Review
Protein Trafficking in Plant Cells

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GUY DELLA-CIOPPA*, GANESH M. KISHORE, ROGER N. BEACHY, AND ROBERT T. FRALEY
Monsanto Company, Plant Molecular Biology Group, St. Louis, Missouri 63198 (G.d.C., G.M.K.,
R.T.F.), and Washington University, Department of Biology, St. Louis, Missouri 63130 (R.N.B.)

ABSTRACT

The cells of higher plants contain distinct subcellular compartments (organelles) that perform specialized functions such as photosynthesis, carbohydrate and lipid metabolism, and so forth. The majoritY of the protein constituents of plant organelles are formed as cytosolic precursors with N-terminal extensions that direct transport across one or more membrane bilayers in a post- or co-translational fashion. Since the majority of proteins in plant cells are products of nuclear gene expression, there must be precise sorting mechanisms in the cytoplasm that direct proteins to their correct cellular locations. Based on recent studies of protein targeting to chloroplasts and vacuoles, the details of these intracellular sorting mechanisms are becoming clear. The ability to direct proteins to specific compartments within cells provides new opportunities for improvement of plants by genetic manipulation.

A characteristic feature of plants is their ability to grow using sunlight as the only source of energy. The internal structure of a plant cell thus requires an elaborate system of energy transducing membranes to carry out photochemical reactions. The complexity of these reactions and the underlying cytoarchitecture necessary to carry them out, is unparalleled in other systems. Despite the existence of two semiautonomous organelles within plant cells, the bulk of the cellular proteins are biosynthesized in the cytoplasm and transported either co- or posttranslationally across one or more membranes. The problem of protein sorting and processing in plants can therefore be intrinsically more demanding than in other eucaryotic systems. Five major targets of protein transport in plant cells are the secretory pathway (ER, Golgi body, vacuole and plasma membrane), the chloroplast, the mitochondrion, the nucleus, and the glyoxysome/peroxisome. Transport of proteins into these cellular compartments is mediated by amino terminal 'topogenic sequences.' For nuclear-encoded mitochondrial and chloroplast proteins, posttranslational targeting to the organelles involves recognition of a sequence of amino acids usually at the amino terminal end of the protein. These amino terminal TP sequences are proteolytically removed after import into the organelle. Proteins imported by chloroplasts are further sorted into suborganelar compartments, and recent evidence indicates that the final targeting information is also present in the TP sequences. Proteins destined for the

CHLOROPLASTS

The chloroplast of a plant cell plays a pivotal role in not only photosynthesis but also in the biosynthesis of almost all key metabolic products such as amino acids, carbohydrates, and lipids. It is probably the most complex of all eucaryotic cell organelles. The vast majority of chloroplast proteins that carry out this complex biochemistry are products of nuclear-encoded genes (15) and as such they must be imported from their site of synthesis in the cytoplasm.

The first hint as to the mechanism of chloroplast import of nuclear-encoded polypeptides came from the finding that the small subunit of RuBPCase from Chlamydomonas reinhardtii is synthesized as a precursor with an N-terminal TP sequence (13). The TP sequence of the RuBPCase small subunit from higher plants was subsequently shown to target the precursor to the chloroplast where it then mediates translocation across the envelope membranes (7, 19). Since these initial observations, a host of other chloroplast preprioproteins with TPs ranging upwards of 33-amino acid sequences have been identified (25). All TPs characterized to date are rich in positively charged amino acids and hydrophilic residues (especially serine and threonine). TP sequences in general lack long hydrophobic stretches and contain few, if any, acidic residues. Chloroplast precursors undergo proteolytic maturation inside the organelle to give a mature protein that lacks the TP sequence; the processed protein is no longer import competent. Import into chloroplasts occurs in an energy dependent, posttranslational fashion that is in most respects similar to the mechanism known for yeast and mammalian mitochondrial import. Although the details of actual import process in chloroplasts are largely unknown, it is now clear that the outer envelope of the organelle plays an important role in precursor recognition (8).

Recent experiments have shown that the TP is both necessary and sufficient to target a foreign protein to the chloroplast compartment. Chimeric proteins made with the TP of the small subunit of RuBPCase fused to the N-terminus of the bacterial protein NPT II were imported and processed in transgenic plant chloroplasts in vivo and in recombinant chloroplast import assays in vitro (26, 30). However, in these experiments no quantitative data were presented to evaluate the efficiency of import of the chimeric protein relative to the RuBPCase small subunit. More recently, Wasmann et al. (31) reported that the RuBPCase small subunit TP will most efficiently target NPT II into chloroplasts in vitro when a portion of the mature small subunit (23 amino acids) is included in the fusion protein. The chimeric protein containing 23 amino acids of the mature small subunit (TP-SS-NPT II) was imported with about the same efficiency as

1 Abbreviations: TP, transit peptide; RuBPCase, ribulose-1,5-bisphosphate carboxylase; NPT II, neomycin phosphotransferase II; EPSP, 5-enolpyruvylshikimate-3-phosphate; SS, small subunit; DHFR, dihydrofolate reductase.
the complete small subunit. The construct containing the TP fused directly to NPT II was imported at about 10% the normal efficiency. These results suggest that amino acid sequences following the TP/mature cleavage site are important for translocation efficiency. In addition, Lubben and Keegstra (24) recently showed that import of a Mr, 17,500 heat-shock protein fused to the RuBPCase small subunit TP is about 40% as efficient as import of the complete small subunit. Although the RuBPCase/heat-shock fusion used in their study included 13 amino acids of the mature small subunit, the results suggest that other determinants responsible for optimal import may lie even further within the mature sequence itself. It appears on the basis of these in vitro experiments that TP sequences alone may not be sufficient for high-level import of all foreign proteins into chloroplasts.

In contrast to the in vitro experiments just described (31), Kuntz et al. (23) studied import into chloroplasts of TP-NPT II fusions in transgenic tobacco in vivo. The results of these experiments were surprising because the mature form of NPT II from the direct fusion (TP-NPT II) was found localized in chloroplasts whereas the construct containing 23 amino acids of the mature small subunit (TP-SS-NPT II) was present primarily in the cytosol. These in vivo results are the opposite of those found in vitro. An explanation for the discrepancy may be due to differential proteolysis of the two fusion proteins by degradative enzymes in the cytosol of the tobacco cells. In vivo experiments may thus reflect the steady state level of precursor protein in the cytosol as opposed to the absolute rate of chloroplast import.

Given the similarities in chloroplast and mitochondrial import mechanisms (e.g. posttranslational import, ATP dependent, basic N-terminal TP sequence, etc.) how are plant cells able to correctly target precursor proteins to the appropriate organelle without significant error? Hurt et al. (21) have addressed this question by fusing a portion of the TP from the small subunit of RuBPCase to mouse DHFR (a cytosolic protein) and yeast cytochrome oxidase subunit IV (an imported mitochondrial protein). Reconstituted import assays with yeast mitochondria in vitro showed that the chloroplast precursor sequence could direct uptake of both proteins into mitochondria. These in vitro uptake results were also confirmed in vivo by complementation of a yeast mutant that is deficient in the nuclear gene for cytochrome oxidase subunit IV. Although the efficiency of the heterologous 'mismatching' was significantly reduced in both assays, it suggests that chloroplasts and mitochondria import precursors by similar, but not identical, mechanisms. If these results observed in the yeast system hold for plant mitochondria, a selective screening mechanism may exist in plant cells to prevent incorrect sorting in vivo. However, before the extent of mismatching can be critically evaluated, it will be necessary to switch TPs from normal plant mitochondrial/chloroplast precursors and then study the targeting in vitro and in transgenic plant tissues in vivo.

Chloroplasts are unique in their organization in that they contain an internal set of thylakoid membranes which enclose a lumen space; thus, imported proteins must be routed to multiple compartments once inside the organelle. Smeekens et al. (29) studied the import of two nuclear-encoded precursors routed to two different locations within the chloroplast. In vitro import assays into pea chloroplasts showed that the thylakoid lumen protein, plastocyanin, had been entirely redirected to the stromal compartment when fused behind the TP of Fd (a stromal protein). On the other hand, the plastocyanin TP when fused to Fd gave rise largely to a processing intermediate within the chloroplast stroma, only a small fraction of which had been directed into the thylakoid lumen. The inefficient targeting of the chimeric fusion to the thylakoid lumen was proposed to be due to rapid association of the imported Fd with other enzymes in the stroma with which it would normally form complexes. Future studies that focus on directing foreign passenger proteins into the thylakoid lumen in order to test for stromal interactions will be of interest. It seems clear from these studies that targeting of proteins to the lumen space is more complex than previously envisaged. Import of plastocyanin has been proposed to involve two domains within the TP, a domain for chloroplast import (the first 38 amino acids) and a domain for thylakoid transfer (the remaining 28 amino acids) (29). This model is supported by identification of a processing intermediate of plastocyanin that is specifically cleaved by a thylakoid protease (18).

Recently, the cDNA for the chloroplast stromal enzyme, EPSP synthase has been obtained from petunia (28). Analysis of the cDNA indicates that EPSP synthase contains a 72-amino-acid TP; this represents the longest chloroplast TP characterized to date. EPSP synthase is of significant agronomic interest because it is the target of the herbicide glyphosate (Roundup®) which is widely used in agriculture throughout the world. The cDNA for EPSP synthase was cloned into an SP6 transcription/translation system and the precursor enzyme was shown to be imported and correctly processed by heterologous chloroplasts in vitro (11). The cDNA encoding the TP sequence of petunia EPSP synthase has also been fused to a gene from Escherichia coli that encodes a glyphosate-resistant form of EPSP synthase (12). Import assays show that the chimeric plant/bacterial EPSP synthase can be efficiently targeted to chloroplasts both in vitro and in vivo (12).

One of the interesting features of EPSP synthase is that the precursor enzyme is catalytically active and has a similar sensitivity to the herbicide glyphosate as the mature enzyme (11). Pre-EPSP synthase thus represents the first example of a catalytically competent chloroplast precursor enzyme. This observation suggests that prior to uptake into the chloroplast, the catalytic site of pre-EPSP synthase assumes a three-dimensional conformation similar to that of the mature enzyme. Furthermore, this suggests that the TP sequence constitutes an independent structural and functional domain within the folded precursor. To our knowledge, none of the mitochondrial precursors has been assayed for catalytic activity. In view of the observation for pre-EPSP synthase, it seems likely that other single-subunit precursors may also be functional enzymes. Since pre-EPSP synthase is catalytically active and subject to inhibition by glyphosate, it will be of interest to determine if inhibitors strong enough to prevent the transport of enzymes in vivo inhibit EPSP synthase when presented as TP-fusions, it seems clear that organelle targeting is dependent only on the TP and the sequences surrounding the TP/mature junction.

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MITOCHONDRIA

Despite the wealth of information on mitochondrial import of proteins there are no studies reported in the literature thus far where plant mitochondria have been tested for their ability to import proteins. Information on nuclear encoded plant mitochondrial genes is just beginning to accrue and in vitro systems should be available in the near future to determine if plant mitochondria import proteins by mechanisms similar to those established for animal and yeast mitochondria. Based on the published cDNA and protein sequence of nuclear-encoded plant mitochondrial ATPase β subunit (5), it appears that the presence of the plant protein is much longer (about 57 amino acids) than yeast and animal mitochondrial presequences (20-40 amino acids). The longer sequence may be required to ensure absolute discrimination between chloroplasts and mitochondria in plant systems. This could be easily verified by studying the import of yeast, animal, and plant mitochondrial precursors by plant mitochondria and chloroplasts in vitro. Despite differences in the size of the TP, other characteristic features such as absence of acidic residues, preponderance of basic and hydrophilic residues, presence of basic residues within the amino terminal end of presequence etc., are all conserved between the plant, yeast, and animal systems.

VACUOLES

The vacuole, the largest organelle in most plant cells, serves primarily as the lysosomal compartment and contains sugar hydrolases, endo- and exo-peptidases, chitinase, and protease inhibitors. Many of these proteins are synthesized in the rough endoplasmic reticulum and pass through the Golgi bodies. A few of these proteins (e.g. proteases and chitinase) are further exported from the vacuole to the plasmalemma. In some tissues, vacuoles differentiate to form protein bodies which store proteins. During this process, hydrolytic enzymes are either inactivated or excluded from the organelle (4).

The most thoroughly studied vacuolar proteins are the legume seed storage proteins that accumulate in the ‘protein bodies’ of developing embryos. Immunocytochemical and biochemical evidence has shown that both 7S and 11S proteins, the major legume seed storage proteins, are produced on membrane-associated polysomes. However, the 7S protein is glycosylated while the 11S protein is not. Very little is known about the primary amino acid sequences or posttranslational modifications that direct these processes. Two approaches are being taken to dissect the pathway(s) of vacuole-bound proteins. One is to express the genes in heterologous systems (nonplant) such as Xenopus oocytes and yeast, and the second is to express genes in transgenic plants. The signal peptides of both 11S and 7S proteins are correctly cleaved in the Xenopus system. However, a significant fraction of the proteins produced in oocytes is secreted to the external medium (1) indicating misdirection of the proteins to the cell surface instead of the central vacuole. The accuracy of posttranslational glycosylation in this system has not been investigated.

Expression of cloned cDNAs of storage proteins in yeast has shown that the 7S seed proteins are glycosylated presumably at the expected amino acid residues (9), although not with the efficiency of the host plant. Mutations in the signal peptide of phaseolin 7S protein affect the posttranslational glycosylation of the mature protein (10) indicating that the amino acid sequence in the signal peptide region influences the posttranslational modification of the protein. Recently, several research groups have reported the expression of genes encoding phaseolin and soybean 7S proteins in petunia and tobacco plants (2, 27). Glycosylation of the proteins appeared to be normal and the signal sequence of the soybean 7S protein was cleaved properly. Immuno-gold localization studies showed that the phaseolin protein had accumulated in the protein bodies of transgenic tobacco seeds (17). Furthermore, the subunits were correctly assembled to multimeric 7S proteins in transgenic petunia seeds (2). It appears, therefore, that the signal sequences and glycosylation sites of seed storage proteins are conserved among different plant species.

To compare the posttranslational modification and stability of soybean 7S seed storage protein subunits in vegetative cells versus seeds, a gene encoding the α subunit was placed under the control of a constitutive plant promoter and cloned into petunia plants (3). No quantitative differences in the amounts of mRNA for this protein could be detected in these tissues. However, the quantity of the protein in the seeds was 20 to 50 times greater than that in vegetative cells, and it had a lower mol wt indicating differential processing of the same protein by the two cell types. At least some of these differences in vegetative cells appeared to be related to the presence of the protein in the ER and Golgi instead of the vacuoles. These results imply that the seed storage proteins are unstable in the leaf vacuoles, but stable in seed vacuoles that differentiate into protein bodies. However, absolute confirmation would require a similar study in a homologous transgenic system.

Protease inhibitors induced by wounding of tomato plants have also been localized in the vacuoles of leaf tissues. Analysis of the cDNA clone of proteinase inhibitor I has revealed that 42 of the 111 amino acids in the preprotein are lost as a result of posttranslational processing: this represents 38% loss of the original molecule (16). It would be interesting to determine if this signal sequence could be used by the seed storage proteins for targeting into ‘protein bodies.’

FUTURE PROSPECTS

It is clear that protein processing and sorting in plants is fundamentally similar to that in mammalian and other eucaryotic cells. However, several questions remain unanswered with respect to organelle import of proteins. For example, how are multisubunit proteins imported? Does the assembly occur in the cytosol or in the organelle, and do oligomeric proteins undergo association in their precursor forms? Furthermore, at what site(s) are the cofactors and prosthetic groups added to enzymes, and are there differences in the rates of import of preholoenzyme versus preapoenzyme?

At the organelle level, it would be interesting to identify receptors and the protease(s) involved in import and maturation of the preproteins. In view of considerable divergences in the primary structure of the presequences as well as the cleavage site, it will not be surprising if multiple receptors and proteases are involved in these processes. And finally, it would be interesting to isolate mutants deficient in the various steps of protein import/processing and determine their effects on cellular metabolism. Such mutants have now been described in yeast and a systematic investigation of these mutants is expected to yield a wealth of information on protein processing in eucaryotic cells.

In addition, very little information is available on the signal sequences used by plants for targeting proteins into the cell wall and extracellular space. Recent analysis of cDNA sequences for pathogenesis related proteins (20) and extensins (6) indicate that extracellular space proteins contain N-terminal signal peptide sequences which are presumably important for targeting. Analysis of the genes for these proteins can therefore be expected to provide information on the nature of topogenic sequences used for extracellular targeting. It is clear that a complete understanding of protein processing in plants requires analysis of additional systems. These studies are important from a plant genetic-engineering standpoint, since it is not only important to express genes in specific tissues but also within specific organelles. Expression of foreign genes in appropriate cellular compartments may be essential for maintaining a stable, functional protein in the plant.
environment. Similarly, manipulation of metabolic pathways in plants requires that engineered proteins be efficiently delivered to the correct location in the cell.

LITERATURE CITED


