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The Molecular Concept of Protein Translocation across the Outer Membrane of Chloroplasts

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Chloroplasts, plant organelles which facilitate photosynthesis, originated when photosynthetic bacteria became a part of non-photosynthetic eukaryotic cell. Chloroplast proteins synthetised in the cytosol have to be transported into the organelle, which is facilitated by the Toc complex on the outer envelope. The preprotein receptors Toc34 and Toc159 associate with the pore-forming Toc75 to form the Toc core complex. Toc64 and Toc12 dynamically associate with the core complex and recruit chaperones, forming the intermembrane space complex. Describing Toc159 as integral membrane protein provides insight into its function. After receiving preproteins from Toc34, it uses GTP hydrolysis to push the precursor into the translocation channel. GTP hydrolysis by Toc34 controls its binding/handover cycle. Moreover, different isoforms of Toc components form complexes with different specificity for photosynthetic and non-photosynthetic preproteins. After initial translocation steps, a series of binding spots seems to constitute an affinity chain that guides preproteins further on their translocation pathway. This model parallels the "acid chain" described in mitochondrial import. Considering recent data, a new "big picture" of chloroplast import begins to emerge.

Keywords
chloroplasts
envelope membranes
GTPase
protein receptors
translocation channel
phosphorylation

INTRODUCTION

Chloroplasts originated in a single primary endosymbiotic event more than 1.2 billion years ago (*e.g*. Gray, 1989; Cavalier-Smith, 1992; Morden *et al*., 1992; Butterfield, 2000; Moreira *et al*., 2000), when a photosynthetic cyanobacterium was taken up by a heterotrophic cell. This event was followed by a massive transfer of genetic material to the host nucleus (Martin, 2003), which led to a problem for the newly established endosymbiotic relationship: proteins encoded by the transferred genes were now being synthesized in the cytosol of the host cell, and had in most cases to be transferred back to the newly acquired chloroplast. Here, the chloroplast outer envelope is the first barrier for the cytosol-synthesized proteins. For a long time, the prevailing opinion was that the outer envelope of chloroplasts is like a molecular sieve – full of holes and leaky, with maybe a limited selectivity

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(Flügge, 2000). However, a significant body of recently accumulated evidence points to a tightly controlled regulation of the traffic across the outer envelope. Thereby, the previously favoured picture of the outer envelope being a molecular sieve has to be revised and has to be atributed to artefacts of at that time widely used patch-clamp technique (Soll *et al*., 2000). One of the molecular gates controlling the entry of molecules into chloroplasts is the protein translocon, responsible for selection and import of protein molecules.

The first task of this complex is the recognition and binding of chloroplastic precursor proteins which have to be imported. Most of such proteins are synthesized with a cleavable N-terminal transit sequence, which is both necessary and sufficient to target them for chloroplast import (Sveshnikova *et al*., 2000; Bruce, 2001). The transit sequence consists of 20 to 150 amino acids, has an overall positive charge and is rich in hydroxylated amino acids (Soll and Schleiff, 2004). Its variable length and divergent primary structure suggest that it is not a specific sequence motif, but rather a certain structural characteristic, which is recognized by the receptors. For a long time it was believed that only outer envelope proteins lack an N-terminal transit sequence and some authors suggest that they might have an internal signal instead (Bruce *et al*., 2001; Schleiff and Klösgen, 2001). However, recent proteomic approaches identified many plastid proteins without an obvious signal with the characteristics outlined above within the coding region (Kleffmann *et al*., 2004). Therefore, the characteristics of the targeting signal and translocation events will be revisited for these proteins in future. The first hint for an alternative route in *Arabidopsis thaliana* came from the observed endoplasmic reticulum intermediate of α-carbonic anhydrase before its translocation into plastids (Villarejo *et al*., 2005).

The Toc Complex

The Toc core complex consists of three types of subunits: the channel protein Toc75, two GTPases with receptor function, Toc34 and Toc159, and two less abundant Toc components, Toc64 and Toc12 (Box 1), which are dynamically associated with the core complex (Figure 1). The latter two proteins are components of an intermembrane space complex, together with imsHsp70 and Tic22 (Becker *et al*., 2004;[15] Qbadou *et al*., 2007). Toc12 is an outer envelope protein which has a C-terminal J-domain protruding into the intermembrane space. The J-domain, so named after its homology with DNA-binding J-proteins, interacts with the intermembrane space chaperone imsHsp70, recruiting it to the complex and stimulating its ATPase activity (Becker *et al*., 2004).[15] Toc12 also interacts with the intermembrane space part of Toc64, another member of the IMS complex, which is less well characterized than Toc core complex components. Toc64 contains three cytosolic exposed tetratricopeptide (TPR) repeats, which form a clamp-type domain (Young *et al*., 2004). This C-terminal TPR domain of Toc64 recognizes Hsp90 delivered precursor proteins via interaction with the Hsp90 chaperone (Qbadou *et al*., 2006). The Tic22 protein, a member of the translocon in the intermembrane space, is thought to mediate interaction between the Toc and the Tic complex (Kouranov *et al*., 1998; Becker *et al*., 2004,[15] Qbadou *et al*., 2007), therefore helping to streamline the import process.

The Toc75 pore belongs to the Omp85 class of proteins found in the outer (lipopolysaccharide) membrane of gram-negative bacteria, as well as in outer membranes of mitochondria and chloroplasts (Schleiff and Soll, 2005; Löffelhardt *et al*., 2007). Indeed, Toc75 has homology to slr1227 from *Synechocystis* (Bölter *et al*., 1998; Reumann *et al*., 1999) and alr2269 from *Anabaena* (Mo-

Box 1. Nomenclature Regarding Protein Translocation

Membrane receptors	
Toc	… translocon on the outer envelope of chloroplasts
Tic	… translocon on the inner envelope of chloroplasts
TocXX	… XX indicates the molecular weight of the first isoform identified
atTocXX	… the species where the isoform originates from is given in two letter code (*e.g. Arabidopsis thaliana:* at)
Soluble proteins	
c/ims/st	… prefix to indicate localization in cytosol / intermembrane space / stroma
Precursor protein	
m(name)	… mature form of the protein (after cleavage of the transit sequence)
p(name)	… precursor form of the protein (cytosolic form before translocation containing the transit sequence)
transit sequence	… The N-terminal section of the precursor proteins, which is essential and sufficient to target the protein and which is cleaved off by a stromal peptidase

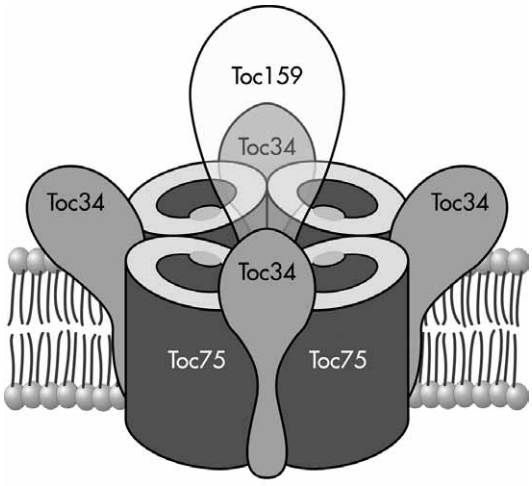

Figure 1. Stoichiometry and topology of the Toc core complex. The Toc core complex consists of four Toc75 and Toc34 subunits, with an additional Toc159 in the centre. According to our current understanding, Toc75 proteins form four separate pores, and a single Toc159 serves all of them. The presented structure was inferred from stoichiometry data and EM images.

slavac *et al*., 2005; Ertel *et al*., 2005; Bredemeier *et al*., 2007). The C-terminal β-barrel domain of Toc75 functions as a translocation pore and shows a high degree of homology to its prokaryotic ancestor, while the N-terminus, responsible for recognition and complex assembly, seems to have been evolutionary shaped in its function after endosymbiosis (Ertel *et al*., 2005; Bredemeier *et al*., 2007). Receptors Toc34 and Toc159 have no known functional homologues in prokaryotes, although some authors point out that related GTPases exist in *Synechocystis* (Reumann and Keegstra, 1999).

The mixed prokaryotic/eukaryotic structure of the Toc translocon gives some insight into its evolutionary origins (Heins and Soll, 1998). Toc components have no homology to the components of the four main prokaryotic protein secretion systems (Reumann and Keegstra, 1999). Instead, the Toc translocon is built around a prokaryotic pore by modification of the regulatory properties of the pore itself and by addition of new receptor proteins of eukaryotic origin to the whole complex, like Toc34 and Toc159 (Löffelhardt *et al*., 2007). It appears that Toc receptor proteins have already adopted their function in the early evolution of land plants. Multiple isoforms of Toc34 are present in plastids of gymnosperms, where they might be involved in biogenesis of different plastid types (Fulgosi *et al*., 2005).

Both Toc34 and Toc159 have a C-terminal transmembrane domain, and a more N-terminally located GTPase domain, which is a region of high similarity between the two receptor proteins. Unlike the smaller Toc34, the Toc159 has an additional domain at its N-terminus, the acidic A-domain. This part of the protein seems not to be essential for its function in protein translocation, at least in *in vitro* experiments (Chen *et al*., 2000), although its presence increases yield in import experiments (Chen *et al*., 2000). Hence, the role of the A-domain is still unknown. Its apparent lack of structure makes it interesting to speculate that the A-domain might function on the same principle as natively unfolded proteins – as a 'protein fishing' string with multiple low-affinity binding sites which 'hooks' its interaction partners (Dafforn and Smith, 2004), or a flexible interaction surface which facilitates molecular recognition by adjusting itself to its ligands (Fink *et al*., 2005). Alternatively, the unstructured A-domain might target Toc159 for degradation (Fink *et al*., 2005), leading to a high turnover of the receptor which could account for a regulatory mechanism of precursor protein translocation.

The G-domain of GTPases carries out nucleotide binding and hydrolysis. This ≈20 kDa domain consists of a mixed six-stranded β sheet and five helices located on both sides (Vetter and Wittinghofer, 2001; Sun *et al*., 2002; Yeh *et al*., 2007). The structural units are organized into three motifs: the switch I, switch II and the P-loop regions, connected to nearby β-strands. The catalytic domain functions as a conformational switch, with significant structural differences between the strained GTP bound and the relaxed GDP bound state. Catalytic domains of G proteins and those of ATP-hydrolyzing motor proteins share a degree of similarity in structure and mechanism of action, with a power stroke in the range of 10 pN in both cases (Kosztin *et al*., 2002). Unlike ATP dependent motor proteins, the known G proteins lack a "lever arm" to produce large-amplitude displacements.

Earlier studies (Hiltbrunner *et al*., 2001) suggested that Toc159 might be a soluble receptor, because it was found in the cytosol as well as in the outer envelope after cell fractionation. At this point it was speculated that the soluble receptor could transfer the guidance complex to the chloroplast surface, thus acting as the primary precursor protein receptor. The soluble receptor hypothesis was brought into question when similar experiments were conducted using a larger number of carefully selected controls (Becker *et al*., 2004).[38] Toc159 presence in the cytosol was shown to be an artefact introduced during isolation. Furthermore, although the Toc159 itself has affinity for the transit sequence, Toc34 seems to be the primary precursor protein receptor, with the polypeptide being transferred to the Toc159 in a later step, as discussed below.

The Toc core complex isolated from pea (*Pisum sativum*) has an apparent molecular mass of ≈ 500 kDa and a molecular stoichiometry of 1 : 4 : (4–5) between Toc159, Toc75 and Toc34 (Schleiff *et al*., 2003).[39] Electron microscopic single particle analysis of isolated Toc complex revealed a toroid-shaped particle measuring 13 nm in diameter, with a protruding finger-like domain in

the centre, giving it a height of 10–12 nm, and dividing the central cavity into four apparent pores. The known component stoichiometry supplemented with structural analyses makes it tempting to speculate about the arrangement of the subunits, four Toc75s and Toc34s forming independent translocation pores assembled around one Toc159 representing the central finger-like domain.

A second layer of complexity of the translocon was found when sequencing of the *Arabidopsis thaliana* genome was completed in the year 2000, as several isoforms were found for each component (Oreb *et al*., 2006; Box 2). Isoforms of the Toc core components found in *Arabidopsis thaliana* and their tissue-specific stoichiometry point to the existence of several Toc "flavours" with different precursor protein specificity (Kubis *et al*., 2003; Vojta *et al*., 2004; Moghadam and Schleiff, 2005). There are two Toc34 homologues in *A. thaliana* (Jelic *et al*., 2003), namely atToc33 and atToc34. The atToc33 is regulated by phosphorylation, similar to the Toc34 from pea, while atToc34 seems to lack such regulation. GTPase activity of both proteins is stimulated by precursor proteins. Different subclasses of precursor proteins bind preferentially to one or the other Toc34 isoform. While atToc33 is highly expressed in leaves and shows strong stimulation of its GTPase activity by photosynthesis-specific precursor proteins, atToc34 is located primarily in roots and strongly activated by non-photosynthetic precursor proteins (Gutensohn *et al*., 2000; Jelic *et al*., 2003). This specialization is not absolute, since a degree of functional overlap has been demonstrated – knockouts of atToc33 with a working copy of atToc34 are viable, although they show a relatively mild phenotype (Jarvis *et al*., 1998). The family of Toc159 proteins in *A. thaliana* consists of atToc159, atToc132, atToc120 and atToc90 (Kubis *et al*., 2004). The atToc159 isoform is highly expressed in photosynthetic tissues, especially during early development, while atToc132 and atToc120 are uniformly expressed, which makes them comparably abundant in non-photosynthetic tissues (Kubis *et al*., 2004). No single knockout mutant has a particularly strong phenotype, and the atToc132/atToc120 double mutant resembles the atToc159 knockout. The pore-forming Toc75 comes in two main varieties in *A. thaliana*: the most abundant outer envelope protein atToc75-III, highly expressed in growing photosynthetic tissues, and the atToc75-IV, which is uniformly expressed at a low level (Baldwin *et al*., 2005). Knockout of atToc75-III is embryo lethal. Additionally, a pseudo-gene named atToc75-I belongs to the Toc75 group, due to its homology with other members. Another Toc75-like channel of the chloroplast outer envelope is the 66 kDa Toc75-V (Eckart *et al*., 2002; Inoue *et al*., 2004). It is phylogenetically more closely related to prokaryotic Toc75 ancestors than to other plant Toc75 proteins.

Box 2. Different Isoforms of the Toc Components

Family	*Proteins in* A. thaliana
Toc159	Toc159, Toc132, Toc120, Toc90
Toc75	Toc75-I, Toc75-III, Toc75-IV, Toc75-V
Toc34	Toc33, Toc34
Toc64	Toc64
Toc12	not yet identified in *A. thaliana*

Translocation across the Outer Envelope

– Recognition

Nascent precursor proteins are prevented from inappropriate interactions with proteins abundant in the cytosol by molecular chaperones from the Hsp70 family. They keep the polypeptide in a soluble, partly unfolded, import competent state. For some proteins with N-terminal transit peptide, early transport events are also assisted by 14-3-3 family proteins which, together with Hsp70s, form a guidance complex (May and Soll, 2000; Fulgosi *et al*., 2002) upon phosphorylation of the transit sequence (Waegemann and Soll, 1996). This assembly is subsequently targeted to the Toc34 receptor on the chloroplast surface (Figure 2). However, whether this transport route is as general as discussed (Soll and Schleiff, 2004) remains under debate, as direct evidence of phosphorylation and 14-3-3 association was presented for only a small number of precursor proteins. Alternatively, a class of non-phosphorylated precursor proteins has been recently found to assemble with Hsp70 and Hsp90, but not with 14-3-3. These nascent precursor proteins are targeted to the cytosolically exposed TPR domain of Toc64 via its interaction with the Hsp90 chaperone (Qbadou *et al*., 2006, Figure 2).

The cytosolically exposed tetratricopeptide domain of Toc64 was found to recognize the C-terminus of Hsp90 (Qbadou *et al*., 2006) in a clamp-type manner. However, at this stage, Toc64 does not interact with the delivered precursor protein itself but the Hsp90 chaperone. The recognition of the Hsp90 by Toc64 is followed by a transient interaction of Toc64 with the GTP-charged G domain of Toc34. Hence, the transfer of a precursor protein to Toc34 takes place in a GTP-dependent manner, even though the molecular mechanism is not yet explained in detail. However, $Toc34_{GTP}$ now recognizes the transit peptide of the Hsp90 delivered precursor protein directly. At this point the 14-3-3 and the Hsp90 dependent routes for precursor protein delivery converge (Figure 2) as Toc34 was found to be the primary receptor of the 14-3-3 delivered precursor proteins as well. Furthermore, most of the components and mechanistic steps of the way from the ribosome to the chloroplast remain to be explored in the future, especially in the light of recently discovered alternative import routes (Villarejo *et al*., 2005; Radhamony and Theg, 2006).

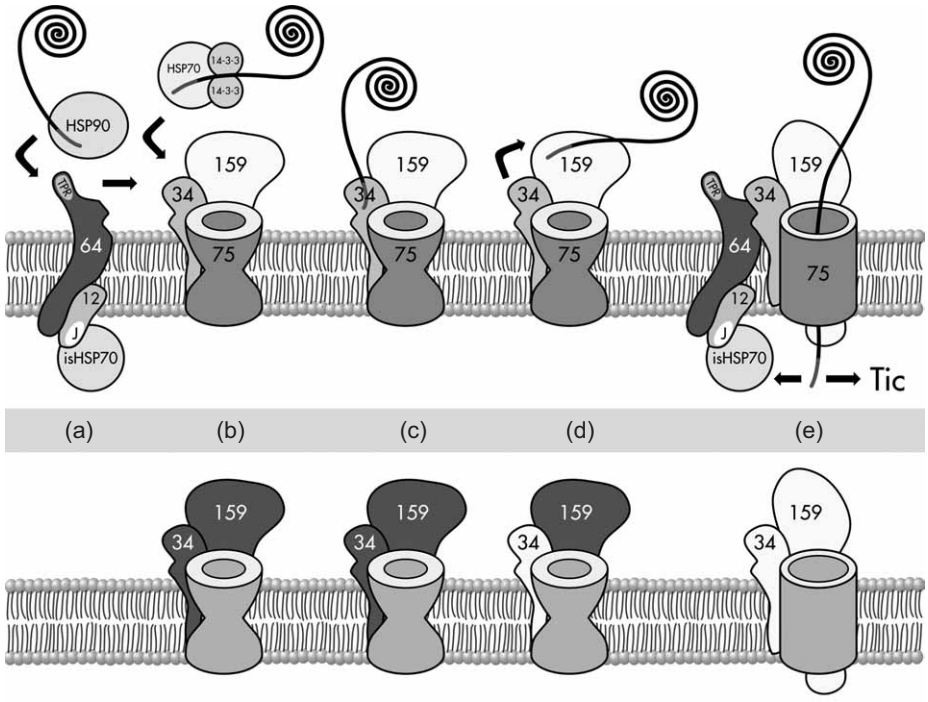

Figure 2. The mechanism of Toc complex action. The top portion gives a scheme of precursor protein translocation by the Toc complex. The bottom portion indicates the nucleotide state of the receptors Toc34 and Toc159 at the according translocation steps. Here the GTP state is indicated by dark grey and the GDP state by light grey. Additionally, the open and closed state of the pore Toc75 is indicated. (a) A precursor protein (curled thick line) containing a transit sequence (grey extension of the line) is delivered to Toc64 by an Hsp90 chaperone molecule. The TPR domain of Toc64 facilitates its interaction with Hsp90. After binding, Toc64 undergoes a transient interaction with a GTP loaded Toc34 molecule, which perceives the precursor protein. (b) A second class of precursor proteins is delivered to the chloroplast surface by a so called guidance complex consisting of one Hsp70 molecule and a 14-3-3 dimer. These precursor proteins are recognised directly by Toc34. (c) After binding the transit sequence (dark gray) of a precursor protein, Toc34 hydrolyzes its bound GTP and (d) transfers the precursor protein to Toc159. Hydrolysis of GTP causes a conformational change in Toc159 (e) and the precursor protein is pushed through the translocation pore. The precursor protein is now taken over by the Tic translocon on the inner envelope. Alternatively, precursor protein could be taken over and pulled by imsHsp70, delivered by Toc64/Toc12 during the interaction of Toc64 and Toc34. Toc12 contains a J domain which facilitates its interaction with chaperones in the intermembrane space (*e.g.* imsHsp70).

– *Transfer*

The Toc34 receptor is the entry point for each precursor protein into the Toc core complex. Toc34 has to be charged with GTP in order to recognize the precursor protein (*e.g.* Sveshnikova *et al*., 2000, Jelic *et al*., 2002, Jelic *et al*., 2003). In case of the model substrate, which is the small subunit of RubisCO, $Toc34_{GTP}$ recognizes the C-terminus of the transit sequence with high affinity (Schleiff *et al*., 2002). At the same time the N-terminal portion of the transit sequence engages with the Toc159 receptor in a nucleotide dependent manner (Becker *et al*., 2004; Smith *et al*., 2004), but only when the transit sequence is de-phosphorylated. Details of the de-phosphorylation are still unknown, and some authors (Nakrieko *et al*., 2004) even doubt the role of precursor protein phosphorylation *in vivo*. However, the interaction of the C-terminal part of the transit sequence withToc34 induces the subsequent hydrolysis of GTP (Jelic *et al*., 2002). Since the GDP loaded form of the receptor Toc34 has a lower affinity for the precursor proteins than the GTP loaded form, this hydrolysis is paralleled by a precursor protein release from its binding pocket (Jelic *et al*., 2003, Figure 2). The released C-terminal section of the transit sequence will now be recognized by the next GTP charged receptor, namely Toc159 (Becker *et al*., 2004).[38] Thereby, the recognition of the C-terminal portion induces the GTP hydrolysis of Toc159 as well.

Recognition of the precursor protein and its processing is modulated by phosphorylation. Both GTPases are dominant phosphor-proteins phosphorylated by two different kinases (Fulgosi and Soll, 2002). For Toc34 it was demonstrated that both GTP binding and precursor protein recognition is impaired after phosphorylation (Sveshnikova *et al*., 2000; Jelic *et al*., 2002; Jelic *et al*., 2003). Interestingly, in *A. thaliana* only Toc33, but not Toc34 (Box 2) can be phosphorylated, suggesting different regulatory mechanisms for the different translocons formed. However, the functional mechanism and the physiologically relevance of the phosphorylation remains elusive, especially since *toc33* knock out plants complemented with a phosphor-mimicking mutant do not show a significant phenotype (Aronsson *et al*., 2006). Hence, the molecular identification of the kinases involved in receptor phosphorylation will challenge this regulatory circuit.

An additional open question is the regulation of the GTPase cycle for the two G proteins in the Toc complex. Crystal structure of Toc34 revealed that its molecules exist as dimers (Sun *et al*., 2002). More recent studies indicate the possibility that Toc34 forms heterodimers with Toc159, the interaction taking place via their homologous GTP-binding domains (Kessler and Schnell, 2002). Such an interaction could provide a means for mutual activation of the two receptors thereby facilitating the precursor protein handover, and could also have a role in the assembly of the Toc core complex. Furthermore, it was suggested that dimerisation might play a role in the regulation of the GTPase cycle. However, final evidence for this notion is still missing.

– *Translocation*

There is strong experimental evidence that precursor protein transfer across the outer envelope of chloroplast is indeed powered by the GTPase action of Toc159, which hydrolyses GTP and operates in a way that could be visualized as being similar to the operation of a sewing machine (Schleiff *et al*., 2003).[62] Toc159 seems to provide the only driving force for the first translocation step. An initial pulling force of chaperones can be ruled out in this phase of translocation since a minimal reconstituted system consisting of Toc159 and Toc75 is able to carry out the import reaction using GTP only (Schleiff *et al*., 2003).[62] However, the intermembrane space localized Hsp70 (imsHsp70, Marshall *et al*., 1990; Waegemann and Soll, 1991; Schnell *et al*., 1994) might take over *in vivo* once the precursor protein reaches the intermembrane space after the initial transfer event catalyzed by Toc159.

The essential role of Toc159 in chloroplast biogenesis (Bauer *et al.*, 2000), and its presence in a »catalytic ratio« in the complex (1:4 to Toc75 and Toc34, Schleiff *et al*., 2003)[39] further support the notion of Toc159 action as a translocation motor. Here, a loose parallel with the ATPase driven SecA-type protein translocation (Manting and Driessen, 2000) can be drawn. In both systems, the receptor itself charges the initial translocation, even though SecA is a soluble protein, while Toc159 is not.

But what happens after the initial stroke? Toc75 itself contains a precursor binding site (Hinnah *et al*., 1997; Ertel *et al*., 2005). This might be the first recognition site in the intermembrane space. Furthermore, Toc64 interacts with the rest of the IMS complex – and directly with the Toc12. Transfer of the precursor protein to the Toc34 induces Toc64 to activate its interaction partner Toc12, which in turn recruits the ATP-loaded imsHsp70 by interaction of its J-domain (Becker *et al*., 2004;[15] Qbadou *et al*., 2007). The chaperone is ready to be transferred to the precursor protein when it emerges from the translocation pore. Upon precursor protein binding, imsHsp70 catalyzes the hydrolysis of its bound ATP, which is stimulated by the action of the J-domain of Toc12. This is followed by the exchange of the bound ADP for a new molecule of ATP and subsequent precursor protein release.

The IMS complex is now ready for the next cycle. It is generally believed that precursor proteins are immediately taken over by the Tic translocon on the inner envelope (Akita *et al*., 1997; Nielsen *et al*., 1997) – however, the close coupling of Toc64 recognition on one side and chaperone recruitment on the other makes it tempting to speculate about an alternative pathway followed by Toc64-recognized precursor proteins. Therefore, the IMS complex might actually represent an alternative import route, perhaps for import of proteins destined for the intermembrane space or one/both of the envelope membranes, as opposed to the 'classical' route through the Tic complex, which would be followed by proteins targeted for stroma or the thylakoid system.

For the complete picture of a feasible mechanistic model of translocation, the question of energetic has to be addressed. What drives the bulk of translocation? For recognition and transfer of mitochondrial precursor proteins at the organelle surface a so-called 'acid chain' hypothesis was formulated (Komiya *et al*., 1998). Here it is suggested that a series of acidic receptor sites interacting with the positively charged targeting signals are strategically placed along the import pathway and drive the first steps of protein import (Schatz *et al*., 1997). In this model the order of binding is thought to be assured by the topological arrangement of these sites (Schatz *et al*., 1997) and a massive short-circuiting of the pathway is probably prevented by cytosolic chaperones associated with the precursor protein. It is appealing to transfer such model to the action of the chloroplast translocon. Here, on the cytosolic face of the outer envelope of chloroplasts an affinity chain is built by different charged G domains, whereas initial recognition is partly facilitated by an interaction with the protein involved in delivery (Qbadou *et al*., 2006). The final intermembrane space localized binding site of this affinity chain might be within Toc75 itself (Ertel *et al*., 2005) or might be presented by the intermembrane space localized domain of Toc64 (Qbadou *et al*., 2006). A precursor protein already transferred into the intermembrane space might be guided further in a similar manner through the Tic complex, which lacks a protein with a motor activity or a proton gradient across the membrane in which it is embedded (Soll and Schleiff, 2004). However, binding spots which would constitute an 'affinity chain' through the Tic complex still need to be identified.

In contrast to the mitochondrial »affinity chain« hypothesis the transfer across the outer envelope itself remains energy dependent. Toc159 probably actively pushes the precursor protein through the translocation pore, enough to expose a string of amino acid residues to the

other side (Schleiff *et al*., 2003).[62] Subsequently, there has to be a mechanism which could provide enough energy to power the complete import reaction *in vivo*. Likely candidates for this mission are molecular chaperones, abundant in both the stroma and the intermembrane space of chloroplasts. The idea is not new – more than a decade ago, researchers envisaged a system similar to a Brownian ratchet which could drive protein import into organelles (Simon *et al*., 1992; Neupert and Brunner, 2002). In this model, the precursor protein inserts reversibly into the translocation channel, and oscillates inwards and outwards due to the thermal motion. Chaperone binding on the inner side would prevent backsliding of the polypeptide chain and thus rectify its movement. However, this model could not explain the rate of translocation of folded proteins (Glick, 1995) and how a hydrophobic interaction between translocation channel and precursor protein could be pried off. Subsequently an alternative so-called power stroke model was proposed (Neupert and Brunner, 2002). This model suggests that membrane anchored or associated Hsp70s undergo a conformational change upon precursor protein binding and subsequent ATP hydrolysis, thus acting much in the way of conventional molecular motors. The power stroke of such anchored chaperones would exert a force enough not only to import the polypeptide chain, but also to unfold protein domains when necessary. Both models, the Brownian ratchet and the power stroke, were extensively theoretically investigated in order to confirm the validity of one or the other based on the kinetic data (Elston, 2002). The more than a decade long debate seems to be settled by reconciliation of the two models proposing a mechanism called entropic pulling (De Los Rios *et al*., 2006). According to this model, pulling force is exerted due to entropy loss caused by excluded volume effects. An unbound polymer can access all available conformations, except those which violate the volume of the membrane or the translocation pore. The large volume of Hsp70 greatly increases the excluded volume constraint, and the number of available conformations is significantly decreased. The number of available conformations is directly related to entropy – less available conformations correspond to a decrease in entropy. After a thermodynamically favourable binding of Hsp70 to its substrate, the excluded volume produces an effective pulling force of entropic origin – 10 to 20 pN when the bound chaperone is 8 to 15 residues away from the membrane. If another chaperone binding site doesn't emerge in the next ≈30 residues, a kind of Brownian ratchet could continue to pull, albeit with lesser force. According to the entropic pulling model, thermal fluctuations are rectified by a free-energy gradient, without a requirement for an anchor or even a pore. This single mechanism is able to explain different functions of Hsp70, functional differences depending on co-chaperones (such as J domain), nucleotide exchange factors or docking. Also, in this model chaperones do not need a molecular fulcrum and the energy produced is sufficient to explain quick import, even of proteins that require partial unfolding. Hence, *in vivo* final translocation after initial push by Toc159 might be assisted by IMS localized Hsp70 (Marshall *et al*., 1990; Waegemann and Soll 1991; Schnell *et al*., 1994).

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SAŽETAK

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Kloroplasti današnjih biljaka potječu od endosimbioze fotosintetskih bakterija i eukariotske stanice, zbog čega se većina proteina kloroplasta sintetizira u citosolu. Preduvjet za funkcioniranje kloroplastnih proteina je transport u organelu, za što je na vanjskoj membrani zadužen Toc kompleks. Receptori Toc34 i Toc159 uz kanal Toc75 čine glavni dio Toc kompleksa, na koji su labavije vezani Toc64 i Toc12. Ove dvije komponente u međumembranskom prostoru vežu molekularni pomagači (engl. *chaperone*) tvoreći tako međumembranski kompleks. Utvrđivanje membranske lokalizacije Toc159 nudi objašnjenje njegove funkcije. Nakon što mu Toc34 preda prekursorski protein, Toc159 koristi hidrolizu GTPa kako bi ga pogurao u translokacijski kanal. Kod Toc34, hidroliza GTPa služi za kontrolu ciklusa vezanja/predaje prekursora. Različite izoforme komponenata Toc translokona udružuju se stvarajući komplekse specifične za fotosintetske ili nefotosintetske prekursorske proteine. Nakon početne faze translokacije, prekursor stupa u kontakt sa serijom veznih mjesta rastućeg afiniteta, koja ga dalje usmjerava na translokacijskom putu. Ovaj model podsjeća na "*acid chain*" hipotezu proteinskog transporta u mitohondrijima. Povezani u cjelinu, noviji podaci počinju otkrivati cjelovitu sliku transporta proteina u kloroplaste.