Chapter 6
The Periplasmic Chaperones Skp and SurA

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Abstract The periplasm of Gram-negative bacteria contains a specialized chaperone network that facilitates the transport of unfolded membrane proteins to the outer membrane as its primary functional role. The network, involving the chaperones Skp and SurA as key players and potentially additional chaperones, is indispensable for the survival of the cell. Structural descriptions of the apo forms of these molecular chaperones were initially provided by X-ray crystallography. Subsequently, a combination of experimental biophysical methods including solution NMR spectroscopy provided a detailed understanding of full-length chaperone–client complexes. The data showed that conformational changes and dynamic re-organization of the chaperones upon client binding, as well as client dynamics on the chaperone surface are crucial for function. This chapter gives an overview of the structure-function relationship of the dynamic conformational rearrangements that regulate the functional cycles of the periplasmic molecular chaperones Skp and SurA.

Keywords Molecular chaperones · Protein folding · Periplasm · Gram-negative bacteria · Protein structure · Protein dynamics

Abbreviations

ATP	Adenosine 5′-triphosphate
BAM	β-barrel assembly machinery
Hsp60	Heat shock protein 60
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser effect

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OMP	Outer membrane protein
PPIase	Parvulin-like peptidyl-prolyl isomerase
PRE	Paramagnetic relaxation enhancement
SAXS	Small angle X-ray scattering
Skp	Seventeen Kilodalton Protein
SurA	Survival factor A

Introduction

For Gram-negative bacteria, such as *E. coli*, around 35% of the proteome is directed to the bacterial cell envelope (Krogh et al. 2001; Orfanoudaki and Economou 2014; De Geyter et al. 2016; Tsirigotaki et al. 2017). The cell envelope is composed of two membranes: the inner membrane and the outer membrane, which are separated by the aqueous periplasm (Van Wielink and Duine 1990). The periplasm is a viscous and oxidizing compartment that contains a thin layer of peptidoglycan playing a structural role as a bacterial cell wall. Periplasmic proteins are synthesized as pre-proteins in the cytoplasm and are subsequently translocated across the inner membrane by either of two secretory pathways, depending on the signal sequence they carry, the Sec machinery and the twin arginine translocation (TAT) system (Wickner et al. 1991; Driessen et al. 2001; Natale et al. 2008; Lycklama et al. 2012; Tsirigotaki et al. 2017).

Thereby, the majority of proteins are processed by the Sec machinery, which translocates its clients in an unfolded conformation. As proteins exit the Sec machinery and enter the periplasm, a complex chaperone network in charge of protein quality control ensures their folding and integrity. The network comprises two main pathways for clients, the protection of protein folding under stress conditions and the transport of client proteins towards the outer membrane. The first pathway includes the chaperones HdeA/HdeB, which are activated by a pH-controlled mechanism that helps to prevent protein aggregation in acidic environment (Hong et al. 2005; Kern et al. 2007; Ding et al. 2015; Zhang et al. 2016; Yu et al. 2017; Salmon et al. 2018; Yu et al. 2018), and the chaperone Spy (Quan et al. 2011; Stull et al. 2016; He et al. 2016). The second pathway, the transport of outer membrane proteins (OMPs) through the periplasm is controlled by the network of two chaperones SurA and Skp (Bitto and McKay 2002; Walton and Sousa 2004; Korndörfer et al. 2004). Additionally, the protease DegP is responsible for the degradation of off-pathway OMPs (Jiang et al. 2008; Clausen et al. 2011; Ge et al. 2014a, b) and has also been shown to display chaperone activity under low temperature conditions (Spiess et al. 1999). Under heat shock conditions, in turn, the chaperone FkpA has been demonstrated to play a key role in OMP biogenesis (Ge et al. 2014a, b). In addition, the biogenesis of OM lipoproteins requires the dedicated chaperone LolA (Tajima et al. 1998; Miyamoto et al. 2001; Taniguchi et al. 2005; Okuda and Tokuda 2009).

Besides these two pathways, the periplasm contains several folding catalysts that are speeding up two slow reaction steps, the cis–trans isomerization of peptidyl-prolyl bonds and the formation of disulfide bonds. Cis–trans isomerization is generally

a slow process that can often be rate-limiting for protein folding and that can be accelerated by enzymes with peptidyl-prolyl cis-trans isomerases (PPIase) activity (Liu and Walsh 1990; Bardwell et al. 1991; Hayano et al. 1991; Scholz et al. 1998). Four proteins with PPIase activity have been identified in the *E. coli* periplasm, FkpA (Arie et al. 2001; Saul et al. 2004; Helbig et al. 2011), PpiA (Liu and Walsh 1990; Hayano et al. 1991), PpiD (Antonoaea et al. 2008; Matern et al. 2010) and SurA (Bitto and McKay 2002), among which SurA and FkpA are the main players. A second slow process in protein folding is the formation of disulfide bonds. In the periplasm, the formation of disulfide bond is catalyzed by the Dsb redox system that comprises the oxidation system DsbA and DsbB and the isomerization system DsbC and DsbD (Joly and Swartz 1997; Messens and Collet 2006; Vertommen et al. 2008; Ito and Inaba 2008; Heras et al. 2009; Denoncin and Collet 2013). In this chapter, we focus on the recent advances of the structural characterization of the chaperones Skp and SurA that transport the OMPs through the periplasm and promote their insertion into the outer membrane by the BAM complex.

The biogenesis of OMPs is a challenging task for the cell, since the proteins have to cross two biophysical barriers to reach the outer membrane: The inner membrane and the aqueous periplasm. On this pathway, the translocation of the OMPs across the inner membrane, from the cytoplasm into the periplasm, is facilitated by the SEC translocase (Driessen et al. 2001; van den Berg et al. 2004; Chatzi et al. 2014; Tsirigotaki et al. 2017). The second biophysical barrier for the insoluble OMPs, the aqueous periplasm, is then overcome by a unique network of molecular chaperones. This network is organized around the chaperones Skp (Seventeen Kilodalton Protein) and SurA (survival factor A), which work on two parallel pathways to target the OMP clients to the β-barrel assembly machinery (BAM) complex, which eventually facilitates their folding and insertion (Fig. 6.1) (Rizzitello et al. 2001; De Geyter et al. 2016).

Understanding the structural biology of these two chaperones is of key scientific interest due to their fundamental role in polypeptide transport and OMP biogenesis. A first milestone has been the determination of the crystal structures of the client-free (apo) forms of SurA and Skp (Bitto and McKay 2002; Walton and Sousa 2004; Korndörfer et al. 2004). These structures provided valuable information about the functional mechanisms of the individual components of the chaperone system in single, individually stabilized states. Subsequently, new developments of biophysical techniques including nuclear magnetic resonance (NMR) spectroscopy allowed the observation of these chaperone systems in aqueous solution and in complex with client proteins, thus providing complete descriptions of the chaperones dynamic functional cycles. These findings have established how structural plasticity allows these chaperones to undergo conformational rearrangements and populate conformational ensemble states that overall regulate their functional cycles. Such conformational flexibility enables the chaperones to adapt to their highly dynamic client proteins as well as to fulfil their roles in a complex interaction network in the periplasm.

The Periplasmic Chaperone Skp

The crystal structure of Skp shows a homo-trimeric assembly where the three subunits are connected by a β-barrel trimerization domain that extends into three α-helical "tentacles" or "arms" that give rise to Skp's characteristic jelly fish-like shape (Fig. 6.2a, b) (Walton and Sousa 2004; Korndörfer et al. 2004). Each monomer contributes three β-strands to the trimerization interface. Importantly, these strands are however not connected within one protomer subunit but form a small β-barrel across the trimer by connecting β-strands intermolecularly from two adjacent subunits (Fig. 6.2a). Each arm is constituted by two long α-helixes in coiled-coil arrangement, extending from the oligomerization domain and forming a central cavity. The presence of a central cavity bears some resemblance to the well-characterized Hsp60 (GroEL, CTT) chaperone, where a central cavity serves as a protective environment for the client protein (Braig et al. 1995; Xu et al. 1997; Ditzel et al. 1998; Horwich et al. 2007). The overall organization and shape of Skp bears further similarity to the ATP-independent holdase chaperone prefoldin, with the difference that the N and C-termini of Skp are located at the β-barrel trimerization domain, while for prefoldin

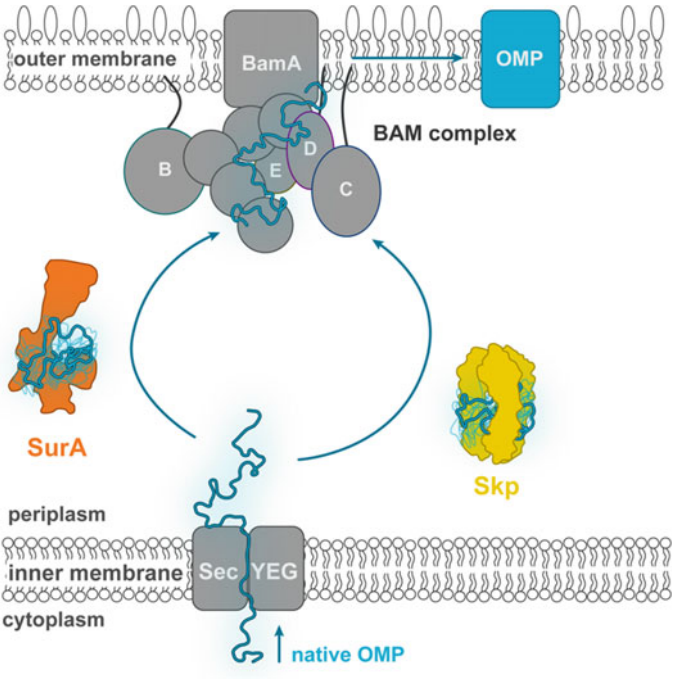

Fig. 6.1 The periplasmic chaperone network in outer membrane biogenesis. After their synthesis in the cytoplasm, unfolded native OMPs are translocated by the SecYEG machinery in a linear fashion across the inner membrane. Subsequently, they are transported by the chaperones Skp or SurA on parallel pathways to the BAM complex for subsequent insertion into the outer membrane

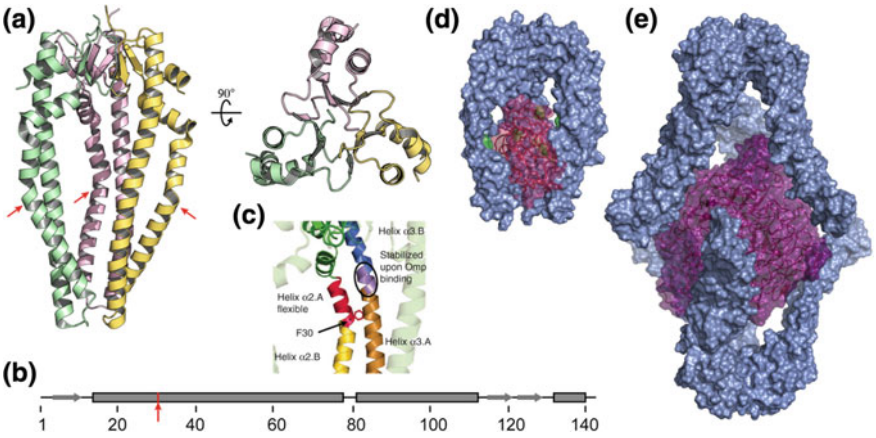

Fig. 6.2 Dynamic adaptation of the periplasmic chaperone Skp and its client protein. **a** Structure of Skp trimer [PDB code 1SG2 (Korndörfer et al. 2004)] with the individual protomers coloured in green, pink and yellow. The position of phenylalanine 30 is highlighted by red arrows. **b** Skp amino acid sequence and secondary structure. The β-sheets and α-helixes are represented by grey arrows and rectangular shapes, respectively. Phenylalanine 30 is highlighted as in (**a**). **c** Close-up on Skp arms highlighting the pivot element in helices α2 and α3. The flexible helix α2.A (red) is linked by the hinge at phenylalanine 30 to the more rigid helix α2.B (yellow). The helicity of segment Ser89–Arg93 (purple) of helix α3.A (orange) is stabilized upon client binding [adapted from (Burmann et al. 2013)]. **d** Structural model of a single conformation from the dynamic Skp(blue)–tOmpA (purple) ensemble. The residues highlighted in green correspond to the alanine identified as interacting with the unfolded tOmpA [adapted from (Callon et al. 2014)]. **e** Model of the multivalent binding of OMPs that are too large to fit in the cavity of one trimeric Skp. The arms are in an open conformation to expand the size of the cavity [adapted from (Holdbrook et al. 2017)]

they are located at the extremity of the arms (Siegert et al. 2000; Martín-Benito et al. 2002; Ohtaki et al. 2008). Just like prefoldin, Skp is classified as an ATP-independent holdase chaperone.

The protein Skp was functionally identified as a binder of OMPs in a pull-down experiment with unfolded OmpF covalently linked to Sepharose beads (Chen and Henning 1996). OmpF is however not the only client protein of Skp, as shown in a subsequent study where the Skp clientome was mapped by a combined strep-tag affinity and proteomic approach (Jarchow et al. 2008). Overall, more than 30 proteins were identified in the Skp clientome, including the OMPs OmpA and LamB, but also several soluble periplasmic proteins, such as MalE and OppA, demonstrating Skp's functional importance for a broad spectrum of clients (Table 6.1). The functional relevance of the interaction of Skp with soluble proteins was additionally highlighted by studies showing that Skp chaperone activity can improve the expression yields of recombinant proteins not only in the periplasm, but also in the cytosol when a Skp variant devoid of its signal-sequence is used (Bothmann and Plückthun 1998; Levy et al. 2001).

Table 6.1 List of unambiguously identified wild-type Skp client proteins with outer membrane localization. Data from Jarchow et al. (2008)

Number	Uniprot entry	Protein name	Function
1	P06129	BtuB	Vitamin B12 transport
2	P17315	CirA	Putative iron transport, colicin IA and IB receptor
3	P13036	FecA	Fe(III) dicitrate transport
4	P05825	FepA	Ferrienterobactin receptor
5	P02943	LamB	Maltose and maltodextrin transport
6	P0A910	OmpA	Outer membrane porin
7	P06996	OmpC	Outer membrane porin
8	P02931	OmpF	Outer membrane porin
9	P0A917	OmpX	Putative defense
10	P75780	Fiu	Catecholate siderophore transport
11	P10384	FadL	Long-chain fatty acid transport
12	P0A921	PldA	Phospholipase
13	P0A927	Tsx	Nucleoside porin
14	P76045	OmpG	Outer membrane porin
15	P02930	TolC	Multidrug exporter
16	P76115	YncD	Putative iron/siderophore receptor

The complex of an OMP client protein and Skp was initially characterized by NMR spectroscopy and fluorescence spectroscopy (Walton et al. 2009; Qu et al. 2009). A full understanding of the dynamic nature of the complexes at atomic resolution was however only obtained subsequently with a complete description of structure and dynamics of Skp–OmpX and Skp–tOmpA (transmembrane domain of OmpA) complexes determined by solution NMR spectroscopy (Burmann et al. 2013; Callon et al. 2014). The OMP polypeptide was found to adopt a disordered conformation that interacts within Skp's central cavity by both hydrophobic contacts and interactions between the charged loops of the OMP and charged parts of the cavity. Notably, these works exploited state-of-the-art NMR experiments to determine for the first time the dynamic properties and structure of a chaperone–client complex with a full-length native client protein with atomic resolution. Moreover, these studies demonstrated the conformational flexibility of Skp in its apo and in its client-bound holo state in solution. In the apo form, a hinge element located at the conserved residue phenylalanine 30 allows the Skp arms to explore a large degree of conformational flexibility (Fig. 6.2a–c). Upon OMP binding, the flexibility of the arms is decreased, leading to a stabilization of the cavity around the bound client protein. While bound, the OMP client protein explores a dynamic landscape of disordered states within the cavity of Skp with no polypeptide segment of OMP stably bound to the chaperone. Thereby, the OMP encapsulated in the cavity has a reduced compactness by approximately a factor of two in comparison to the disordered OMP

in denaturant solution. The local lifetime of the individual conformations of at most 1 ms is in stark contrast to the long global lifetime of the complex in the range of hours. This discrepancy arises by avidity from a combination of multiple weak local interactions with a short lifetime. A detailed analysis of a combination of distance-based NMR experiments (NOE and PRE) confirmed the contact interface between Skp and the bound OMP in the cavity (Fig. 6.2d).

The observed position of the OMP client protein in the Skp cavity also raised the question by which mechanism Skp adapts to a wide range of different clients ranging in size from 150 residues (OmpX) to more than 700 residues (FhuA). A study combining molecular modelling, small angle X-ray scattering (SAXS) and NMR spectroscopy confirmed that the hinges on the arms permit to dramatically extend the size of the cavity in an ATP-independent manner to adapt to the size of client proteins (Fig. 6.2e) (Holdbrook et al. 2017). A complementary model was established by systematically analysing the binding capacity of a large variety of OMPs with a size range from 8 to 16 strands to Skp (Schiffrin et al. 2017). These data demonstrated that a higher Skp:OMP ratio is required to bind larger OMPs (16-stranded), while for smaller OMPs the Skp cavity can be expanded to adapt to the size of the client proteins. One common characteristic of Skp is that it exists only as a multiple of the trimeric state, indicating that this stoichiometry corresponds to the active form of the chaperone. Thereby, a recent study established that at physiological concentration Skp exists in an equilibrium between a trimeric and a monomeric state (Sandlin et al. 2015). However, the conformation of the monomeric state is still controversial as Sandlin et al. hypothesized a folded conformation by the mean of a van't Hoff analysis, while Burmann et al. (2013) observed a disordered conformation. To date no study has been able to shed light on characteristics and functionality of the Skp monomeric state.

While structure and dynamics of Skp in apo and holo states have been extensively characterized, the mechanism allowing the release of OMPs from Skp into the outer membrane is less clear. Burmann et al. (2013) have proposed a mechanism hypothesizing that in a ternary Skp–OMP–BAM transition complex the weak local affinity and the rapid structural interconversion of the OMP within the Skp cavity would allow client release by dynamic rearrangements of the polypeptide while it remains bound to Skp. According to this model, the OMP bound to Skp might be inserted sequentially one β-hairpin at a time into the lipid membrane. Furthermore, the BAM complex could guide the unfolded OMP, by transient contacts with BamA POTRA domains to the membrane entry point.

Taken together, the key element regulating Skp's functional cycle is its dynamic nature, which allows both to operate large motion of its arms and to adapt to highly dynamic client proteins. The fast dynamics of the encapsulated OMP client permit its release in an ATP-independent manner. The recent demonstration of the biological relevance of a second non-characterized monomeric state could lead to additional critical insights into the functional cycle of Skp.

The Periplasmic Chaperone SurA

The periplasmic chaperone SurA is generally considered the main pathway for the transport of OMPs to the BAM complex (Sklar et al. 2007; Lazar and Kolter 1996; Rouvière and Gross 1996). Deletion of surA results in a lower OMP density and accumulation in the periplasm of the major OMPs OmpA, OmpF, and LamB in an unfolded form (Rouvière and Gross 1996). The expression of these proteins is downregulated upon induction of the σ^E-dependent cytoplasmic stress response, which is activated when mis- or unfolded proteins are accumulating in the periplasm (Lazar and Kolter 1996; Missiakas et al. 1996; Rouvière and Gross 1996). The drastically decreased levels of outer membrane-inserted OMPs in SurA mutant strains result in phenotypes characterized by a defective cell envelope resulting in an increased sensitivity to hydrophobic agents such as SDS-EDTA and to antibiotics such as novobiocin (Lazar and Kolter 1996).

SurA is composed of four distinct domains: a large N-terminal domain, two parvulin-like peptidyl-prolyl isomerase (PPIase) domains and a short C-terminal helix followed by a short β-strand (Fig. 6.3a, b) (Bitto and McKay 2002). The combination of the PPIase 1 domain (P1) with the N and C-terminal domains form the core of the protein and the PPIase 2 domain (P2) is flexibly connected to this core by two linkers. The C-terminal 10 last residues form a short β-strand that forms an antiparallel β-sheet with an N-terminal β-hairpin. While the exact amino acid sequence of the C-terminus is not essential, its shortening drastically affects chaperone activity (Chai et al. 2014). A minimal construct of SurA, composed of the N-terminal domain directly linked to the C-terminal domain, displays activity in vitro and can complement the SurA deletion phenotype in vivo (Behrens et al. 2001; Webb et al. 2001). This suggests that the N/C-domain pair is responsible for the chaperone activity and corroborates the initial observation of a large cavity on the N-terminal domain that could be a binding site for unfolded clients (Fig. 6.3a, b) (Bitto and McKay 2002).

How the PPIase domains are relevant for the function of SurA is still unclear, especially as several SurA homologues have only one or no PPIase domain (Alcock et al. 2008). In general, parvulin-like domains feature enzymatic activity to catalyze the cis–trans conversion of peptide bonds preceding prolyl residues (Rahfeld et al. 1994; Rudd et al. 1995). For SurA only the P2 domain exhibits significant PPIase activity and both PPIase domains can simultaneously be deleted without significant loss of function (Rouvière and Gross 1996; Behrens et al. 2001). Isolated PPIase domains 1 and 2 do not exhibit chaperone activity and do not complement activity in a surA depletion mutant (Behrens et al. 2001). However, it cannot be excluded that their PPIase activity is important for specific client proteins and/or under certain conditions. In this context, it has recently been demonstrated that while the deletion of the P2 domain does not completely abolish the chaperoning activity it is significantly decreasing it (Soltes et al. 2016). This discovery could potentially be explained by a large dynamic conformational rearrangement of the P2 domain that allows closure of the N-terminal domain by a clamp-like mechanism. Soltes et al. also showed that the binding of the P1 domain to the core stabilizes the protein but inhibits the access to the

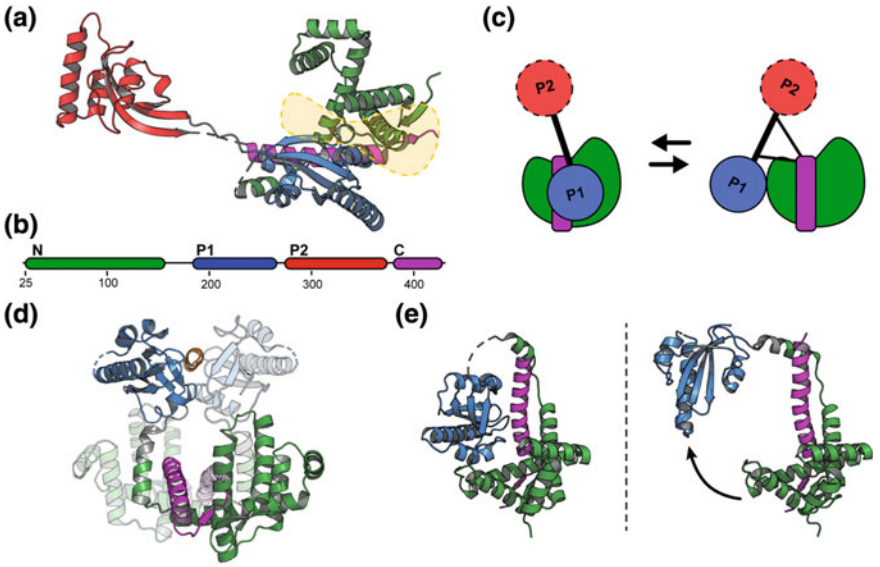

Fig. 6.3 Conformational rearrangements of the periplasmic chaperone SurA regulate its function. **a** Crystal structure of apo SurA (PDB 1M5Y). The core of the protein is formed by the N-terminal domain (green) together with the PPIase domain I (P1) (blue) and the C-terminal helix (magenta). The PPIase domain II (P2) (red) is connected by two flexible linkers and is thus located 30 Å away from the core. The hypothesized client binding site on the N-terminal domain is highlighted in yellow. **b** SurA domain composition coloured as in (**a**). **c** Schematic illustration of the regulation of SurA functional cycle by conformational dynamics of the two PPIase domains relatively to the core, coloured are as in (**a**) [adapted from (Soltes et al. 2016)]. **d** Structure of the dimeric complex of SurAΔP2 (SurA lacking the domain P2) bound to a peptide mimicking a client protein (PDB 2PV3), coloured as in (**a**). The peptide is coloured in orange. **e** Different conformations of the P1 domain in the apo state (left) and in the monomer extracted from the dimeric complex with a peptide (right). The formation of the complex triggers a major conformational change that leads to the dissociation of the P1 domain from the core domain

binding site, while the flexible unbound state increases the chaperone activity at the cost of a decreased stability (Fig. 6.3c). The combination of the studies above suggests that the N/C-domain could represent a platform providing the basal chaperone activity that then has been evolutionarily diversified by addition of accessory domains.

The involvement of SurA in the assembly and transport of β-barrel OMPs to the outer membrane is clearly demonstrated by multiple studies (Rizzitello et al. 2001; Sklar et al. 2007). A specific set of OMPs that require SurA for their proper maturation has been revealed by a differential proteomics experiment including the formerly known SurA client proteins OmpF, OmpA, FhuA, and LamB as well as newly discovered client proteins such as LptD (Vertommen et al. 2009). Some motifs determining client specificity of SurA were identified using phage display experiments (Bitto and McKay 2003; Xu et al. 2007). These studies have shown that SurA recognizes peptide sequences rich in aromatic amino acids and arranged in the specific pattern

Ar–X–Ar, where Ar is an aromatic residue and X can be any residue. The Ar–X–Ar sequence is found with increased abundance at the C-terminus of transmembrane β-barrel OMPs, indicating a possible role as recognition signal for SurA. This study lead to the development of a high affinity peptide mimicking the C-terminus of OMPs and the crystal structure of SurA with this peptide bound could be solved (Xu et al. 2007) (Fig. 6.3d). The structure shows a hydrophobic binding site on the P1 domain, however, not only one but two P1 domains are binding to one peptide, thus triggering a 2:1 SurA:peptide stoichiometry. Thereby, the P1 domain undergoes large conformational changes, which result in its separation from the core domain, while the structures of the N- and C-terminal domains remain unaffected compared to the apo form (Fig. 6.3e). Overall, the biological relevance of this structure remains unclear, since the binding of a short peptide is not necessarily representative for a full-length client protein. Furthermore, the position of the binding site contradicts in vivo data showing that the chaperoning activity is not affected by P1 deletion. This discrepancy could perhaps be explained by a decoupling of the sites of substrate recognition and chaperone activity. In the structure by Xu et al. the peptide bound to the P1 domain is oriented with its N-terminus towards the SurA N/C—domain. A full-length client protein could then bind the N/C-domain via a non-specific interaction.

Overall, the structural properties of SurA–OMP complexes are by far not as well characterized as the Skp–OMP complex, as no atomic resolution information is available. To obtain a complete picture it will be necessary to determine, among others, which functional role the conformational rearrangements of the domains have. A complete characterization of the structure and dynamics of SurA in its apo and holo states, is highly desired in order to draw a complete the picture of the molecular mechanism underlying the function of SurA.

Skp- and SurA-Mediated Stabilization and Folding of Client Proteins

The increasingly detailed structural knowledge of the periplasmic molecular chaperone network is accompanied by several studies characterizing how these chaperones interact with and influence the folding behaviour of client OMPs in vitro. Trimeric Skp has been found to form stable 1:1 (Skp_3:OMP) complexes with small OMP clients such as unfolded OmpA, but also complexes in 2:1 (Skp_3:OMP) ratio with larger OMPs such as unfolded BamA and FhuA (Schiffrin et al. 2016; Thoma et al. 2015). Thereby, Skp can bind unfolded client OMPs with high affinities, corresponding to dissociation constants in the low nM range, as determined for multiple unfolded OMP clients, such as OmpA, OmpG and BamA as well as OmpLA, OmpW and PagP (Qu et al. 2007; Moon et al. 2013). While the stoichiometry of SurA:OMP complexes has not been studied in detail, it has been proposed to bind OmpC as well as FhuA in a 2:1 ratio (Thoma et al. 2015; Li et al. 2018). The affinity of SurA to OMP clients is generally much weaker than of Skp. While SurA's affinity to full-length nascent

client proteins has been determined to a lesser extent, it has been found to bind unfolded OmpG and OmpF with affinities in the low μM range (Bitto and McKay 2004). Similar values were found for its affinity to heptapeptide WEYIPNV, which contains the Ar–X–Ar motif frequently found in OMPs (Bitto and McKay 2003).

A higher client affinity of Skp compared to SurA is also consistent with experiments showing Skp binding to unfolded OmpC at faster rates than SurA as well as 6-fold longer lifetimes of chaperone-client complexes formed by Skp with unfolded FhuA compared to SurA-FhuA complexes (Wu et al. 2011; Thoma et al. 2015). Moreover, Skp has been shown to outcompete SurA in equimolar mixtures of both chaperones when binding unfolded FhuA or OmpA (Thoma et al. 2015; Schiffrin et al. 2017). The client OMP either remains bound to Skp when preassembled Skp—client complexes are added to apo SurA, or transfers to Skp when preassembled SurA–client complexes are added to apo Skp. Differences in client binding were further elucidated by a recent study reporting a mild disaggregase function for Skp, which could break down scant aggregates containing up to 5 OmpC monomers, whereas such behavior could not be observed for SurA (Li et al. 2018). These data are also in full agreement with the notion that Skp binds its clients in a tighter and more compact conformation than SurA.

While chaperones can maintain client OMPs dynamically unfolded in solution over prolonged time periods, proper folding of the client requires the presence of a lipid membrane which provides the thermodynamic stabilization of the native form (Moon et al. 2013; Burmann et al. 2013; Thoma et al. 2015). Thereby, the transition from a chaperone-stabilized state to a folded, membrane-embedded state depends on the biophysical properties of the lipid bilayer. For example, OmpA and PagP fold into negatively charged lipid bilayers from solutions containing Skp, but not into neutral bilayers (Bulieris et al. 2003; Patel et al. 2009; Patel and Kleinschmidt 2013; McMorran et al. 2013). Moreover, the presence of additional factors such as LPS, transmembrane proteins in general and in particular the presence of BamA appears to influence the transition from a chaperone-stabilized state into the lipid bilayer (Bulieris et al. 2003; Patel et al. 2009; Patel and Kleinschmidt 2013; Schiffrin et al. 2017). Especially client OMPs folding from complexes with SurA appear to profit greatly from the presence of membrane-embedded BAM, but show relatively little folding into empty lipid bilayers (Schiffrin et al. 2017). Despite the influence of different membrane composition, it evolves as a common theme from these studies that elevated concentrations of Skp prevent folding and membrane insertion of client OMPs (Bulieris et al. 2003; Schiffrin et al. 2016). The inhibitory effect of Skp was also observed when looking at chaperone assisted OMP folding on the single molecule level. The large OMP FhuA has a strong tendency to misfold in the absence of periplasmic chaperones, whereas in the presence of either of the chaperones Skp or SurA, misfolding is prevented. In agreement with the bulk measurements, folding of FhuA is largely abolished in the presence of Skp, however, FhuA can reinsert into the lipid bilayer in a series of folding steps shaped by individual beta-hairpins in the presence of SurA (Fig. 6.4a) (Thoma et al. 2012, 2015). The sequence in which individual β-hairpins insert into the membrane can be random, while a certain tendency is observed to progress from the C-terminal toward the N-terminal end of the

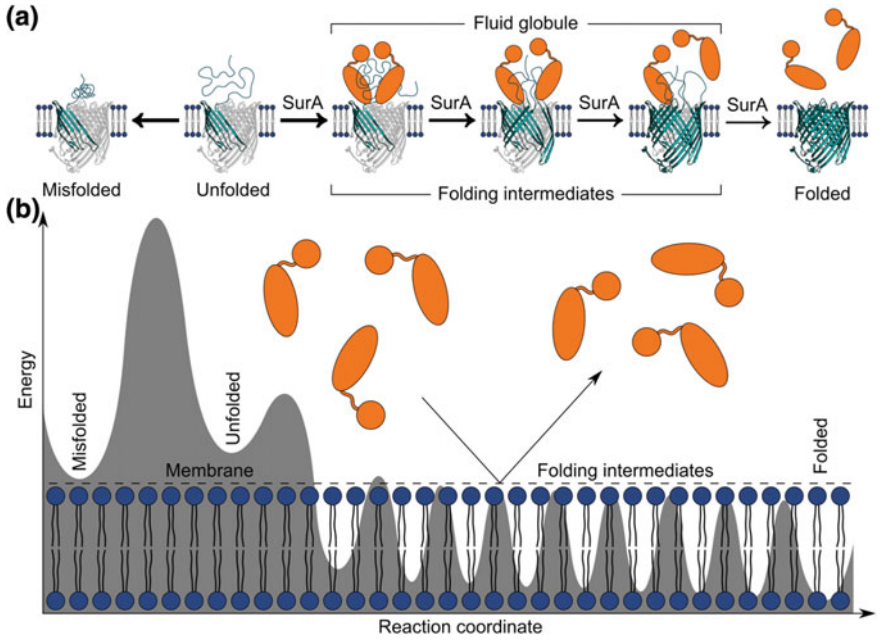

Fig. 6.4 Folding and membrane insertion model of client OMP FhuA from a SurA-FhuA complex. **a** In the absence of chaperones, the folding pathway of unfolded FhuA (cyan) is directed towards a misfolded state. The presence of SurA (orange) stabilizes the unfolded state of FhuA and promotes stepwise insertion and folding of β-hairpins into the lipid membrane until folding is completed, upon which SurA is released. **b** The hypothetical folding free-energy landscape of FhuA in the presence of SurA is characterized by a series of free-energy wells corresponding to β-hairpins stably inserted in the lipid membrane. SurA (orange) prevents misfolding of FhuA in solution but is spatially excluded from the lipid membrane (blue) and thus cannot interact with folded intermediates [adapted from (Thoma et al. 2015)]

protein. Thereby, the yet unfolded segments of the nascent FhuA polypeptide remain protected from misfolding over the entire time of the folding process. In agreement with the higher binding affinity of Skp, in an equimolar mixture of both chaperones, the inhibitory effect Skp exerted on the folding behaviour dominates over the folding promoting effect of SurA (Thoma et al. 2015). Consequentially, these experiments indicate that both chaperones could interact effectively with partially inserted client OMPs, strengthening the concept of the lipid membrane acting not only as a free-energy sink but also as a physical barrier separating partially folded segments from the chaperones (Fig. 6.4b).

In summary, these in vitro findings indicate that Skp binds clients with high affinity and a strong tendency to prevent their folding whereas SurA binds unfolded OMPs with lower affinity while promoting their folding. Together with in vivo findings, which indicate severely reduced OMP levels in SurA-depleted strains, but only mild phenotypes upon Skp depletion, the available data indicate a role of SurA acting

as the main periplasmic pathway for OMP folding, while Skp forms a secondary backup pathway (Silhavy et al. 2010; Sklar et al. 2007; Wu et al. 2011). The concept of SurA acting as the primary pathway is further substantiated by the observation that the delivery of major OMPs, as well as certain large OMPs such as LptD and FhuA seem to rely particularly on the presence of SurA (Vertommen et al. 2009). However, it remains to be elucidated precisely how the differential effects of both chaperones observed in vitro are modulated in vivo to promote efficient delivery of client OMPs to the OM.

Concluding Remarks

During the last decade, ground-breaking works have drastically improved our understanding of the molecular mechanism regulating the periplasmic chaperones Skp and SurA and highlighted the major importance of conformational flexibility in their mechanisms of action. These latest advancements have been made possible by the technical development of SAXS, molecular dynamics and NMR spectroscopy methods, in addition to the ground-laying crystal structures, highlighting the critical importance of the integration of the different methods. The structure and dynamics of the periplasmic chaperone Skp in its apo and holo states have shown that large conformational rearrangements of its cavity are the key factors of its functional cycle, giving Skp the capacity to adapt in response to the properties of its client proteins. Similarly, preliminary results on the chaperone SurA highlight the role played by the dynamic rearrangements of its domains to regulate its functional cycle.

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