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Structural diversity of signal recognition particle RNAs in plastids

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One of the pathways for protein targeting to the plasma membrane in bacteria utilizes the co-translationally acting signal recognition particle (SRP), a universally conserved ribonucleoprotein complex consisting of a 54 kDa protein and a functional RNA. An interesting exception is the higher plant chloroplast SRP, which lacks the otherwise essential RNA component. Furthermore, green plant chloroplasts have an additional post-translational SRP-dependent transport system in which the chloroplast-specific cpSRP43 protein binds to imported substrate proteins and to the conserved 54 kDa SRP subunit (cpSRP54). While homologs to the bacterial SRP protein and RNA component previously have been identified in genome sequences of red algae and diatoms, a recent study investigated the evolution of the green plant SRP system.[1] Analysis of hundreds of plastid and nuclear genomes showed a surprising pattern of multiple losses of the plastid SRP RNA during evolution and a widespread presence in all non-spermatophyte plants and green algae. Contrary to expectations, all green organism groups that have an identified cpSRP RNA also contain a cpSRP43. Notably, the structure of the plastid SRP RNAs is much more diverse than that of bacterial SRP RNAs. The apical GNRA tetraloop is only conserved in organisms of the red lineage and basal organisms of the green lineage, whereas further chloroplast SRP RNAs are characterized by atypical, mostly enlarged apical loops.

Keywords: signal recognition particle, SRP RNA, chloroplast, plastid, protein transport, cpSRP evolution, photosynthetic organisms

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The co-translational transport of membrane proteins to the plasma membrane in bacteria or the endoplasmic reticulum of eukaryotes requires the universally conserved cytosolic signal recognition particle (SRP).[2,3] The minimal core of all cytosolic SRPs consists of 2 essential conserved components, a SRP RNA and a ~54 kDa protein (SRP54). The bacterial SRP RNA is characterized by a conserved elongated structure containing one asymmetrical, one symmetrical, and an apical GNRA tetra loop (N: A, C, G, or U; R: A or G). Intense work to study the molecular function of the SRP RNA assigned precise functions to various regions of the SRP RNA. The asymmetrical and symmetrical loops mediate the binding to the 54 kDa subunit, whereas the apical loop plays a critical role in establishing the contact to the bacterial SRP receptor, FtsY. Here, electrostatic interactions between the apical loop and a conserved lysine residue of FtsY (Lys399 in *E. coli*) stabilize an early complex intermediate which results in an at least ~100-fold acceleration of FtsY/SRP complex formation (transient tether model).[4] Remarkably, recent studies showed that the FtsY/SRP complex travels subsequently from the apical loop region to the distal end of the SRP RNA, which leads to a stimulation of the FtsY/SRP GTPase activity.[5,6] With regard to the highly conserved structure and essential function of the SRP RNA in bacteria, the identification of a chloroplast SRP system in higher plants that functions without a SRP RNA was surprising. In higher plants, chloroplast (cp) SRP54 is tightly associated with the chloroplast-specific cpSRP43 and mediates the post-translational targeting of the light harvesting chlorophyll a,b-binding proteins (LHCPs) to the thylakoid membrane.[7-9] Analogous

Table 1. Variety of apical loops of plastid SRP RNAs

		apical loop		
phylogenetic position	**no. org.**	**length (no. org.)**	**GNRA tetra loop; no. org.**	**other loops (max. Two examples)**
red lineage	16	4 nt (16)	15	AUAC
chlorophytes				
prasinophyceae	7	4 nt (7)	7	
trebouxiophyceae	7	4 nt (7)	5	GAUA; AAAA
ulvophyceae	4	4 nt (1)	-	CUGA
		5 nt (1)		CUAAA
		6 nt (1)		UUAAUA
		7 nt (1)		UUUAAGU
chlorophyceae	4	4 nt (2)	1	AUAU
		1 nt (1)		U
		3 nt (1)		UUU
streptophytes				
charophytes	5	4 nt (2)	-	UAAA
		9 nt (2)		UACGUUCUA; UACGUUCCA
		10 nt (1)		UAGUUUGAUA
bryophytes	12	9 nt (1)	-	UAUAAAAUA
		10 nt (11)		AACUAAAUUA; UAUCAAACUA
lycophytes	2	5 nt (2)	-	UAAUA; UAGUA
monilophytes	28	10 nt (26) 11 nt (2)	-	GAGUAUCAUA; UAACAAACUA UAAUUAAUCUA
2° endosymbiosis				
chlorarachniophyceae	1	4 nt (1)	-	AAAA

For each phylogenetic position (red lineage and green lineage with different subgroups of chlorophytes, streptophytes, and secondary plastids-containing organisms) the indicated number of organisms, which are described to contain a plastid SRP RNA, have been analyzed regarding the predicted structure of the apical loop.[1] The loop length (nt, nucleotides) is given together with the number of organisms having these loop kinds. In addition, the number of organisms containing the conserved GNRA tetra loop is indicated. Maximal 2 examples of atypical loops are given for each loop type and subgroup.

to the bacterial SRP system, an interaction between cpSRP54 and a homolog of the bacterial SRP receptor, cpFtsY, is required for the insertion of the LHCPs into the membrane in vitro.[10,11] As in bacteria, a second cpSRP43-free but ribosome-associated pool of cpSRP54 mediates the co-translational transport of at least some plastid encoded thylakoid membrane proteins (e.g., D1).[12-14] Some insight into the "strategy" of higher plants cpSRP54 and cpFtsY to work efficiently together without a SRP RNA came from recent studies showing that: 1) the structure of cpFtsY has a so-called "preformed" conformation that alleviates an efficient interaction with cpSRP54,[15,16] and 2) the C-terminal M-domain of cpSRP54 catalyzes the cpSRP54/cpFtsY complex formation in a similar range as the SRP RNA in the bacterial system.[17] Interestingly, however, we recently reported that the SRP RNA-free SRP system is only conserved within the spermatophytes, whereas all other branches of the green and red plant lineage show a widespread presence of plastid SRP RNAs.[1] Recently published genomes have confirmed these patterns (data not shown).

There are several reasons that the plastid SRP RNAs were not identified previously. First, most of the sequence may be changed while preserving key elements of the structure, thus rendering simple primary sequence comparisons impossible except for phylogenetically close homologs. Second, a conserved apical tetraloop was always expected to be part of the structure. Third, the low number of available chloroplast genomes in non-spermatophyta organisms made comparative genomics speculative. Furthermore, had the *Codium fragile* 4.5S RNA not been mis-annotated as a rRNA in 1987,[18] these cpSRP RNAs would naturally have been found much earlier. A confounding factor was the discovery of the unique post-translational cpSRP43 in both land plants and green algae. This finding seemed to indicate that cpSRP43 had taken over the functions of the cpSRP RNA, although no evidence existed showing the protein being part of the co-translational SRP.

Here, we like to emphasize that the structure of the plastid SRP RNAs is much more diverse than that of bacterial SRP RNAs, which becomes most evident by the impressive variety of apical loops (**Table 1**). The typical apical GNRA tetraloop is highly conserved in the red lineage with only 1 exception in 16 analyzed sequences and in basal organisms of the green lineage (prasinophyceae and trebouxiophyceae) with only 2 exceptions in 14 analyzed sequences, whereas all other chloroplast SRP RNAs are characterized by atypical apical loops ranging from 1 to 11 nucleotides (**Table 1**). As it is conceivable to speculate that the bacterial transient tether model might be at least transferable to those plastid SRP systems having the bacterial-type SRP RNA, we analyzed 5 plastid FtsY sequences of the red lineage and 7 basal green algae for presence of the among bacteria highly conserved Lys399. The sequence alignment suggests that this amino acid is indeed conserved in plastid FtsY of the red lineage, whereas this was not the case in cpFtsY of the basal green algae (**Fig. 1**). Although Lys399 was identified in cpFtsY of the trebouxiophyceae,

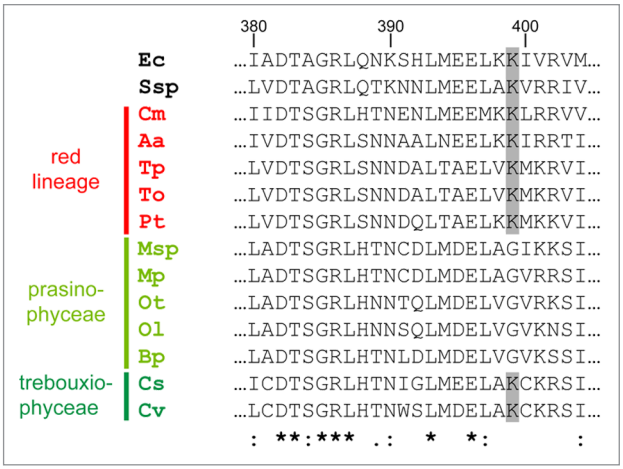

Figure 1. Phylogenetic distribution of the among bacteria conserved FtsY-Lys399 in plastid FtsY proteins. Sequence alignment of plastid FtsY receptor proteins from several organisms of the red lineage (marked red – Cm: *Cyanidioschyzon merolae,* Aa: *Aureococcus anophagefferens,* Tp: *Thalassiosira pseudonana* CCMP1335, To: *Thalassiosira oceanica,* Pt: *Phaeodactylum tricornutum* CCAP 1055/1), the prasinophyceae (marked light green – Msp: *Micromonas* sp RCC299, Mp: *Micromonas pusilla* CCMP1545, Ot: *Ostreococcus tauri,* Ol: *Ostreococcus lucimarinus* CCE9901, Bp: *Bathycoccus prasinos*), and 2 trebouxiophyceae (marked green – Cs: *Coccomyxa subellipsoidea* C-169, Cv: *Chlorella variabilis*) compared with 2 bacterial FtsY sequences (Ec: *Escherichia coli,* Ssp: *Synechococcus* sp RCC307). The lysine residue (Lys399 in *E. coli*) important for the transient tether model[4] is highlighted in gray. The residue numbering refers to *E. coli* FtsY.

this residue was replaced with glycine in all cpFtsY sequences of the prasinophyceae. Therefore, prasinophyte cpFtsYs resemble in this respect the orthologous proteins in higher plants, which were shown previously to contain an uncharged amino acid instead of the basic Lys399.[4] As our data show that the presence of GNRA tetraloop containing SRP RNAs is not strictly correlated with the presence of Lys399 in FtsY in chloroplasts of the green lineage, the molecular function of the chloroplast GNRA tetraloop SRP RNAs probably differ from the *E. coli* system. This seems to be even more likely for chloroplast SRP systems that contain SRP RNAs with deteriorated apical loops. However, it should be noted that even bacterial SRP RNAs do not always contain the usual GNRA tetraloop. Another widespread alternative loop is composed of URRC. Furthermore, few examples of bacteria with a smaller or bigger apical loop (AA loop: *Truepera radiovictrix, Gemmata obscuriglobus;* CCGAA loop: *Gemmatimonas aurantiaca*) or a URRU tetraloop (>10 members of bacteroidetes, firmicutes) have been identified.[19] Understanding of the precise molecular functions of typical and atypical SRP RNAs in chloroplast post– and/or co-translational SRP dependent protein transport will be an exciting task for future studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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