Accelerated Publication

The L18 Domain of Light-harvesting Chlorophyll Proteins Binds to Chloroplast Signal Recognition Particle 43

Received for publication, February 17, 2000, and in revised form, March 15, 2000
Published, JBC Papers in Press, March 21, 2000, DOI 10.1074/jbc. C000108200

Chao Jung Tu‡, Eric C. Petersen§, Ralph Henry¶, and Neil E. Hoffman
From the ‡Carnegie Institution of Washington, Department of Plant Biology, Stanford, California 94305, the §Biological Sciences Department, University of Arkansas, Fayetteville, Arkansas 72701, and ¶Paradigm Genetics, Research Triangle Park, North Carolina 27709

Chloroplast signal recognition particle (cpSRP) is a novel type of SRP that contains a homolog of SRP54 and a 43-kDa subunit absent from all cytoplasmic SRPs but lacks RNA. It is also distinctive in its ability to post-translationally interact with light-harvesting chlorophyll proteins (LHCP), hydrophobic proteins synthesized in the cytoplasm and targeted to the thylakoid via the stroma. LHCP integration into thylakoid membranes requires the two subunits of cpSRP, cpFtsY, and the membrane protein ALB3. It had previously been shown that the L18 domain, an 18-amino acid peptide between the second and third transmembrane domains, and a hydrophobic domain are required for interaction with cpSRP. In the present study we used a pull-down assay, with cpSRP43 or cpSRP54 fused to glutathione transferase (GST) to study interactions between cpSRP43, cpSRP54, LHCP, and cpFtsY. cpFtsY was not observed to form significant interactions with any of the proteins even in the presence of nonhydrolyzable GTP analogs. Our data indicate that cpSRP43 binds to the L18 domain, that cpSRP54 binds to the hydrophobic domain, and that LHCP and cpSRP54 independently bind to cpSRP43. These data confirm that the novel post-translational interaction between LHCP and cpSRP is mediated through binding to cpSRP43.

SRP is a ubiquitous cytoplasmic ribonucleoprotein that mediates the co-translational targeting of endomembrane and secretory proteins to the endoplasmic reticulum in eukaryotes and of polytopic membrane proteins to the cytoplasmic membrane in prokaryotes (reviewed in Refs. 1 and 2). All cytoplasmic forms of SRP contain an RNA and a 54-kDa GTPase, SRP54. SRP54 plays a major role in SRP-dependent targeting, where it binds to nascent chains via an interaction with hydrophobic domains of signal sequences. The bound ribosome-nascent, chain mRNA is piloted to the membrane in part because of the affinity of SRP for its membrane-bound receptor. Upon binding its receptor, SRP dissociates from the nascent chain, and translation resumes at the membrane.

A specialized organellar SRP identified in chloroplasts (cpSRP) (3) contains an SRP54 homolog (cpSRP54) (4) but differs from cytoplasmic forms in that it lacks an RNA (5, 6), contains a novel 43-kDa subunit (7), and binds substrates post-translationally (8). The known substrates of cpSRP are the LHCPs, hydrophobic proteins that are synthesized in the cytoplasm and post-translationally transported to the internal membranes of the chloroplast via a soluble pathway that proceeds through the stroma (9, 10). The solubility of LHCP is maintained in the stroma by its binding to cpSRP to form the targeting intermediate termed the transit complex (8, 11). Localization of LHCP to the thylakoid membrane further requires two additional soluble components, GTP (12) and chloroplast FtsY. The latter is a homolog of the SRP receptor α subunit (6, 13). Recent evidence indicates that the thylakoid membrane localized translocon needed for translocation of LHCP into the lipid bilayer is composed minimally of the integral membrane protein ALB3 (14).

The unique ability of cpSRP to bind LHCPs post-translationally prompted a comparison of mammalian and chloroplast SRPs. In co-translational assays, both SRPs exhibited similar substrate binding properties in which signal peptide hydrophobicity played an important role (15). However, DeLille et al. (16) found that the bovine preprolactin (PPL) signal sequence, which acts as an efficient substrate for cpSRP binding in cotranslational assays (15), lacks the recognition elements necessary to support post-translational binding to cpSRP. This finding suggested that different recognition elements present in LHCP are required for the formation of an LHCP/cpSRP transit complex (16). An investigation of the LHCP structural properties required for post-translational binding to cpSRP determined that two domains of LHCP are important for binding to cpSRP, a hydrophobic domain and a unique recognition element that is used to promote post-translational interaction (16). The latter element was determined to be an 18-amino acid hydrophilic domain (L18) located in the stroma between the second and third transmembrane domains. In the present study, we have examined interactions between LHCP, cpSRP54, cpSRP43, cpFtsY, and cpSRP reconstituted from individual subunits. Our data indicate that the post-translational interaction between LHCP and cpSRP largely involves binding of the L18 domain to cpSRP43. The second interaction between a hydrophobic domain of LHCP and cpSRP is shown to involve cpSRP54.

MATERIALS AND METHODS

DNA Constructs—The construction of the 54HIS translation vector (pAF1) and G43 expression vector (pGEX4Tchaos(m)) were described by Schuenemann et al. (3). The construction of G54 expression vector...
(pGTK-54HIS) and FtsY translation vectors (pTu1) were described in Tu et al. (6). Preprolactin and the LHCP preprolactin translation vectors H*, L18PPL, and L33PPL were described by DeLille et al. (16).

Reconstitution of Transit Complex—The collection of pea stroma and the methods for formation of the transit complex were described previously (5, 6). Radiolabeled LHCP precursor, H*, and L33PPL (0.15 μCi) synthesized in wheat germ extracts (17) were mixed with either pea stroma (equivalent to 80 μg of chlorophyll), 160 ng of cspSRP54 synthesized in wheat germ extract (3), or recombinant cspSRP43 (50 ng) in 10 mM HEPES (pH 8.0), 55 mM sorbitol, 10 mM MgCl₂, and 1 mM ATP in a final volume of 15 μl for 15 min at 25 °C. The transit complex was fractionated on a 6% undenaturating polyacrylamide gel (11) and detected by fluorography using Amplify (Amersham Pharmacia Biotech) according to the manufacturer’s instructions.

Recombinant Protein Over-expression and Purification—Recombinant G43 was over-expressed from pGEXchaos(m) in the *Escherichia coli* strain BL21, whereas G54 was over-expressed from pAF1 in the *E. coli* strain XL1-Blue (MRB). The over-expression and purification of recombinant G43 (4) has been described (3). For over-expression of G54, cells were grown in LB medium containing 100 μg Ampicillin to an A₆₀₀ of 0.8 at 30 °C. The expression was induced by adding 0.3 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 3 h. Cells were collected, sonicated in lysis buffer (50 mM Tris-HCl, pH 8.0, 300 mM NaCl, 1 mM DTT, 1 mM EDTA, 1 mM Phenylmethylsulfonylfluoride (PMSF), 1 μg/ml leupeptin, and 1 μg/ml Pefabloc), and the extract incubated with Glutathione-Sepharose (Amersham Pharmacia Biotech). The over-expressed G54 proteins were eluted in 10 mM glutathione in lysis buffer.

Protein Binding Assay—Recombinant G43 protein (0.1 μg), recombinant G54 (0.1 μg), radiolabeled LHCP precursor (2.4 μCi), 54HIS synthesized in wheat germ extract (0.6 μCi), and in vitro translated FtsY protein (2.4 μCi) were combined as indicated. The proteins were diluted into incubation buffer (final concentration: 20 mM HEPES-KOH, pH 8.0, 50 mM KCl, 10 mM MgCl₂, 1 mM ATP, and 0.1 mM GTP*), and the extract incubated with Glutathione-Sepharose (Amersham Pharmacia Biotech). The over-expressed G54 proteins were eluted in 10 mM glutathione in lysis buffer.

Peptide (L18) Competition Binding Assay—The L18 peptide sequence corresponding to the sequence NH₂-VDLPYGGSGFDPLGLADD-COO from LHCP residues 189–206 was synthesized by Regev and colleagues (18). The L18 peptide, respectively.

RESULTS

LHCP Binds to cspSRP43—To look at interactions between cspSRP subunits, cpFtsY, and LHCP, we developed a pull-down assay using glutathione S-transferase fused to cspSRP43 (G43) or cspSRP54 (G54). The GST-fusion proteins were expressed in *E. coli* and purified to near homogeneity. Purified GST was used as a control. GST or GST-fusion protein was incubated with the indicated radiolabeled translation products, and the resulting solution was passed over glutathione-Sepharose. The fusion protein and material bound to it were eluted with glutathione, and the eluates were analyzed on SDS-PAGE (Fig. 1A, lanes 1–5). None of the translation products bound to the column in the presence of GST alone (for example, see Fig. 2). In Fig. 1A, lanes 1–5, shows the extent of binding of cpSRP54, cpFtsY, and LHCP to G54. Consistent with previous reconstitution experiments (3, 6), more than 30% of the cspSRP54 bound to G54. Unexpectedly, a significant amount of LHCP also directly bound to G43. The amount of cpFtsY interacting with G43 under the conditions of the assay was low but detectable. Binding of cpFtsY to G54 was also minimal (Fig. 1A, lane 1). Some LHCP was found to interact directly with G54; however, the amount bound was less than 2% of that which bound to G43 (Fig. 1A, lane 3 versus lane 5). Pairwise combinations of cpSRP54, LHCP, and cpFtsY were simultaneously incubated with G43, and little to no variation in binding was observed compared with incubations with G43 and the individual proteins (Fig. 1, B, lanes 1–3 versus A, lanes 1–3). When LHCP and cpFtsY were simultaneously incubated with G54, the level of LHCP binding to GST54 was similar compared with binding in the absence of cpFtsY (Fig. 1, B, lane 4 versus A, lane 5). When all four proteins were present simultaneously, there was no further change in the amount of binding (Fig. 1B, lane 5). The cpFtsY binding was minimal in all cases tested (Fig. 1A, lanes 2 and 4 and B, lanes 2–5). The binding data were unaffected by the presence of GTP, GTPγS, and GMP-PNP (data not shown), suggesting that the binding of cpSRP54 and LHCP to cspSRP43 is unaffected by the presence of GTP or GTP analogs. Together, these data indicate that cpSRP43 and, to a lesser extent, cpSRP54 directly bind to LHCP.

**FIG. 1.** LHCP binds to cspSRP43. A, radiolabeled cspSRP54his (0.6 μCi), cpFtsY (2.4 μCi), or LHCP (2.4 μCi) were mixed with either G43 (0.1 μg) or G54 (0.1 μg) and the binding assayed as described under “Materials and Methods.” B, same as in A, except multiple translation products were added to the assay. Proteins were separated by SDS-PAGE on 13% acrylamide gels and detected by fluorography.

**FIG. 2.** The L18 domain of LHCP binds to cspSRP43. A, diagram of constructs made between LHCP and preprolactin. The sequence of the LHCP domain from the LHCP, Lhcb1, is shown; 1 PPL, only the first 30 residues of preprolactin are displayed; 2 L18-PPL, residues 189–206 of LHCP fused to L13 of preprolactin; 3 L33-PPL, residues 189–222 of LHCP fused to V18 of preprolactin. The signal sequence of preprolactin is underlined. B, results of a pull-down assay in which 3 μCi of the indicated radiolabeled translation product is incubated with 0.1 μg of the indicated glutathione S-transferase protein, as described under “Materials and Methods.” Proteins were separated by SDS-PAGE on 13% acrylamide gels and detected by fluorography.
second and third transmembrane domains of LHCP (Fig. 2A), has been shown to be important for interaction with cpSRP (16). In this context, pull-down assays were utilized to investigate the interactions between each subunit of cpSRP and the L18 domain, using constructs illustrated in Fig. 2A. LHCP and bovine PPL were used as the positive and negative controls, respectively. LHCP contains three transmembrane domains, whereas PPL contains a single hydrophobic domain in the N-terminal signal sequence. For the construct L18PPL, the L18 domain of LHCP was fused to the N terminus of full-length PPL. When incubated with stroma, this construct forms a transit complex with cpSRP (16). A second construct, L33PPL, which contains the L18 domain plus an additional 15 amino acids downstream from L18 fused to PPL beginning at the PPL signal peptide hydrophobic domain, also forms the transit complex when incubated with stroma (16). H* is similar to L33PPL with the exception that the first five leucines in the H-domain of the PPL signal sequence have been deleted. This type of mutation destroys the co-translational interaction between the signal sequence and cytoplasmic SRP (18). Similarly, H* does not form a transit complex when incubated with stroma (16). The binding of LHCP, PPL, H*, and L18PPL to GST, G43, and G54 are shown in Fig. 2B. As also observed in Fig. 1A, LHCP binds G43 more extensively than it binds G54. The interaction with G54 appears to be significant, as no LHCP binds to GST alone. The negative control, PPL, does not bind to any of the three GST proteins. In contrast, the two PPL constructs containing the L18 domain, H* and L18PPL, bind G43 nearly as well as LHCP binds. The two PPL constructs bind to G54 less effectively than LHCP. These data clearly demonstrate that cpSRP43 binds the L18 domain. Furthermore, a hydrophobic sequence in the substrate protein is not required for this binding.

**A Hydrophobic Domain and cpSRP54 Are Required to Form Transit Complex**—The transit complex formed between cpSRP and LHCP can be demonstrated as a soluble form of LHCP that migrates into non-denaturing gels (11). In the absence of cpSRP, LHCP aggregates and remains at the top of the gel (Fig. 3 lane 1; Refs. 3 and 8). The finding that H* and PPL did not form transit complexes when incubated with stroma provided strong evidence that both a hydrophobic domain and the L18 domain are required for transit complex formation (16). The fact that a transit complex can be reconstituted from cpSRP43, cpSRP54, and LHCP allows us to test this hypothesis explicitly (6). The transit complex formed between LHCP and pea cpSRP migrates more slowly than the complex formed from native or reconstituted Arabidopsis cpSRP (Fig. 3, lane 2 versus lane 3, and Ref. 3). Despite the efficient binding of cpSRP43 with LHCP, no complex is formed when cpSRP54 is lacking (Fig. 3, lane 4). Likewise, the complex does not form in the absence of cpSRP43. Reconstitution assays with L33PPL and H* are consistent with previous observations (16). The transit complex can be reconstituted with L33PPL, cpSRP43, and cpSRP54 but not when the substrate is H*. As H* efficiently interacts with cpSRP43 but not with cpSRP54, these data clearly demonstrate that a hydrophobic domain and cpSRP43 are required to form a transit complex.

**LHCP and cpSRP54 Bind to Distinct Sites on cpSRP43**—The binding sites for LHCP and cpSRP54 on cpSRP43 have not been mapped. To examine whether distinct binding sites are present on cpSRP43, we added L18 peptide to compete with either LHCP or cpSRP54 for binding to G43 in a pull-down assay. The cpSRP54 used was a 1:4 mixture of wheat germ translation products translated in the presence of [35S]methionine and cold methionine, respectively. The LHCP was radiolabeled by translation in wheat germ extracts containing [35S]methionine. The G43 was expressed in E. coli and detected by specific antisera. Fig. 4 shows that LHCP binding to cpSRP54 is competed by as little as 5 μM L18 peptide. In contrast, no competition of cpSRP54 binding to G43 is observed with as much as 100 μM L18. L18 peptide had no effect on the binding of G43 to the glutathione-Sepharose column (Fig. 4, lower panel). Together these data clearly demonstrate that LHCP and cpSRP54 bind distinct sites on cpSRP43.

**DISCUSSION**

One distinctive feature of the LHCP targeting reaction is the post-translational interaction between LHCP and cpSRP. The novel interaction has been attributed to the unique presence of the 43-kDa subunit in the chloroplast SRP (3, 7). This notion has been further substantiated by the results of the present study. Two cpSRP binding domains have previously been identified in LHCP, a hydrophobic domain and the L18 domain (16). Using a post-translational pull-down assay, we show that the L18 domain is necessary and sufficient for binding to cpSRP43. By comparison, the interaction of cpSRP43 with the L18 domain is negligible and likely to be insignificant. It was previously observed that Arabidopsis plants lacking cpSRP43 exhibit a specific defect in LHCP biogenesis (7, 19). This specificity can now be attributed to the binding of cpSRP43 to LHCP proteins containing an L18 domain.

As cpSRP43 binds two distinct proteins, LHCP and cpSRP54, the possibility arose that the cpSRP43 binds these two proteins at independent binding sites. A competition study using L18 peptide revealed that the two proteins indeed bind cpSRP43 independently. The L18 peptide has been successfully used to compete with LHCP transit complex formation and integration reactions containing stroma (16). However, in these
studies L18 peptide was 10–100 times less effective than LHCP, which is an effective competitor at 1–2 μM levels. A possible explanation, that the L18 peptide is unstable in stroma, is corroborated by the present study, where we observed competition of LHCP using 5 μM concentrations of L18 peptide in the absence of stroma. Thus, we have further substantiated the utility of L18 peptide as a tool for the study of LHCP biogenesis.

Although cpSRP43 can efficiently bind to LHCP, from prior reconstitution studies it is clear that a functional cpSRP requires cpSRP54. Although direct evidence is lacking that cpSRP54 binds the hydrophobic sequence, the following indirect evidence strongly suggests this possibility. First, a hydrophobic domain on the substrate and cpSRP54 are required for the formation of a transit complex (Ref. 16 and this work). Second, substrates lacking the hydrophobic domain still bind efficiently to cpSRP43 (this work). Third, cross-linking studies indicate that cpSRP54 directly binds to LHCP (8). Fourth, it is well known that cytoplasmic SRP54 binds to hydrophobic sequences (20).

As very little post-translational binding occurs between cpSRP54 and LHCP (or PPL), we favor the idea that the initial interaction between LHCP and cpSRP occurs through the binding of the L18 domain to cpSRP43. cpSRP43 was found to be a dimer (6); however, it is not known whether LHCP binding to cpSRP43 requires the dimeric state. The initial binding between LHCP and cpSRP43 should facilitate an interaction between the hydrophobic domains of LHCP and cpSRP54. Although only one hydrophobic domain is required for a productive interaction between cpSRP and LHCP, LHCP has three such domains. It remains to be determined whether all three hydrophobic domains of LHCP are sequestered from the aqueous phase by interacting with cpSRP. If so, cpSRP has additional distinctive properties compared with cytoplasmic SRP. By interacting with its substrate co-translationally, cytoplasmic SRP54 only binds to a single sequence. In the case of cpSRP, additional hydrophobic binding sites could be contributed by cpSRP43. However, because L18 quantitatively competed for the LHCP binding site, we consider this possibility unlikely. Alternatively, if the binding of the hydrophobic domains is exclusively the role of cpSRP54, it raises the possibility that this protein is capable of binding to multiple domains on the substrate or to a single preferred domain that induces a partially folded and stable conformation of LHCP.

By interacting with the hydrophobic domains of LHCP, cpSRP presumably functions as a chaperone that maintains the integration competence of LHCP. A second role may be to pilot LHCP to the thylakoid membrane. The role of cpFtsY remains poorly understood, although it and GTP are clearly required for the integration of LHCP into thylakoid membranes (6, 12, 13). We observed negligible binding between cpSRP and cpFtsY even in the presence of nonhydrolyzable analogs of GTP. In this regard the interaction between cpFtsY and cpSRP is distinguishable from that of SRP and its receptor (21). This apparent difference may be a reflection of the fact that the chloroplast proteins have evolved to accommodate cpSRP43.

REFERENCES
1. Walter, P., and Johnson, A. E. (1994) Annu. Rev. Cell Biol. 10, 87–119
2. Rapoport, T. A., Jungnickel, B., and Kutay, U. (1996) Annu. Rev. Biochem. 65, 271–303
3. Schuenemann, D., Gupta, S., Persello-Cartieaux, F., Klimyuk, V. I., Jones, J. D. G., Nussaume, L., and Hoffman, N. E. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 10312–10316
4. Franklin, A. E., and Hoffman, N. E. (1993) J. Biol. Chem. 268, 22175–22180
5. Schuenemann, D., Amin, P., and Hoffman, N. E. (1999) Biochem. Biophys. Res. Commun. 254, 253–258
6. Tu, C. J., Schuenemann, D., and Hoffman, N. E. (1999) J. Biol. Chem. 274(38), 27219–27224
7. Klimyuk, V. I., Persello-Cartieaux, F., Havaux, M., Contard-David, P., Schuenemann, D., Meidnerhoff, K., Gozet, P., Jones, J. D., Hoffman, N. E., and Nussaume, L. (1999) Plant Cell 11, 87–100
8. Li, X., Henry, R., Yuan, J., Cline, K., and Hoffman, N. E. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3789–3793
9. Cline, K., Pulsom, D. R., and Viitanen, P. V. (1989) J. Biol. Chem. 264, 14225–14232
10. Reed, J. E., Cline, K., Stephens, L. C., Bacot, K. O., and Viitanen, P. V. (1990) Eur. J. Biochem. 194, 33–42
11. Payan, L. A., and Cline, R. (1991) J. Cell Biol. 112, 603–613
12. Hoffman, N. E., and Franklin, A. E. (1994) Plant Physiol. 105, 295–304
13. Kogata, N., Nishio, K., Hirohashi, T., Kikuchi, S., and Nakai, M. (1999) FEBS Lett. 447, 329–333
14. Moore, M., Harrison, M. S., Peterson, E. C., and Henry, R. (2000) J. Biol. Chem. 275, 1529–1532
15. High, S., Henry, R., Mould, R. M., Valent, Q., Meacock, S., Cline, K., Gray, J. C., and Luirink, J. (1997) J. Biol. Chem. 272, 11622–11628
16. Adam, Z., and Hoffman, N. E. (1993) Biochemistry 32, 1529–1534
17. Adam, Z., and Hoffman, N. E. (1993) Plant Physiol. 102, 35–43
18. High, S., Flint, N., and Dobberstein, B. (1991) J. Cell Biol. 113, 25–34
19. Amin, P., Sy, D. A., Pilgrim, M. L., Parry, D. H., Nussaume, L., and Hoffman, N. E. (1999) Plant Physiol. 121, 61–70
20. Kurzchalia, T. V., Wiedmann, M., Girshovich, A. S., Bochkareva, E. S., Bielka, H., and Rapoport, T. A. (1986) Nature 320, 634–636
21. Connolly, T., Rapiejkow, P. J., and Gilmore, R. (1991) Science 252, 1171–1173
The L18 Domain of Light-harvesting Chlorophyll Proteins Binds to Chloroplast
Signal Recognition Particle 43
Chao Jung Tu, Eric C. Peterson, Ralph Henry and Neil E. Hoffman

J. Biol. Chem. 2000, 275:13187-13190.
doi: 10.1074/jbc.C000108200 originally published online March 21, 2000

Access the most updated version of this article at doi: 10.1074/jbc.C000108200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 21 references, 15 of which can be accessed free at
http://www.jbc.org/content/275/18/13187.full.html#ref-list-1