Transit Peptides Play a Major Role in the Preferential Import of Proteins into Leucoplasts and Chloroplasts*

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Jiangxin Wan†, Stephen D. Blakeley, David T. Dennis, and Kenton Ko‡

From the Department of Biology, Queen’s University, Kingston, Ontario, Canada K7L 3N6

The in vitro import characteristics of six different precursors of plastid proteins were assessed to determine differences in the protein import pathways of leucoplasts and chloroplasts. Five of these precursor proteins are destined to different subchloroplast sites, and one is a leucoplast stromal precursor protein. The results indicate that some of these precursors can be imported equally into both plastid types and others preferentially into one type of plastid versus the other. The ability of plastids to import different proteins correlates with the in vitro steady state levels of these proteins. Additional differences were also observed in the intranuclear portion of the translocation pathway for two thylakoidal proteins. The differences in import characteristics were found to be predominantly governed by information in the transit peptides, since attachment of the various transit peptides to different plastid and foreign proteins demonstrated that the import behavior of the proteins is transferable with the transit sequence. These results indicate that the import mechanisms of leucoplasts and chloroplasts are sufficiently different such that the plastids respond differently to the information present in the transit peptides.

The NH₂-terminal transit peptide extension of nuclear-encoded precursors of plastid proteins is considered the primary signal for directing posttranslational import of the precursor (see reviews by Keegstra, 1989; Keegstra et al., 1989). The transit peptide generally possesses sufficient information for the correct targeting of proteins to the plastid compartment and for subsequent intranuclear sorting. Targeting of stromal-destined precursor proteins appears to be directed by information in a contiguous segment of NH₂-terminal residues (Smeekens et al., 1986, 1987; Schmidt and Mishkind, 1986; Van den Broeck et al., 1986), whereas thylakoid lumen proteins, e.g. plastocyanin and Oee1,1 are directed by bipartite transit peptides (Smeekens et al., 1986, 1987; Weisbeek et al., 1987; de Boer et al., 1988; Ko and Cashmore, 1989). There are also examples of proteins such as Cab and Ferredoxin binding subunit of photosystem I that possess suborganellar targeting information within the mature region of the protein rather than within the transit peptide (Cline, 1988; Lampa, 1988; Van den Broeck et al., 1986, 1988; Viti et al., 1988; Hand et al., 1989; also see review by Theg and Scott, 1993). In these cases, the NH₂-terminal transit peptide acts only to import the protein into the stromal compartment.

The targeting of proteins into plastids other than chloroplasts and the additional information accompanying this process are even more complex when it is considered that plastids can vary enormously in function. Most of the evidence to date indicates that targeting information for other types of plastids can be transposed from chloroplast precursor proteins without apparent effect. For instance, the chloroplast Rubisco precursor proteins can be imported into leucoplasts (Boyle et al., 1986; Halpin et al., 1989), etioplasts, and amyloplasts (Schindler and Soll, 1986; Strzalka et al., 1987). Conversely, the amyloplast-targeting transit peptide of the maize waxy protein is able to mediate protein transport into chloroplasts (Klosgen et al., 1989; Klosgen and Weil, 1991). These results collectively suggest that a very similar import mechanism operates in all types of plastids and recognizes different transit peptides despite the plastid type-specific nature of the passenger protein. The uniformity of the import mechanisms is not unexpected, since all types of plastids are derived from the same progenitor, the proplastid, and are usually developmentally linked.

Although the central mechanism of protein import appears functionally similar in different plastid types, there are reported variations that occur in different parts of the translocation pathway. Chloroplast proteins located in thylakoids, such as plastocyanin and Oee1, can be imported into leucoplasts and amyloplasts, respectively, but are processed only to the intermediate forms (Halpin et al., 1989; Strzalka et al., 1987). These results indicate that the internal protein transport and maturation system present in the chloroplast is absent or at least different in the leucoplast and amyloplast. Furthermore, an amyloplast porin and a leucoplast pyruvate kinase (Pka) are not able to bind to chloroplasts under conditions in which other precursors bind, suggesting that there may be selectivity in their import into different types of plastid (Fischer et al., 1994; Wan et al., 1995). In the case of Pka, the NH₂-terminal transit peptide directs proteins to both leucoplasts and chloroplasts in the same manner. However, the presence of a 19-amino acid “import-modifying domain” downstream of the transit peptide abolishes its typical binding behavior toward chloroplasts and confers a requirement for a higher level of ATP for its uptake into leucoplasts. These latter studies suggest that both the transit peptide and the protein translocation apparatus of the different types of plastid contribute to the mechanistic differences observed in the import of proteins.

In this study, we expand on this type of phenomenon to include a number of other plastid precursor proteins. The in vitro import characteristics of a number of chloroplast and leucoplast precursor proteins were assessed to define in more

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† Present address: Dept. of Molecular Genetics and Cell Biology, The University of Chicago, 920 E. 58th St., Chicago, IL 60637.
‡ To whom correspondence should be addressed. Tel.: 613-545-6155; Fax: 613-545-6161; E-mail: kolk@biology.queensu.ca.

1 The abbreviations used are: Oee1, 33-kDa oxygen-evolving protein; Pka, pyruvate kinase A; Pkg, leucoplast pyruvate kinase G; Dhfr, mouse cytosolic dihydrofolate reductase; Rubs, small subunit of ribulose-1,5-bisphosphate carboxylase; Cab, chlorophyll a- and b-binding protein; Rca, ribulose-1,5-bisphosphate carboxylase activase; Fnr, ferredoxin-NADP-reductase.
detail mechanistic differences in the import pathways of leucoplasts and chloroplasts. The results indicate that some of these precursor proteins can be preferentially imported into one type of plastid versus the other and that this difference is reflected in the in vivo steady state levels of the proteins in question. The differences in import characteristics were found to be predominantly influenced by the transit peptide.

MATERIALS AND METHODS

Subfractionation and Protein Analysis—Subfractionation of leucoplasts and chloroplasts was according to the methods of Smeekens et al. (1986). Total plastid protein samples, membrane fractions, and stromal extracts were subjected to denaturing SDS-polyacrylamide gel electrophoresis (Laemmli, 1970) and electrophoretically transferred onto nitrocellulose membranes (Towbin et al., 1979). The protein blots were processed according to the method of Hoffman et al. (1987). Primary antibody reactions were detected using alkaline phosphatase-conjugated anti-rabbit IgGs (Promega). Antibodies against tobacco Pkg and pea RbcS were generated from fusion proteins as reported earlier (Wan et al., 1995). The antibodies for Cab were made against pea Cab proteins.

Construction of Chimeric Fusion Proteins—All of the chimeric gene constructs were analyzed with restriction enzymes and sequencing to ensure the fusion points were in frame, using established protocols. In vitro transcription of the fusion constructs was initiated from the SP6 promoter.

**Pkg-RbcS**—Tobacco Pkg was subcloned into pGEM5Zf+ (Promega) as a 2,000-base pair pSstI-NoI DNA fragment and was designated pTPkg (Wan et al., 1995). pTPkg was digested with EcoRI and EcoRV and religated to isolate the DNA sequence encoding the Pkg transit peptide. The EcoRI site was converted into a blunt end. A SpII DNA fragment encoding the mature part of the pea RbcS sequence was joined to the Pkg transit peptide via the SpHII site.

**Pkg-Dhfr**—A 455-base pair SolI-EcoRI DNA fragment encoding the transit peptide of tobacco Pkg was inserted into pGEM11Zf+ (Promega). This plasmid was then used to construct Pkg-Dhfr by inserting a SmaI-HindIII DNA fragment of Dhfr (retrieved from Pka1-Dhfr; Wan et al., 1995) into the EcoRI site after the HindIII and EcoRI sites had been converted into blunt ends.

**RbcS-Dhfr**—A HindIII-BamHI DNA fragment encoding the pea RbcS transit peptide was retrieved from pSSNPT and subcloned into the SmaI-BamHI sites of pGEM4. The HindIII site was rendered blunt by Klenow. The resulting vector was designated pSSTP (Ko and Cashmore, 1989). A XbaI DNA fragment encoding Dhfr was retrieved from Pka1-Dhfr and inserted into the XbaI site of pSSTP after the XbaI sites had been converted into blunt ends.

**RbcS-Cab and Cab-RbcS**—A XbaI-PstI DNA sequence encoding pea Cab was retrieved from pDX90 (a gift from A. Cashmore, University of Pennsylvania) and inserted into pSSTP via the corresponding sites to construct RbcS-Cab. The Cab-RbcS fusion was constructed as described by Hand et al. (1989).

**Protein Targeting to Leucoplasts and Chloroplasts**—The growth conditions for castor plants (Ricinus communis L. cv Baker 296) and isolation of intact leucoplasts were as described by Boyle et al. (1986). Castor seeds (stages 4 – 6) were selected according to the developmental profile described by Greenwood and Belew (1982). The protein content of the isolated leucoplasts was determined using the BCA protein assay procedure (Sigma). The number of leucoplasts in the suspension was determined using a Coulter counter (Coulter Electronics Inc.). Approximately 8 × 10^7 leucoplasts were used for each import reaction, which is equivalent to about 200 μg of protein.

The growth conditions for pea plants were as described by Ko and Cashmore (1989). Intact pea chloroplasts were isolated from 14-day-old seedlings as described by Bartlett et al. (1982). Approximately 8 × 10^7 chloroplasts were used for each import reaction, which is equivalent to 100 μg of chlorophyll.

Import assays were assembled in 0.3 ml as described in Bartlett et al. (1982). A typical import reaction contained intact plastids, 35S-labeled translation products, 10 mM methionine, 10 mM cysteine, 50 mM Heps/KOH, pH 8.0, 0.33 M sorbitol, and ATP. Radiolabeled precursors were prepared as described by Wu et al. (1994). For binding assays, the ionophore nigericin (400 nM final concentration) was used in place of ATP (Cline et al., 1985). The assays were carried out in the dark at room temperature for 30 min. Any modifications to the typical import reaction conditions are noted in the text. Protease treatment, plastid reisolation, and subfractionation of the organelles were performed according to the methods of Smeekens et al. (1986). The total plastid membrane fractions contained both envelopes and thylakoids and were not separated. Many assys were analyzed by SDS-polyacrylamide gel electrophores and prepared for fluorography using EN' HANCE (DuPont NEN) and exposed to Kodak XAR x-ray films. The LKB Ultrascan XL laser densitometer was used to quantitate and analyze the resulting fluorograms.

RESULTS

**Targeting of Fnr, Rca, RbcS, Cab, and Oee1 to Leucoplasts and Chloroplasts**—The import characteristics of five different chloroplast precursor proteins (Fnr, Rca, RbcS, Cab, and Oee1) were assessed using in vitro import assays with leucoplasts and chloroplasts. The polypeptide precursors tested are proteins destined to import suborganellar compartments of the chloroplast, such as the stroma (Rca and RbcS), the stromal face of the thylakoid membrane (Fnr), the thylakoid membrane (Cab), and the thylakoid lumen (Oee1). Treatment of the plastids with thermolysin or trypsin was used to distinguish between externally located and imported proteins (Joyard et al., 1983; Cline et al., 1984). The formation of translocation intermediates at the envelope was additionally monitored using the combination of protease treatment and subfractionation of plastids into total organelar membrane (envelope and internal membranes) and stroma. Translocation intermediates may be in the form of protease-resistant full-length precursors or partly exposed precursors with limited accessibility to exogenously added proteases (Wu et al., 1994) that still fractionate with the membranes. The presence of translocation intermediates thus reflects subtle changes in the protein import process at the envelope.

All of the precursor proteins tested displayed typical behavior in terms of binding to leucoplasts or chloroplasts in the presence of nigericin (Fig. 1, A and B, lanes 2 and 3). All bound precursors were sensitive to thermolysin, indicating an external cytoplasmic-accessible location. Differences in import behavior between leucoplasts and chloroplasts were observed for some of the precursors in assays conducted with exogenously added ATP. Most of the Fnr and Rca precursors were imported into the stromal compartment of the leucoplast (Fig. 1A, lane 4) and were resistant to both proteases (Fig. 1A, lanes 5–10). The import of Fnr and Rca into leucoplasts appears to be the same as that into chloroplasts, and the level of import appears comparable (Fig. 1, A versus B).

Even though RbcS is another stromal protein that functions in the same biochemical pathway as Rca, only 30–40% of RbcS was imported into the stroma of the leucoplast in the presence of ATP and processed to the mature form (Fig. 1A, lane 9). Most of the RbcS was present as full-length precursors and intermediate forms (Fig. 1A, lane 4) which co-fractionated with the membranes (Fig. 1A, lane 10). A substantial portion of the precursor forms appeared to be resistant to thermolysin (Fig. 1A, lane 5) although some of the intermediate forms were cleaved by thermolysin, resulting in distinct smaller size products (Fig. 1A, lane 5) that also co-fractionated with the membranes (Fig. 1A, lanes 7 and 8). The precursor and the intermediate forms were, however, trypsin-sensitive (Fig. 1A, lane 6), whereas the mature form was resistant. These protease-accessible precursor and intermediate forms most likely represent translocation intermediates in the envelope, such as those described for chloroplasts by Wu et al. (1994) and, more recently, by Scott and Theg (1996). Although RbcS is imported by leucoplasts, the uptake is distinct from that observed in chloroplasts (Fig. 1, A versus B), suggesting that even in the presence of high levels of ATP, RbcS is imported into leucoplasts in an import competent chimeric relative to chloroplasts. The efficiency results in the appearance of translocation intermediates.

An even lower level of import into leucoplasts was observed with Cab, in which less than 10% of the precursor was im-
The import level and processing of precursors were followed for chloroplasts (Fig. 1B, lanes 4–10) and leucoplasts (Fig. 1B, lanes 1–4). The import level of Rbcs, Cab, and Oee1 was generally higher in chloroplasts than in leucoplasts (based on the ratio of precursor to mature forms observed). Intermediates of Rbcs and Oee1 were not detected in either plastid type. The import level of Rbcs, Cab, and Oee1 was generally high in chloroplasts, whereas less than 10% of the imported Oee1 precursors were observed between 0 and 0.5 mM ATP for leucoplasts (Fig. 3A, lanes 4–10). The level of imported Oee1 was detected at 1 mM ATP for chloroplasts (Fig. 3A, lanes 1–4) and 0 to 0.1 mM ATP for chloroplasts (Fig. 3B, lane 1). Higher ATP concentrations resulted in a decrease of bound precursors with a concomitant increase in the level of imported products in both plastid types except for Rbcs, Cab, and Oee1 in the leucoplast assays. These precursors appear to accumulate on the leucoplast despite higher ATP levels. Imported mature forms began to appear at 1 mM ATP for leucoplasts (Fig. 3A, lane 5) and at 0.25 mM for chloroplasts (Fig. 3B, lane 3). The level of imported products reached a maximal state beyond 2 mM ATP for leucoplasts (Fig. 3A, lanes 6 and 7) and 1 mM ATP for chloroplasts (Fig. 3B, lanes 5–7). There were also a number of other differences. Regardless of the ATP concentration in the reactions, the import level of Rbcs, Cab, and Oee1 was generally higher in chloroplasts than in leucoplasts (based on the ratio of precursor/mature forms observed). Intermediates of Rbcs and Oee1 were clearly evident at all ATP levels in leucoplasts but not in chloroplasts, as is apparent in Fig. 1. Conversely, intermediate forms of Fnr and Rca were not detected in either plastid type. Like Pkg, both Fnr and Rca were imported at comparable levels into both leucoplasts and chloroplasts. The import behavior of Pkg has been examined in detail in a previous study (Wan et al., 1995) and is used here for comparative purpose.

**Steady State Levels of Native Plastid Proteins in the Leucoplast and Chloroplast**—The in vivo levels of five native proteins (Rbcs, Pkg, Oee1, Cab, and Rbcs) were measured by immuno-

**Fig. 2. Localization of imported Oee1 in the leucoplast.** Leucoplasts were fractionated into stroma and membranes following the import of Oee1 with 3 mM ATP. The translation product of Oee1 and the mature form were co-fractionated with stroma and membranes as indicated in Fig. 1. + and −, treatments with and without thermolysin and sonication. P, I, and M, precursor, intermediate, and mature forms, respectively.
The import reactions were conducted under a dim green light with increasing levels of ATP (0–3 mM; lanes 1–7). Note that as in Fig. 1, the indicated level of exogenous ATP added to each reaction was in addition to the 25 μM ATP contributed by the translation mixture.

blot analysis to determine whether there was a correlation between native protein levels and the amount of in vitro import. An immunoreactive Rbcs protein band was detected in the stroma of both leucoplasts and chloroplasts (Fig. 4, lanes 1, 3, 4, and 6), but the amount of Rbcs was about 10 times higher in the chloroplast than in the leucoplast. Since there are equal amounts of Rbcs and RbcL, the large subunit of rubisco, the same samples were immunoprobed with anti-RbcL IgGs to determine the level of RbcL. The results show that RbcL is present in both plastids at the same level as Rbcs (Fig. 4). Similarly, a weak single immunoreactive Cab protein band was detected in the membrane fraction of leucoplasts (Fig. 4, lanes 1 and 2), whereas a much more abundant doublet was present in the chloroplast membranes (Fig. 4, lane 5). A similar pattern was detected for Oee1, low levels in leucoplasts and higher amounts in chloroplasts (Fig. 4). In contrast to Rbcs, Cab, and Oee1, immunoreactive Pkg protein bands were present at similar levels in the stroma of both leucoplast and chloroplast (Fig. 4, lanes 1, 3, 4, and 6). These results suggest that the level of native protein in the two types of plastid correlates with the level of import observed in vitro for the corresponding precursor protein.

**Import of Chimeric Fusion Proteins Rbcs-Cab, Cab-Rbcs, and Pkg-Rbcs**—The import behavior displayed by Rbcs and Cab toward the two plastid types indicates that the precursors of these proteins import more readily into chloroplasts, based on the presence of translocation intermediates. Translocation intermediates are used as indicators of differences in the import process. To investigate whether the transit peptides determine the import characteristics of the passenger polypeptide, we assessed the import behavior of three chimeric fusion proteins. Rbcs-Cab and Cab-Rbcs fusion proteins were used to determine whether the observed import behavior, manifested by translocation intermediates, is a function of the transit peptide. Since the Pkg transit peptide is capable of directing proteins into chloroplasts and leucoplasts at similar levels (Wan et al., 1995), we also replaced the Rbcs transit peptide with the one from Pkg (Pkg-Rbcs) first to determine whether the import behavior of Pkg can be transposed to Rbcs and, second, to differentiate effects due to the transit peptide versus effects originating from the mature region of the protein.

The import behavior of Rbcs-Cab resembled Rbcs rather than Cab (see Figs. 1, 3, and 5). The precursors bound to leucoplasts in the presence of nigericin (Fig. 5A, lanes 2 and 3) and were imported into the stroma at a level comparable to Rbcs (approximately 40%; Fig. 5A, lane 4). The imported form was processed to the size expected for Cab. However, as in the case of Rbcs, most of the Rbcs-Cab was present as full-length precursors and intermediate forms, which co-fractionated with the membranes (Fig. 5A, lane 10). A portion of the precursor form of Rbcs-Cab appeared to be completely resistant to thermolysin (see Rbcs in Fig. 1A, lane 3, for comparison). Another portion of the precursor form as well as the intermediate forms were digested by thermolysin, giving rise to distinct, smaller size products (Fig. 5A, lane 5). One major thermolysin-generated product was only slightly larger (approximately 1–2 kDa) than mature Cab and co-fractionated with the membranes (Fig. 5A, lanes 5 and 8). Both precursor and intermediate forms were
trypsin-sensitive (Fig. 5A, lane 6), whereas the mature form of Rbcs-Cab was resistant to both proteases (Fig. 5A, lanes 5 and 6). Rbcs-Cab was imported into chloroplasts at a level comparable to that of Rbcs and responded to increasing ATP concentrations in both plastid types in a manner similar to Rbcs (Figs. 3 and 5, C and D).

In contrast to Rbcs-Cab, the Cab-Rbcs precursor behaved more like Cab than Rbcs. Cab-Rbcs bound to leucoplasts in the presence of nigericin (Fig. 5A, lanes 2 and 3) and imported at a very low level, which could only be observed when the sample received a longer exposure (Fig. 5A, lane 11). Most of the Cab-Rbcs accumulated on the surface of the leucoplast as full-size precursors (Fig. 5A, lane 10). These precursor forms were sensitive to both thermolysin and trypsin (Fig. 5A, lanes 5–8). The import of Cab-Rbcs into chloroplasts was, however, as efficient as Cab and responded similarly to increasing ATP levels in both plastid types (Figs. 3 and 5, C and D).

Exchanging the Rbcs transit peptide for the one from Pkg (Pkg-Rbcs) altered the import profile of Rbcs to resemble Pkg. Pkg-Rbcs bound to leucoplasts in the presence of nigericin (Fig. 5A, lanes 2 and 3). Approximately 60% of Pkg-Rbcs was imported into leucoplasts and processed to a polypeptide 6 kDa smaller than the precursor at 3 mM ATP (Fig. 5D, lane 4). Intermediate and protease-resistant precursor forms were not evident. The level of Pkg-Rbcs imported into leucoplasts was similar to that of unaltered Pkg (Fig. 3, also see Wan et al., 1995). The import of Pkg-Rbcs into chloroplasts also gave a result similar to that of Pkg (Fig. 5B), in which more than 80% of Pkg-Rbcs was imported into the chloroplasts and processed to the size expected for Rbcs (Fig. 5B, lane 4). Furthermore, Pkg-Rbcs responded to increasing ATP concentrations in the same manner as Pkg for both types of plastid (Figs. 3 and 5, C and D).

**Import of Chimeric Constructs Rbcs-Dhfr and Pkg-Dhfr**—To further test the role of the transit peptide in determining the import behavior of the proteins toward the two different types of plastid, the import characteristics of two chimeric constructs between the respective transit peptides and the mouse cytosolic dihydrofolate reductase (Rbcs-Dhfr and Pkg-Dhfr) were determined.

Rbcs-Dhfr bound to both leucoplasts and chloroplasts in the presence of nigericin (Fig. 6, A and B, lanes 2 and 3). Less than 30% of Rbcs-Dhfr was imported into the stroma of the leucoplast at 3 mM ATP. Most of the Rbcs-Dhfr was present as full-size precursors and as intermediate forms that co-fractionated with the membranes (Fig. 6A, lanes 4–10). However, in the case of the chloroplast, more than 60% of Rbcs-Dhfr was imported into the stroma (Fig. 6B, lanes 4–10). As with unaltered Rbcs, translocation intermediates were not evident with the chloroplast assays. The response of Rbcs-Dhfr toward the two plastid types with increasing ATP levels was also similar to that of Rbcs (Figs. 3 and 6, C and D). Therefore, the import characteristics of Rbcs-Dhfr resemble Rbcs, indicating that the Rbcs transit peptide is capable of directing a foreign protein into the two plastid types in a manner resembling unaltered Rbcs precursors.

The import characteristics imparted by the Pkg transit peptide were also evident with the Pkg-Dhfr fusion. Pkg-Dhfr bound to leucoplasts and chloroplasts in the presence of nigericin (Fig. 6, A and B, lanes 2 and 3). Approximately 40 and 50% of Pkg-Dhfr was imported into leucoplasts and chloroplasts at 3 mM ATP, respectively (Fig. 6A, lanes 4–10, and B–D). As with unaltered Pkg, translocation intermediates were not evident in either case. These results demonstrate that the Pkg transit peptide is able to target the same foreign protein as in the above construct in a manner resembling the unaltered Pkg precursor.

**DISCUSSION**

Although the overall protein translocation mechanism of different plastid types is functionally similar and can, to a large extent, recognize and import most types of plastid precursor proteins, there also appear to be some variations in the import pathways in different types of plastids. Variations that occur during precursor protein binding and recognition or during translocation across the plastid envelope may reflect significant mechanistic changes to the import process at the membrane. The 30-kDa outer envelope porin protein from pea root plastids can only be targeted to nongreen plastids and not to chloroplasts (Fischer et al., 1994), suggesting at least three
possibilities: 1) porin contains plastid-specific targeting information; 2) the protein import machinery of chloroplasts does not recognize the porin precursor; and 3) a combination of the above two possibilities may exist. In another example, a leucoplast pyruvate kinase (Pka) binds only to leucoplasts but not to chloroplasts (Wan et al., 1995), even though the Pka transit peptide itself displays typical binding and import behavior in both plastid types. The difference in binding characteristics in this latter case was partly attributed to an import-modifying domain located in the mature segment of Pka. The import-modifying domain appears to influence recognition of the Pka transit peptide by the chloroplast at the binding step as well as conferring a higher ATP requirement for uptake into the leucoplast. In this study, we expand on this type of phenomenon to include targeting information contained within the transit peptides of a number of other plastid precursor proteins. The import results and the appearance of translocation intermediates by chimeric fusion precursor proteins provide strong evidence that the import characteristics displayed toward chloroplasts and leucoplasts are influenced by information contained in the transit peptide, since the effects are transposable with the transit sequence.

The in vitro import characteristics of five plastid precursor proteins provide evidence that there can be major differences in the way precursor proteins are imported into different plastid types. The most significant differences observed are at the level of import and in the subsequent internal processing events. In the case of the two chloroplast precursors that are targeted to the stroma, Fnr and Rca, there was no observable difference in their import behavior toward the two plastid types. Fnr and Rca also behaved in a manner similar to the leucoplast pyruvate kinase Pkg, which was also imported at comparable efficiencies by both leucoplasts and chloroplasts (Wan et al., 1995). Fnr and Rca were both imported at comparable levels into leucoplasts and chloroplasts in the presence of 3 mM ATP. Maximal import levels were obtained when the ATP concentration was elevated to greater than 1 mM in the leucoplast import assays and greater than 500 μM in the chloroplast import reactions. In contrast to Fnr, Rca, and Pkg, three other precursor proteins, Rbcs, Cab, and Oee1, were imported preferentially into chloroplasts. Even though all three precursor proteins are imported by both leucoplasts and chloroplasts, the levels of import are substantially lower in leucoplasts, since only one-third of the Rbcs precursors were imported and processed by leucoplasts relative to that observed for chloroplasts. The lower level of Rbcs import is also reflected by the substantial presence of translocation intermediates in the membrane fraction. The lower level of import in leucoplasts is even more pronounced with Cab. In contrast to chloroplasts, only a very small amount of Cab is imported by leucoplasts. The imported form appears only as a single band in both the stroma and membranes, as opposed to the doublet typically observed in the thylakoids of the chloroplast. The incomplete intraorganellar sorting of Cab may be due to the absence of efficient integration mechanisms associated with photosynthetically active thylakoids. In the case of Oee1, import occurs fairly efficiently in the leucoplast import machinery of both plastid types. The overall import behavior and the information in the transit peptide is perceived by the import machinery in place. The overall import behavior and the presence or absence of translocation intermediates governed by the Rbcs and Pkg transit peptides can also be transposed to the foreign protein Dhfr. The Rbcs-Dhfr and Pkg-Dhfr fusion proteins respond to both types of plastids in a manner similar to that of native Rbcs and Pkg. The lower levels of import observed relative to their unaltered counterparts in cases such as Pkg-Dhfr (import levels, 40% in the leucoplast and 50% in the chloroplast), Pkg-Rbcs (about 60% in the leucoplast and 80% in the chloroplast) and Cab-Rbcs (less than 5% in the leucoplast and about 60% in chloroplasts) suggest that information in the passenger protein can also have some influence on the import properties via “interactions” with the transit peptide. Taken together, transit peptides appear to play a major role in determining the preferential import of the proteins into different types of plastid. In addition to the regulation of gene expression, adjustments to the import mechanism may also represent another level of control to accommodate the requirements of the tissue under various environmental parameters and developmental status of the tissue.

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