The chloroplast signal recognition particle (cpSRP) consists of an evolutionarily conserved 54-kDa subunit (cpSRP54) and a dimer of a unique 43-kDa subunit (cpSRP43). cpSRP binds light-harvesting chlorophyll proteins (LHCPs) to form a cpSRP/LHCP transit complex, which targets LHCP to the thylakoid membrane. Previous studies showed that transit complex formation is mediated through the binding of the L18 domain of LHCP to cpSRP43. cpSRP43 is characterized by a four-ankyrin repeat domain at the N terminus and two chromodomains at the C terminus. In the present study, we used the yeast two-hybrid system and in vitro binding assays to analyze the function of different domains of cpSRP43 in protein complex formation. We report here that the first ankyrin repeat binds to the 18-amino acid domain on LHCP that binds to cpSRP43, whereas the third and fourth ankyrin repeats are involved in the dimerization of cpSRP43. We show further that the interaction of cpSRP43 with cpSRP54 is mediated via binding of the methionine-rich domain of cpSRP54 to the C-terminally located chromodomains of cpSRP43. Both chromodomains contain essential elements for binding cpSRP54, indicating that the closely spaced chromodomains together create a single binding site for cpSRP54. In addition, our data demonstrate that the interaction of cpSRP54 with the chromodomains of cpSRP43 is enhanced indirectly by the dimerization motif of cpSRP43.

The cpSRP43 sequence is characterized by the presence of two types of motifs mediating protein-protein interactions (9). The N-terminal region of cpSRP43 contains four ankyrin repeats, and the C-terminal region contains two closely spaced chromodomains (9). The ankyrin repeat, an ~33-amino acid-long motif, is found in a very large number of proteins (reviewed in Refs. 19 and 20). The diversity of biological roles of ankyrin repeat-containing proteins indicates that the ankyrin repeat serves as a versatile module generating the dimerization interface for a variety of different protein substrates. The chromodomain is a 30–70-amino acid motif that was first identified in the Drosophila chromatin-binding proteins polycomb and heterochromatin protein (21, 22). Subsequently, chromodomains were found in various proteins involved in the regulation of chromatin structure by protein-protein interactions (23, 24). Interestingly, cpSRP43 is the first example of a non-nuclear chromoprotein. In the present work, we used the yeast two-hybrid system to analyze the function of the ankyrin repeats and the chromodomains of cpSRP43 in self-dimerization and heterodimerization with cpSRP54 and LHCP. The binding properties of the minimal interaction domains identified by the yeast two-hybrid system were confirmed using an in vitro binding assay.
Table I

| Primers used for PCR amplification |
|------------------------------------|
| Forward primers                     |
| 1. cpSRP4(Asp)                      |
| 2. cpSRP4(Asp)                      |
| 3. cpSRP4(Asp)                      |
| 4. cpSRP4(Asp)                      |
| 5. cpSRP4(Asp)                      |
| 6. cpSRP4(Asp)                      |
| 7. cpSRP4(Asp)                      |
| 8. cpSRP4(Asp)                      |
| 9. cpSRP4(Asp)                      |
| 10. cpSRP4(Asp)                     |
| 11. cpSRP4(Asp)                     |
| 12. cpSRP4(Asp)                     |
| 13. cpSRP4(Asp)                     |
| 14. cpSRP4(Asp)                     |
| Reverse primers                     |
| 15. cpSRP4(Asp)                     |
| 16. cpSRP4(Asp)                     |
| 17. cpSRP4(Asp)                     |
| 18. cpSRP4(Asp)                     |
| 19. cpSRP4(Asp)                     |
| 20. cpSRP4(Asp)                     |
| 21. cpSRP4(Asp)                     |
| 22. cpSRP4(Asp)                     |
| 23. cpSRP4(Asp)                     |
| 24. cpSRP4(Asp)                     |
| 25. cpSRP4(Asp)                     |
| 26. cpSRP4(Asp)                     |
| 27. cpSRP4(Asp)                     |
| 28. cpSRP4(Asp)                     |
| 29. cpSRP4(Asp)                     |
| 30. cpSRP4(Asp)                     |

Experimental Procedures

Plasmid Construction for the Yeast Two-hybrid System—Plasmid pAS2 (CLONTECH) was used to construct the bait plasmids encoding the Gal4 DNA binding domain hybrid protein. Plasmid pACT2 (CLONTECH) was used to construct the prey plasmids encoding the Gal4 activation domain hybrid protein. All constructs encode an additional hemagglutinin epitope tag. All cDNAs encoding the L33 domain of LHCP (LHCP-(189–222)), the mature form or truncations of cpSRP43 and cpSRP54, were obtained by PCR amplification with Pfu polymerase (Stratagene) or Pwo polymerase (Peqlab) using the plasmids pAB80 (25), pSPUTKGStChao (15), and pAF1 (6) as templates. The cDNA coding for mature cpSRP43 (residues 61–376) was obtained by using primers 3 and 17 (Table I). Coding sequences for the truncations of cpSRP43 or mature cpSRP43 and cloned into pAS2 showed no blue color development after 1.5 h. Yeast cells cotransformed with pAS2 and the constructs cloned into pACT2 showed only background growth on −Leu/−Trp/−His medium. However, all of these yeast clones showed no blue color development after 1.5 h. Yeast cells cotransformed with pAS2 and the pACT2 constructs encoding mature or deletions of cpSRP45 showed only background growth on −Leu/−Trp/−His plates and had no β-galactosidase activity even after 24 h of color development. Constructs encoding either deletions of cpSRP43 or mature cpSRP43 and cloned into pAS2 showed slight self-activation of the −his reporter, which led to some cell growth (+) on −Leu/−Trp/−His medium. However, all of these yeast clones showed no blue color development after 1.5 h. Yeast cells cotransformed with pAS2 and the pACT2 constructs encoding mature or deletions of cpSRP45 showed only background growth on −Leu/−Trp/−His plates and had no β-galactosidase activity even after 24 h of color development. LHCp-(189–222) cloned into pAS2 showed strong self-activation of the −his-lacZ reporter, such that only the pACT2 version of this construct was used. For fusion proteins that showed only weak or no interaction in the yeast two-hybrid experiments, an expression level comparable with full-length cpSRP43 or cpSRP54 was verified by Western blot analysis using antibodies against the hemagglutinin tag (Roche Molecular Biochemicals). All experiments were performed at least in duplicate.

Plasmid Construction for Protein Binding Assays—The cDNAs coding for cpSRP43-(190–350), cpSRP43-(259–350), cpSRP43-(61–159), and the M domain of cpSRP54 (residues 371–564) were obtained by PCR amplification as described above. Primer combinations 12/26 and 7/27 were used to yield cpSRP43-(190–258) and cpSRP43-(190–358), respectively. The reverse primer for the amplification of cpSRP43-(180–258) was designed to introduce two additional methionine residues at the C terminus. The PCR products encoding cpSRP43-(190–258), cpSRP43-(259–350), and cpSRP43-(61–159) were digested with the restriction enzymes BamHI and Clal and cloned into the vector fragment from the in vitro translation vector pSPUTKGStChao (15) digested with BamHI and Clal. The resulting constructs encode cpSRP43-(190–258), cpSRP43-(259–350), and cpSRP43-(61–159) as GST fusion proteins. The in vitro translation vector
Function of the Ankyrin Repeats and Chromodomains of cpSRP43

RESULTS

Dimerization of cpSRP43 Is Mediated via the Ankyrin Repeat Domain of cpSRP43—In a previous study, it was demonstrated that cpSRP43 occurs as a homodimer (15). This result was based on the observation that cpSRP43, from the stroma of an Arabidopsis mutant that lacks cpSRP4 (ifc1–2), elutes from a gel filtration column at 70 kDa, which is twice the predicted monomer molecular mass. In addition, homodimerization of cpSRP43 was shown by cross-linking experiments with highly purified recombinant cpSRP43 (15). Sequence analysis of cpSRP43 has shown that the protein contains four ankyrin repeats at the N terminus and two chromodomains at the C terminus (9). A schematic presentation of the exact location of these sequence motifs is shown in Fig. 1. To determine which domains of cpSRP43 mediate its dimerization, we cloned the mature protein and various deletion constructs (Fig. 2) into the bait plasmid pAS2 and the prey plasmid pACT2. Yeast cells were cotransformed with the indicated plasmid combinations. The ability of the cotransformed yeast cells to grow on medium lacking histidine (−His) and to express β-galactosidase indicates an interaction of the tested proteins. Table II shows that bait cpSRP43 interacts with prey cpSRP43, confirming our previous conclusion that cpSRP43 forms a homodimer (15). As a first step toward analyzing which domain of cpSRP43 mediates dimerization, the interaction between mature cpSRP43 and the ankyrin repeat-containing N-terminal part of cpSRP43 (cpSRP43A-(61–258)) versus the chromodomains-containing C-terminal part of cpSRP43 (cpSRP43C-(259–376)) was tested. Table II shows that mature cpSRP43 interacts with cpSRP43A-(61–258) but not with cpSRP43C-(259–376). The notion that the self-dimerization of cpSRP43 is mediated via the ankyrin repeat domain was further confirmed by the observation that bait and prey cpSRP43A-(61–258) interact but that no interaction was seen between the two cpSRP43C-(259–376) constructs.

To define more clearly the region of the ankyrin repeat domain involved in dimerization, a series of truncations of cpSRP43, removing the N-terminal region outside the ankyrin repeats (cpSRP43-(125–376)), followed by successive removal of three ankyrin repeats (cpSRP43-(156–376), cpSRP43-(190–376), and cpSRP43-(223–376)), was constructed (Fig. 2). Table II shows that mature cpSRP43 and cpSRP43-(125–376) both dimerized with cpSRP43-(125–376), cpSRP43-(156–376), cpSRP43-(190–376), and cpSRP43-(223–376) without any obvious loss of interaction strength. These data suggest that the fourth ankyrin repeat by itself is sufficient to mediate dimerization. This result was corroborated by the finding that cpSRP43-(223–376) interacts strongly with its corresponding prey construct. The third ankyrin repeat is also capable of mediating dimerization as evidenced by the extreme interaction of cpSRP43-(61–227), lacking the fourth ankyrin repeat, with cpSRP43 (Table II). When the third ankyrin repeat is also removed (cpSRP43-(61–193)), dimerization is essentially abolished. No binding was detected between cpSRP43 and cpSRP43-(61–159) containing the first ankyrin repeat only. These data together with the results from Table II indicate that...
the third and fourth ankyrin repeats each are capable of mediating the dimerization of cpSRP43.

The M Domain of cpSRP43 Interacts with cpSRP43—Like cytoplasmic SRP54, chloroplast SRP54 is also composed of an N-terminal G domain (residues 76–370) and a C-terminal M domain (residues 371–564). To analyze which domain of cpSRP54 mediates binding to cpSRP43, mature cpSRP54 and its G and M domain were cloned in pAS2, and the interaction with cpSRP43 as prey was tested. The results show that cpSRP54 interacts strongly with cpSRP43 in the yeast two-hybrid system and that the same strength of interaction is obtained between the M domain of cpSRP54 and cpSRP43 (Table III). No binding was observed between the G domain of cpSRP54 and cpSRP43. These data show clearly that the interaction between cpSRP43 and cpSRP54 is mediated via the M domain of cpSRP54.

The Chromodomains of cpSRP43 Mediate Binding to cpSRP54—To characterize the binding site of cpSRP43 for cpSRP54, we tested the binding of cpSRP43 to a series of N-terminal deletion constructs of cpSRP43. No obvious change in binding intensity was observed between cpSRP43 and mature cpSRP43, cpSRP43A-(125–376), cpSRP43A-(156–376), cpSRP43A-(190–376), or cpSRP43A-(223–376) (Table IV). A pronounced lesser, but still significant, binding was detected between cpSRP43A-(190–376) and cpSRP43A-(223–376) more precisely, we tested the interaction of LHCP-(189–222) and the C-terminal chromodomains-containing C-terminal part of cpSRP43 (cpSRP43C-(259–376)) (Table IV). If the loss of binding activity between cpSRP43 and cpSRP43C-(259–376) versus cpSRP43 and cpSRP43C-(223–376) is caused by a direct interaction of cpSRP54 with the fourth ankyrin repeat, we reasoned that cpSRP54 should interact with the ankyrin repeat-containing N-terminal part of cpSRP43. However, binding of cpSRP54 to cpSRP43A-(61–258) or even cpSRP43A-(61–305) was not observed. Together with the above-mentioned observation that cpSRP43-(223–376) forms a dimer (Table II), these binding data indicate that cpSRP54 binds cpSRP43 via the chromodomains and suggest that binding is promoted by the dimerization of cpSRP43. Alternatively, the dimerization motif of cpSRP43 may stabilize or enhance cpSRP54 binding independently of dimerization.

To define the binding site of cpSRP43 for cpSRP54 in more detail, N- and C-terminal truncations of cpSRP43C-(259–376) were constructed, and the interaction with cpSRP54 was tested. Removal of amino acids 259–282 (cpSRP43-(283–376)), containing the first conserved region of the first chromodomain, completely abolished binding to cpSRP54 (Table IV). The C-terminal deletion of amino acid 351–376 (cpSRP43-(259–350)) had no effect on cpSRP54 binding, but additional removal of the second strongly conserved region of the second chromodomain (cpSRP43-(259–339)) resulted in a complete loss of binding activity. These data demonstrate clearly that both chromodomains contain essential elements for binding cpSRP54.

The L18 Domain of LHCP Binds to the First Ankyrin Repeat of cpSRP43—Recent studies have shown that the L18 domain, a hydrophilic region located between the second and third transmembrane domain of LHCP (Fig. 2), can efficiently bind cpSRP43 (17, 18). We observed a strong interaction between a 33-amino acid-long region containing L18 (LHCP-(189–222)) and cpSRP43 by using the yeast two-hybrid assay (Table V). Further binding tests showed that LHCP-(189–222) interacts with the ankyrin repeat-containing region of cpSRP43 (cpSRP43A-(61–258)), but no binding was detected between LHCP-(189–222) and the C-terminal chromodomains-containing domain of cpSRP43 (cpSRP43C-(259–376)) (Table V). To map the binding site of cpSRP43A-(61–258) for LHCP-(189–222) more precisely, we tested the interaction of LHCP-(189–

### Table II

| Bait (pAS2) | Growth on −His | Activity of β-galactosidase |
|------------|----------------|----------------------------|
| 43         | + + +          | −                          |
| 43A-(61–258)| + + +          | −                          |
| 43C-(259–376)| +             | −                          |
| Empty      | + + +          | −                          |

| Prey (pACT2) | Growth on −His | Activity of β-galactosidase |
|-------------|----------------|----------------------------|
| 43          | + + +          | +                          |
| 43A-(125–376)| + + +          | +                          |
| 43-(156–376)| + + +          | +                          |
| 43-(190–376)| + + +          | +                          |
| 43-(223–376)| + + +          | +                          |
| 43-(61–227)| + + +          | +                          |
| 43-(61–193)| + + +          | +                          |
| Empty       | + + +          | +                          |

### Table III

| Bait (pAS2) | Growth on −His | Activity of β-galactosidase |
|-------------|----------------|----------------------------|
| 54          | + + +          | +                          |
| 54-M        | + + +          | +                          |
| 54-G        | − + +          | /                          |
| Empty       | + + +          | +                          |

Mature cpSRP54, its M and G domain (54-M, 54-G), and mature cpSRP43 were cloned into the yeast two-hybrid plasmids pAS2 and pACT2, respectively. Yeast strain Y190 was cotransformed with the indicated combination of bait and prey plasmids, and the activation of the −his-lacZ reporter was measured as described under “Experimental Procedures.”

#### TABLE II

The ankyrin repeat domain of cpSRP43 mediates its self-dimerization

Mature cpSRP43 or the indicated deletions of cpSRP43 were cloned into the yeast two-hybrid plasmids pAS2 and pACT2. Yeast strain Y190 was cotransformed with the indicated combinations of bait and prey plasmids, and the activation of the −his-lacZ reporter was measured as described under “Experimental Procedures.” A schematic presentation of the used constructs is given in Fig. 2. /, not measured.
Mature cpSRP54 or the indicated deletions of cpSRP43 were cotransformed into the yeast two-hybrid plasmids pAS2 and pACT2. Yeast strain Y190 was cotransformed with the indicated combination of bait and prey plasmids, and the activation of the −his-lacZ reporter was measured as described under “Experimental Procedures.”

| Table IV |
| --- |
| Both chromodomains of cpSRP43 are involved in the binding of cpSRP43 |
| | Prey (pACT2) | Bait (pAS2) | Growth on −His | Activity of β-galactosidase |
| | | | 54 | Empty | 54 |
| 43 | + + + | − | + + + |
| 43-(125–376) | + + + | − | + + + |
| 43-(156–376) | + + + | − | + + + |
| 43-(190–376) | + + + | − | + + + |
| 43-(223–376) | + + + | − | + + + |
| 43A-(61–258) | − | − | − |
| 43-(61–305) | − | − | − |
| 43C-(259–376) | + | + | + |
| 43-(283–376) | − | − | − |
| 43-(296–376) | + | + | + |
| 43-(259–350) | + | + | + |
| 43-(259–339) | + | + | + |
| 43-(259–320) | + | + | + |
| Empty | − | − | − |

| Table V |
| --- |
| The L18 domain of LHCP binds to the first ankyrin repeat of cpSRP43 |
| | Prey (pACT2) | Bait (pAS2) | Growth on −His | Activity of β-galactosidase |
| | | | LHCP189–222 | Empty LHCP189–222 | Empty |
| 43 | + + + | + | + + + | − |
| 43A-(61–258) | + + + | + | + + + | − |
| 43C-(259–376) | + + + | + | + + + | − |
| 43-(125–376) | + + + | + | + + + | − |
| 43-(156–376) | + + + | + | + + + | − |
| 43-(190–376) | + + + | + | + + + | − |
| 43A-(61–258) | + + + | + | + + + | − |
| 43-(61–159) | + + + | + | + + + | − |
| Empty | − | − | − | − |

FIG. 3. In vitro protein binding assays confirm the results obtained by the yeast two-hybrid system. A, first and second lanes, radiolabeled cotranslation products (Co-TP) of cpSRP43-(190–258) and GST-cpSRP43-(190–258) or cpSRP43-(190–258) and GST. Third–fifth lanes, results of a pull-down assay in which radiolabeled in vitro translated GST-cpSRP43-(190–258) were used as described under “Experimental Procedures.” B, first lane, radiolabeled in vitro translated cpSRP54-(371–564) (54-M). Second–fourth lanes, results of a pull-down assay in which radiolabeled in vitro translated cpSRP54-(371–564) was incubated with equal picomoles of in vitro translated GST-cpSRP43, GST-cpSRP43-(259–350), or GST as described under “Experimental Procedures.” C, first lane, radiolabeled in vitro translated LHCP-(189–206)-preprolactin (L-18). Second–fourth lanes, results of a pull-down assay in which radiolabeled in vitro translated LHCP-(189–206)-preprolactin was incubated with equal picomoles of in vitro translated GST-cpSRP43, GST-cpSRP43-(61–159), or GST as described under “Experimental Procedures.”

222) with the indicated N-terminal deletion constructs of cpSRP43 (Table V). LHCP-(189–222) binds cpSRP43-(125–376) with the same efficiency as cpSRP43, indicating that the N-terminal region outside the ankyrin repeat domain is not involved in binding (Table V). No binding was detected between LHCP-(189–222) and the constructs cpSRP43-(156–376), cpSRP43-(190–376), and cpSRP43-(223–376) (Table V). These data suggest that the first ankyrin domain mediates binding to LHCP-(189–222). This assumption was confirmed by the observation that LHCP-(189–222) interacts with the cpSRP43-(61–159) containing the extreme N terminus and the first ankyrin repeat only. This interaction was weaker than the interaction with the complete ankyrin repeat domain. However, the differences in binding strength were not distinctive enough to decide clearly whether LHCP binding needs a dimorphic state of cpSRP43.

The Results of the Yeast Two-hybrid System Are Confirmed by in Vitro Protein Binding Assays—We next sought to confirm the binding properties of the minimal identified interaction domains characterized by the yeast two-hybrid system by in vitro fusion protein binding assays. First, we verified that the region of cpSRP43 containing the third and fourth ankyrin repeat (cpSRP43-(190–258)) is able to form homodimers. To test this notion we cotranslated a GST-cpSRP43-(190–258) fusion protein together with cpSRP43-(190–258) and checked whether cpSRP43-(190–258) is co-precipitated in a pull-down assay using glutathione-Sepharose. Control samples contained a cotranslation of GST and cpSRP43-(190–258) and a single translation of GST-cpSRP43-(190–258). Results of the binding reaction show that cpSRP43-(190–258) is specifically co-precipitated by GST-cpSRP43-(190–258) and not by the unfused GST protein (Fig. 3A). The sample containing the precipitation of GST-cpSRP43-(190–258) by itself did not contain a radiolabeled protein of the size of cpSRP43-(190–258). Therefore it can be ruled out that the radiolabeled protein in the co-precipitation of GST-cpSRP43-(190–258) and cpSRP43-(190–258) is generated by the degradation of GST-cpSRP43-(190–258). Second, we wanted to confirm that the interaction of cpSRP54 with cpSRP43 is mediated via binding of the M domain of cpSRP54 (cpSRP54-(371–564)) to cpSRP43-(259–350). To test that, in vitro translated GST-cpSRP43-(259–350), GST-cpSRP43, or GST were incubated with radiolabeled in vitro translated cpSRP54-(371–564), and binding was analyzed using a pull-down assay. Fig. 3B shows that cpSRP54-(371–564) is specifically co-precipitated by GST-cpSRP43 and GST-cpSRP43-(259–350) and not by the unfused GST protein. The binding of cpSRP54-(371–564) to GST-cpSRP43-(259–350) is
Ankyrin repeats have been described to be involved in binding heterologous proteins (19) as well as in mediating homodimerization (28, 29). The ankyrin repeats of cpSRP43 exhibit both types of binding. Interestingly, repeats 3 and 4 are involved in forming cpSRP43 homodimers and seem fundamentally different from repeat 1, which cannot homodimerize but can bind the L18 domain. The molecular basis for these different binding properties of the two kinds of domains is an interesting question for future studies. Our results do not specify a role for the ankyrin repeat 2. This domain may be involved in binding other factors or may have subtle influences over binding that are not recognized with the present assay.

The chromodomain of cpSRP43 is distinctive from previously characterized chromodomain proteins. The latter fall into three subgroups: those containing just a single chromodomains, those containing two chromodomains, and those containing a chromodomains and a shadow chromodomain (23, 24). Shadow chromodomains are closely related to chromodomains but form a distinct subgroup (23). Chromodomains found in pairs are typically separated by a flexible linker and function independently. For example, the chromodomain of heterochromatin protein-1β functions as a protein interaction motif for heterologous proteins, whereas the shadow domain of heterochromatin protein-1β mediates homodimerization (30–32).

Fig. 4. Model of the transit complex. cpSRP consists of one cpSRP43 dimer and one cpSRP54 monomer. The homodimerization of cpSRP43 is mediated via the third and fourth ankyrin repeat of cpSRP43. The heterodimerization between cpSRP43 and cpSRP54 occurs between the M domain of cpSRP54 and both chromodomains of cpSRP43. The cpSRP complex is presumed to interact with a single molecule of LHCP to form the transit complex. In this complex, the L18 domain of LHCP interacts with the first ankyrin repeat of cpSRP43, and a hydrophobic domain of LHCP contacts cpSRP54, presumably within the M domain. It can be speculated that cpSRP forms a cavity to shield LHCP from the aqueous phase. tm1, tm2, tm3, transmembrane domains 1, 2, and 3, respectively.

significantly weaker than the binding to full-length GST-cpSRP43, reflecting very well the results obtained with the yeast two-hybrid system.

Third, we confirmed that the first ankyrin domain of cpSRP43 binds to the L18 domain of LHCP. In vitro translated GST-cpSRP43-(61–159), GST-cpSRP54, or GST was incubated with radiolabeled in vitro translated LHCP-(189–206). Fig. 3C shows that LHCP-(189–206) binds to GST-cpSRP43-(61–159), supporting the result obtained by the yeast two-hybrid system. As in the in vitro system, this interaction was slightly weaker than the binding to the full-length GST-cpSRP43.

DISCUSSION

In the present study we used the yeast two-hybrid system and in vitro binding assays to define the interacting domains among cpSRP43, cpSRP54, and LHCP. These proteins comprise the transit complex, an intermediate in the integration of LHCP into the thylakoid membrane. Although it was shown previously that the L18 domain of LHCP binds to cpSRP43 (17, 18), neither the interacting domains of cpSRP43 that bind LHCP nor the interacting domains mediating homodimerization of cpSRP43 or heterodimerization of cpSRP were identified previously. We tested whether these interactions occurred through the predicted protein interaction motifs within cpSRP43, namely the four ankyrin repeats and two chromodomains. Four clear conclusions emerge from these studies. First, the binding between LHCP and cpSRP43 occurs through the first ankyrin repeat of cpSRP43 and the L18 domain. Second, homodimerization of cpSRP43 occurs through both the third and fourth ankyrin repeats. Third, heterodimerization between cpSRP43 and cpSRP54 is mediated via the M domain of cpSRP54 and requires both chromodomains of cpSRP43. Fourth, the ankyrin repeats comprise at least two types of nonoverlapping binding sites. One mediates homodimerization of cpSRP43, and the other mediates the binding of cpSRP43 with LHCP.

Like cytosolic SRP54, cpSRP54 consists of two separate compact domains: an N-terminal G domain and a C-terminal M domain. The G domain of cpSRP54 is 70% similar to the prokaryotic protein, indicating that both domains have an analogous function. The similarity between the prokaryotic and chloroplast M domain (50%) (7) is less pronounced. In cytosolic SRP54, the M domain mediates the binding of the RNA component of SRP and also recognizes the signal sequence of the protein being translated (3, 4). Although not explicitly shown, the M domain of cpSRP54 is also likely to bind to substrate because direct interaction between cpSRP54 and LHCP has been shown to occur via chemical cross-linking (8), and the hydrophobic sequence in LHCP is required for transit complex formation (18). In this work, we show that the interaction between cpSRP54 and cpSRP43 is mediated via the M domain of cpSRP54. Thus binding of cpSRP54 to cpSRP43 and cytosolic SRP54 to SRP-RNA both occur through their respective M domains. In a previous report we showed that cpSRP54 does not bind prokaryotic SRP-RNA and that prokaryotic SRP54 can not bind to cpSRP43 (6). Together these data support the idea that the M domain of cpSRP54 has evolved a fundamentally different binding function from its cytosolic predecessor. The exact nature of the motif mediating the binding to cpSRP43 remains to be resolved.

It had been determined previously that each complex of cpSRP contains two cpSRP43 subunits/subunit of cpSRP54

E. Klostermann, E. Jonas-Straube, and D. Schünemann, unpublished results.
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(15). However, it was not known whether the dimerization of cpSRP43 promoted binding to cpSRP54. In the current work we observed that constructs of cpSRP43 lacking the dimerization domain bound cpSRP54 with strongly reduced efficiency. Thus it seems that dimerization of cpSRP43 indeed stabilizes cpSRP. In the current work we also observed that constructs of cpSRP43 lacking the dimerization domain also bound LHCP less effectively. Thus the dimerization of cpSRP43 may also promote the binding of substrate. From these observations, we predict that mutations preventing cpSRP43 dimerization will lack activity in LHCP integration. This hypothesis is currently being tested.

Our data suggest that in the transit complex, cpSRP43 acts as a molecular adaptor with the contact sites for dimerization in the middle of the protein and the N- and C-terminal flexible arms providing independent binding sites for LHCP and cpSRP54. The arms may form a cradle around LHCP where the N terminus of cpSRP43 binds to the hydrophilic part of LHCP, and the C terminus covers the hydrophobic portion of LHCP via mutual binding to cpSRP54. A diagram depicting this putative arrangement is shown in Fig. 4.

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