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Review

Targeting and biogenesis of transporters and channels in chloroplast envelope membranes: Unsolved questions

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ABSTRACT

Chloroplasts produce carbohydrates, hormones, vitamins, amino acids, pigments, nucleotides, ATP, and secondary metabolites. Channels and transporters are required for the movement of molecules across the two chloroplast envelope membranes. These transporters and channel proteins are grouped into two different types, including β-barrel proteins and transmembrane-domain (TMD) containing proteins. Most β-barrel proteins are localized at the outer chloroplast membrane, and TMD-containing proteins are localized at the inner chloroplast membrane. Many of these transporters and channels are encoded by nuclear genes; therefore, they have to be imported into chloroplasts after translation on cytosolic ribosomes. These proteins should have specific targeting signals for their final destination in the chloroplast membrane and for assembly into specific complexes. In this review, we summarize recent progress in the identification, functional characterization, and biogenesis of transporters and channels at the chloroplast envelope membranes, and discuss outstanding questions regarding transporter and channel protein biogenesis.

1. Introduction

Chloroplasts have a special function in plant cells as the site of photosynthesis and carbohydrate biosynthesis. Many compounds are synthesized from photosynthetic products in chloroplasts [1]. These include plant hormones, amino acids, pigments, nucleotides, fatty acids, and other secondary metabolites [2]. Chloroplasts also store ions such as Fe^{2+} and K^+ [3,4], and metal ions such as Cu^{2+}, Mn^{2+}, Mg^{2+}, and Co^{2+} are imported as cofactors of chloroplast proteins involved in photosynthetic and metabolic reactions [1]. Nuclear-encoded proteins are imported from the cytosol into chloroplasts; more than 95% of all chloroplast proteins (>3000 proteins) are estimated to be nuclear-encoded [5–7]. Therefore, chloroplasts should have close communication with the cytosol and surrounding compartments [8–10] to regulate the heavy traffic between the cytosol and chloroplasts through the two chloroplast membranes.

In this review, we summarize recent advances in the identification, functional characterization, and biogenesis of transporters and channels in the chloroplast inner and outer envelope membranes. We also discuss unresolved questions regarding the biogenesis of these proteins.

2. Transporters and channels at the chloroplast envelope membranes

2.1. β-Barrel proteins at the outer chloroplast membrane

The chloroplast outer membrane is not considered to act as a permeability barrier, and solute movement across the outer membrane is limited solely by size. Thus, the outer membrane functions as a selective molecular sieve [1,8]. Electrophysiological studies identified four voltage-gated channels at the outer envelope membrane [11], including outer membrane protein 16 (OEP16), OEP21, OEP24, and OEP37 [1,12–15] (Table 1). OEP16 is a cation-selective conductance channel for the transport of amino acids [16] and also plays a role in the import of NADPH:protochlorophyllide oxidoreductase A (pPORA) precursor [14]. OEP21 is an anion-selective channel for specific inorganic phosphates, triose phosphates, and 3-phosphoglycerates [15]. OEP21, OEP24, and OEP37 are ATP-regulated selective solute channels [1,14,15]. OEP16 contains four helical transmembrane domains (TMDs), whereas OEP21, OEP24, and OEP37 are β-barrel proteins [17] (Table 1). Toc75 is another β-barrel protein that is a core component of the translocon complex

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Table 1
Outer membrane proteins of chloroplasts.

Type	TP[a]	Protein name	Function
Multi-pass[b]	–	Outer envelope pore protein16-1	Amino acid-selective channel protein
Multi-pass	–	Outer envelope pore protein16-2	Amino acid-selective channel protein
Multi-pass	–	Outer envelope pore protein16-3	Amino acid-selective channel protein
Multi-pass	–	Outer envelope pore protein16-4	Amino acid-selective channel protein
Multi-pass	–	Outer envelope protein64	Protein transport
β-Barrel	–	Trigalactosyldiacylglycerol3 (TGD4)	Lipid transport
β-Barrel	+	TOC75-3	Protein transport
β-Barrel	–	TOC75-4 (OEP80)	Protein transport
β-Barrel	–	Outer envelope pore protein21A	Voltage-gated anion channel
β-Barrel	–	Outer envelope pore protein21B	Voltage-gated anion channel
β-Barrel	–	Outer envelope pore protein24A	Voltage-gated anion channel
β-Barrel	–	Outer envelope pore protein24B	Voltage-gated anion channel
β-Barrel	+	Outer envelope pore protein37[c]	Cation transport

[a] TP, transit peptide.
[b] Multi-pass, multi α-helical TMD protein.
[c] OEP37 localization is still controversial. *Arabidopsis thaliana* chloroplast outer membrane proteins were detected using Uniprot (http://www.uniprot.org/). Only reviewed proteins were used.

Table 2
Distribution of chloroplast multi-pass membrane proteins.

Membrane type	Total (multi-pass)[a]	With TP[b]	Without TP	β-Barrel proteins
Outer membrane	13	1 (7.7%)	12 (92.3%)	12 (92.3%)
Inner membrane	36	33 (91.6%)	4 (8.4%)	1 (2.7%)
Thylakoid membrane	53	49 (92.4%)	4 (7.6%)	0 (0%)

[a] *Arabidopsis thaliana* contains 173 chloroplast multi-pass membrane proteins in Uniprot (http://www.uniprot.org/). Of these, the localization of 79 proteins is unknown.
[b] TP, transit peptide.

and plays a crucial role in the import of proteins containing transit peptides into chloroplasts [18,19]. OEP80 is a paralog of Toc75 (translocon at the outer membrane of chloroplasts 75), but its function is not clearly understood. OEP80 loss-of-function mutation produces an embryonic lethal phenotype, supporting its essential role [20,21]. β-Barrel proteins are the major constituents in the chloroplast outer membrane (Table 2).

2.2. Transporters and channels at the inner chloroplast membranes

Photosynthetic reactions assimilate and fix carbon dioxide, and the chloroplast provides the cell with reduced carbon compounds. Sugar transporters localize to the inner envelope membrane to export photoassimilates from chloroplasts to the cytosol [1,22]. These include the triose phosphate/phosphate translocator (TPT), glucose transporter (pGlcT), and maltose transporter (Table 3). Plastids in sink tissues import various metabolites from the cytosol using transporters such as glucose 6-phosphate translocator, phosphoenolpyruvate (PEP)/phosphate translocator, and xylulose 5-phosphate/phosphate translocator [23] (Table 3). These metabolites are imported into the stroma in exchange with inorganic phosphate (P_i). Secondary metabolites are exported from chloroplasts to the cytosol by transporters such as the bile acid:Na symporter (BASS) [24,25] and the folate-biopterin transporter [26]. Phosphate is imported into chloroplasts by the plastid phosphate translocator (pPHT) family that contains four members of the drug/metabolite transporter (DMT) superfamily [27]. These antiporters catalyze the exchange of phosphorylated C3-, C5-, and C6-compounds for P_i. The chloroplast envelope membrane contains two dicarboxylate transporters (DiTs) for the exchange of ammonia assimilates between the chloroplast and cytosol; DiT1 imports the precursor of ammonia assimilation, 2-oxoglutarate, in exchange with malate, whereas DiT2 exports the end product of ammonia assimilation, glutamate, to the cytosol and imports malate [28]. Lipids also are imported into chloroplasts through the inner membrane by specific transporters. Trigalactosyl diacylglycerol 1 (TGD1), TGD2, and TGD3 localized to the inner membrane form an ATP binding cassette (ABC) transporter that imports lipids into chloroplasts [29] (Table 3).

Another group of transporters are those involved in ion translocation through the inner membrane. Chloroplasts need ions including Ca^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, and Fe^{2+}. Many of these ions are used as protein cofactors. Permease in chloroplast 1/translocon of inner chloroplast envelope 21 (PIC1/TIC21) localizes in the inner envelope and functions in iron transport into chloroplasts [3,4]. The antibiotic transporter multiple antibiotic resistance 1 (MAR1) is also postulated to transport iron into chloroplasts [30]. Iron is transported in chelated forms. A significant amount of K^+ is stored in chloroplasts. K^+-efflux antiporter 1 (KEA1) and KEA2 at the inner envelope membrane transport K^+ into chloroplasts, which are crucial for regulating K^+ homeostasis and chloroplast development [31] (Table 3).

The third group of transporters mediates the import of nuclear-encoded proteins into chloroplasts. Translocon at the inner envelope membrane of chloroplasts 110 (Tic110) is a prominent protein translocation channel at the inner membrane [32]. Tic20 is proposed to form a protein import channel that may function together with or independently of Tic110 [33] (Table 3). Tic110 contains two hydrophobic transmembrane helices at its N-terminus, which anchor the protein in the membrane [34,35], and four amphipathic α-helices in the large C-terminal domain, which are responsible for channel formation [18]. Tic20 is predicted to have four α-helical TMDs [33].

3. Targeting signals of transporters and channels at the chloroplast envelope membranes

3.1. Targeting signals of β-barrel proteins

Many channels and transporters localized to the chloroplast outer and inner membranes are encoded by the nuclear genome and translated on cytosolic ribosomes [5]. Thus, they should be targeted to their final destination after translation in the cytosol.