Protein translocation in chloroplasts

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Contents

Abstract

Photosynthesis in chloroplasts is essential for life. It involves a number of nuclear- and plastome-encoded membrane proteins. Nuclear-encoded choloroplast proteins with an N-terminal stroma transit peptide are translocated from the cytoplasm into the stroma by the TOC/TIC (translocon of outer membrane complex/translocon of inner membrane complex) machinery, where the stromal transit peptide is removed by the stromal processing peptidase. Translocation of proteins from the stroma into or across the thylakoid membraneis fulfilled by the Sec, the twin-arginine (TAT), SRP or a 'Spontaneous' machinery. In the review, we will present the recent progress of the protein import and sorting in chloroplasts.

Introduction

Photosynthesis is a process by which plants, algae and photosynthetic bacteria like cyanobacteria use the energy from sunlight to convert carbon dioxide (CO_2) and water to glucose and oxygen (Niel 1932).

In plant, photosynthesis is fulfilled in chloroplasts. They are believed to have evolved from cyanobacteria. This suggested by the endosymbiotic theory, because they have their own genome (RIS and PLAUT 1962), this genome has high similarity to bacterial DNA and chloroplast have typical bacterial porin like membrane proteins (Flügge and Benz 1984). Chloroplasts are similar in structure to cyanobacteria. They have three basic compartments: the envelope, the thylakoid and the stroma.

The chloroplast envelope consists of an outer layer, an intermembrane space and an inner layer. The outer layer consists of 48% phospholipids, 46% galactolipids and 6% sulfoplipids. The inner membrane consists of 16% phospholipids, 79% galactolipids and 5% sulfoplipids (Block et al. 1983). The distinct compositions result in a big difference in the membrane strength and permeability. The outer membrane is permeable to a lot of ions and metabolites, while the inner membrane needs additional transport proteins for translocation. The stroma in chloroplasts is similar to the cytoplasm of cyanobacteria. The chloroplast DNA, replication and transcription machineries and other components that are located in the cytoplasm in cyanobacteria, are localized in the stroma of chloroplasts. The thylakoids are enclosed compartments inside the stroma, consisting of the thylakoid lumen and the thylakoid stroma. In higher plants the thylakoids are ordered in grana, which are big structures consisting of stacks of granal thylakoids and are connected by stromal thylakoids. All the thylakoids membranes are connected with each other to the inner membrane. It is believed that during chloroplast diversion the thylakoids are formed from the inner envelop membrane by invagination (Boardman and Anderson 1964). The thylakoid lumen is a fluid enclosed by the thylakoid membrane. The thylakoid lumen is a fluid enclosed by the thylakoid membrane. The primary function of the lumen is photophosphorylation. During photosynthesis a proton motif force is created over the thylakoid membrane which is used to assimilate organic carbon.

Proteomic analyses show that the thylakoid proteome is composed of 384 different proteins: 89 are luminal proteins; 116 are integral membrane proteins; 62 are peripheral membrane proteins at the stroma side and 68 are peripherally bound to the membrane at the luminal side (Peltier et al. 2000b). In this review, it will be discussed how proteins are imported into and sorted in the stroma and the thylakoid.

Translocation of nuclear-encoded proteins into the stroma

Most of chloroplast proteins are synthesized in the nucleus. Two-dimensional gel electrophoresis analysis of the chloroplast proteome reveals over 250 different spots. This likely underestimates the total proteomic diversity (Peltier et al. 2000). It is estimated that about 100 of these proteins are encoded by the chloroplast genome. This means that the majority of proteins in the chloroplast are nuclear-encoded proteins that need to be translocated across the dual envelope membranes of the chloroplast to their destination (Sugiura 1989).

The inner and outer membranes of the chloroplast are suggested to connect at specific points, referred to as contact points (Cline, Keegstra, Staehelin 1985). The outer membrane of the chloroplast contains porin-like proteins. They allow smaller proteins including some proteases and molecules with a size up to 7-13 kDa to move freely into the intermembrane space, without the requirement for the specific chloroplast translocation machinery. These proteins are transported to their destinations by passive transport depending on the information stored in the proteins (Cline and Henry 1996). The large nuclear encoded proteins that are unable to reach their destinations through the passive transport pathways are synthesized with an N-terminal stroma targeting domain (STD), which varies between 20-150 amino acids in length and is responsible for targeting and translocation of preproteins into stroma. Interestingly, there seems to be very little similarity in the sequences of STDs. They only share some general features like the hydrophilic characteristic, no acidic residues and a high content of hydroxylated residues (HEIJNE, STEPPUHN, HERRMANN 1989). STDs seem to lack secondary structure; this enables an easy accessibility for chaperones (Ivey and Bruce 2000). After arrival in the stroma, STD is removed by stromal processing peptidases (SPP) (Richter and Lamppa 1998). Proteins that are destined for the thylakoid system have an additional N-terminal signal sequences downstream of the STD.

Two protein translocons, TOC (translocon at the outer membrane of chloroplasts) and TIC (translocon at the inner membrane of chloroplasts), work together to achieve translocation of nuclear-encoded preproteins into the stroma (Keegstra and Froehlich 1999). TOC transports preproteins through the outer membrane into the inner membrane space (Andrès, Agne, Kessler 2010). Translocation of preproteins is initiated by the receptor at the membrane interface (Heijne et al. 1989), and translocation through the TOC complex is accomplished in a GTP and ATP dependent matter. Two different pathways for migration of proteins through the TOC complex have been uncovered. The main difference between the two pathways is whether preproteins are phosphorylated before binding with TOC or not. Further transport of preproteins through the TIC complex into the stroma requires stromal ATP. The TOC/TIC translocation pathway is often referred to as the general import pathway (Kovács-Bogdán, Soll, Bölter 2010, Heijne et al. 1989). Preproteins translocation into the stroma by the general import pathway happens in three steps (Heijne, Steppuhn, Herrmann 1989).

1) The first step is the binding of preproteins to the outer envelope. This process is reversible and energy independent.
2) The second step is the insertion of preproteins across the outer membrane. This is usually referred to as the early intermediate stage. It is irreversible and requires ATP while it is also inhibited by non-hydrolysable GTP analogues (Inoue and Akita 2008). This stage can be subdivided into three steps based on the requirements for temperature and energy: the association stage, the insertion stage and penetration stage. All three stages appear to be ATP dependent (Perry and Keegstra 1994b).
3) The final step is the translocation of preproteins across the membrane and the removal of the transit peptide, usually referred to as the late intermediate stage. This stage requires ATP concentration (≥1mM) in the stroma (Inoue and Akita 2008) .

Chaperones

To prevent aggregation due to a lack of secondary structure, the newly synthesized preproteins in the cytoplasm interact with molecular chaperones like Hsp70 and Hsp90 to be kept in a soluble conformation.

Hsp70

Hsp70 belongs to the well conserved heat shock protein family. The main function of the Hsp70 protein family is to facilitate the correct folding of proteins. Members of the Hsp70 family have three major functional domains. An N-terminal ATPase domain that exchanges the chemical energy stored in ATP into mechanical energy thereby leading to conformational changes in the other two domains. The second domain is a substrate binding domain. This domain contains a groove that binds to hydrophobic amino acids. The third domain is a C-terminal domain that is rich in alpha helices and regulates the activity of Hsp70. When bound by ADP, the C-terminal alpha helices forms a lid, closing the substrate binding domain. When bound by ATP, the C-terminal lid opens to allow for substrate binding. When bound ATP is hydrolysed the proteins traps the substrate in a partially folded state (Pain and Blobel 1987).

In chloroplast, Hsp70 is present in the cytoplasm, the inner membrane space and the stroma. This makes it one of the most widely employed chaperones in chloroplasts. The Hsp70 pathway is the quicker of the two pathways. Some preproteins can be phosphorylated to form a guidance complex with Hsp70, giving them a high translocation speed. Disabling the ability of a preprotein to be phosphorylated reduces its translocation speed but does not compromise translocation. It is assumed that disabled phosphorylation enables proteins to be translocated by the Hsp90 dependent pathway. Hsp70 dependent pathway is mostly used by photosynthetic proteins that have a high turnover speed (Qbadou et al. 2006).

Hsp90

Another guidance complex is formed by preproteins associated with Hsp90. This pathway is slower but does not require ATP (Waegemann and Soil 1991).

Hsp90 show similarities in function, mechanism and structure with Hsp70. Hsp90 consists of four structural domains. The first is a highly conserved N-terminal region, which functions as an ATP targeting domain similar to the N-terminal domain in Hsp70. The second domain is a linker domain that is strongly charged and links the N-terminal domain to the middle domain. The middle domain binds substrates in a mechanism similar to that of Hsp70. Upon binding to the substrate, the protein

Table 1. Protein families of the TOC complex and their function.

Protein family	Function	References
Toc159	Import receptor or motor protein	(Pearl and Prodromou 2006)
Toc75	Main channel forming protein	(Wang et al. 2008)
Toc64	Import receptor and forms a contact site with Tic22, Toc12 and Hsp70	(Eckart et al. 2002)
Toc34	Import receptor and GTPase	(Qbadou et al. 2006)
Toc12	Forms a contact site with Tic22, Toc64 and Hsp70	(Jelic et al. 2002)

binding domain is open and Hsp90 hydrolyses ATP to trap substrates. Hsp90 has an extra ATP site at the C-terminus, of which the function remains unclear (Waegemann and Soil 1991).

The TOC complex

The TOC complex consists of three core subunits, Toc34, Toc159 and Toc75 and accessory proteins. Each of them represents a protein family. In *A. thaliana,* Toc34 has two homologs, atToc33 and atToc34. Toc75 has four homologs: Toc75-I Toc75-V, Toc75-III and Toc75-IV (initially discovered as Oep80). The Toc159 family comprises of atToc159, atToc132, atToc120 and atToc90. It has been observed that the Toc paralogues assemble into distinct TOC complexes (Becker et al. 2004b), which is assumed to identify a wide variety of precursor proteins.

The receptors, Toc34 and Toc159

Toc34 and Toc159 are GTPases. They are both localised in the chloroplast outer membrane and work as the main receptors for the complex. They are responsible for the initial binding of the target proteins that is GTP dependent (Ivanova et al. 2004). Non-hydrolysable GTP analogues have been shown to inhibit the forming of the early intermediate stage. Toc34 and Toc159 function together with Toc75, a member of the Omp85 family (Seedorf, Waegemann, Soll 1995). In vitro experiments show that the presence of members of the three core Toc families is sufficient for preprotein translocation into lipid vesicles (Gentle et al. 2005). The Toc complex has been estimated to be around 500-1000kDa in *Pisum sativum* and 500 kDa in *A. thaliana.* The wide range of the weight indicates that the complex might differ, making it difficult to determine a clear stoichiometry.

Toc34 and Toc159 share a common structural organization. They have a C-terminal domain which anchors the protein in the membrane, a highly acidic A-domain at the N-terminal which hangs in the cytosol and a GTP binding domain called the G-domain (Schleiff, Jelic, Soll 2003b). The G-domains of Toc34 and Toc159 can react together. Analysis in Toc34 by point mutations (R130A and F67A) have shown that disabled receptor dimerization does not affect the binding of preproteins, which is important for initiating the translocation across the outer membrane. Normally, GTPases can be activated or deactivated by binding to GTP or GDP, respectively. This is commonly facilitated by GTPase Activating Proteins (GAP) or Guanine Exchange Factors (GEF) (Richardson et al. 2009). No such factors have been found for the TOC GTPase, but the presence of preproteins stimulates the action of GTP bound to Toc34, suggesting that the preproteins function as a GAP to activate Toc34 (Gasper et al. 2009).

Toc34

The first discovered member of the Toc34 family is psToc33 (*Pisum sativum* Toc*,* 33kDa*).* The *ppi1* mutant *(*plastid protein import 1*, A. thaliana ΔToc33)* has a pale green colour indicating an abnormal chloroplast with smaller and less developed thylakoids than that in the wild-type (Jelic et al.

2002).The *ppi3* mutant (plastid protein import 3, *A. thaliana ΔToc34)* showed a decreased root length. The double *ppi1-ppi3* mutant was embryo lethal. This means that the Toc34 family is essential for development, with an overlapping function among homologues (Jarvis et al. 1998).

Toc159

The first member of the Toc159 family was discovered as an 86kDa protein from *P. sativum* called psToc86. It was later discovered that this protein missed it's the N-terminal A-domain and is actually 159kDa (Constan et al. 2004). atToc120, atToc132 and atToc159 lack typical the hydrophobic transmembrane helices, yet they are insensitive to protease treatment, suggesting that they are well protected in the membrane. Deletion studies have shown that atToc159 is important for normal development. atToc120 and atToc132 double mutant is either albino or seedling lethal, while single mutants have no clear physiological effect. These results suggest that atToc120 and atToc132 have an important overlapping function independent of atToc159. It has also been observed that atToc34 is present in complexes with atToc120 and atToc132, and that atToc34 can communicate with atToc159. This strengthens the hypothesis that the variety of Toc complexes is responsible for recognizing different preproteins (Jarvis et al. 1998).

The channel component, Toc75

Toc75 is the most abundant protein in the chloroplast outer membrane, forming a pore-like channel that has been estimated to be 14 Å at the narrowest part (Cline 2000). It plays a central role in chloroplast protein import. It has been shown to be in the close vicinity of the preprotein during import (Hinnah et al. 2002). Toc75 is a typical Omp85 family member of bacterial outer membrane proteins and consists of two domains. The N-terminal domain has three POTRA (polypeptide transported associated) domains. The C-terminal domain is deeply embedded in the membrane with a 16-18 beta sheets folding into a beta barrel. Toc75 is the only protein in the TOC complex that has been found to have a cleavable N-terminus. Toc75 is firstly translocated to the stroma by the TOC/TIC translocon and is then processed by SPP before re-enter into the chloroplast inner membrane.

A. thaliana has three members of the Toc75 family: Toc75-III, Toc75-IV and Toc75-V and one pseudogene called Toc75-I. Toc75-III is the closest homolog of *P. sativum* Toc75 and is most likely the channel forming protein of the *A. thaliana* TOC complex. Deletion studies have shown that *A. Thaliana* Δtoc75-III strains are seedling lethal (Perry and Keegstra 1994). *A. thaliana* Δ*toc75-IV* mutant only exhibited a visible phenotype under dark grown conditions, which suggests that toc75-IV functions in etioplasts but is not essential for normal growth (Hust and Gutensohn 2006). Toc75-IV has a shortened N-terminal domain, and it misses the POTRA domain and five of the predicted beta sheets. Toc75-V (80kDa) is firstly identified as Oep80. It functions independent of the TOC complex and is necessary for the correct insertion of beta barrels into the membrane (Baldwin et al. 2005). OEP80 and other members of the Toc75 family have different functions in both cyanobacteria and chloroplasts, implying that they have diverged prior to the endosymbiotic event (Inoue and Potter 2004). The fourth Toc75 homolog is Toc75-I, which is encoded by a pseudogene interrupted by a gypsy transposon insertion (Inoue and Potter 2004).

It is shown that the Toc75 family is the only component of the TOC complex with cyanobacterial origin. It is believed that the rest of the TOC complex formed around Toc75 after the endosymbiotic event. This must have happened in an early stage of evolution, as all higher plants, mosses and green algae have a Toc complex (Baldwin et al. 2005).

Accessory subunits

Toc12

Toc12 is a small protein that is located at the inner leaflet of the outer membrane. It contains a J-domain and interacts with Hsp70 and enhances ATP hydrolysis by DnaK. Two different roles have been proposed for Toc12. Toc12 is believed to trap Hsp70 at the TOC complex to ease the binding with incoming preproteins and prevent preproteins from slipping back into the cytosol. A disulphide bridge might be involved in the binding between Toc12 and Hsp70. Another function for Toc12 is the mediation of contact sites between the inner and outer membrane. It is believed that Toc12, Tic22, Toc64 and Hsp70 together form a bridge between the two translocon complexes (Kalanon and McFadden 2008).

Toc64

Toc64 has three different functional regions: an N-terminal region that contains a transmembrane region, a C-terminal region that contains three TPR (tetratricopeptide) motifs and a central region that has a strong homology with amidases. Toc64 has been shown to interact with Toc12 and loosely attach to the core of the TOC complex (Becker et al. 2004). *P. sativum* has one Toc64 version, *A. thaliana* has three paralogs of Toc64: Toc64-I, Toc64-III and Toc64-V. Toc64-III is targeted to the chloroplasts outer membrane. The other two paralogs are non- chloroplastic. Toc64-I has no function in chloroplast translocation and Toc64-V is a mitochondrial protein. Toc64-III has been shown to bind Hsp90 and is believed to help the Hsp90-preprotein complex to the TOC complex. While triple mutants of the Toc64 proteins in *A. thaliana* had no clear importing deficiency, suggesting an unessential role of Toc64-III in translocation (Sohrt and Soll 2000).

Mechanism of TOC translocation

There are two functional models for the translocation mechanism of the TOC complex: the targeting model and the motor model. The main difference between the two theories is the primary preprotein acceptor and the role of GTP hydrolysis.

The targeting model

The targeting model employs Toc159 as the primary receptor of preproteins (Hofmann and Theg 2005). The main clue for this is that Toc159 and Toc75 react with preproteins in the early intermediate stage. Toc159 enters the TOC complex together with the preproteins, and forms heterodimers with Toc34. Triple point mutations in the GTP-binding site led to accumulation of soluble Toc159. It is likely that Toc159 is dependent on its GTP binding site for reintegration into the outer envelope. This system shows similarity with the signal recognition particle (SRP) pathway (Hiltbrunner et al. 2001; Smith et al. 2004) by assuming that Toc159 and Toc34 fulfil the function of SRP and SRP-receptors, respectively (Aronsson and Jarvis 2008).

The motor model

The motor model considers Toc34 is permanently associated with the core of the TOC complex as the primary receptor for preproteins. In the motor model, preprotein recognition is GTP dependent. Upon hydrolysis of the GTP, the preprotein is transferred from Toc34 to Toc159 at the expense of one or more GTP (Schnell, Kessler, Blobel 1994, Schleiff, Jelic, Soll 2003), while preproteins using the Hsp90 targeting pathway will be dephosphorylated before transit from Toc34 to Toc159.

Table 2. The main protein families of the TIC translocon. Protein family

Protein family	Function	references
Tic110	Main channel forming protein	(Becker et al. 2004)
Tic62	NADP/NADP$^+$ sensing and regulation	(Balsera et al. 2009)
Tic55	Possible redox regulation but not necessary for normal development	(Stengel et al. 2008)
Tic40	The motor	(Boij et al. 2009b)
Tic32	NADP/NADP$^+$ and Ca^{2+}/CaM sensing and regulation	(Stahl et al. 1999)
Tic22	Form a bridge with Toc64, Toc12 Hsp70	(Chigri et al. 2006, Kouranov et al. 1998; Stahl et al. 1999)
Tic21	Linking TOC and TIC translocons	(Kouranov et al. 1998; Stahl et al. 1999)
Tic20	Independent channel protein	(Kouranov et al. 1998)
Hsp93	Motor ATPase	(Teng et al. 2006)

Mutagenesis studies reveal that A864R of *A. thaliana* Toc159 exhibits GTP binding but does not hydrolyze GTP while K868R mutation abolished Toc159 GTP binding and hydrolysis. Neither of the two mutations has a visible effect on the biogenesis of chloroplasts (Kovacheva et al. 2005). However, Toc159 A864R is more efficient in importing preproteins into isolated chloroplasts, while Toc159 K868R is less efficient compared to WT. This data indicate that Toc159 functions as a switch and is switched on in a GTP bound state, while to hydrolysis of GTP is not necessary for functioning. Despite that, non-hydrolysable GTP analogues will inhibit preprotein import. It suggests that other GTPases, than Toc159, such as Toc34, contribute to preprotein import (Agne et al. 2009).

The TIC complex

After the preproteins are translocated to the inner membrane space by the TOC complex, further translocation across the inner envelope membrane of chloroplasts is fulfilled by the TIC complex. Eight components of the TIC complex have been identified so far: Tic110, Tic62, Tic55, Tic40, Tic32, Tic22, Tic21 and Tic20. Tic22 is the only soluble part of the complex and functions in the inner membrane space. Tic110 and Tic20, Tic22 and Tic21 form the membrane translocation channel. Tic40 associates with Hsp93 to provide the driving force during translocation by ATP hydrolysis. Tic62, Tic55 and Tic32 regulate the complex via redox sensitive groups (Young et al. 1999)

Channel forming components

Tic110 is the main channel forming component of the TIC complex. Electrophysiological measurements have shown that 53% of the protein consists of alpha helices that assemble into a cation-sensitive channel with the diameter of the inner pore estimated to be 1.7nm (Kovács-Bogdán, et al. 2010, van den Wijngaard and Vredenberg 1999). Tic110 is translocated into chloroplasts by the general import route. After processed to the mature form in the stroma, Tic110 is reinserted into the inner membrane of chloroplasts. The N-terminus of Tic110 has two transmembrane helices (Heins et al. 2002), consisting of four amphipathic helices that can form into a cation selective channel (Kessler and Blobel 1996). The large domains of Tic110 facing the stroma

are responsible for the recruitment of Hsp93, Cpn60 and/or other chaperones to provide the driving force for translocation and correct folding of the preproteins (Balsera et al. 2009). The loops of other domains of Tic110 contact the TOC complex and the imported preproteins in the inner membrane space. Tic110 has no clear origin and is not present in cyanobacteria or any other prokaryotes (Kessler and Blobel 1996). *A. thaliana* has one paralogue of Tic110, and a *Tic110*-null mutant is seedling lethal (Reumann and Keegstra 1999). It has been found that Tic110 is expressed in root, stem, leaf and flower tissue of *A. thaliana*, suggesting that it not only has a role in chloroplasts but also in other plastids.

Tic20 is present in all plant tissue and is most abundant during fast tissue growth. It likely supports Tic110 in its function and might form additional membrane pores. Tic20 is inserted in the inner membrane by four predicted alpha helices (Inaba et al. 2003). *A. thaliana* has four paralogues of Tic20, while their concentrations are much lower than that of Tic110. Reduction of the amount of Tic20 causes a pale green phenotype. Some similarities have been found between Tic20 and the mitochondrial Tim17 and Tim23, which function in translocation in the inner mitochondrial envelop translocation (Kouranov et al. 1998).

Other channel forming proteins are Tic21/PIC1 and Tic22. These proteins are believed to be very similar in topology. Tic21 appears to be essential for normal function of the Toc/Tic machinery and is thought to link the two complexes in the inner membrane space (Kalanon and McFadden 2008). Tic22 helps to bridge Toc64, Toc12 and the Hsp70 proteins (Teng et al. 2006)

The Motor complex

The process of translocation through the Inner envelope membrane (IEM) is fuelled by ATP dependent Hsp93/ClpC. Hsp93 forms a ternary complex with Tic110 and Tic40.

Tic40 is a single-spanning integral inner membrane protein anchored by the N-terminal transmembrane domain. The C-terminus projects into the stroma consisting of a TRP-like domain and an α domain similar to mammalian Hip (Hsp70-interacting protein) and Hop protein (Hsp70/Hsp90 organizing protein) (Kouranov et al. 1998). Tic40 is proposed to be important for coordinating the sequential steps of translocation (Stahl et al. 1999). The atTic40 depletion strain shows a pale green and slow growing phenotype. This suggests that Tic40 plays an important role in regulating the chaperones.

Regulation, Tic62, Tic32 and Tic55

The regulation of the TIC complex is believed to be achieved by three proteins: Tic62, Tic32 and Tic55. Two parameters are involved during regulation: The $NADPH/NADP^+$ concentration and the Ca^{2+}/CaM concentration.

Tic62 and Tic32

Tic62 can be found as isoforms both in the IEM and the stroma of chloroplasts. Tic62 is capable of rapidly adapting to the $NADPH/NADP^+$ pool in the stroma. Low stromal NADPH concentrations lead to conformational changes in Tic62 and Tic32, rendering them more affinity for the TIC complex. When the concentration of NADPH increases, Tic62 loses affinity for the Tic complex and a large portion of Tic62will diffuse into the stroma. It seems that Tic32 is affected by the Ca^{2+}/calmodulin(CaM) ratio. Calcium is a common secondary messenger in a wide variety of biochemical processes. CaM is a common calcium sensing protein in calcium regulation in

eukaryotes. Calcium ionophores as well as CaM inhibitors interact with the imported proteins in the chloroplast. It appears that the binding of CaM to the C-terminus and NADPH at the N-terminus of Tic32 are mutually exclusive for the binding of Tic32 to the rest of the Tic complex (Bedard et al. 2007).

Tic55

Tic55 is believed to have a regulatory role like Tic62 and Tic32. There are two Tic55 paralogs in *A. thaliana*: Tic55-II and PTC-52 (Protochlorophyllide-dependent translocon component, 52kDa). Due to the low similarity in sequence (26%), they are expected to perform different functions in chloroplasts (Chigri et al. 2006b; Stengel et al. 2008). Deletion of Tic55-II and/or PTC-52 has no effect on phenotype and the import rates in chloroplasts. It suggests that Tic55 plays an unessential role in protein translocation (Boij et al. 2009).

Protein translocation from the stroma to the thylakoid

Membrane targeting

After preproteins arrival in the stroma, the N-terminal transit peptide is cleaved by SPP. Stromal proteins can be targeted to the thylakoid membrane via four pathways (Table 3). Proteins can be targeted to the thylakoid membrane in a SRP dependent or a spontaneous manner (Caliebe et al. 1997, Eichacker and Henry 2001). The Sec translocon or twin-arginine translocon (TAT) are used to translocate proteins across the thylakoid membrane into the lumen (Knott and Robinson 1994, Robinson, Thompson, Woolhead 2001). Considering the cyanobacterial lineage in chloroplasts, it is explainable how the two machineries that are very similar to bacterial systems are used in eukaryotic cells.

SRP-insertion

In bacteria, SRP binds to the signal sequence as it emerges from the ribosome, and directs the SRP-ribosome nascent chain complex (RNC) to the membrane bound SRP receptor FtsY, which is in the vicinity of the SecYEG translocon. The compositions of the SRP complex are variable in organisms, but usually with a conserved RNA-component. However, chloroplasts have no RNA moiety in SRP but a 43 kDa protein (cpSRP43) that has no homologues in non chloroplastic SRP and is believed to replace the function of the RNA component(Woolhead et al. 2001). In chloroplast, membrane targeting of proteins is fulfilled by cpSRP53, an *E. coli* SRP45 orthologue. cpSRP uses two distinct pathways to target proteins to the thylakoid membrane. One is the co-translational pathway where SRP associates with the translating chloroplast ribosome. It is for the translocation of chloroplast encoded proteins to the thylakoid lumen. The second is the post-translational pathway that is dedicated for the light harvesting chlorophyll binding proteins (LHCPs). After processing by SPP, nuclear-encoded LHCP (Light Harvesting Chlorophyll binding Protein) is bound to cpSRP53 and cpSRP43. The SRP43-SRP53-LHCP transit complex is then bound to the membrane receptor cpFtsY (a homologue to *E. coli* FtsY) where after LHCP is inserted into the thylakoid membrane by the *E. coli* YidC homolog Alb3 (Jonas-Straube et al. 2001).

Table 3. The identified components of translocation pathways in stroma of chloroplast.

Pathway	Identified components	Function	References
SRP	atSRP54, SRP43, ctFtsY and Alb3	Insertion of LHCP's into the thylakoid membrane	(Woolhead et al. 2001)
Spontaneous	None	Insertion of Sec/Alb3 independent proteins into the membrane	(Eichacker and Henry 2001)
Sec	ctSecA, ctSecE, ctSecY	Translocating Stromal proteins into the thylakoid lumen	(Woolhead et al. 2001)
Tat	Tha4, Hcf106, ctTatC	Translocation of folded proteins from the Stroma into the thylakoid lumen	(Mori and Cline 2001)

Alb3-dependent membrane insertion

In *E. coli*, YidC can function as an independent insertase or a membrane chaperone in the co-translational protein insertion pathway. Deletion of YidC results in defects in the membrane insertion of some integral membrane proteins (Mori and Cline 2001). Similar to *E. coli* YidC, its homologs Oxa1 in mitochondria and SpoIIIJ/YqjG in *B. subtillus* are also functionally important in membrane protein biogenesis (Samuelson et al. 2000). In chloroplasts, there are two YidC homologues Alb3 and Alb4 that are suggested to be involved in the membrane protein biogenesis. It has shown that LHCPs are strictly dependent on Alb3 for membrane insertion (Hell, Neupert, Stuart 2001; Saller, Fusetti, Driessen 2009). Deletion of Alb3 caused a defective insertion of LHCPs into the thylakoid membrane, but had no effect on other integral membrane proteins (Moore et al. 2000). In addition, LHCPs can be correctly inserted into the membrane when the Sec and Tat pathways are disabled. The data suggest that LHCPs are Alb3 dependent.

On the other hand, some proteins such as PsbX, PsbW, and PsbY are suggested to be integrated into the thylakoid membrane in a spontaneous manner, because their insertion does not seem to be dependent on any factors known to be involved in insertion (Woolhead et al. 2001).

The Sec translocon

The Sec translocon is evolutionarily conserved translocation machinery in the eukaryotes, archaea and eubacteria. The Sec translocon is composed of three core components: SecY, SecE and SecG and accessory proteins such as SecDFYajC (Woolhead et al. 2001b), (Hartmann et al. 1994). In *E. coli*, preproteins are bound by the chaperone SecB to be kept in a translocation competence state in the post-translational pathway prior to be targeted to the SecA-bound SecYEG translocon (Duong and Wickner 1997). Alternatively, the ribosome nascent chain complex is bound by SRP in the co-translational pathway, and then the RNC-SPR complex is guided to the membrane receptor FtsY and the Sec translocon (Driessen et al. PW 1998). In chloroplasts, the Sec translocon seems to be a minimalistic version, with the identified components SecA, SecE and SecY. The Sec translocon in chloroplasts is suggested to function in a similar way and translocates proteins in an unfolded state. The basic mechanism of the Sec system in chloroplasts is the same as that in *E. coli,* and several stromal proteins might perform functions related to the missing Sec components. Interestingly, it has been shown that the chloroplast SecA is not stimulated by the *E. coli* Sec signal peptides, suggesting its chloroplast specific characteristic (Angelini et al. 2005). In addition, the *A. thaliana* Δ*secA* mutant

cannot perform photosynthesis. Therefore, a complete Sec translocon is necessary for normal chloroplast development in spite of its minimalistic design (Sun et al. 2007).

The TAT translocon

The TAT system is a well conserved and common translocon in chloroplasts, bacteria and archea (Knott and Robinson 1994; Mori and Cline 2002). It is characterized by transporting proteins across membrane in a folded state, which allows folded redox enzymes to be translocated across the membrane with their necessary cofactors attached (Sargent, Berks, Palmer 2006). The TAT system depends purely on a ΔpH gradient (Palmer, Sargent, Berks 2005). The N-terminal signal for the TAT system is characterized by a highly conserved twin arginine motif on the sequence. It is believed that some proteins in chloroplasts fold too quickly to be translocated by the Sec system and thus need the TAT translocon. There are major differences in function and components of the Tat systems in Gram-negative and Gram-positive bacteria. Generally Gram-negative bacteria (like cyanobacteria) have the TAT system with a 10kDa TatA, 18kDa TatB and 30kDa TatC(Cline, Ettinger, Theg 1992). Gram-positive bacteria often have no TatB and a multifunctional TatA. The chloroplast TAT system shows similarity with its counterpart in gram-negative bacteria. It has three components Tha4, Hcf106 and cpTatC, which are homologues to bacterial TatA, TatB and TatC respectively (Mori, Summer, Cline 2001). The Tat components are present at two different locations in chloroplasts. Hcf106 and cpTatC associate with each other to form a ~700kDa Hcf106-cpTatC complex. Tha4 is only associated with the Hcf106-cpTatC complex in the presence of a ΔpH and a functional precursor protein. Despite that there is no structural data of the chloroplast TAT complex, it is believed that the Hcf106-cp-TatC complex only interact with Tha4 during active translocation, after which they directly dissociate (Cline and Mori 2001b). A possible explanation for this could be that maintaining a large translocation channel open for a longer time might compromise the integrity of the membrane. Opening the channel only when needed might reduce this risk. A noticeable difference between bacterial and thylakoid TAT system is the signal consensus sequence. The TAT signal consensus of bacteria and thylakoids are ((S/T)RRXFLK) and (RRXXφ), respectively, where φ is usually Leucine, phenylalanine, valine or methionine and X can be any amino acid in the signal consensus sequence(Cline and Mori 2001a; Mori and Cline 2002). Despite these differences plant and bacterial TAT translocation pathways seem to have an overall similar mechanism. *P. sativum* thylakoid TAT translocon has even been shown to translocate a variety of proteins with *E. coli* signals very efficiently.

After arrival in the thylakoid lumen, proteins are processed into their mature form by thylakoid luminal processing peptidase (TPP) to execute their functions in photosynthesis (Albiniak, Baglieri, Robinson 2012).

Conclusion

The ability of chloroplasts to produce organic molecules from carbon dioxide, water and light known as photosynthesis is essential for life on earth. A variety of proteins are involved in the process. A lot of progress has been made to elucidate how nuclear- and chloroplast- encoded proteins are imported into stroma of chloroplasts and destined for their final destinations to fold into a functional conformation. Here we summarize the general knowledge about the translocation systems in chloroplasts. Considering the endosymbiont event of chloroplasts, it is explainable that the translocation systems in chloroplast show similarity with that in gram-negative bacteria. Future study on a molecular level might shed more light on the exact mechanisms of translocation.

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