Two Distinct Sites of client protein interaction with the chaperone cpSRP43

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ABSTRACT

Integral membrane proteins are prone to aggregation and misfolding in aqueous environments and therefore require binding by molecular chaperones during their biogenesis. Chloroplast signal recognition particle 43 (cpSRP43) is an ATP-independent chaperone required for the biogenesis of the most abundant class of membrane proteins, the light-harvesting chlorophyll a/b-binding proteins (LHCPs). Previous work has shown that cpSRP43 specifically recognizes an L18 loop sequence conserved among LHCP paralogs. However, how cpSRP43 protects the transmembrane domains (TMDs) of LHCP from aggregation was unclear. In this work, alkylation-protection and site-specific crosslinking experiments found that cpSRP43 makes extensive contacts with all the TMDs in LHCP. Site-directed mutagenesis identified a class of cpSRP43 mutants that bind tightly to the L18 sequence but are defective in chaperoning full-length LHCP. These mutations mapped to hydrophobic surfaces on or near the bridging helix and the β-hairpins lining the ankyrin repeat motifs of cpSRP43, suggesting that these regions are potential sites for interaction with the client TMDs. Our results suggest a working model for client protein interactions in this membrane protein chaperone.

Proper protein folding and localization are critical for cellular protein homeostasis. The post-translational targeting of integral membrane proteins poses an acute challenge to protein homeostasis. Before arrival at the target membrane, nascent membrane proteins are highly prone to aggregation in the cytosol and other aqueous cellular compartments. Thus, effective molecular chaperones or chaperone networks are required to minimize improper exposure of the transmembrane domains (TMDs) on newly synthesized membrane proteins and to maintain them in a soluble, translocation-competent conformation. Many examples illustrate the intimate link between chaperone function and membrane protein biogenesis, including SecB, Skp, and SurA that protect bacterial outer membrane proteins, and Hsp70 homologues implicated in the import of precursor proteins to the endoplasmic reticulum, mitochondria or chloroplast (1-7).

The light-harvesting chlorophyll a/b-binding proteins (LHCP) comprise over 50% of the protein content on the thylakoid membrane of green plants and form the most abundant family of membrane proteins on earth (8). LHCPs are nuclear encoded, initially synthesized in the cytosol, and imported across the chloroplast envelope in a largely unfolded state (8). In the chloroplast stroma, LHCPs are protected in a soluble ‘transit complex’ by the chloroplast signal recognition particle (cpSRP), comprised of the cpSRP43 and cpSRP54 protein subunits (9-12). Via interactions between the GTPase domains of cpSRP54 and its receptor cpFtsY, LHCPs are delivered to the Alb3 translocase and inserted into the thylakoid membrane (11,13-20). Previous work showed that the cpSRP43 subunit binds tightly to and quantitatively prevents the aggregation of multiple members of the LHCP family, and that it is necessary and sufficient to chaperone LHCPs (21,22). Although the chaperone activity of cpSRP43 is allosterically regulated by additional components in the cpSRP pathway, such as cpSRP54 and Alb3 (19,22-24), the simple composition of the cpSRP43-LHCP chaperone-client pair and the robustness of cpSRP43’s chaperone activity make this pair an excellent system to understand the interaction and regulation of membrane protein chaperones.

A long-standing question about the cpSRP43 chaperone is the mechanism by which it prevents the hydrophobic TMDs on its substrate proteins from aggregation. The substrate-binding domain (SBD) of cpSRP43 is comprised of ankyrin repeat motifs 1-4, capped at the N-terminus by a chromodomain (CD1) and at the C-terminus by a bridging helix (BH) (21,22,25). Biochemical and crystallographic analyses showed that a conserved Tyr204 in the third ankyrin repeat motif recognizes an FDPGL motif in L18, a conserved 18-amino acid sequence between the second and third TMDs of LHCP (12,22,25-27). However, interaction with a soluble loop sequence is unlikely to be sufficient to protect LHCPs, which contain three TMDs, from aggregation. The ability of cpSRP43 to quantitatively prevent full-length LHCPs from aggregation is highly suggestive of additional interactions between cpSRP43 and the substrate TMDs. Moreover, a recent study showed that cpSRP43 also protects aggregation-prone regions of glutamyl-tRNA reductase to enhance the stability of this enzyme (28), indicating that cpSRP43 can contact hydrophobic segments on client proteins independently of L18 recognition. Nevertheless, deletion of individual TMDs in LHCP or replacement with the TMDs from unrelated membrane proteins did not severely disrupt the cpSRP43-LHCP interaction.
(26); this lack of specificity rendered the putative TMD interactions of cpSRP43 particularly challenging to demonstrate and identify. Although a crosslinking study identified three additional residues in TM3 of LHCP that can crosslink to cpSRP43 (27), the study did not identify additional possible interactions with the remainder of LHCP, nor the sites on cpSRP43 that interact with the substrate protein. Further, conformational rearrangements occur in the cpSRP43 SBD upon substrate binding (22), making it particularly challenging to define the interaction of this chaperone with the substrate TMDs.

In this work, we used a combination of chemical modification-protection, crosslinking, and site-directed mutagenesis studies to understand the interaction between cpSRP43 and its client protein. The results showed that cpSRP43 can interact with LHCP across all three TMDs, and identified a set of mutant cpSRP43s that are specifically disrupted in their ability to chaperone LHCP without affecting recognition of the L18 motif. These observations suggest potential sites for TMD interactions on this membrane protein chaperone.

RESULTS
Mapping the interaction sites of cpSRP43 on Lhcb5 through alkylation protection and site-specific crosslinking. Lhcb5 is a member of the LHCP family that strongly depends on cpSRP for its biogenesis. Previous work showed that Lhcb5 forms a tight complex with cpSRP43, with an apparent $K_d$ value of $\sim 10$ nM (26). To define the sites on Lhcb5 involved in complex formation with cpSRP43, we tested the ability of cpSRP43 to protect individual residues in Lhcb5 from alkylation by N-ethylmaleimide (NEM). To this end, we purified a set of Lhcb5 variants in which single cysteines were engineered at every 5-10 residues across the entire sequence of Lhcb5 (29). To ensure that all Lhcb5 are bound by the chaperone, we tested the efficiency of complex formation between each single cysteine variant of Lhcb5 and cpSRP43 (Table 1) and used cpSRP43 concentrations that are saturating for each Lhcb5 mutant during assembly of the respective cpSRP43•Lhcb5 complexes. The previously identified FDPLGL interaction motif in the L18 sequence was not tested, as point mutations at any of these residues severely impaired complex formation with cpSRP43 (25,26).

The efficiency of NEM alkylation was quantified by intact mass spectrometry and provides a direct measure for the solvent accessibility of individual cysteine residues in Lhcb5 (29). Previous work showed that LHCPs are imported into the chloroplast in a largely unfolded state (8), and its folding requires the lipids and binding of photosynthetic pigments in the thylakoid membrane (30-32). This loosely folded state of Lhcb5 in the stroma is mimicked by denaturation in 6M GdmCl or 8M urea, and the NEM alkylation of the engineered single cysteines in denaturant solubilized Lhcb5 were used as a control to correct for the intrinsic differences in the reactivity of cysteines at different positions (29). Comparison of the alkylation efficiency in the cpSRP43•Lhcb5 complex to that of chemically denatured Lhcb5 provides a measure for the degree to which individual residues in Lhcb5 are protected by interaction with cpSRP43.

Representative data for the complexes of two Lhcb5 variants, V135C and E156C, are shown in Figures 1A and 1B, respectively. Deconvolution and quantification of the m/z spectrum showed that for Lhcb5(E156C), a single alkylated species was present after a 10 minute alkylation reaction, indicating that this site was fully alkylated and thus solvent exposed in the complex (Fig. 1B). By contrast, the m/z spectrum of Lhcb5(V135C) contained both the unalkylated and alkylated species (Fig. 1A), indicating that this site was protected by cpSRP43.

The results of the alkylation-protection experiments for all the Lhcb5 variants are shown in Figure S1 and summarized in Figure 1C. Mapping of the alkylation protection efficiencies at 10 minutes onto the sequence of Lhcb5 (Fig. 1D) revealed several patterns. Residues 70–143, which span the first two TMDs of Lhcb5 and their intervening loop, were modestly to heavily protected, suggesting that they were contacted by cpSRP43 in the cpSRP43•Lhcb5 complex. Residues 190–200, which form the C-terminal part of TM3, were also extensively protected, consistent with the results of a previous crosslinking analysis (27). The C-terminal loop of Lhcb5 was also modestly protected. In contrast, residues in the N-terminal loop of Lhcb5 were highly accessible. In addition, residues in the loop connecting TM2 and TM3 of Lhcb5 were accessible, consistent with crystallographic analysis showing that the L18 peptide is bound at a solvent accessible site on the surface of cpSRP43 (25). Together, these results show that cpSRP43 induced protections of Lhcb5 including all its TMDs, the TM1-TM2 loop, and the C-terminus.

To independently probe for the interaction of the LHCP TMDs with cpSRP43, we incorporated a photoinducible crosslinker, $p$-benzoyl-L-phenylalanine (pBpa), into specific positions of Lhcb5 via amber suppression coupled to the S30 in vitro translation system (33-35). Crosslinking occurs when
the ketone oxygen of the incorporated pBpa is within 3.1 Å of an interaction partner (36). To specifically examine the interactions between cpSRP43 and Lhcb5, we used a supermutant of cpSRP43 (intein-cpSRP43) that has been shown to mimic the conformation and activity of cpSRP54-activated cpSRP43 in both NMR spectroscopy and biochemical assays (22). We observed UV-induced, cpSRP43-dependent high molecular weight crosslinking products for pBpa incorporated at all three TMDs as well as the L18 motif of Lhcb5 (Fig. 2). Western blot analyses with anti-Strep (for Lhcb5) and anti-cpSRP43 antibodies of both the in vitro translation reaction and affinity-purified cpSRP43-Lhcb5 complex confirmed that the high-molecular weight band(s) contained both cpSRP43 and Lhcb5 (Fig. 3, A-C). MS analysis of the crosslinked bands for two Lhcb5 variants, with Bpa incorporated at residues 162 and 180, further confirmed that both bands contained cpSRP43 and Lhcb5 at a roughly 1:1 molar ratio (Fig. 3, D-F). These results indicate that all three of the TMDs of Lhcb5 can come into close contact with cpSRP43 in the complex. Crosslinking efficiency was highest with pBpa incorporated near the DPLG motif in the L18 sequence (Figs. 2, residues 162 and 164), consistent with specific recognition of this motif by cpSRP43. In comparison, the crosslinked bands between cpSRP43 and pBpa incorporated in the TMDs of Lhcb5 were weaker and more diffuse. The observation of two crosslinked bands, both containing equimolar cpSRP43 and Lhcb5 (Fig. 3D-F), further suggest the presence of alternative conformations in the cpSRP43-Lhcb5 complex. These observations suggest that the interactions of Lhcb5 with cpSRP43 are potentially dynamic.

Collectively, the combination of crosslinking and alkylation protection experiments in this section provides strong evidence that, in addition to the L18 motif, cpSRP43 can make contacts with and induce protection of all three of the TMDs in its client protein.

cpSRP43’s substrate binding domain is highly sensitive to point mutations. Previous work established that CD1, the ankyrin repeat motifs, and the BH together form a structural and functional unit that comprises the SBD of cpSRP43, which is sufficient to chaperone LHCPS (22). To establish which sites of cpSRP43 are involved in complex formation with LhCP, we mutated all solvent-exposed hydrophobic residues (Leu, Ile, Val, and Trp) in the SBD, as well as additional residues on the β-hairpins of the ankyrin repeat motifs and on the BH (highlighted in blue in Fig. 4A). Each residue was mutated to cysteine in an otherwise cysteineless cpSRP43 (C118A, C240S). Cys-less cpSRP43 is 5-fold reduced in binding and chaperoning LHCP compared to wildtype cpSRP43 because it is shifted to a less active conformation (22), but otherwise behaves analogously to wildtype cpSRP43.

We tested each single cysteine mutant of cpSRP43 for its ability to bind and protect LHCP from aggregation using a well-established light scattering assay (Figs. 4B and 4C). In this assay, LHCP denatured and solubilized in 8M urea was added to a solution containing either buffer, cys-less cpSRP43 (referred to as WT), or the mutant cpSRP43 of interest, and the turbidity of the solution was monitored in real time. In the absence of cpSRP43, LHCP aggregated extensively in aqueous solution (Figs 4B and 4C, green lines). The presence of 2.5 µM cys-less cpSRP43 prevented the aggregation of ~55% LHCP (Figs. 4B and 4C, black lines); this cpSRP43 concentration thus provides the most sensitive condition to screen for mutant cpSRP43s defective in chaperone activity.

We found that single point mutations of a surprisingly large number of residues in the cpSRP43 SBD compromised its chaperone activity. Of the 33 single cysteine mutants tested, only 10 mutants exhibited chaperone activities within three-fold of that of cys-less cpSRP43 (Fig. 4B and 4D). Six mutants exhibited 3-5 fold reductions in the solubilization of LHCP compared to cys-less cpSRP43, and chaperone activity was undetectable for 17 mutants (Fig. 4C and 4D). The sites of mutations that induced modest or severe defects in chaperone activity span almost an entire surface of the cpSRP43 SBD (see Fig. 8 below). Thus, cpSRP43 is highly sensitive to conservative perturbations in its SBD.

To independently test the chaperone activity of cpSRP43 mutants, we used an alternative sedimentation assay (21). LHCP denatured in 8M urea was added to either cys-less (WT) or mutant cpSRP43, and the mixture was separated into soluble and insoluble fractions by sedimentation followed by analysis on SDS-polyacrylamide gel electrophoresis PAGE (Fig. 5A). Qualitatively, most mutants displayed changes in chaperone activity in the sedimentation assay (Fig. 5B) that are consistent with the results of the turbidity assay. Nevertheless, a smaller mutational defect was observed in the sedimentation assay compared to the turbidity assay (cf. Fig. 5B versus 4D). Control experiments with a number of mutants for which this discrepancy is most pronounced revealed two major contributing factors:
(i) the higher protein concentration used in the sedimentation than the turbidity assay, which provided a more favorable binding equilibrium for cpSRP43 variants with binding defects (Fig. 5C); (ii) even at the same protein concentrations, the sedimentation assay showed a smaller mutational defect compared to the light scattering assay (Figs. 4D and 4E). This is likely due to less accurate quantification in western blot analysis, especially for mutants with large defects (the band in either the soluble or pellet fraction is outside the linear range of quantification). Thus, the sedimentation assay corroborated the defects of many cpSRP43 variants in chaperone activity, but the light scattering assay allowed a more sensitive and accurate detection of mutational defects.

Previous work showed that the ability of cpSRP43 to bind LHCP and generate a soluble transit complex is integral to the subsequent targeting and insertion of LHCP (10-14,37); this was the case for both in vitro translated as well as chemically denatured LHCP (21,38). To further assess the relationship between the ability of cpSRP43 to solubilize LHCP and the efficiency of LHCP targeting and integration, we measured and compared the two activities in parallel. LHCP unfolded in 8 M urea were either prevented from aggregation by dilution into a solution containing cpSRP43 (Fig. 6A, lane 1), or allowed to aggregate by dilution into buffer (Fig. 6A, lane 2). As cpSRP43 is also able to reverse LHCP aggregation (21,26,39), preformed LHCP aggregates were also incubated with increasing concentrations of cpSRP to allow re-solubilization by the chaperone (Fig. 6A, lanes 3-5). These samples were tested for the extent of LHCP solubilization (by the sedimentation assay) and for the efficiency of LHCP integration into thylakoid membrane (Fig. 6). Successful integration leads to protection of LHCP from thermolysin, giving rise to two protease-protected bands (Fig. 6A, DP1 and DP2). We observed a strong correlation between the degree of LHCP solubilization by cpSRP43 and the efficiency of LHCP insertion (Fig. 6B). As a constant amount of targeting factors (cpSRP43, cpSRP54, and cpFtsY) was present in all the integration reactions regardless of the conditions of pre-incubation, the observed differences in LHCP integration efficiency do not arise from the differences in the concentration of targeting factors, but rather from differences in the conformation of LHCP prior to initiation of the integration reaction. Together with previous work(10-14,37), these results strongly suggest that the ability of cpSRP43 to solubilize LHCP is required for its proper targeting and integration into the thylakoid membrane.

Two distinct classes of defective cpSRP43 SBD mutants. The large number of surface residues that exhibit a mutational defect in substrate binding could arise from an extensive interaction surface of cpSRP43 with LHCP, or from perturbations of the global conformation of the SBD by the mutations. Recent NMR studies showed that the SBD of apo-cpSRP43 intrinsically samples active and inactive conformations with equal probability (22), supporting the possibility that the activity of cpSRP43 could be susceptible to mutations that shift the conformational equilibrium. To control for mutational effects on the global conformation of the SBD, we tested the ability of cpSRP43 mutants to bind the L18 recognition motif of LHCP. All the chaperone-defective mutations examined here are located away from the crystallographically identified L18 binding site of cpSRP43 (Y204 highlighted in Fig. 8 below; (25)); thus, a defect in L18 binding caused by these mutations most likely arises from a global structural defect of the SBD, rather than disruption of a direct interaction with L18.

The binding affinity of cpSRP43 for L18 was measured based on the cpSRP43-induced increase in the fluorescence anisotropy of a HiLyte-Fluor488-conjugated L11 peptide, which represents the minimal sequence in L18 required for high affinity binding to cpSRP43 (26,29). Representative equilibrium titrations for L11-cpSRP43 binding are shown in Figures 6A and 6B. The equilibrium dissociation constants ($K_d$) for L11 binding to WT and mutant cpSRP43’s derived from the equilibrium titrations are summarized in Table 2. The anisotropy change of L11 induced by a sub-saturating concentration (0.19 μM) of each mutant cpSRP43 relative to that of WT cpSRP43 are summarized in Figure 6C.

We found that mutation of a large number of residues affected the interaction of cpSRP43 with the L18 motif. Eight mutants bound the L11 peptide an order of magnitude more weakly than WT cpSRP43 ($K_d ~ 0.8 – 3.5$ μM; Fig. 7B and Table 2, yellow), and three mutants exhibited ~100-fold weakened binding to L11 ($K_d > 10$ μM; Table 2, red). In contrast, eleven mutant cpSRP43s bound the L11 peptide with $K_d$ values within 5-fold of that of WT cpSRP43 ($K_d < 0.6$ μM; Fig. 7A and Table 2, green). We designated ten of these mutants as Class I: they either bind L11 with similar affinity compared to WT cpSRP43 but are defective in chaperoning LHCP (L103C, W106C, V124C, L228C, I237C, N260C, and E263C), or the
modest reductions in L11 binding observed with these mutants were insufficient to account for their complete loss of chaperone activity towards LHCP (V156C, G193C, L231C). Thus, Class I mutants specifically disrupt the ability of cpSRP43 to protect LHCP from aggregation. The remainder of the chaperone-defective mutants were designated as Class II. Although a direct involvement in TMD binding cannot be excluded, much of the defects of these mutants can be attributed to disruptions in interaction with the L18 motif. Since these mutations are located away from the vicinity of the L18 binding site, Class II mutants disrupt L18 binding allosterically by altering the conformation of the SBD.

**DISCUSSION**

cpSRP43 is a small, ATP-independent chaperone with an SBD comprised mostly of ankyrin repeat motifs. At a size of 25 kD, the cpSRP43 SBD is able to effectively chaperone multi-pass membrane protein substrates comparable to its own size, providing an attractive system to understand how a small protein scaffold interacts with and provides protection for large client proteins. Previous understanding of the cpSRP43-LHCP interaction was limited to recognition of the L18 loop sequence in LHCP by cpSRP43-Tyr204 (21,25,26,37). In this work, the results of alkylation-protection and crosslinking experiments showed that LHCP interacts more extensively with cpSRP43; the regions of interaction and/or cpSRP43-induced protection span all three TMDs of LHCP as well as the TM1-TM2 loop and the C-terminus. Furthermore, site-directed mutagenesis of the cpSRP43 SBD identified two classes of mutant cpSRP43’s: Class I, which disrupts cpSRP43’s ability to protect LHCP from aggregation without affecting high-affinity recognition of L18; and Class II, which allosterically disrupts binding of the L18 motif. Together, these results provide evidence for much more extensive cpSRP43-client interactions than previously recognized, and suggest potential sites of cpSRP43 that bind and protect the TMDs of LHCP.

When mapped onto the crystal structure of the cpSRP43 SBD, the two classes of mutants are enriched in different regions of the cpSRP43 SBD, suggesting that different surfaces in the SBD mediate distinct functions. The residues that give rise to Class II mutants are primarily located on the helices in the ankyrin repeat motifs (Fig. 8B, magenta). As the sites of Class II mutations are away from the previously identified L18 binding site (Tyr204; highlighted in blue), we attribute their defects to disruption of the active conformation of the cpSRP43 SBD (see discussion in the next paragraph). In contrast, residues that give rise to Class I mutants are enriched in the bridging helix, the β-hairpins in the ankyrin repeat motifs, and a hydrophobic surface in CD1 (Fig. 8A, orange), suggesting that these regions may either form or are in close vicinity of the TMD binding sites in cpSRP43. In support of this notion, Class I mutations cluster on or near major hydrophobic surfaces on the cpSRP43 SBD (Fig. 8C). This model is also consistent with the general structural and functional features of ankyrin repeat proteins, which are formed by individual repeats of helix-loop-helix folds connected by β-hairpins. Structural, computational, and protein engineering studies showed that intra- and inter-repeat interactions between the helices allow ankyrin repeat proteins to cooperatively fold into concave L-shaped structures; in contrast, the loops and β-hairpins, which project outward from the helices, often form the recognition site for interaction partners (40-43). We therefore propose that client recognition by cpSRP43 may occur analogously, with the L18 sequence specifically recognized by the loop in Ank3, while the TMDs in LHCP are bound and protected by the hydrophobic surfaces on Ank4 and BH, and on or near the β-hairpins.

The large number of residues in the cpSRP43 SBD, at which a single conservative mutation away from the direct L18 interaction site severely disrupts substrate binding and chaperone activity, is extraordinary. This behavior is characteristic of molecular systems that sample inactive conformations with a high probability, such that small perturbations are sufficient to drive the molecule or complex into the inactive state (44-47). Likewise, the sensitivity of cpSRP43 to point mutations suggests that its SBD is at the threshold of a cooperative conformational change required to attain a chaperone-active conformation. This model is consistent with recent NMR data that detected distinct conformational states in the cpSRP43 SBD that are equally populated in apo-cpSRP43 (22). As observed previously, this property of cpSRP43 may be particularly useful in enabling regulation, allowing cpSRP43 to be readily switched ‘on’ and ‘off’ by its regulators in the stroma and at the target membrane, respectively (22). The precise nature of the conformational changes in cpSRP43 remains an important question for future investigations.

**EXPERIMENTAL PROCEDURES**

*Protein Expression and Purification.* Single cysteine mutants of Lhcb5 and cpSRP43 were constructed
using the QuikChange Mutagenesis procedure (Stratagene) according to manufacturer’s instructions. WT and mutant cpSRP43, LHCP, and wildtype and mutant Lhcb5 were overexpressed and purified as previously described (20,21).

**Alkylation.** Single-cysteine mutants of Lhcb5 solubilized in 8M urea was treated with 4 mM TCEP overnight. Lhcb5 was rapidly diluted to a final concentration of 1 µM in Buffer D (200 mM NaCl, 50 mM KHEPES, pH 7.5) containing 4 µM cpSRP43 and incubated for 10 min to allow complex formation. The sample was centrifuged at 13,000 rpm for 30 min to remove aggregated proteins, and the soluble fraction was subject to NEM alkylation as described (29). Briefly, the pre-formed Lhcb5-cpSRP43 complex was incubated with 100 µM N-ethylmaleimide (NEM) for 2 or 10 minutes and quenched with 50 mM DTT. Quenched samples were treated with 0.2% formic acid and analyzed on an LC-MSD SL 1100 series (Agilent) using a 2.1 x 150 mm Zorbax 300SB-C3 column (Agilent) and a gradient consisting of 0.2% formic acid as solvent A and 0.2% formic acid in acetonitrile (89.8%) and methanol (10%) as solvent B. Intact masses were determined in the single quadrupole. Chemstation software (Agilent) was used to deconvolute the masses and quantify the proteins. The accessibility of each site (1- protection) was calculated from the alkylation efficiency of each cysteine mutant observed in the cpSRP43-Lhcb5 complex after subtracting that of the same mutant denatured in 6M GdmCl.

**Photoinducible crosslinking.** Amber stop codons were introduced into Lhcb5-coding plasmids at the indicated sites using the QuikChange Mutagenesis procedure (Stratagene). To incorporate pBpa, Lhcb5 harboring the amber codon at specific positions were in vitro translated using an S30 translation extract coupled to amber suppression, as previously described (35), except that an evolved aminoacyl-tRNA synthetase specific for pBpa was used in place of that for coumarin (34). Translation reactions were incubated for 90 minutes at 30 °C in the presence or absence of 20 µM intein-cpSRP43. Crosslinks were induced by exposure to 365 nm light for 2 hours at 4 °C. 10 µL reactions were mixed with an equal volume of 2X SDS and 4M Urea and analyzed by SDS-PAGE. Gels with 35S-labeled Lhcb5 were visualized by autoradiography using a Typhoon scanner. Western blots with 1XStrep-tagged Lhcb5 were immunoblotted with either anti-Strep or anti-cpSRP43 antibodies and visualized on a LI-COR Odyssey imaging system at 800 nm. Bands were quantified using the GelAnalyzer software. Crosslinking efficiency was determined by the ratio of the high-molecular weight crosslinked Lhcb5 bands to total Lhcb5 after subtracting the cpSRP43-independent crosslinks in the corresponding area in control reactions lacking cpSRP43.

To purify cpSRP43-Lhcb5 after crosslinking, translation reactions were scaled up to 1 mL and incubated for 60 minutes at 30 °C in the presence or absence of 5 µM intein-cpSRP43. Crosslinks were induced by exposure to 365 nm light for 45 min at 4 °C. After centrifugation at 16,000g for 15 min, the supernatant was loaded onto 100 µL Strep-Tactin resin pre-equilibrated with Strep Wash Buffer (20 mM HEPES, 500 mM NaCl, pH 7.5), or 100 µL of NiNTA resin pre-equilibrated with NiNTA Wash Buffer (20 mM HEPES, 300 mM KCl, 20 mM imidazole, pH 7.5). The resin was washed five times with 500 µL of Strep or NiNTA Wash Buffer and eluted with 500 µL of Strep Elution Buffer (20 mM HEPES, 500 mM NaCl, 20 mM Biotin, pH 7.5) or NiNTA Elution Buffer (20 mM HEPES, 300 mM KCl, 200 mM imidazole, pH 7.5). The final wash and elution fractions from Strep-Tactin purification were immunoblotted with anti-cpSRP43 antibody as previously described. The wash and elution fractions from NiNTA purification were stained with Coomassie blue and sent for MS analysis.

**Mass spectrometry analysis of cpSRP43-Lhcb5 crosslinks.** Samples were separated by SDS-PAGE using a 4–12% gradient NuPAGE gel (Invitrogen) with NuPAGE MES running buffer (Invitrogen) for 35 minutes at 200 V. The gel was stained with colloidal Coomassie stain (Invitrogen) and de-stained with water and ammonium bicarbonate. The desired bands were excised and digested by trypsin overnight at 37 °C. Digested peptides were extracted from gel and lyophilized. Digested samples were subjected to LC-MS/MS analysis on a nanoflow LC system, EASY-nLC 1200 (Thermo Fisher Scientific) coupled to a QExactive HF Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a Nanospray Flex ion source. Samples were directly loaded onto a 20cm x 50µm ID PicoFrit column (New Objective, Woburn, MA) packed in house with ReproSil-Pur C18AQ 1.9 um resin (120Å pore size, Dr. Maisch, Ammerbuch, Germany) and heated to 60 °C. Peptides were separated with the following gradient at a flow rate of 220 nL/min: 2–6% Solvent B (3 min), 6–25% B (40 min), 25–40% B (17 min), and 100% B (9min). Solvent A consisted of 97.8% H2O, 2% ACN, and 0.2% formic acid, and solvent B consisted of 19.8% H2O, 80% ACN, and 0.2% formic acid. The QExactive HF Orbitrap was

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*Interactions in A Membrane Protein Chaperone*
operated in data dependent mode with the Tune (version 2.7 SP1build 2659) instrument control software. Spray voltage was set to 2.5 kV, S-lens RF level at 50, and heated capillary at 275 °C.

Raw files were searched against the protein sequences using MaxQuant (48,49) (ver. 1.6.0.16) assuming trypsin digestion with up to two missed cleavages. Precursor mass tolerances were less than 4.5 ppm after mass recalibration and fragment ion tolerances were 20 ppm. Protein abundances were estimated by iBAQ (50).

**Chaperone Activity of cpSRP43.** The ability of cpSRP43 to prevent LHCP aggregation was measured as described (21). cpSRP43 were ultracentrifuged in a TLA-100 rotor (Beckman Coulter) at 100,000 rpm for 30 min at 4 °C prior to the experiment. Light scattering experiments were performed by addition of 3 μL of 50 μM LHCP denatured in 8 M urea to 150 μL buffer D (50 mM KHEPES, pH 7.5, 200 mM NaCl) or 2.5 μM cpSRP43 in buffer D. Assays at higher protein concentrations were performed by addition of 6 μL of 125 μM LHCP to 150 μL of 15 μM cpSRP43. Light scattering was monitored at 360 nm on a UV-Vis spectrometer (Beckman Coulter) over time until equilibrium was reached. The percentage of soluble LHCP (% soluble) at 300 s was calculated from:

\[
% \text{ soluble} = 1 - \frac{A_{\text{obsd}}}{A_0}
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in which \(A_0\) and \(A_{\text{obsd}}\) are the optical density readings in the absence and presence of cpSRP43, respectively. cpSRP43 itself does not contribute to the optical density reading (21).

Sedimentation experiments were performed by addition of 1 μL of 100 μM LHCP denatured in 8M urea to 19 μL of 15 μM cpSRP43 in buffer D. Sedimentation assays at lower concentrations were performed by adding 1 μL of 20 μM LHCP to 19 μL of 5 μM cpSRP43. The mixtures were incubated at room temperature for 30 minutes, and the soluble and pellet fractions were separated by centrifugation at 18,000 g for 30 minutes at 4 °C. The pellet was resuspended in 8M Urea, and both the pellet and soluble fractions were analyzed by SDS-PAGE. Gels were stained with Coomassie Blue (when using 5 μM LHCP) or western blot using an anti-LHCP antibody (when using 1 μM LHCP), and were imaged on a LI-COR Odyssey imaging system at 700 nm (for Coomassie stain) or 800 nm (for western blots). The intensity of LHCP bands was quantified using the GelAnalyzer software.

**Measurement of L18 binding.** The L18 binding affinity of cpSRP43 was measured using L11 (GSFDPLGLADD), the minimal binding motif in L18, conjugated to HiLyte-Fluor488. Anisotropy measurements were conducted in Buffer D on a Fluorolog 3-22 spectrofluorometer (Jobin Yvon), using 100 nM HiLyte-Fluor488-labeled L11 and varying concentrations of cpSRP43. Samples were excited at 500 nm and fluorescence anisotropy was recorded at 527 nm, as previously described (21,22). The data were fit to Eq 1,

\[
A_{\text{obsd}} = A_0 + \frac{\Delta A}{\frac{[L11]+[\text{pro}]+K_d-\sqrt{([L11]+[\text{pro}]+K_d)^2-4[L11][\text{pro}]}}{2[L11]}}
\]

in which [pro] is cpSRP43 concentration, \(A_0\) is the observed anisotropy value, \(A_{\text{obsd}}\) is the anisotropy value without cpSRP43, \(\Delta A\) is the change in anisotropy at saturating cpSRP43 concentrations, and \(K_d\) is the equilibrium dissociation constant for the interaction of cpSRP43 with L11-HiLyte-Fluor488.

**LHCP Integration Assay.** Thylakoids were collected from chloroplasts of 9-12 days old pea leaves (Laxton Progressive 9) hypotonically lysed in lysis buffer (10 mM KHEPES, pH 7.5, 10 mM MgCl2) as described (51). Thylakoids were further salt-washed and resuspended to a concentration of 1 mg/mL chlorophyll (1X). Each 150 μL LHCP integration reaction contained 20 μL of 35S-methionine-labeled LHCP pre-incubated with cpSRP43 (generated as described in legends to Fig. 6), 50 mL of 1X salt-washed thylakoid, 2 mM ATP, 2 mM GTP, 3 μM cpFtsY, and cpSRP43/54 supplemented to a final concentration of 3 μM. Integration reactions were incubated at 25 °C for 30 min and quenched on ice. The reaction mixtures were thermolysin-treated for 40 min and centrifuged to isolate the thylakoid membrane as described (51). The resulting pellets were resuspended in 2X SDS and analyzed by SDS-PAGE and quantified using Storm 840 (Molecular Dynamics) and ImageQuant (GE Healthcare).

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**Conflict of Interest:** The authors declare no competing financial interests.

**Author Contributions:** S.-o.S. conceived and coordinated the study. C.Z.M. and A.S. designed
performed, and analyzed the experiments in Fig. 5; A.S. and M.Y. designed, performed, and analyzed the experiments in Figs. 2 and 3; C.Z.M., and E.M. designed, performed, and analyzed the experiments in Figs. 4, 7, and Table 2, S.P. and S.H. designed, performed, and analyzed the experiments in Fig. 1 and Table 1 with help from A.M. and M.S., T.X.N. performed the experiments in Fig. 6. S.-o.S., C.Z.M. and A.S. wrote the paper with edits from T.X.N and S.H., A.M. and M.S. All authors reviewed the results and approved the final version of the manuscript.

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13. Tu, C.-J., Schunemann, D., and Hoffman, N. E. (1999) Chloroplast FtsY, Chloroplast Signal Recognition Particle, and GTP are required to reconstitute the soluble phase of


PMID: 21832051


Table 1. Chaperone activity of cpSRP43 towards individual Lhcb5 single-cysteine mutants (columns 2 and 3), and NEM alkylation efficiency at each cysteine in the presence of WT cpSRP43 (columns 4 and 5).

<table>
<thead>
<tr>
<th>Lhcb5 Construct</th>
<th>1 µM cpSRP43 Activitya</th>
<th>5 µM cpSRP43 Activitya</th>
<th>Alkylationb at 2’</th>
<th>Alkylationb at 10’</th>
</tr>
</thead>
<tbody>
<tr>
<td>I40</td>
<td>ND</td>
<td>ND</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>G50</td>
<td>ND</td>
<td>ND</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Q70</td>
<td>ND</td>
<td>ND</td>
<td>0.55 ± 0.02</td>
<td>0.67 ± 0.10</td>
</tr>
<tr>
<td>I75</td>
<td>84.48</td>
<td>ND</td>
<td>0.53 ± 0.05</td>
<td>0.66 ± 0.10</td>
</tr>
<tr>
<td>A85</td>
<td>102.15 ± 0.66</td>
<td>ND</td>
<td>0.33 ± 0.00</td>
<td>0.39 ± 0.02</td>
</tr>
<tr>
<td>P90</td>
<td>57.06 ± 31.69</td>
<td>ND</td>
<td>0.19</td>
<td>0.37 ± 0.14</td>
</tr>
<tr>
<td>C100 (WT)</td>
<td>95.59</td>
<td>ND</td>
<td>0.62 ± 0.16</td>
<td>0.66 ± 0.17</td>
</tr>
<tr>
<td>G110</td>
<td>ND</td>
<td>ND</td>
<td>0.29 ± 0.05</td>
<td>0.35 ± 0.03</td>
</tr>
<tr>
<td>N120</td>
<td>ND</td>
<td>ND</td>
<td>0.47 ± 0.04</td>
<td>0.52 ± 0.09</td>
</tr>
<tr>
<td>N125</td>
<td>74.62</td>
<td>ND</td>
<td>0.47 ± 0.00</td>
<td>0.56 ± 0.11</td>
</tr>
<tr>
<td>L130</td>
<td>68.18 ± 0.24</td>
<td>ND</td>
<td>0.28 ± 0.00</td>
<td>0.32 ± 0.01</td>
</tr>
<tr>
<td>V135</td>
<td>89.89 ± 14.78</td>
<td>ND</td>
<td>0.13</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>G143</td>
<td>89.20</td>
<td>ND</td>
<td>0.34</td>
<td>0.45 ± 0.01</td>
</tr>
<tr>
<td>T150</td>
<td>95.84</td>
<td>ND</td>
<td>0.80</td>
<td>0.88 ± 0.03</td>
</tr>
<tr>
<td>E156</td>
<td>80.74</td>
<td>ND</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>D157</td>
<td>86.34</td>
<td>ND</td>
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<tr>
<td>G162</td>
<td>95.39</td>
<td>ND</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>A171</td>
<td>86.33</td>
<td>ND</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>L180</td>
<td>89.85</td>
<td>ND</td>
<td>0.91 ± 0.12</td>
<td>0.91 ± 0.13</td>
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<tr>
<td>I185</td>
<td>82.86 ± 5.02</td>
<td>ND</td>
<td>0.91 ± 0.12</td>
<td>0.86 ± 0.02</td>
</tr>
<tr>
<td>L190</td>
<td>59.79</td>
<td>95.04</td>
<td>0.44 ± 0.10</td>
<td>0.48 ± 0.11</td>
</tr>
<tr>
<td>M195</td>
<td>74.87 ± 23.58</td>
<td>95.50</td>
<td>0.17 ± 0.02</td>
<td>0.24 ± 0.05</td>
</tr>
<tr>
<td>I200</td>
<td>77.24</td>
<td>ND</td>
<td>ND</td>
<td>0.25 ± 0.10</td>
</tr>
<tr>
<td>V210</td>
<td>92.27</td>
<td>ND</td>
<td>0.81</td>
<td>0.91 ± 0.13</td>
</tr>
<tr>
<td>P220</td>
<td>88.82</td>
<td>ND</td>
<td>0.44</td>
<td>0.50 ± 0.17</td>
</tr>
<tr>
<td>A230</td>
<td>87.60</td>
<td>ND</td>
<td>0.56</td>
<td>0.64 ± 0.14</td>
</tr>
</tbody>
</table>

a cpSRP43 chaperone activity was measured using 1 µM Lhcb5 in the light scattering assay. % soluble Lhcb5 at indicated cpSRP43 concentrations are reported. b Fraction of NEM modified Lhcb5 in the cpSRP43•Lhcb5 complex after indicated times of the alkylation reaction. All values represent mean ± S.E.M., with n = 2. N.D., not done.
Table 2. $K_d$ values for binding of HiLyte-conjugated L11 to individual cpSRP43 mutants. All cpSRP43 mutants shown in this table are derived from cys-less cpSRP43 (denoted as WT). Green highlights mutants that exhibit $K_d$ values within 3-fold of WT cpSRP43; yellow highlights mutants exhibiting 3-5 fold defects in L11 binding; and red highlights mutants that are severely defective in L11 binding. * indicates that saturation could not be reached with the mutant during equilibrium titrations, and their $K_d$ values for L11 were estimated assuming the same end point in the titration curve as cys-less cpSRP43.

<table>
<thead>
<tr>
<th>Construct</th>
<th>Average $K_d$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0.12 ± 0.029</td>
</tr>
<tr>
<td>L103C</td>
<td>0.14 ± 0.052</td>
</tr>
<tr>
<td>W106C</td>
<td>0.19 ± 0.027</td>
</tr>
<tr>
<td>W114C</td>
<td>3.03 ± 0.61</td>
</tr>
<tr>
<td>V124C</td>
<td>0.18 ± 0.058</td>
</tr>
<tr>
<td>V125C</td>
<td>0.78 ± 0.058</td>
</tr>
<tr>
<td>W133C</td>
<td>17.5* ± 3.65</td>
</tr>
<tr>
<td>V156C</td>
<td>0.55 ± 0.17</td>
</tr>
<tr>
<td>T162C</td>
<td>12.2* ± 0.44</td>
</tr>
<tr>
<td>F166C</td>
<td>3.53 ± 0.44</td>
</tr>
<tr>
<td>G193C</td>
<td>0.53 ± 0.16</td>
</tr>
<tr>
<td>G194C</td>
<td>2.38 ± 0.61</td>
</tr>
<tr>
<td>L195C</td>
<td>14.8* ± 5.26</td>
</tr>
<tr>
<td>T196C</td>
<td>0.87 ± 0.14</td>
</tr>
<tr>
<td>V222C</td>
<td>0.83 ± 0.22</td>
</tr>
<tr>
<td>L228C</td>
<td>0.083 ± 0.066</td>
</tr>
<tr>
<td>L231C</td>
<td>0.41 ± 0.029</td>
</tr>
<tr>
<td>I237C</td>
<td>0.17 ± 0.078</td>
</tr>
<tr>
<td>L238C</td>
<td>2.05 ± 0.19</td>
</tr>
<tr>
<td>R252C</td>
<td>0.42 ± 0.18</td>
</tr>
<tr>
<td>E256C</td>
<td>0.64 ± 0.017</td>
</tr>
<tr>
<td>I259C</td>
<td>0.94 ± 0.26</td>
</tr>
<tr>
<td>N260C</td>
<td>0.062 ± 0.0035</td>
</tr>
<tr>
<td>E263C</td>
<td>0.22 ± 0.063</td>
</tr>
</tbody>
</table>
Figure Legends

Figure 1. Alkylation pattern of Lhcb5 in the cpSRP43•Lhcb5 complex show cpSRP43-induced protection on the substrate protein. (A, B) Mass spectrum (upper left), deconvolution (lower panels), and component analysis (upper right) for a partially alkylated Lhcb5 residue, C135 (part A) and a completely alkylated Lhcb5 residue, C156 (part B). (C) Summary of the NEM alkylation efficiencies at individual sites in Lhcb5. Alkylation reactions were carried out for 10 minutes. For each engineered cysteine, ‘Fraction Accessible’ was calculated from the ratio of the fraction of alkylation in the cpSRP43•Lhcb5 complex relative to that of Lhcb5 dissolved in 6M GdmHCl. Error bars indicate S.E.M., with n = 2. (D) The alkylation protection pattern of Lhcb5 in complex with cpSRP43 is mapped onto the sequence of Lhcb5. Colored triangles denote the extent of protection, with white denoting the least protection (0% protection, or 100% alkylation) and blue denoting the highest observed protection (>50% protection, or <50% alkylation).

Figure 2. Site-specific crosslinking suggests extensive contacts between Lhcb5 and cpSRP43. (A) SDS-PAGE analysis of 35S-labeled Lhcb5 containing a photocrosslinker, pBpa, at indicated positions. Purified superactive cpSRP43 was present (+ lanes) or absent (− lanes) during translation, and samples were protected from light (− lanes) or exposed to UV light to induce pBpa crosslinks (+ lanes). Marked bands indicate the crosslinked cpSRP43•Lhcb5 complex (red), free Lhcb5 (green), and Lhcb5 crosslinked to an unknown protein in the translation extract (yellow). (B) Summary of crosslinking efficiencies between cpSRP43 and Lhcb5 with pBpa incorporated at different sites. Crosslinking efficiency was calculated from the ratio of the cpSRP43•Lhcb5 band to total Lhcb5 (after subtraction of background from the corresponding locations in the +UV, −cpSRP43 lane) for the SDS-PAGE analysis in part A and replicates (not shown). Data are reported as mean ± S.E.M., with n = 2.

Figure 3. Analysis of the high molecular weight crosslinked bands between cpSRP43 and Lhcb5. The color-marked bands in A-D indicate the crosslinked cpSRP43•Lhcb5 complex (red), free Strep-Lhcb5 (green), free cpSRP43 (blue), and Strep-Lhcb5 crosslinked to an unknown protein (yellow). (A-B) Representative western blot analyses of the crosslinking reactions and their controls from Figure 2A using anti-Strep (for Strep-tagged Lhcb5; part A) or anti-cpSRP43 (part B) antibodies. The lower MW band for Lhcb5 is likely a C-terminally proteolyzed product of full-length Lhcb5. (C) Representative western blot of affinity purification of the cpSRP43-Lhcb5 complex based on Strep-tagged Lhcb5. The final wash (W) and elution (E) from Strep-Tactin resin of Strep-Lhcb5 were shown for reactions with (+ lanes) and without (− lanes) intein-cpSRP43 and exposure to UV light. (D) Representative Coomassie-stained SDS-PAGE gels for the same crosslinking reactions purified based on His6-tagged cpSRP43, with Bpa incorporated at the indicated residues of Lhcb5. (E) NuPAGE gels showing the purified (as in C and D) cpSRP43-Lhcb5(162Bpa) and cpSRP43-Lhcb5(180Bpa) crosslinking reactions. The two labeled bands (A and B) were digested and sent for mass spectrometry analysis. (F) Representative results for MS analysis of the abundance of cpSRP43 and Lhcb5 in bands A and B excised the gel in part E for Lhcb5(162Bpa).

Figure 4. Single-cysteine mutants across the cpSRP43 SBD exhibit defects in chaperone activity in the light scattering assay. (A) Structure of cpSRP43 indicating all sites where cysteine mutations were made (blue). (B, C) Representative data showing the chaperone activity of neutral (part B) and defective (part C) cpSRP43 mutants. Light scattering time traces are shown for LHCP diluted into aqueous buffer (green), into a solution containing cyst-less WT (black), and into solutions containing the indicated cpSRP43 mutants. (D) Summary of the chaperone activity for all the single cysteine mutants of cpSRP43 measured by light scattering. Mutants exhibiting chaperone activity within three-fold of that of cyst-less cpSRP43 are considered neutral (above dashed line), whereas mutants with lower activity are considered defective (below dashed line). Error bars indicate S.E.M, with n = 3-13.

Figure 5. Analysis of the chaperone activity of mutant cpSRP43 using the sedimentation assay and comparison with the results of light scattering assay. (A) Representative Coomassie-stained gels for analysis of the chaperone activity of cpSRP43 mutants using the sedimentation assay. ‘C’ denotes lanes with cpSRP43 only; ‘S’ denotes the soluble fraction; ‘P’ denotes pellet. (B) Summary of the relative chaperone activity of all
Interactions in A Membrane Protein Chaperone

cpSRP43 mutants measured by the sedimentation assay. Values are reported relative to cys-less cpSRP43 (WT). (C) Comparison of the chaperone activity of cpSRP43 mutants measured at high (green) and low (blue) protein concentrations using the light scattering assay. (D) Representative western blot images for analysis of the chaperone activity of cpSRP43 mutants using the sedimentation assay at low protein concentrations. ‘C’ denotes lanes with cpSRP43 only; ‘S’ denotes the soluble fraction; ‘P’ denotes pellet. ‘LHCP’ denotes controls where indicated concentrations of purified LHCP were loaded to assess the dynamic range of western blot. (E) Comparison of the chaperone activity of cpSRP43 variants measured by the sedimentation (red; data from part D and replicates) and light scattering (blue) assays at the same concentration. Data were reported as mean ± S.E.M, with n = 2 – 9.

Figure 6. Solubilization of LHCP by cpSRP43 correlates with LHCP targeting and integration. (A) 35S-methionine labeled LHCP (lane 6, ‘Load’) were pre-incubated under different conditions with cpSRP43 and tested for targeting and insertion into thylakoid membrane in the presence of 3 μM cpSRP43, cpSRP54, and cpFtsY. Lanes 1-2: 2 μL of 35S-LHCP in 8 M urea was added to 40 μL Buffer D with (lane 1) or without (lane 2) 3 μM cpSRP43/54 and incubated for 60 min. Lanes 3-5, 2 μL of 35S-LHCP in 8 M urea was added to 33.6 μL of Buffer D and allowed to aggregate at RT for 60 sec, followed by addition of an equimolar ratio of cpSRP43/54 to final concentrations of 5, 15 and 30 μM in a final volume of 40 μL. 20 μL of the preincubated sample was used for the LHCP integration assay. DP1 and DP2 (25 and 18.5 kDa) are the protease-protected fragments of integrated LHCP (51). The remaining 20 μL was subjected to the sedimentation assay as described under Experimental Procedures, except that LHCP bands were quantified by autoradiography using Storm 840 (Molecular Dynamics) and ImageQuant (GE Healthcare). Details of the LHCP integration assay are described under Experimental Procedures. (B) Correlation of the translocation efficiency of LHCP with the degree to which LHCP is solubilized by cpSRP43.

Figure 7. Characterization of the interaction of mutant cpSRP43s with the L18 motif. (A, B) Representative equilibrium titrations for the binding of WT and mutant cpSRP43s to Hylite-Fluor488 labeled L11. Representative data for cpSRP43 mutants that can bind L11 with high affinity are shown in part A, and those for mutants exhibiting weakened L11 binding are shown in part B. (C) Summary of the cpSRP43-induced changes in the fluorescence anisotropy of L11 at 0.19 μM, which is subsaturating for binding of cys-less cpSRP43 to L11. The data for all mutants are normalized to that of cys-less cpSRP43 (denoted as WT). All data are reported as mean ± S.E.M., with n ≥ 2.

Figure 8. Mapping two classes of cpSRP43 mutants onto the crystal structure of the cpSRP43 SBD (PDB# 3dep). (A) Residues whose mutations led to defective chaperone activity for LHCP but did not disrupt L18 binding are categorized as Class I and colored in orange. (B) Residues whose mutations disrupted both cpSRP43’s chaperone activity and its interaction with the L18 motif are categorized as Class II and colored in magenta. (C) A putative model for the interaction surfaces of cpSRP43 with LHCP, with Tyr204 (blue) interacting with the L18 sequence, and the hydrophobic surfaces formed by Ank4, BH and the β-hairpins along the ankyrin repeat motifs involved in protection of the TMDs of LHCP. The electrostatic surface potential of the cpSRP43 SBD was generated using Adaptive Poisson-Boltzmann Solver (52) and visualized in PyMOL.
Interactions in A Membrane Protein Chaperone

Figure 1

A

V135

B

E156

C

10 min Alkylation

D

Protection

0%

>50%
Figure 2

A

B

Crosslinking efficiency

Fraction of total Lhcb5 crosslinked to cpSRP43

0 0.1 0.2 0.3

75 77 83 91 130 138 147 162 164 180 190 194 200 WT

N L18 L162 L164 L165 C
Figure 3

A: IB: anti-Strep

B: IB: anti-cpSRP43

C: UV +/−

D: cpSRP43, LHCb

E: Lad. 162, 180

F: Protein abundances from MS analysis
Figure 5

A

B

C

D

E
Figure 6

A

B

% translocation

% soluble LHCP

Interactions in A Membrane Protein Chaperone
Figure 7

Interactions in A Membrane Protein Chaperone
Two Distinct Sites of client protein interaction with the chaperone cpSRP43
Camille McAvoy, Alex Siegel, Samantha Piszkiewicz, Emily Miaou, Mansen Yu, Thang Nguyen, Annie Moradian, Michael J. Sweredoski, Sonja Hess and Shu-ou Shan

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