

Review

Protein Trafficking in Plant Cells

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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 TP¹ sequences are proteolytically removed after import into the organelle. Proteins imported by chloroplasts are further sorted into suborganelle compartments, and recent evidence indicates that the final targeting information is also present in the TP sequence. Proteins destined for the

secretion pathway in plants are inserted co-translationally into the ER and then further processed and sorted by mechanisms that are fundamentally similar in all eucaryotic cells.

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 preproteins with TPs ranging upwards of 33-amino acids 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 reconstituted 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, Wassmann *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.

Protein Trafficking In Plant Cells

Arturo Cuomo



Protein Trafficking In Plant Cells:

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Dashek,2010-03-09 While there are a few plant cell biology books that are currently available these are expensive methods oriented monographs The present volume is a textbook for upper undergraduate and beginning graduate students This textbook stresses concepts and is inquiry oriented To this end there is extensive use of original research literature As we live in an era of literature explosion one must be selective These judgements will naturally vary with each investigator Input was sought from colleagues in deciding the literature to include In addition to provision of select research literature this volume presents citations and summaries of certain laboratory methods In this connection the textbook stresses quantitative data to enhance the student s analytical abilities Thus the volume contains computer spread sheets and references to statistical packages e g Harvard Graphics and Statistica **Plant Cell Biology** ,2020-08-31 Plant Cell Biology volume 160 in Methods in Cell Biology includes chapters on modern experimental procedures and applications developed for research in the broad area of plant cell biology Topics covered in this volume include techniques for imaging and analyzing membrane dynamics and movement across membranes cell wall composition structure and mechanics cytoskeleton dynamics and organization cell development ion channel physiology cell mechanics and methods related to quantifying cell morphogenesis Provide in depth procedures and application notes from selected experts who developed the methods Each chapter will include figures and movies as appropriate to explain complex techniques Chapters will include caveats of techniques and future prospects

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