Protein Transport into Mitochondria Is Conserved between Plant and Yeast Species*

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Protein targeting into plant mitochondria was investigated by in vitro translocation experiments. The precursor of the mitochondrial F1-ATPase β subunit from Nicotiana plumbaginifolia was synthesized in vitro, translated to, processed, and assembled in purified Vicia faba mitochondria. Transport (but not binding) required a membrane potential and external nucleotides and was conserved among plant species. β subunit precursors from the yeasts Saccharomyces cerevisiae and Schizosaccharomyces pombe were imported and correctly processed in plant mitochondria. This translocation used protease-sensitive components of the outer membrane. Conversely, the N. plumbaginifolia β subunit precursor was efficiently translocated and cleaved in yeast mitochondria. However, a precursor for a chloroplast protein was not targeted to plant or yeast mitochondria. We conclude that the machinery for protein import into mitochondria is specific and conserved in plant and yeast organisms. These results are discussed in the context of a poly- or monophyletic origin of mitochondria.

Plant mitochondria and chloroplasts contain their own genome which has a limited coding capacity. Most of mitochondrial and chloroplast proteins are encoded in the nucleus, synthesized as precursors on cytoplasmic ribosomes, and subsequently transported into organelles. Precursors generally contain an amino-terminal extension which directs the protein to the target membrane and is removed after import into the organelle.

The chloroplast protein import has been better studied than the targeting process into plant mitochondria (for review, see Keegstra et al., 1989; Weisbeek et al., 1989). However, the mechanism of mitochondrial import has been well characterized in fungal (Saccharomyces cerevisiae and Neurospora crassa) and mammalian cells (for review, see Rosenberg et al., 1987; Attardi and Schatz, 1988; Hartl et al., 1989). The majority of mitochondrial targeting precursors share a positively charged amphiphilic structure (von Heijne, 1986) involved in binding to proteinaceous receptors on the mitochondrial surface (Pfaller et al., 1988). Translocation into mitochondria requires a membrane potential across the inner membrane (Schleyer et al., 1982; Gasser et al., 1982) and in addition ATP, possibly for the maintenance of precursors in an unfolded form (Pfanner et al., 1987; Chen and Douglas, 1987; Verner and Schatz, 1987). Once introduced in the mitochondrial matrix, precursors are removed by a processing machinery whose components have been identified (Hawlischek et al., 1988; Witte et al., 1988; Yang et al., 1988). Processed proteins are finally directed to their submitochondrial compartment.

The presence of chloroplasts in plant cells may require a more stringent sorting machinery. This raises the question whether proteins are imported into plant mitochondria as in fungal and mammalian mitochondria. We have previously shown that the β subunit precursor of mitochondrial F1-ATPase from Nicotiana plumbaginifolia is synthesized as a larger precursor which is processed during or after mitochondrial uptake (Boutry et al., 1987a). Similar results were obtained with other precursors introduced in maize (White and Scandalios, 1987), broad bean (Whelan et al., 1988), or pea (Unger et al., 1989) mitochondria. The precursor of the F1-ATPase β subunit from N. plumbaginifolia contains a presequence which is rich in basic and hydroxylated amino acids but poor in acidic residues (Boutry and Chua, 1985). It could possibly form a positively charged amphiphilic α helix (von Heijne, 1986). Its involvement in mitochondrial targeting was demonstrated by showing that a NH2-terminal amino acid residue of the precursor were capable of specifically targeting the passenger protein, bacterial chloramphenicol acetyltransferase, into mitochondria in transgenic plants (Boutry et al., 1988/b). The presence length of the N. plumbaginifolia β was estimated to be 55 residues long from comparison with the sequence of the mature β subunit of sweet potato (Kobayashi et al., 1986).

In the present work, we report in vitro experiments demonstrating that the β subunit precursor is correctly translocated in plant mitochondria, cleaved, and assembled in the F1-ATPase complex. This import process requires both energized membranes and external nucleoside triphosphates. We compared in vitro targeting of the β subunit from plant and yeast species into either plant or yeast mitochondria and found that the process was conserved between those organisms.

MATERIALS AND METHODS

Strains and Media—The Escherichia coli strain used for plasmid selection and propagation was 71–18 (SupE7, thi, Δlac-proAB, [F', proAB, lacFZΔM15]). Cells were grown at 37 °C in LB medium (1% bactotryptone, 0.5% yeast extract, 1% NaCl, pH 7.5 (NaOH)). Ampicillin-resistant transformants were selected on solid medium supplemented with 50 µg/ml ampicillin.

The yeast strain D73-10B was used for mitochondrial preparations. Cells were grown at 30 °C in YPD medium (2% yeast extract, 1% bacto-peptide, 2% glucose).

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DNA Construction—The vector used for in vitro transcription was pTZ18 (United States Biochemical Corporation) which positions the T7 RNA polymerase promoter adjacent to the multilinker region. The EcoRI cDNA fragment containing the F1-ATPase \( \beta \) subunit gene of N. plumaginifolia (Boutry and Chua, 1985) was inserted into the EcoRI site of pTZ18U (pTZ2-ATP2). A Sall-HindIII fragment containing the coding sequences of T. cerevisiae ATPIP2 gene (Takeda et al., 1985) was inserted into the corresponding sites of the polylinker region of pTZ18U (pTZ2-ATP2-C). An AclI fragment containing the Schizosaccharomyces pombe atp2 gene was filled in with the Klenow DNA polymerase and inserted into the SmaI site of pTZ18R. This fragment contains the whole coding sequence with 114 nucleotides of the 5' non-coding-transcribed sequence. HindIII restriction analysis confirmed the fragment orientation (pTZ-ATPB-P). A XmnI fragment containing a 3'-noncoding sequences of the precursor was inserted into the SmaI site of pTZ18R. This fragment contains the whole coding sequence with 114 nucleotides of the 5'-noncoding-transcribed sequence. SacI restriction analysis confirmed the fragment orientation (pTZ-ab).

In Vitro Transcription Translation—The plasmids pTZ2-ATP2, pTZ2-ATP2-C, and pTZ2-ab were linearized with HindIII. The plasmid pTZ2-ATP2-C was linearized with XbaI. The translational reaction included a 50-μl reaction: 40 mM Tris-HCl (pH 8.0), 15 mM MgCl2, 10 mM dithiothreitol, 0.5 mM each of ATP, CTP, UTP, GTP, 0.5 mM GpppG, 50 units of RNasin, 300 μg/ml bovine serum albumin, 5 μg of linearized DNA, and 12 units of T7 RNA polymerase. Following an incubation of 2 min at 37 °C to allow RNA capping, GTP was added to 0.1 mM and the mixture was incubated 30 min at 37 °C. The RNA was then purified through a 1-ml Sephadex G-50 column equilibrated with 10 mM Tris-HCl (pH 7.5), 1 mM EDTA. Five μl of RNA were translated for 1 h at 30 °C in 44 μl of a nuclease-treated reticulocyte lysate supplemented with 100 μCi of [35S]methionine.

In the case of chloroplast import, 5 μl of RNA were translated for 1 h at 30 °C in 36 μl of a wheat germ extract supplemented with 100 μCi of [35S]methionine.

In Vitro Mitochondrial Import—Vicia faba mitochondria were isolated from dark-grown hypocotyls as previously outlined (Boutry et al., 1987b). Mitochondria were resuspended in suspension medium (0.4 mM mannitol, 10 mM KH2PO4, pH 7.5 (KOH)). Yeast mitochondria were isolated as described (Genga et al., 1986). In vitro import reactions were performed in 160 μl of a medium containing 200 μM mannitol, 20 mM Hepes-KOH (pH 7.5), 50 mM KCl, 2 mM MgCl2, 1 mM ATP, 1 mM KH2PO4, 1 mM dithiothreitol, 20 μM ADP, 10 mM malate, 5 μl of translation mix and 20–40 μg of mitochondria. The mixture was incubated for 30 min at 28 °C. Modifications of the reaction were as described in the legend to figures. Proteinase K digestion (2.4 μg/ml), where indicated, was performed for 15 min at 0 °C. Mitochondria, after addition of 1 mM PMSF, were reisolated by pelleting through a 1-ml mannitol cushion (0.6 M mannitol, 10 mM KH2PO4 (pH 7.5) (KOH), 100 mM KCl, 1 mM PMSF) and washed in 0.5 ml of suspension medium supplemented with 1 mM PMSF. The resultant products were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis through 9–13% gradient gels as described (Laemmli, 1970). Proteins were then transferred to nitrocellulose membranes (Towbin et al., 1979) which were subsequently exposed to x-ray films. The volume of reticulocyte lysate loaded as a control on the gels was 3-fold lower than the volume added in the import reaction.

In Vitro Chloroplast Import—Chloroplast isolation from pea leaf and import were as described by Cline et al. (1985).

F1-ATPase Purification—Mitochondrial membrane fractions were obtained from purified mitochondria which were diluted (5 mg/ml) in hypotonic buffer (4 mM Tris, 2 mM ATP, 1 mM EDTA (pH 8.0) (HCl)) and centrifuged at 100,000 × g for 1 h in a Kontron TST-60.4 rotor. The pellet was resuspended in the same buffer at a concentration of 10 mg/ml at room temperature, mixed in an Eppendorf tube with an equal volume of chloroform neutralized by 100 mM Tris (pH 8.0) (HCl). The two phases were mixed by vortexing for 20 s. The aqueous phase was separated by 30 min centrifugation at 80,000 × g. The aqueous phase was centrifuged again for 30 min at the Beckman Airfuge rotor. The pellet was resuspended in the same buffer at a concentration of 4 mg/ml Tris, 1 mM EDTA, 2 mM ATP (pH 8.0) (HCl) and centrifuged for 4 h in a Kontron TST-60.4 rotor at 50,000 rpm (260,000 × g) at 20 °C. Fractions (20 in total) were collected from the bottom, and 75-μl aliquots were tested for ATPase activity (Pullman et al., 1960). The peak of ATPase activity normally sedimented in fractions 8–10 from the bottom.

RESULTS

The \( \beta \) Subunit Precursor Is Imported and Processed in Purified Mitochondria Isolated from Both Dicotyledon and Monocotyledon Species—A complete atp2-1 cDNA clone encoding the mitochondrial F1-ATPase \( \beta \) subunit of N. plumaginifolia (Boutry and Chua, 1985) was placed under the control of a T7 polymerase promoter in the expression vector pTZ18. In vitro transcribed RNA was subsequently translated in a rabbit reticulocyte lysate into a \( \beta \) polypeptide precursor with the expected size of \( M_s = 59,000 \) (Fig. 1A, lane 1). The labeled precursor was incubated with purified V. faba mitochondria in the presence of a respiratory substrate (malate) and ATP and found to be partly cleaved to a mature form (lane 2) which had the same electrophoretic mobility as the mature \( \beta \) subunit of a partially purified V. faba F1-ATPase.

![Fig. 1](http://www.jbc.org/)
was thus located inside the organelle. On the contrary, the precursor as well as two unidentified translated products of lower apparent size were not incorporated into the organelle since they were degraded by the protease treatment (lanes 3–5). Interestingly, the β precursor synthesized in a wheat germ extract was binding to mitochondria but was poorly translocated into the organelle (results not shown). Our import experiments were conducted with mitochondria isolated from *V. faba* hypocotyls because this material facilitated the isolation of intact and functional mitochondria as indicated by their high respiratory controls (not shown). The import reaction was also observed with *Nicotiana tabacum* mitochondria isolated from green leaves although with a much lower efficiency (Boutry *et al.*, 1987a). This probably reflects the reduced respiratory activities of mitochondria obtained from green material. Finally, the β precursor, obtained from a dicotyledon species, was also translocated and correctly processed in mitochondria isolated from *Allium porrum*, a monocotyledon species (Fig. 1B). This result indicates that plant import and processing machinery are well conserved in the plant kingdom.

The Imported and Processed β Subunit Is Assembled into F$_1$-ATPase Complex—We tested whether the mature β subunit was assembled into an F$_1$-ATPase complex. Mitochondria were lysed by osmotic shock after *in vitro* import of the labeled precursor. The F$_1$-ATPase was solubilized by a chlo-roform treatment of the membrane fraction and purified by centrifugation on a sucrose gradient (Boutry *et al.*, 1983). Electrophoretic analysis of the different fractions revealed the presence of an F$_1$-ATPase in the fractions 8–10 (Fig. 2A) where the ATPase activity sedimented (not shown). The autoradiograph (Fig. 2B) shows that the mature β subunit peaked in the same fractions. An additional peak of mature β subunit was observed in the top fractions of the gradient and was probably associated with the F$_1$-ATPase not totally assembled. Thus, once imported into the mitochondrial matrix, the β subunit precursor from *N. plumbuginifolia* was cleaved and assembled into a functional F$_1$-ATPase complex associated with the inner membrane.

Import of β Subunit Precursor into Plant Mitochondria Requires Both Transmembrane Potential and External Nucleoside Triphosphates—The mitochondrial import process was found to be sensitive to reagents abolishing the membrane potential across the mitochondrial inner membrane. Indeed, the addition of 2.5 μM antimycin A, an inhibitor of the respiratory chain, dramatically reduced the amount of imported protein (Fig. 3, lane 2). Addition of 1 μM of an oxidative phosphorylation uncoupler (carbonylcyanide m-chlorophenylhydrazone) or a K$^+$ ionophore (valinomycin) completely blocked the translocation of the β subunit precursor to mitochondria (lanes 3 and 4). Translocation of Mn-superoxide dismutase into maize mitochondria was also found to be sensitive to valinomycin (White and Scandalios, 1987). However, in the latter case, valinomycin also abolished binding of the precursor to mitochondria. This data contrasts with our results and those reported for other organisms.

In order to study nucleotide requirements, the translation mixture was passed through a Sephadex G-50 column to remove small molecules. In the absence of nucleotides, the import and cleavage of the β precursor was severely reduced (Fig. 4A, lane 1) or almost completely abolished (Fig. 4B, lane 1). When increasing levels of ATP were added, import process resumed to a maximal efficiency at about 1 mM ATP (lanes 2–7). Requirement was not specific for ATP since the β precursor was translocated and cleaved to the mature form as well in the presence of 1 mM of GTP, CTP, or UTP (Fig. 4B). Thus, by the requirement of a membrane potential and external nucleoside triphosphates, protein translocation in plant mitochondria is similar to mitochondrial import in *N. crassa* (Shleyer *et al.*, 1982; Pfanner and Neupert, 1986) and *S. cerevisiae* (Gasser *et al.*, 1982; Chen and Douglas, 1987; Eilers *et al.*, 1987).

Both Plant and Yeast β Precursors Are Translocated into Mitochondria of Both Organisms—In order to evaluate whether the mitochondrial import machinery was conserved among various organisms, we compared the *in vitro* mitochondrial targeting of the precursor for the F$_1$-ATPase β subunit from *N. plumbuginifolia* and two yeast species, *S. cerevisiae* (Takeda *et al.*, 1985) and *S. pombe*.

Although the amino acid sequences of the three mature

![Fig. 2. Imported β subunit is assembled into a F$_1$-ATPase complex. Reticulocyte lysate containing radiolabeled precursor protein was incubated with *V. faba* mitochondria as described under "Materials and Methods." Mitochondria from 20 import reactions (±1 mg) were used for F$_1$-ATPase purification ("Materials and Methods"). The fractions collected from sucrose gradient were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Coomassie Blue staining of a gel obtained in a control experiment is shown in panel A. The fractions resulting from the import experiment are shown in panel B (autoradiograph). The ATPase activity sedimented in the fractions labeled with a dot. Stars indicate the F$_1$-ATPase subunits: α, β, γ, δ and δ'.](http://www.jbc.org/)

![Fig. 3. Import of F$_1$-ATPase β subunit into plant mitochondria requires a membrane potential. Reticulocyte lysate containing radiolabeled precursor was incubated with *V. faba* mitochondria as described under "Materials and Methods" in the absence (lane 1) or in the presence of 2.5 μM antimycin A (lane 2), 1 μM carbonylcyani-
de m-chlorophenylhydrazone (CCCP) (lane 3), or 1 μM valinomycin (lane 4). The products were analyzed as in Fig. 1.](http://www.jbc.org/)

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4. Import of F1-ATPase β subunit into plant mitochondria requires nucleoside triphosphates. Reticulocyte lysate containing radiolabeled precursor protein was passed through a Sephadex G-50 column equilibrated with import buffer ("Materials and Methods") lacking ATP and ADP. The protein fraction was then added in import reactions containing V. faba mitochondria and nucleotides. Panel A, import reactions in the presence of increasing amounts of ATP. Lanes: 0 = 0 mM; 1 = 0.05 mM; 2 = 0.1 mM; 3 = 0.25 mM; 4 = 0.5 mM; 5 = 1 mM; 6 = 1.5 mM; 7 = 2 mM. Panel B, import reaction in the presence of different nucleotides. Lanes: 1 = without nucleotide; 2 = 1 mM ATP; 3 = 1 mM GTP; 4 = 1 mM CTP; 5 = 1 mM UTP. The products were analyzed as in Fig. 1.

FIG. 5. Comparison of the amino-terminal sequences of the F1-ATPase β subunit precursors from different organisms. The amino-terminal sequences of F1-ATPase β subunit precursor from N. plumbaginifolia (Boutry and Chua, 1985), S. pombe and S. cerevisiae (Vassarotti et al., 1987) are depicted. The beginning of the conserved sequences of the mature part is underlined. Arrows indicate with the sequence of the mature β subunit from sweet potato (Kobayashi et al., 1986). Charged residues are indicated (+ or -).

proteins share over 72% homology, the amino-terminal extensions of the precursors are quite different in length and primary structure (Fig. 5). They exhibit, however, common features of mitochondrial precursors: they are rich in positively charged and hydroxylated residues, lack acidic amino acids, and are capable of forming an amphipathic helix (von Heijne, 1986).

The three β subunit precursors were synthesized in vitro and incubated with V. faba mitochondria. Each precursor was partly processed to a mature form (Fig. 6A, lanes 2, 5, and 8) which was inaccessible to externally added protease (lanes 3, 6, and 9). The radiolabeled mature yeast β subunits possessed the same electrophoretic mobilities as the β subunits of the purified F1-ATPase from both yeast strains (lanes 6' and 9') indicating that in both cases, the cognate cleavage site was recognized by the plant matrix protease. However, it appeared that the S. cerevisiae precursor import was less efficient (lanes 8 and 9). Reciprocally, plant and yeast precursors were incubated with isolated S. cerevisiae mitochondria. The precursors were all imported and cleaved to the correct mature form protected from external protease (Fig. 6B).

An early step in the mitochondrial import process is the interaction between precursors and protease sensitive components, later identified as receptors and which appear to be responsible for the specificity of the imported precursor (Gasser et al., 1982; Zwitzinski et al., 1983; Pfaller et al., 1988). In our study, trypsin pretreatment of V. faba mitochondria reduced import of plant and yeast β subunit precursors (Fig. 7) without modifying the integrity of mitochondria as monitored by respiratory controls (results not shown). Thus, the translocation of the plant and yeast β subunits in plant mitochondria requires a protease-sensitive component of the outer membrane. In both cases, a low residual translocation was observed. This was also the case for the N. crassa proteins translocation (Pfaller et al., 1988) and interpreted as a bypass: once receptors have been destroyed by protease treatment, import can still occur but with low efficiency (Pfaller et al., 1988). Bypassing showed little specificity since import of a chloroplast precursor into mitochondria (Hurt et al., 1986) uses this pathway (Pfaller et al., 1988). However, other chloroplast precursors were not found translocated in yeast mitochondria (Smeekens et al., 1987). In order to investigate this phenomenon in plant mitochondria, we performed import reactions with the light harvesting chlorophyll a/b-binding (Cab) precursor of N. plumbaginifolia. This precursor contains a transit peptide which directs the protein into the thylakoid membranes (Schmidt et al., 1981). Indeed, the Cab precursor synthesized in a wheat germ extract was imported and processed in chloroplast isolated from pea (Fig. 8A). Different mature forms were observed as already reported for other species (discussed in Clark et al., 1990). However, when the Cab precursor was synthesized in a reticulocyte lysate followed by incubation with plant (Fig. 8B) or yeast (Fig. 8C) mitochondria, no processed form was observed, and the precursor was completely degraded by external protease indicating that, in our experimental conditions, this chloroplast precursor was not imported into mitochondria. A similar result was obtained when the precursor was synthesized in a wheat germ extract and incubated with plant mitochondria (results now shown).

DISCUSSION

The β subunit precursor of mitochondrial F1-ATPase from N. plumbaginifolia was imported, processed in purified V. faba mitochondria, and assembled into an F1-ATPase complex (Figs. 1 and 2). In addition to a membrane potential (Fig. 3), the transport of the F1-ATPase β subunit precursor into plant mitochondria requires ATP in the external medium (Fig. 4A). This is also the case for other organisms (for review, see Hartl et al., 1989). Other nucleoside triphosphates could also sustain this process (Fig. 4B). This observation confirms those of Chen and Douglas (1987) who explained the lack of nucleotide specificity by the presence of a nucleoside diphosphokinase associated with the mitochondrial outer membrane and intermembrane space.

Several reports suggest that a function of ATP (or analogues) in mitochondrial protein import is to confer import competence to precursor proteins (Pfanner et al., 1987; Verner...
Fig. 6. F1-ATPase β subunits from plant and yeast are imported into V. faba and S. cerevisiae mitochondria. Reticulocyte lysates containing radiolabeled β precursor from N. plumbaginifolia (lane 1, N. pl), S. pombe (lane 4, S. po.), and S. cerevisiae (lane 7, S. ce) were incubated with V. faba (V. fa) (panel A) or S. cerevisiae (panel B) mitochondria (Mito) as described under “Materials and Methods” (lanes 2, 3, 5, 6, 8, and 9). After reaction, the mixtures were treated with 2.4 μg/ml proteinase K (Prot. K) for 15 min at 0 °C (lanes 3, 6, and 9). The products were analyzed as in Fig. 1. Purified F1-ATPases from S. pombe (lane 6′) and S. cerevisiae (lane 9′) were analyzed in the same gel as in panel A and stained by Amido black after transfer to nitrocellulose membrane. Precursor (closed circle) and mature (closed square) forms are indicated.

Fig. 7. Trypsin pretreatment of plant mitochondria inhibits import of plant and yeast F1-ATPase β subunit. V. faba mitochondria isolated as described under “Materials and Methods” were pretreated for 20 min at 0 °C with the indicated amounts of trypsin as described by Pfaller et al. (1989). After incubation, trypsin was inhibited by the addition of soybean trypsin inhibitor (30-fold weight excess over trypsin) and 0.5 mM PMSF. Mitochondria were washed by pelleting through a 1-ml mannitol cushion (0.6 M mannitol, 10 mM KH₂PO₄ (pH 7.5) (KOH), 100 mM KCl) and washed in 0.5 ml of suspension medium (0.4 M mannitol, 10 mM KH₂PO₄ (pH 7.5) (KOH)). Integrity of the inner membrane was controlled by oxygen consumption. Respiratory controls (around 5 with malate) did not vary with trypsin treatment. Import reactions were performed as described under “Materials and Methods.” The products were analyzed as in Fig. 1. The mature F1-ATPase β subunit was quantified by densitometry of autoradiographs.

Fig. 8. A chloroplast precursor is not imported into plant and yeast mitochondria. Wheat germ (panel A) or reticulocyte lysate (panels B and C) containing the radiolabeled precursor of chlorophyll a/b-binding protein (lane 1), was incubated with pea chloroplast (Chloro) (panel A, lanes 2 and 3), V. faba mitochondria (panel B, lanes 2 and 3) or S. cerevisiae mitochondria (panel C, lanes 2 and 3) as described under “Materials and Methods.” After reaction, the mixtures were treated with 1.7 (panel A, lane 3) or 2.4 (panels B and C, lane 3) μg/ml proteinase K (Prot. K) for 15 min at 0 °C. The products were analyzed as in Fig. 1 except for panel A where the products were resolved on a 11–15% polyacrylamide gel.
another enzyme involved in the early steps of translocation. Finally, the enzymatic machinery responsible for processing the precursor seems conserved since the yeast and plant β precursors were correctly cleaved in heterologous systems.

These observations are confirmed by in vivo experiments. A plant Mn-superoxide dismutase was efficiently imported and correctly processed by yeast mitochondria (Bowler et al., 1989) and inversely, the presence of the mitochondrial tryptophanyl-tRNA-synthase from yeast imported the bacterial β-glucuronidase into mitochondria of transgenic plants (Schmitz and Lonedal, 1989).

These results have an important implication concerning the origin of mitochondria. The endosymbiotic origin of mitochondria (and chloroplasts) is now widely favored. However, the possibility of a multiple origin for mitochondria has been raised (Raven, 1970; Stewart and Mattox, 1984). For instance, the major differences in mitochondrial structure and expression between plant and other organisms together with rRNA sequence comparison support the hypothesis that mitochondria are at least partly of polyphyletic origin (i.e. that the mitochondrial genomes from plants and other organisms partly originated from distinct endosymbiotic events) (Gray et al., 1989). The uniqueness of the mitochondrial protein translocating system, which could have developed only after endosymbiosis of a bacteria-like progenitor of mitochondria, strongly supports a unique origin of mitochondria before fungi and plants diverged. However, this scenario does not exclude the possibility of an additional and more recent symbiotic event in the plant phylum leading to a transfer of genetic information (e.g. of rRNA) from the new endosymbiont to the established mitochondrion (Gray et al., 1989). Alternatively, fusion of mitochondrial membranes with the plasma membranes of the new endosymbiote would have readily provided the latter with the receptors necessary for protein import from the cytosol and thus allowed a rapid evolution of the new endosymbiote interactions which eventually led to the disappearance of the original mitochondrion.

Although chloroplast and mitochondrial protein targeting systems share common properties (for review see Keegstra et al., 1989; Weisbeek et al., 1989), both processes are expected to be specific. However, a chloroplast precursor from *Chlamydomonas reinhardtii* was demonstrated to direct passenger proteins into yeast mitochondria (Hurt et al., 1986) but this import process occurred with low efficiency and seemed to bypass protease sensitive receptors (Pfaller et al., 1989). Interestingly, structure analysis of this and other precursors from *C. reinhardtii* indicated that they are more similar to mitochondrial than to chloroplast targeting precursors from higher plants (Franzen et al., 1990). In other experiments, several chloroplast precursors from higher plants did not import different proteins into yeast mitochondria (Smeekens et al., 1987). Moreover, no misrouting was observed in transgenic plant cells in which a mitochondrial or a chloroplast precursor from *N. plumbaginifolia* specifically addressed the chlorophenolic acetyltransferase into mitochondria or chloroplasts, respectively (Boutry et al., 1987b). Our *in vitro* experiments showed that the chloroplast precursor to the chlorophyll a/b-binding protein was not imported into plant or yeast mitochondria (Fig. 8). Thus, it appears that chloroplast and mitochondrial targeting precursors must contain distinct information required for specific addressing. Indeed, analysis of primary and predicted structures of chloroplast and mitochondrial targeting precursors indicated distinct properties for both types (von Heijne et al., 1989).

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