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Chloroplast signal recognition particle (cpSRP) and ALB3: New insights into structure and function in LHCP targeting

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Introduction

Recently it was shown that chloroplasts contain a specialized type of SRP (cpSRP) that contains an evolutionary conserved 54 kD subunit (cpSRP54), but differs from cytoplasmic SRPs, as it contains a novel 43 kD subunit (cpSRP43), and lacks RNA (Franklin and Hoffman, 1993; Schuenemann et al., 1998; Schuenemann et al., 1999). CpSRP is also distinctive in its ability to interact with its substrate LHCP post-translationally to form a cpSRP/LHCP transit complex, which targets members of the LHCP family to the thylakoid membrane (Li et al., 1995; Schuenemann et al., 1998; Klimyuk et al., 1999; Kim et al., 1999). Studies on the subunit stoichiometry and structure of cpSRP have demonstrated that cpSRP is a trimer composed of one cpSRP43 dimer and one cpSRP54 monomer (Tu et al., 1999). Analysis of the post-translational interaction between cpSRP and LHCP have shown that this interaction is mediated through binding between cpSRP43 and the L18-domain of LHCP, an 18 amino acid peptide located between the second and third transmembrane domains (DeLille et al., 2000; Tu et al., 2000). The cpSRP43 sequence is characterized by the presence of two types of motifs mediating protein-protein interactions. The N-terminal region of cpSRP43 contains four ankyrin repeats and the C-terminal region contains two closely spaced chromodomains.

Besides cpSRP the insertion of LHCP to the thylakoid membrane requires GTP, a chloroplast homolog of the SRP receptor (cpFtsY), and the integral membrane protein ALB3 (Hoffman and Franklin, 1994; Tu et al., 1999; Moore et al., 2000). ALB3 is a homolog of the bacterial plasmamembrane protein YidC. Recent studies indicate at least two different roles of YidC for membrane protein insertion. Firstly, YidC is associated with the bacterial SecYEG complex and secondly, YidC is also involved in the insertion of Sec-independent membrane proteins (Scotti et al., 2000; Samuelson et al., 2000).

In this report we summarize data analysing the function of different domains of cpSRP43 in self-dimerization and heterodimerization with cpSRP54 and LHCP by using the yeast two-hybrid system and *in vitro* binding assays (Jonas-Straube et al. 2001). In addition we provide initial data indicating that ALB3 functions in LHCP integration by a direct interaction of ALB3 with LHCP. Analog to the bacterial YidC chloroplast ALB3 is also associated with the cpSecYE-complex.

Methods

Plasmid construction for the yeast two-hybrid system
Plasmid construction was done as described in Jonas-Straube et al., 2001.

Yeast two-hybrid assay
The yeast two-hybrid assay was performed as described in Jonas-Straube et al., 2001.

Gel filtration analysis
In 10 mM Hepes-KOH pH 8.0, 200 mM NaCl, 1.5 % dodecyl-β-maltosid, 1 mM PMSF solubilized thylakoid membrane proteins (200 µl, 1 mg chl./ml) were separated on a Superose 6 HR column in 20 mM Hepes-KOH pH 8.0, 200 mM NaCl, 0.1 % dodecyl-β-maltosid at a flow rate of 0.4 ml/min. Fractions of 0.8 ml (Fig. 3 upper panels) or 0.3 ml (Fig. 3 lower panels) were collected. The fractions were separated on a SDS- polyacrylamide gel and ALB3 and cpSecY were detected by immunoblot analysis using anti-ALB3 or anti-cpSecY antibodies.

Coimmunoprecipitation
In 10 mM Hepes-KOH pH 8.0, 200 mM NaCl, 1 % digitonin, 1 mM PMSF solubilized thylakoid membrane proteins (500 µl, 1 mg chl./ml) were incubated overnight with anti-ALB3 antibodies cross-linked to 10 mg protein A-Sepharose beads at 4 ^{o}C. The beads were transferred into Wizard minicolumns (Promega) and washed with 4 ml 20 mM Hepes-KOH pH 8.0, 0.5 M NaCl, 0.5 % digitonin. Excess fluid was removed by centrifugation in a microfuge, and the proteins were eluted with 30 µl of 8 M urea in sample buffer.

Results and Discussion

To determine which domains of cpSRP43 mediate its dimerization and the heterodimerization with cpSRP54 and LHCP we tested the interaction of the full-length proteins and various deletion constructs using the yeast two-hybrid system (Jonas-Straube et al., 2001). A schematic presentation of the domain organization of these proteins is shown in Fig. 1. In summary we demonstrate that the third and fourth ankyrin repeats are involved in dimerization of cpSRP43, whereas the first ankyrin repeat binds to the L18-domain of LHCP. We show further that the interaction of cpSRP43 with cpSRP54 is mediated via binding of the methione rich domain (M-domain) of cpSRP54 to the C-terminal 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 binding properties of the minimal interaction domains identified by the yeast two-hybrid

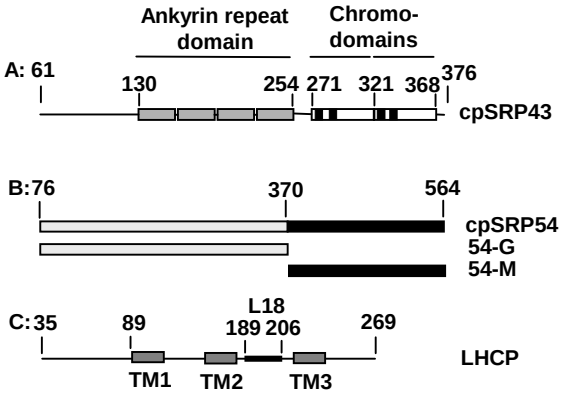

Fig.1 Scheme of the domain organization of cpSRP43, cpSRP54, and LHCP

system were confirmed using an *in vitro* binding assay (Jonas-Straube et al., 2001). 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 model depicting this putative arrangement is shown in Fig.2.

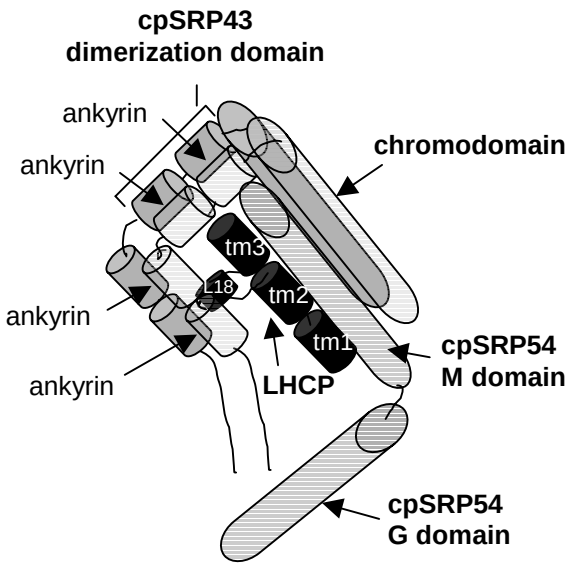

Fig. 2 Model of the transit complex

The transit complex represents an intermediate in the targeting of LHCP to the thylakoid membrane. Integration of LHCP in the membrane further requires the soluble components GTP and chloroplast FtsY. Recently ALB3 was identified as the first membrane component required for LHCP integration (Moore et al., 2000). However, the exact role of ALB3 in LHCP integration is not yet known. As a first step to elucidate the function of ALB3 we analysed whether proteins of the transit complex or cpFtsY are able to interact with ALB3 by using the yeast two-hybrid assay. Table 1 shows that ALB3 is able to interact with LHCP. No binding was detected between ALB3 and cpSRP43, cpSRP54 or cpFtsY. Based on these results it can be concluded that LHCP integration requires a direct interaction between LHCP and ALB3. Further work is in progress to analyse the interaction between these proteins in more detail.

Table 1: ALB3 interacts with LHCP

	bait		
	-his		β-gal. act.
prey	ALB3	empty	ALB3
cpSRP54	-	-	-
cpSRP43	-	-	-
cpFtsY	-	-	-
LHCP	+	-	+
empty	-	-	-

ALB3 is homologous to the bacterial plasmamembrane protein YidC. It has been reported that YidC is associated with the bacterial SecYEG complex and is involved in the insertion of Sec-dependent membrane proteins into the plasmamembrane

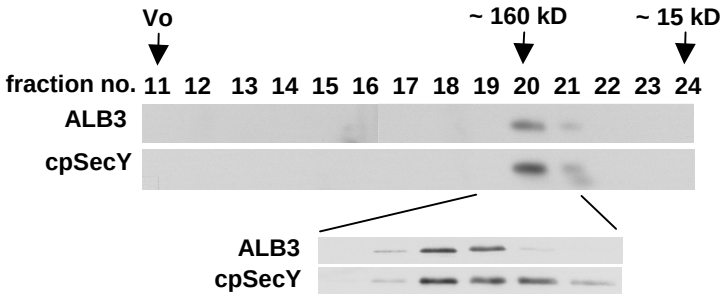

Fig. 3 ALB3 and cpSecY co-fractionate during gel filtration analysis

(Scotti et al., 2000; Samuelson et al., 2000). Therefore we sought to test whether ALB3 is associated with the cpSecYE complex located in the thylakoid membrane. To address this question we examined whether ALB3 and cpSecY co-chromatographed on a gel filtration column after detergent solubilization of thylakoid membranes. As shown in Fig. 3 both proteins co-eluted in a single peak as higher molecular mass species of approximately 160 kDa from a gel filtration column. These data suggest that ALB3 is associated with cpSecYE. However, it cannot be ruled out that ALB3 belongs to a different protein complex that has a similar molecular mass as cpSecYE. To support the assumption that ALB3 is associated with cpSecYE we checked whether antibodies directed against ALB3 co-precipitate cpSecY in an coimmuno-precipitation experiment using solubilized thylakoid membranes. It is shown in Fig 4 that both proteins can be precipitated with anti-ALB3 antibodies, whereas none of the proteins were precipitated by the preimmuneserum.

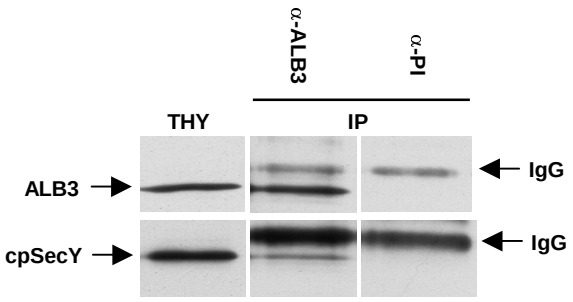

Fig. 4 Coimmunoprecipitation of ALB3 and cpSecY

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