Chloroplasts contain a novel type of signal recognition particle (cpSRP) that consists of two proteins, cpSRP54 and cpSRP43. cpSRP is involved in the post-translational targeting of the nuclear encoded light-harvesting chlorophyll-binding proteins (LHCPs) to the thylakoid membrane by forming a soluble cpSRP-LHCP transit complex in the stroma. Despite high sequence homology between chloroplast and cytosolic SRP54 proteins, the 54-kDa subunit of cpSRP is unique in its ability to bind cpSRP43. In this report, we identified a 10-amino acid long segment of cpSRP54 that forms the cpSRP43-binding site. This segment is located at position 530–539 close to the C terminus of cpSRP54. In addition, we demonstrate that arginine at position 537 is essential for binding cpSRP43 and that mutation of arginine 536 drastically reduced cpSRP43 binding. Mutations within the cpSRP43-binding site of cpSRP54 that reduced or completely abolished cpSRP complex formation also did inhibit transit complex formation and integration of LHCP into the thylakoid membrane, reflecting the importance of these residues for LHCP targeting. Alignment studies revealed that the cpSRP43-binding site is conserved in chloroplast SRP54 proteins and is not present in any SRP54 subunit of cytosolic SRPs.

The cytosolic signal recognition particle (SRP) is part of a ubiquitous protein-targeting machinery that mediates the cotranslational insertion of membrane proteins into the endoplasmic reticulum of eukaryotes and the cytoplasmic membrane of prokaryotes. All of the known cytosolic SRPs are ribonucleoproteins, and their minimal functional core is formed by an RNA component and a conserved 54-kDa protein (SRP54). SRP54 consists of an N-terminal NG-domain encoding a GTPase function and a C-terminal located M-domain, which binds to the signal sequence of the elongating substrate protein (1–3). Recently, it was demonstrated that chloroplasts contain an SRP that is involved in the post translational targeting of members of the nuclear encoded light-harvesting chlorophyll-binding protein family (LHCPs) to the thylakoid membrane (4–6). LHCPs form the peripheral antenna of photosystems I and II and comprise approximately one-third of the thylakoid membrane proteins. Similar to all of the known cytosolic SRPs, chloroplast SRP contains a 54-kDa subunit (cpSRP54). Interestingly, in contrast to cytosolic SRPs, chloroplast SRP does not contain a RNA but rather a novel protein subunit of 43 kDa (cpSRP43) (5, 6). Although cpSRP54 and bacterial SRP54 (Ffh) show high sequence similarity, the chloroplast protein is clearly distinguishable from Ffh because Ffh cannot bind to cpSRP43 (7). In the current model of LHCP targeting to the thylakoid membrane of higher plants, the nuclear encoded LHCP is imported across the envelope membranes into the chloroplast stroma. Here, the transit peptide is cleaved off and LHCP is bound to the chloroplast stroma. By this, the cpSRP complex formation with cpSRP43 (6). Besides cpSRP (cpSRP54 and cpSRP43), a chloroplast homologue of the bacterial SRP receptor (cpFtS-Y) and GTP are required as soluble components for LHCP integration (8–10). Recently, the integral thylakoid membrane protein Alb3 was identified as the first transmembrane protein required for LHCP integration (11).

Several thylakoid membrane proteins (e.g. D1, D2, PSI-A, PSI-B) are encoded by the chloroplast genome and cotranslationally inserted into the thylakoid membrane. Recent reports describe that cpSRP54 is involved in the cotranslational targeting of D1 to the thylakoid membrane by binding to the first transmembrane domain of the elongating nascent chain of D1 (12, 13). Notably, no interaction of cpSRP43 with the D1 protein was detected (12). Further evidence for an involvement of cpSRP54 in cotranslational targeting of thylakoid membrane proteins came from the analysis of Arabidopsis mutants lacking functional cpSRP54. The young leaves of these plants showed a reduced level of the plastid-encoded photosystem I and II reaction center proteins (14, 15). These results supported the earlier observation that the chloroplast stroma contains two different pools of cpSRP54. One pool was bound to cpSRP43 and active in transit complex formation with LHCP, whereas a second pool of cpSRP54 was found to be associated with 70 S ribosomes in the absence of cpSRP43 (6, 16).

These findings raise the central question of how cpSRP54 is recruited for functioning in either the cpSRP43-dependent posttranslational pathway or the cpSRP43-independent cotranslational cpSRP pathway. To answer this question, it is important to know the exact nature of protein-protein interactions occurring during the posttranslational and cotranslational modes of action of cpSRP54. As a first step to solve this question and to identify specific sequence characteristics of...
cpSRP54 that makes cpSRP54 unique among the SRP54 subunits in its ability to bind cpSRP43, we aimed to characterize the binding site of cpSRP54 for cpSRP43. Recently, it was shown that cpSRP43 binds to the M-domain of cpSRP54 (17) and a report by Groves et al. (18) describes that the 26 C-terminal amino acids of cpSRP54 are essential for binding cpSRP43. Here, we show that a 10-amino acid long segment of cpSRP54 forms the cpSRP43-binding site and demonstrate that two amino acids within this region are crucial for binding cpSRP43. These residues are located close to the C terminus of cpSRP54 but not within the last 26 amino acids. The cpSRP43-binding site identified in this report is conserved in all of the chloroplast SRP54 proteins and is not present in any SRP54 subunit of cytosolic SRPs.

**EXPERIMENTAL PROCEDURES**

**N- and C-terminal Deletion Constructs of cpSRP54 for the Yeast Two-hybrid System**—All of the cDNAs encoding the C-terminal M-domain (residues 371–564) of cpSRP54 (cpSRP54M) or various truncations thereof were obtained by PCR amplification with the QuickChange XL site-directed mutagenesis kit (Stratagene) according to the manufacturer's protocol. The primer sets used for the construction of the mutant constructs are listed in Table II. Plasmid pGBKT7-cpSRP54M was used as template DNA to generate the single point mutation constructs cpSRP54M(R536G), cpSRP54M(R537G), cpSRP54M(K538M), cpSRP54M(R539G), and cpSRP54M(K539R) and the deletion constructs cpSRP54M-((371–543); primers 1 and 9 to yield cpSRP54-(371–541); primers 1 and 10 to yield cpSRP54-(371–539); primers 11 and 11 to yield cpSRP54-(371–538); and primers 1 and 12 to yield cpSRP54-(371–537). All of the PCR products were digested with the restriction enzymes NcoI and HindIII and cloned into the pGBKT7 constructs described above as template. The correct sequence of all of the constructs was verified by sequencing (Seqlab).

**Site-directed Mutagenesis Constructs for the Yeast Two-hybrid System**—All of the site-directed mutagenesis constructs were generated using the QuikChange XL site-directed mutagenesis kit (Stratagene) according to the manufacturer's protocol. The primer sets used for the construction of the mutant constructs are listed in Table II. Plasmid pGBKT7-cpSRP54M was used as template DNA to generate the single point mutation constructs cpSRP54M(R536G), cpSRP54M(R537G), cpSRP54M(K538M), cpSRP54M(R539G), and cpSRP54M(K539R) and the deletion constructs cpSRP54M-((371–543); primers 1 and 9 to yield cpSRP54-(371–541); primers 1 and 10 to yield cpSRP54-(371–539); primers 11 and 11 to yield cpSRP54-(371–538); and primers 1 and 12 to yield cpSRP54-(371–537). All of the PCR products were digested with the restriction enzymes NcoI and HindIII and cloned into the pGBKT7 constructs described above as template. The correct sequence of all of the constructs was verified by sequencing (Seqlab).

**Yeast Two-hybrid Assay**—The yeast two-hybrid assays were done as described by Jonas-Straube et al. (17) with the exception of the following modifications. pGBK7T7 constructs (see above) were used instead of pAS2 constructs as prey plasmids. For pGBK7T7 constructs that showed weak or no interaction with cpSRP43 in the yeast two-hybrid system, expression levels comparable with full-length cpSRP54 were verified by Western blot analysis using antibodies against the c-Myc epitope (BD Biosciences). The growth of the yeast cells on medium lacking leucine, tryptophan, and histidine (leu-, trp-, his-) was classified in (+), (+), and (−), whereby (+) means that most clones have a diameter of >1.5 mm and (−) indicates normal background growth (white colonies <0.8 mm). The filter lifts to measure the cpSRP54 translation in a wheat germ extract (Promega) under native conditions as suggested by the manufacturer. The corresponding constructs encoding cpSRP54M(R536K), cpSRP54M(R537K), cpSRP54M(R537N), cpSRP54M(K538K), and cpSRP54M(R538K) were obtained by site-directed mutagenesis (see above) using the primer sets, as listed in Table II, and pGem4SS6.5-cpSRP54M as template DNA. Each construct was confirmed by sequencing. The translation vectors pSPUTK-GST-chaos encoding GST-cpSRP43 and pSPUTK-GST encoding GST were described by Tu et al. (10) and Jonas-Straube et al. (17).

**Protein Pull-down Assays**—GST-cpSRP43, GST, and the indicated constructs of cpSRP54M were obtained by in vitro transcription and in vitro translation in a wheat germ extract (Promega) under native conditions as suggested by the manufacturer. Protein pull-down assays were performed using equimolar amounts of wheat germ extract. The proteins were translated in a wheat germ extract and incubation buffer (20 mM Hepes-KOH, pH 8.0, 50 mM KOAc, 10 mM MgCl₂) in a total volume of 120 μl. Control reactions were performed with in vitro translated GST instead of GST-cpSRP43. All of the binding reactions contained equal amounts of wheat germ extract. The proteins were incubated at 25 °C for 20 min, and the precipitation of the GST fusion protein was performed as described by Tu et al. (10). The eluted samples were analyzed on 15% acrylamide gels and detected by radiolabelling on a PhosphorImager.

**Transit Complex Formation**—Transit complex formation was measured as described previously (6) with the following modifications. 15 μl of in vitro translated GST-cpSRP54 and equal pmol of radiolabeled in vitro translated cpSRP54M or the indicated constructs were diluted with incubation buffer (20 μM Hepes-KOH, pH 8.0, 50 mM KOAc, 10 mM MgCl₂) in a total volume of 120 μl. Control reactions were performed with in vitro translated GST instead of GST-cpSRP43. All of the binding reactions contained equal amounts of wheat germ extract. The proteins were incubated at 25 °C for 20 min, and the precipitation of the GST fusion proteins with glutathione-Sepharose was performed as described by Tu et al. (10). The eluted samples were analyzed on 15% acrylamide gels and detected by radiolabelling on a PhosphorImager.

**Protein Expression and FTIR Spectroscopy**—Recombinant cpSRP54M and cpSRP54M-((371–543); primers 1 and 13 (Table I) and the corresponding pGBK7T7 constructs described above as template. The PCR products were digested with NcoI and HindIII and cloned into the NcoI-HindIII site of the overexpression vector pET-29b(+). The correct sequence of all of the constructs was verified by sequencing (Seqlab).

**Metabolites**

**Plasmid Construction for Protein Pull-down Assays**—The cDNAs encoding cpSRP54M, cpSRP54M(R536G), cpSRP54M(R537G), cpSRP54M(K538M), cpSRP54M(R539G), cpSRP54M(K540M), and cpSRP54M(K538M, K539M, and K540M) were obtained by PCR amplification using primer combination 1 and 13 (Table I) and the corresponding pGBK7T7 constructs described above as template. The PCR products were digested with NcoI and HindIII and cloned into the NcoI-HindIII site of the overexpression vector pET-29b (+). The correct sequence of all of the constructs was verified by sequencing (Seqlab).

**Protein Expression**—The cDNAs encoding cpSRP54M and cpSRP54M-((371–543); primers 1 and 13 (Table I) and the corresponding pGBK7T7 constructs described above as template. The PCR products were digested with NcoI and HindIII and cloned into the NcoI-HindIII site of the overexpression vector pET-29b(+) (Novagen). Each construct was confirmed by sequencing.

**Protein Expression and FTIR Spectroscopy**—Recombinant cpSRP54M and cpSRP54M-((371–543); primers 1 and 13 (Table I) and the corresponding pGBK7T7 constructs described above as template. The PCR products were digested with NcoI and HindIII and cloned into the NcoI-HindIII site of the overexpression vector pET-29b(+) (Novagen). Each construct was confirmed by sequencing.

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FTIR spectroscopy was carried out at 22 °C on a Bruker IFS 88 spectrometer. For each spectrum, a 256-scan interferogram was collected at single beam mode with a 2-cm⁻¹ resolution and a 1-cm⁻¹ interval from the 2000 to 1000 cm⁻¹ region. Reference spectra were recorded under identical conditions with solute buffer only. Each measurement was repeated three times. The resultant protein absorbance spectrum was smoothed to a 4-cm⁻¹ resolution with a Fourier self-deconvolution algorithm to reduce background. Signals originating from side chains were corrected using side chain absorbance bands corresponding to the putative cpSRP43-binding site. The amino acid sequence and the position of this putative cpSRP43-binding site are in boldface (this region required for binding to cpSRP43 is underlined). The cpSRP43-binding site within the M-domain of cpSRP54 are shown in Fig. 1.

The indicated deletions of cpSRP54M were cloned into the yeast two-hybrid plasmid pGBKTK7. Yeast strain Y190 was cotransformed with the combination of each pGBKTK7-54M construct and pACT2-43 encoding mature cpSRP43. The activation of the -his- and lacZ-reporter was measured as described under “Experimental Procedures.”

### RESULTS

Previously, it was demonstrated that the interaction of cpSRP43 and cpSRP54 is mediated via the C-terminal located M-domain of cpSRP54 (cpSRP54M; residues 371–564) (17). To define more clearly the region of cpSRP54M that mediates binding to cpSRP43, serial deletions from either the N- or the C-terminal end of cpSRP54M were cloned into the bait plasmid pGBKTK7. The interaction of cpSRP54M and the various deletion constructs with mature cpSRP43 was tested in the yeast two-hybrid system using pACT2-43 as prey plasmid (Table III). No obvious change in binding intensity was observed between cpSRP54M and the N-terminal deletion constructs cpSRP54M-(485–564), cpSRP54M-(521–564), and cpSRP54M/(530–564) in comparison with full-length cpSRP54M (Table III). A weaker binding was detected between cpSRP43 and cpSRP54M-(533–564), and no binding was observed for cpSRP54M(535–564). Yeast two-hybrid experiments testing the interaction of C-terminal deletion constructs of cpSRP54M with cpSRP43 demonstrated that the last 25 residues can be removed from the C terminus without having an obvious influence on the binding intensity of the resulting construct cpSRP54M-(371–539) to cpSRP43. However, further removal of two additional C-terminal residues, generating cpSRP54M-(371–537), led to a complete loss of interaction with cpSRP43 (Table III). Taken together, these results suggested that the region comprising residues 530–539 of cpSRP54 is involved in the formation of the cpSRP43-binding site by demonstrating that a deletion construct of cpSRP54M lacking this region is not able to bind cpSRP43 (Table III).

A noticeable feature of the amino acid region of cpSRP54 comprising the cpSRP43-binding site is the presence of the positively charged pentapeptide (RRKRR) located at amino acid position of 536–540. To determine whether this region is involved in the binding of the highly negatively charged cpSRP43, we constructed cpSRP54M-(Δ536–540) and tested its ability to bind cpSRP43 by yeast two-hybrid experiments and in vitro pull-down assays. As shown in Table III and Fig. 4, no interaction was observed between cpSRP54M-(Δ536–540) and cpSRP43. We proved that the removal of the positively charged pentapeptide did not lead to an overall structural rearrangement by performing FTIR spectroscopy of highly purified recombinant cpSRP54M and cpSRP54M(Δ536–540). Both proteins generated very similar spectra indicating that their secondary structure is almost identical (Fig. 3). This result, together with the above mentioned observation that a synthetic peptide containing the pentapeptide RRKRR inhibits the binding of cpSRP54 to cpSRP43, demonstrates clearly that residues within this motif are essential for the formation of the cpSRP43-binding site.

We next aimed to define the role of the individual amino acids within the cpSRP43-binding site in boldface (this work). Crucial amino acids within the cpSRP43-binding site are underlined (this work).
acids within the pentapeptide RRKKR (536–540) in binding cpSRP43. Therefore, site-directed mutagenesis was used to exchange one or more of these positively charged residues into uncharged amino acids. The ability of the generated cpSRP54M mutants to bind cpSRP43 was initially tested in yeast two-hybrid experiments (Table IV). No significant change in binding intensity to cpSRP43 was observed when using the construct cpSRP54M(K538M), cpSRP54M(R539G), or cpSRP54M(K540M). Even the simultaneous change of the positively charged residues Lys-538, Arg-539, and Lys-540 (cpSRP54M(K538M,K539M,K540M)) or the deletion of these amino acids (cpSRP54M(Δ538–540)) did not lead to a measurable loss of interaction with cpSRP43 in this system. However, the single mutation R537G caused a complete loss of binding of the corresponding construct cpSRP54M(R537G) to cpSRP43 because no β-galactosidase activity was detectable in the yeast two-hybrid system. Consistently, all of the other tested constructs containing the R537G mutation (cpSRP54M(R537G,K538M), cpSRP54M(R537G,K539M), cpSRP54M(R537G,K540M), K538M, K539M, K540M) were also unable to interact with cpSRP43 (Table IV). Introduction of the mutation R536G reduced the binding intensity, and no interaction with cpSRP43 was observed for the construct cpSRP54M(R536G, K538M, R539G, K540M). These results suggested that residues Arg-537 and Arg-536 of cpSRP54 are critical for the constitution of the cpSRP43-binding site, whereas Lys-538, Arg-539, and Lys-540 are not or only slightly involved in binding cpSRP43.

To further support these results and to quantify them, we measured the differences in the binding of radiolabeled cpSRP54M or various constructs containing mutations within the RRKKR(536–540) motif to GST-cpSRP43 by in vitro pull-down experiments (Fig. 4A). In accordance with the yeast two-hybrid experiments, the results of the binding reactions show that cpSRP54M constructs containing the mutation R537G did not interact with cpSRP43 and that the binding of cpSRP54M(R536G) to cpSRP43 was reduced on average by ~90% compared with cpSRP54M. The mutations K540M and K538M did not influence binding significantly, whereas the conversion of Arg-539 into Gly-539 resulted in a considerable reduction of binding by ~45%. This reduction value was not detected in the semi-quantitative yeast two-hybrid system.

We next sought to analyze whether the positive charge or the specific structure of the arginine side chain at position 536, 537, or 539 is required for the interaction of cpSRP54 with cpSRP43. Therefore, arginines at these positions were replaced individually with lysine and the interaction of the resulting constructs with cpSRP43 was tested by pull-down experiments. As shown in Fig. 4B, cpSRP54M(R536K) and cpSRP54M(R537K) were not able to bind cpSRP43. A reduced binding efficiency was observed for cpSRP54M(R539K). Hence, the constructs containing the Arg/Lys mutations behaved in the same way as the Arg/Gly mutants, demonstrating that a positive charge at position 536, 537, or 539 is not sufficient to mediate interaction with cpSRP43. We then analyzed whether the polar-charged side chain of Arg-537 can be functionally replaced by the polar side chains of glutamine or asparagine. As shown in Fig. 4B, no binding was observed between the constructs cpSRP54M(R537Q) or cpSRP54M(R537N) and cpSRP43. These results indicated that the interaction of cpSRP43 with cpSRP54 specifically requires an arginine residue at position 537.

Previous experiments demonstrated that both subunits of cpSRP are required for the formation of the transit complex with LHCP (6). Therefore, mutations within cpSRP54 that
abolish or diminish binding to cpSRP43 should also have a negative effect on transit complex formation, assuming that a complex formation between cpSRP43 and cpSRP54 is required for the interaction with LHCP. To test the function of Arg-536, Arg-537, and Arg-539 of cpSRP54 in transit complex formation, radiolabeled LHCP was mixed with recombinant cpSRP43 and the indicated cpSRP54M constructs and assayed for transit complex formation. The transit complex represents a soluble form of LHCP and can be detected on non-denaturing gels. As shown in Fig. 5, those mutations (Δ536–540, R537G, R537G/K538M, R538G, and R539G), which reduced binding of cpSRP54 to cpSRP43, inhibited transit complex formation to approximately the same extent as binding to cpSRP43. As expected from the above results, the mutations K538M and K540M did not reduce the formation of transit complex.

To further examine the role of the cpSRP43-binding site of cpSRP54 in LHCP biogenesis, the integration of radiolabeled LHCP into thylakoid membranes was measured in the presence of recombinant cpSRP43, in vitro translated cpFtsY, GTP, and in vitro translated cpSRP54 or the constructs cpSRP54(R537G), cpSRP54(R537K), and cpSRP54(R539K). Fig. 6 shows that the integration activity in assays containing the mutated forms of cpSRP54 is strongly reduced compared with the assay containing cpSRP54. Taken together, these data demonstrate clearly that those amino acids of cpSRP54, which are essential for the cpSRP complex formation, are also crucial for the transport and insertion of LHCP into the thylakoid membrane.

**DISCUSSION**

Chloroplast SRP involved in the posttranslational targeting of LHCP represents a specialized type of SRP, because it consists of the subunits cpSRP54 and cpSRP43 and lacks an RNA moiety. Interestingly, cpSRP54 is also involved in the cotranslational targeting of the chloroplast-encoded D1 to the thylakoid membrane. Recently, some progress has been made to analyze the molecular nature of the interaction between cpSRP54 and cpSRP43. Yeast two-hybrid experiments in combination with in vitro pull-down experiments demonstrated that the C-terminal M-domain of cpSRP54 mediates binding to cpSRP43 (17), and another report (18) using a pepscan approach extended this observation by finding that the extreme C-terminal region of cpSRP54 interacts strongly with cpSRP43. This report describes that the C-terminal 26 residues of cpSRP54 are essential for complex formation with cpSRP43. In the present study, we performed a detailed analysis of the cpSRP43-binding site of cpSRP54. We demonstrated that the essential binding site is located within residues 530–539 of cpSRP54. Furthermore, we showed that two residues, Arg-536 and Arg-537, are crucial for binding cpSRP43. Arg-539 is also expected from the above results, the mutations K538M and R537G did not reduce the formation of transit complex. The transit complex represents a soluble form of LHCP and can be detected on non-denaturing gels. As shown in Fig. 5, those mutations (Δ536–540, R537G, R537G/K538M, R538G, and R539G), which reduced binding of cpSRP54 to cpSRP43, inhibited transit complex formation to approximately the same extent as binding to cpSRP43. As expected from the above results, the mutations K538M and K540M did not reduce the formation of transit complex.

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mediating binding to cpSRP43 are located in the C-terminal region of cpSRP54. However, they are not located within the last 26 amino acids (residues 539–564) of cpSRP54, explaining our initial finding that the removal of these amino acids did not completely abolish binding to cpSRP43.

Based on the above results, one would expect that the cpSRP43-binding region (residues 530–539) including the essential amino acids, Arg-536 and Arg-537, of Arabidopsis cpSRP54 is conserved in all of the chloroplast SRP54 proteins. In Fig. 7, an alignment of the C termini of the known chloroplast SRP54 proteins is shown. As expected, the double arginine motif is conserved in these sequences. In addition, in all of the species, this motif is followed by two positively charged amino acids (Lys-Arg or Lys-Lys). Two other residues that are conserved throughout all of the sequences (Pro-532 and Gly-533) are identified by asterisks and are exclusively located within the cpSRP43 binding motif (underlined residues). Residues corresponding to Arg-536 and Arg-537 of cpSRP54 of Arabidopsis are indicated in boldface.

![Fig. 7. C-terminal section of an alignment of the E. coli SRP54 homologue and chloroplast SRP54 of various organisms. The SRP54 homologue of E. coli and the chloroplast SRP54 homologues of Oryza sativum (Or. sat.), Zea mays (Z. m.), Lycopersicon esculentum (Lyc. esc.), Pisum sativum (Pis. sat.), Chlamydomonas reinhardtii (Chlamy.), and Arabidopsis thaliana (Ar. th.) were aligned using ClustalW, version 1.8. Chloroplast SRP54 proteins are distinguished from the E. coli SRP54 homologue by a C-terminal extension containing the cpSRP43-binding site. Conserved amino acids within this C-terminal extension are indicated by an asterisk and are exclusively located within the cpSRP43 binding motif (underlined residues). Residues corresponding to Arg-536 and Arg-537 of cpSRP54 of Arabidopsis are indicated in boldface.](image)

In *E. coli*, the Ffh protein is involved in the cotranslational transport of membrane proteins to the plasma membrane. During this process Ffh, which is bound to the bacterial SRP-RNA, interacts with the signal sequence of the nascent protein and also contacts the ribosome at the ribosomal subunit L23 that is located close to the nascent chain exit site (21–23). In plastids, cpSRP54 is a component of two fundamentally different mechanisms because it is involved in posttranslational and cotranslational targeting pathways. From these observations, the question concerning the molecular details of switching between the posttranslational and cotranslational modes of action of cpSRP54 arises. Provided that the cotranslational targeting mechanism in chloroplasts is similar to that in bacteria, the results from the present work show that the cpSRP43-binding site is located at a position of cpSRP54, which is apparently not required for the cotranslational pathway. However, because no RNA was identified yet as a component of the cotranslationally acting cpSRP, it might be possible that the cotranslational targeting mechanisms in chloroplasts and bacteria exhibit substantial differences. Therefore, more work is necessary to analyze the molecular details underlying the recruitment of cpSRP54 for functioning in the posttranslational targeting of LHCP and the cotranslational targeting of chloroplast-encoded proteins to the thylakoid membrane.

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