Minireview

Protein Translocation at the Envelope and Thylakoid Membranes of Chloroplasts*

Andrei Kouranov and Danny J. Schnell‡

From the Department of Biological Sciences, Rutgers, The State University of New Jersey, Newark, New Jersey 07102

Endosymbiotic evolution has resulted in the transfer of genes encoding the vast majority of the protein components of plastids to the nuclear genome. In response to this displacement of genetic material, plastids have evolved a system to post-translationally import nuclear encoded preproteins from their site of synthesis on cytoplasmic ribosomes. The protein import process can be viewed as a cascade of protein targeting events that are governed by a hierarchy of topogenic sequences. The targeting sequences are sequentially decoded resulting in the localization of the polypeptide to the appropriate organellar subcompartment (for review see Refs. 1 and 2).

Recent studies have begun to uncover the components that underlie the mechanism of targeting and translocation at the envelope and thylakoid membranes of chloroplasts. These studies suggest a single, common mechanism for recognition and translocation of cytoplasmic preproteins across the double membrane of the envelope. With the exception of two members of the hsp70 family of molecular chaperones, the translocation components of the envelope that have been identified and sequenced are unique and, surprisingly, show no similarity in primary structure to the known components of the mitochondrial import apparatus. In contrast, the thylakoid membrane appears to have evolved a variety of targeting pathways with certain pathways retaining elements that are closely related to bacterial and endoplasmic reticulum translocation systems.

The General Pathway of Envelope Translocation

Protein import across the chloroplast envelope consists of the specific recognition of preproteins at the outer envelope and subsequent translocation of the protein simultaneously across both the outer and inner membranes (for review see Refs. 3 and 4). Envelope translocation is facilitated by the coordinate interaction of protein-conducting machineries in the outer and inner membranes at contact zones where the two membranes are held in close apposition (5). The targeting signal for envelope translocation resides within an amino-terminal extension of the preprotein designated the transit sequence. Although there is no apparent similarity in primary sequence among the transit sequences of different precursor proteins, import competition studies and transit sequence swapping experiments support the proposal that all preproteins use the same mechanism for envelope translocation (3).

Two general stages in envelope translocation can be distinguished by their distinct energy requirements (Fig. 1). The first stage represents the high affinity association of the precursor protein with the outer envelope (6, 7) and requires the hydrolysis of low concentrations (<100 μM) of both ATP (8, 9) and GTP (10) in the cytoplasm or intermembrane space. At this stage, the precursor is irreversibly bound to the envelope and has been designated an early translocation or import intermediate (5, 11). The second stage represents the complete translocation of the precursor into the stroma and requires the hydrolysis of higher concentrations of ATP (>1 mM) within the stromal compartment (12, 13). Unlike protein import into mitochondria, a membrane potential is not involved in envelope translocation. Recent studies suggest that each energy-dependent stage of envelope translocation corresponds to sequential insertion of the preprotein across the outer and inner membranes, respectively (14, 15).

Upon entering the stromal compartment, the transit sequence is removed by a specific metalloendopeptidase called the general stromal processing peptidase (SPP) (16, 17). The SPP is a soluble stromal protein of 140 kDa that contains a zinc-binding domain that is conserved in several metalloendopeptidases including the β subunit of the processing peptidase involved in the maturation of proteins imported into mitochondria (18).

The simultaneous translocation of preproteins across the outer and inner envelope membranes is facilitated by contact zones (5). These zones correspond to specialized membrane domains where the outer and inner membranes come into close contact. A recent study suggests that energy-dependent insertion of a precursor across the outer membrane is accompanied by the engagement or formation of contact sites (14). However, it is apparent that the outer and inner import machineries can function independently if the two membranes are physically separated by subjecting chloroplasts to a hypertonic shock (15). Therefore, it is unlikely that the import machineries are permanently linked but that their interactions at contact zones are dynamic. The biochemical nature of these zones remains to be established, but it is clear that these structures hold clues to the coordinate interactions of the import machineries in the outer and inner membrane.

Components of the Envelope Translocation Machinery

Five proteins of the outer envelope import machinery have been identified (Fig. 1). Three of these components, IAP34 (OEP34), IAP75 (OEP75), and IAP86 (OEP86), form a complex in the outer membrane that stably associates with early translocation intermediates (19, 20). All three IAPs are integral membrane proteins (10, 21–23). IAP34 and IAP86 are related in primary sequence, and both contain cytoplasmically exposed GTP-binding domains (10, 21, 22). The latter characteristic presumably accounts for the GTP requirement in import. Protease sensitivity and membrane extraction experiments have shown that IAP75 is deeply embedded in the outer membrane (19, 23); however, its primary structure is remarkably hydro-

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‡ To whom correspondence should be addressed: Dept. of Biological Sciences, Rutgers, The State University of New Jersey, 101 Warren St., Newark, NJ 07102. Tel.: 201-648-1082; Fax: 201-648-1007. E-mail: schnell@andromeda.rutgers.edu.

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hsp70s that are essential for protein import into mitochondria. Features distinguish it from the soluble cytoplasmic and matrix proteins. The outer membrane with the bulk of the protein apparently located in the intermembrane space of the envelope (19). These observations suggest that an initial, reversible interaction of transit sequences with the lipid component of the bilayer may induce regular structure, thereby facilitating the binding of the transit sequence to receptor components of the import machinery.

Recent studies have suggested that the unique lipid composition of the envelope may play an important role in the specific targeting of precursor proteins to the outer membrane. Model membrane studies have shown that chloroplast preproteins and transit peptides interact specifically with outer membrane lipids and that this binding is specific for the transit sequence (for a summary see Ref. 29). Of particular interest is the observation that the lipid-protein interactions induce regular secondary structure into the normally disordered structure of transit sequences observed in aqueous solutions (30). These observations suggest that an initial, reversible interaction of transit sequences with the lipid component of the bilayer may induce regular structure, thereby facilitating the binding of the transit sequence to receptor components of the import machinery.

Late stage import intermediates have been identified that span the inner envelope membranes (5, 15, 26), and several candidates for components of the inner membrane import machinery have been identified (Fig. 1). Two envelope components, IAP100 (Cim97) (5, 26) and IAP36 (5), copurify with late stage import intermediates. IAP36 has not been characterized, but IAP100 is an integral inner membrane protein with a single apparent transmembrane domain (31, 32). The IAP100 associates with the plastid hsp60 homolog in an ATP-dependent manner and has been proposed to function in recruiting the hsp60 chaperone to the site of import, thereby facilitating folding of newly imported proteins (31). Two additional proteins, IAP21 and IAP25, recently have been shown to cross-link directly to the transit sequence of an early import intermediate (14). The localization of IAP25 has not been confirmed, but IAP21 appears to be an inner membrane protein. The function of neither protein has been investigated in detail, but their interactions with the transit sequence make them candidates for components that mediate the presentation of the precursor to the import machinery of the inner membrane.

A set of at least two immunologically related envelope proteins of 44 kDa, designated Com44/Cim44, has been detected in a covalently cross-linked aggregate from envelope membranes that contain a partially translocated import intermediate (26). Variants of the Com44/Cim44 have been localized to both the outer and inner envelope membrane (33), but their role in translocation remains to be investigated in detail.

The Multiple Pathways for Thylakoid Targeting

Nuclear encoded thylakoid proteins contain dual targeting signals (for review see Ref. 1) that direct a two-step targeting process. The primary signal is a cleavable stromal targeting domain (STD) that directs the proteins across the envelope into the stroma and is removed by the SPP. These sequences are structurally and functionally equivalent to the transit sequences of stromal proteins. SPP processing generates a stromal intermediate that is targeted to the thylakoid membrane or lumen by a secondary targeting signal. Lumenal proteins contain a bipartide transit sequence (34) composed of an STD followed in tandem by a cleavable thylakoid lumen targeting

Several groups have proposed that the two proteins function as molecular chaperones on either side of the outer membrane (19, 26). The presence of the chaperones would serve to maintain the import competence of the precursor as it enters the import machinery on the cis side of the membrane (Com70) and as it emerges into the intermembrane space on the trans side of the membrane (hsp70-IAP) prior to engaging the inner membrane import machinery. This proposal is attractive as it is consistent with models in which hsp70 chaperones provide the driving force for membrane translocation (27, 28) and also because the binding of the chaperones would account for the ATP requirement for the formation of early import intermediates.

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to the thylakoid lumen. Both proteins contain bipartide, cleav-

g-of the oxygen-evolving complex (OE33) and plastocyanin (PC)


distinct pathways for thylakoid targeting (Fig. 2). These path-


te result in the definition of at least three, and perhaps four,


assays using intact chloroplasts or isolated thylakoids have


ing that they are not obligatorily coupled processes.


doing is analogous to the protein translocon in the thylakoid membrane.


S. Barkan, personal communication.


targeting of the stromal intermediates of OE33 and PC to


targeting has now been confirmed. A chloroplast homolog of the


e. coli SecY protein (CPSecY) has been


targeting has been localized to the


targeting has been shown not to stimulate translocation of the OE17 or


OE33 stromal intermediates (38, 40, 46). In addition, CPSecA


suggests that these precursor proteins may use a CPSecA path-


way, their translocation into the lumen requires only a \( \Delta \)pH at


the thylakoid membrane and is not competed by the PC or


OE33 stromal intermediates (38, 40, 46). In addition, CPSecA


has been shown not to stimulate translocation of the OE17 or


OE23 intermediates into isolated thylakoids (43). The require-


ment for only a \( \Delta \)pH is unique among known protein translo-


cation systems, but the electrochemical potential is an essen-


tial component of bacterial protein export suggesting that the


\( \Delta \)pH pathway also may retain elements in common with this


system.


The third pathway for thylakoid targeting is represented by


the insertion of the integral, major light-harvesting complex


protein (LHCP) into the thylakoid membrane. LHCP integra-


tion is directed by one or more regions within the transmem-


brane domains of the protein, is GTP-dependent, and is stimu-


lated by the pH gradient at the thylakoid membrane (47).


Partial reconstitution of the LHCP integration using stromal


extracts and isolated thylakoids has identified one of the es-


sential components of the transit complex (48). Remarkably,


this component is a chloroplast homologue (54CP) of the 54-


dkDa subunit of the signal recognition particle (SRP) and the E.


coli SRP (49). The discovery of CP54 suggests that it may


mediate the delivery of the LHCP transit complex to the thyl-


akoid membrane through a GTP binding and hydrolysis cycle


similar to that used by SRP in targeting the nascent chain-


ribosome complex to the ER (24). Thus, LHCP integration


represents a second conservative targeting pathway that is


homologous to protein targeting pathways that function at


the endoplasmic reticulum and the bacterial inner membrane.


The existence of a fourth pathway for thylakoid targeting has


been suggested by the recent discovery that the integral mem-


brane protein, CF \(_{0}\) II, integrates into the membrane independ-


et of a \( \Delta \)pH, nucleoside triphosphates, or stromal factors (50).


The mechanism of CF \(_{0}\) II integration remains to be examined in
detail, but the observations have prompted the investigators to
propose that this protein inserts into the thylakoid membrane
independent of proteinaceous factors.


In Vivo Models for Thylakoid Protein Targeting


The assignment of distinct thylakoid targeting mechanisms


and studies of their biochemical nature are supported by the


recent development of \( \textit{in vivo} \) genetic models in higher plants


and green algae. Two maize mutants have been identified that


specifically affect distinct targeting pathways (51). The \( \textit{tha}1 \)


mutant is specifically affected in the OE33 and PC targeting


pathway and not in the other pathways. This mutant also


affects the targeting of a plastid-encoded protein, cytochrome \( f \),


indicating that plastid and nuclear encoded proteins can share
a targeting mechanism. Cytochrome \( f \) is synthesized on plastid
ribosomes as a precursor with a typical amino-terminal LTD.


The \( \textit{tha}1 \) gene has recently been cloned and has been shown to


be the maize CPSecA (1).\(^2\) A second mutant, \( \textit{hcf106} \), is defective


only in OE17 and OE23 targeting. Neither mutant affects the
integration of LHCP.


The analysis of a class of mutations in the LTD of cytochrome \( f \)
of \( \textit{Chlamydomonas} \) suggests that although certain elements
of the targeting pathways of each class of thylakoid proteins
may be unique, they may also share common components (\( e.g. \),
the components of the membrane translocation machinery) (52).
One of these mutants showed a dominant-negative effect on
the the integration of LHCP and a second plastid-encoded
protein, D1. These results suggest that cytochrome \( f \), D1, and
LHCP may share common elements at one point in their trans-
location process. Suppressors of the cytochrome \( f \) mutants have


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been identified, and their analysis should provide important in vivo evidence that complements the biochemical studies of thylakoid targeting.

Perspective

Research over the last 2 years has provided a bountiful harvest of information on the pathways and components of protein targeting at the chloroplast envelope and the thylakoid membrane. The components that have been identified provide the needed markers with which to identify additional components. Within the not-so-distant future, it should be possible to reconstitute part or all of these targeting reactions and, thereby, address more fundamental questions such as the exact roles of nucleoside triphosphates or the ΔpH in regulating and/or driving membrane transport. The identification of mutants affected in thylakoid targeting provides encouraging signs that in vivo models to address such fundamental questions can now be applied to protein targeting in chloroplasts. Molecular genetic systems aimed at manipulating components of the import apparatus are now being developed to provide in vivo models for envelope translocation. The knowledge obtained from the combination of these approaches will be essential for understanding plastid biogenesis but should also have important implications for understanding the general principles that govern protein translocation in other systems. For example, the relationship among the CPSecA and 54CP pathways and their bacterial and ER counterparts should provide knowledge of the essential, conserved elements of membrane translocation in these systems. In contrast, the apparent lack of similarity between chloroplast and mitochondrial import systems should provide insight into the distinct evolution of translocation systems as a consequence of dual endosymbiotic events.

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