecYEG heterotrimer. Second, in the 'front-to-front' model would the SecA helicase domain be positioned in such a way as to face head-on the presumed 'non-translocating' SecYEG trimer expected to act as a docking station. In the 'back-to-back' model only minor contacts of the SecA helicase motor would be possible laterally with the 'non-translocating' SecYEG. From either arrangement, it is difficult to rationalize the high binding affinity of the isolated SecA helicase motor with SecYEG and their extensive interactions (Ramamurthy and Oliver, 1997; Dapic and Oliver, 2000; Vrontou *et al*., 2004; Karamanou *et al*., 2008) and hence difficult to explain the role of an extra copy of SecYEG as a docking station for a single SecA. If SecA is considered to bind to SecYEG dimers as a dimer (not shown) then it would acquire a 'head-to-tail' (i.e. helicase motor to C-domain) anti-parallel dimer in the 'back-to-back' SecYEG arrangement or a 'head-to-head' (i.e. helicase motor to helicase motor) facing opposite orientations. SecYEG-bound SecA may be further stabilized by additional contacts to SecD/SecF/YajC (Economou *et al*., 1995) that have not been visualized yet and are absent from the current models.

The future now: proceed with caution

The oligomerization state of SecA and of SecYEG remains perplexing. This is partly due to the idiosyncrasies of the proteins involved and the dynamic nature of their interactions. It is also partly due to the use of low-resolution methods that fail to detect multiple states and leave room for data over-interpretation and detergent solubilization that may break up native complexes and promote others. In our opinion, the field requires a fresh influx of novel, higher-resolution, more accurate and less disruptive technologies. Less disruptive methods such as cryo-EM, freeze-fracture electron microscopy and the currently emerging cryo-electron tomography are some examples. In cryo-EM, samples are rapidly frozen, embedding proteins into a layer of vitreous ice that is very near to the native environment of the protein (Jonic and Venien-Bryan, 2009). Controlling the freezing times is the key for a successful outcome but this technique has such high powers of resolution that it can be used to even follow conformational rearrangements during catalysis (Miyazawa *et al*., 2003). In freeze-fracture electron microscopy, the sample, usually whole cells or membrane preparations, is frozen and the surface is broken to reveal underlying structures (Severs, 2007). Cryo-electron tomography coupled with appropriate 3-D image processing uses frozen samples at near native conditions and low doses of electrons to preserve the integrity of the structures observed. It can determine the structure of membrane protein assemblies in intact cells. The current low resolution (down to ~2 nm) is only appropriate for large macromolecular complexes.

Additional tools such as atomic force microscopy (Muller, 2008) and single molecule spectroscopy (Garcia-Saez and Schwille, 2007) might be employed to gain insight in the physiological quartenary state of the translocase while it is maintained intact and functional. All these methods will prove powerful when combined with the already established crystallography and NMR tools. For example docking techniques can be used to fit high-resolution static (X-ray) or dynamic structures (NMR) into low-resolution 'envelopes' that have been obtained under non-disruptive conditions in membranes (e.g. cryo-EM, SANS, AFM, etc.). This would be particularly important for resolving detergent-sensitive dynamic quartenary complexes.

Conclusions

Oligomerization is a strategy employed by many proteins to enhance their functional capabilities. SecA, the ATPase nanomotor of the bacterial protein translocase, forms dimers in solution. However, these dimers are of variable spatial arrangements, leaving us to ponder on which of these faithfully depict the native quaternary conformation. Crystallization artefacts apart, SecA protomers are highly charged and flexible and might indeed exploit more than one electrostatic/hydrophobic complementarity strategy. Irrespective of this, we should also reflect on the possibility that even if we go to great lengths to elucidate the oligomeric state of SecA in solution, we might not be any wiser about what happens upon translocase assembly and how these events are modulated to a remarkable extent by preproteins, nucleotides, phospholipids, chaperones and the channel itself. Other than a larger collection of static structural states obtained by traditional methods and rigorous testing of current models, we also need to make better use of non-disruptive and dynamic biophysical methods. Whatever the technical routes chosen, one thing is certain: this totally unique molecular machine is full of surprises and will keep us excited (but sleepless) for years to come.

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