Review

Cell PRESS

Redox extends its regulatory reach to chloroplast protein import

Mónica Balsera[1], Jürgen Soll[2] and Bob B. Buchanan[3]

[1] Departamento de Estrés Abiótico, Instituto de Recursos Naturales y Agrobiología de Salamanca (IRNASA-CSIC), Salamanca, Spain
[2] Department of Biology I, Botany, Ludwig Maximilians University, Martinsried, Germany
[3] Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA

The import of chloroplast proteins synthesized in the cytosol of a plant cell is mediated by two multiprotein complexes or translocons located at the outer and inner membranes of the chloroplast envelope, respectively, TOC and TIC. These complexes integrate different signals to assure the timely transport of proteins into the chloroplast in accordance with the metabolic and developmental needs of the cell. The past few years have witnessed the emergence of redox as a regulator of the protein transport process. Here, we discuss evidence that the metabolic redox state of the chloroplast regulates the import of preproteins by altering either the activity or composition of participating transport components. It appears that, through these redox changes, chloroplasts communicate with other compartments of the plant cell.

Chloroplast proteome: need for coordination of different factories within the plant cell

Because the vast majority of chloroplast proteins are nuclear-encoded, plastid biogenesis relies on the efficient sorting of proteins imported from the cytosol and on their coordinated assembly with the smaller number of proteins synthesized within the organelle, for example photosystem multicomplexes or RuBisCO (Figure 1 and Box 1) [1–3]. Owing to changes in metabolism linked to the environment or stage of development, chloroplasts require adaptive responses. The proficient coordination of plastid function with activities of the plant cell requires rapid and efficient reciprocal communication. Plastid signaling is currently an area of intensive research that includes, among others, a coordination of plastid protein import and nuclear gene expression [4,5]. Herein, we discuss emerging evidence for the role of redox in regulating protein import as a function of the needs of the cell under changing conditions.

Original evidence for regulation of protein import by redox

Some years ago, the effect of light on protein import into chloroplasts was analyzed [6]. The authors performed *in vitro* import studies with precursors of the photosynthetic and non-photosynthetic isoforms of ferredoxin (Fdx) and ferredoxin–NADP reductase (FNR), FdxI/FdxIII and FNRI/FNRII, respectively. Interestingly, light had a strong, specific effect on import of the non-photosynthetic isoforms. Whereas the photosynthetic FdxI and FNRI isoforms were properly transported to the stroma with concomitant development of mature proteins after cleaving off the transit peptide in light and darkness, the non-photosynthetic FdxIII and FNRII precursors failed to enter the stroma and the preproteins accumulated in the chloroplast intermembrane space (IMS) in the light. The mistargeting to the IMS was not reversed when chloroplasts were transferred back to dark. However, if the precursor proteins were reisolated and imported into non-light treated chloroplasts, they were properly processed. The effect was ascribed to misfolding in the IMS and to the possibility that the translocation of certain precursor proteins across the envelope was regulated by light. The molecular determinants behind this observation have, however, remained elusive.

Other lines of evidence point to thiol-based redox as a regulator of protein import

In recent years, diverse mechanisms have been invoked to explain the import of proteins into chloroplasts. In an attempt to dissect the participating factors and characterize the energetic and protein requirements, *in vitro* import assays have been developed with the isolated organelle [7]. Following this approach and using the cysteine (Cys)-modifying reagent *N*-ethylmaleimide, earlier investigators concluded that functionally important Cys residues are involved in the formation of early import intermediates as well as in the import process itself [8,9]. These results were in line with independent research that showed a stimulation of import by dithiothreitol (DTT) or reduced glutathione added to the import reaction mixture [10]. A different approach demonstrated that the *in vitro* modification of Cys to yield a disulfide bridge inhibited the formation of early import intermediates and subsequent import [11]. Inhibition was relieved by DTT, thus demonstrating reversibility of the redox effect. Molecular analysis prompted the conclusion that members of the TOC machinery participated in the crosslinking reaction, namely the receptors Toc159 and Toc34 and the channel Toc75. These findings have been confirmed very recently [12]. The authors analyzed the effect on import not only of dithiol reagents, such as DTT, but also monothiols such as β-mercaptoethanol and reduced glutathione. It was concluded that, although both types of reducing agents enhanced protein import, dithiols were much more effective. Moreover, tris(2-carboxyethyl)phosphine (TCEP), a phosphine reagent that seems not to cross the chloroplast envelope and exert its effect on the outside, confirmed that

Corresponding author: Buchanan, B.B. (view@berkeley.edu).

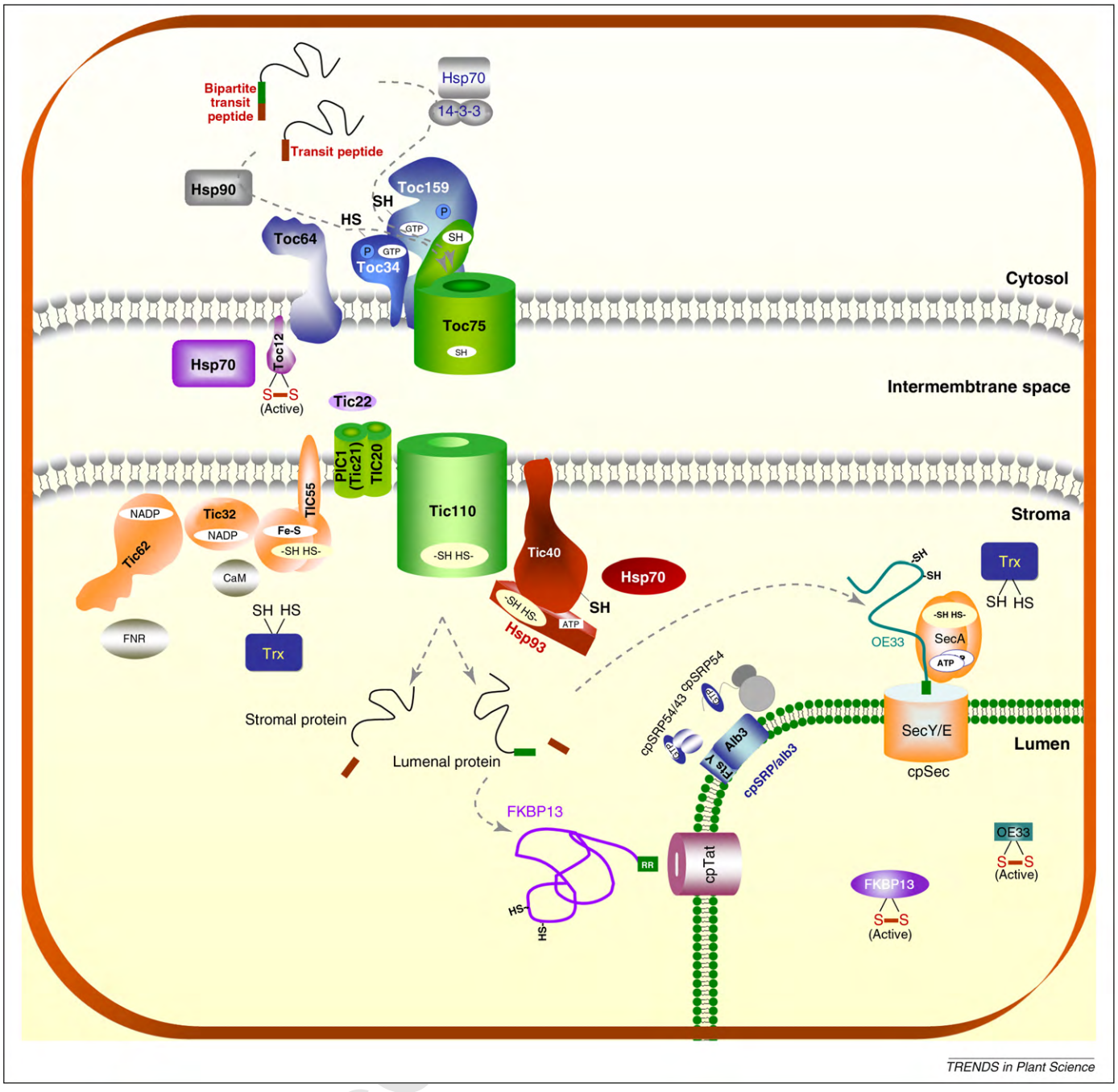

Figure 1. Schematic representation of known components of the protein import machineries in chloroplast membranes from *Pisum sativum*. Most chloroplast proteins synthesized in the cytosol contain a removable N-terminal sequence (transit peptide) for proper plastid targeting and translocation via the general import pathway (Box 1; [1,2]). According to the general view, cytoplasmic chaperones such as Hsp70/14-3-3 or Hsp90 bind to preproteins to keep them unfolded in an import competent state and guide them to the receptors (Toc159 and Toc34) of the outer membrane of the chloroplast. It has been shown that the N-terminal region alone is sufficient for successful targeting of certain proteins. After crossing the outer membrane, the preprotein passes through the inner membrane until it reaches the stroma, where the transit peptide is proteolytically removed to yield a functional protein. Stromal chaperones such as Hsp93 and/or Hsp70 participate in this latter transport stage. It has been found that hydrolysis of ATP and GTP is required for translocation of the preproteins across the two membranes of the plastid envelope. Thylakoidal proteins are further inserted or translocated through the photosynthetic membrane [43]. Lumenal proteins contain a bipartite transit peptide that is sequentially removed for further translocation across the cpSec or cpTat thylakoid translocons. Nuclear- and chloroplast-encoded thylakoid protein biogenesis is dependent on the Alb3 insertase, cpSec translocase or both. However, not all chloroplast proteins follow the general import pathway and other translocation mechanisms are currently being described (not shown). Recently, an ER-dependent pathway for chloroplast import has been described for several substrates and details of the transport mechanism are awaited with interest [50–52]. In addition, several chloroplast proteins do not contain a cleavage transit peptide and their import pathways remain largely unknown [53,54]. Some outer envelope proteins apparently do not use proteinaceous machinery for membrane insertion and others do not require the participation of the TOC primary receptors [55,56].

members of the outer translocon facing the cytosol contain Cys essential for protein import. It might well be that those subunits participate in a thiol–disulfide redox switch that governs import. By contrast, a comparison of the DTT and TCEP effects suggests that components of the inner envelope membrane might be affected.

During evolution, chloroplasts developed an intricate regulatory network based on the modification of Cys according to the changing redox status of the organelle [13]. A growing body of evidence indicates that certain components of TOC and TIC complexes have redox-active Cys with the potential to regulate protein function and,

Box 1. The general import pathway into chloroplasts

Several cytosolic factors have been invoked to facilitate the guidance of presequences to the TOC receptors and enhance the efficiency of translocation (Figure 1). The cytosolic Hsp70/14-3-3 complex might escort preproteins to the TOC GTPase receptors, Toc159 and Toc34 [57]. Another set of precursors seems to be guided by cytosolic Hsp90 that, after interaction with Toc64, delivers the precursor to Toc34 [58]. TOC GTPase receptors, whose GTPase activity is regulated by transit peptide binding, play a primary role in chloroplast targeting [3]. Although their relative contribution to preprotein recognition is a subject of intense debate, Toc159 and Toc34 probably heterodimerize in the docking stage with the transit peptide, contributing to the activation of membrane translocation. At the expense of ATP, the preprotein is transferred and transported through Toc75, a β-barrel transmembrane protein that constitutes the TOC channel [59]. Components of the chaperone system in the intermembrane space, composed of the DnaJ-like Toc12 and imsHsp70, seem to be recruited to the outer envelope by Toc64 [17,60]. Together with Tic22, a soluble TIC component in the IMS, these keep the preprotein in a competent unfolded state and coordinate the translocation to the TIC channel, probably Tic110 [19,61]. Concomitant with preprotein reception, Tic110 recruits components of the import motor (chaperone Hsp93 together with co-chaperone Tic40 and/or chaperone Hsp70) at the stromal side of the envelope [26,62]. Tic20 is also described as a protein-conducting channel. New experimental evidence obtained from a Tic20 homolog in apicomplexa suggests a regulatory role of Tic20 in protein import [63]. Functional complementation studies are in order to determine a conserved role of Tic20 in plants and apicomplexa. Another putative protein-conducting channel, Tic21, might compensate for Tic20 at different stages of leaf development [64]; an independent study has, however, classified PIC21/Tic21 as a permease [65]. Three redox-sensing auxiliary components transiently interact with the TIC core to modulate preprotein import according to the metabolic redox state of the chloroplast. Several modes of regulation affecting preprotein import efficiency have been identified: (i) modulation of the GTPase activity of the TOC receptors [66]; (ii) intra- or intermolecular covalent interactions in TOC components taking place under oxidizing conditions [11,12,15,17]; (iii) modification of TOC isoform composition according to preprotein and/or plastid type [67–70]; (iv) variation in TIC protein composition as a reflection of chloroplast metabolic redox status [33,71]; and (v) modulation of activity of TIC components by regulatory Cys [19,28] (Figure 1).

consequently, import into chloroplasts (Table 1) [14]. A recent study documents the ability of the single conserved Cys in the Toc34 family to undergo disulfide bridge formation under oxidizing conditions both *in vitro* and *in organello* [15]. The current view of receptor binding invokes a transient dimerization of receptors (Box 1), although stable dimers cannot be ruled out [16]. The formation of Toc159–Toc34 heterodimer via Cys crosslinking has been proposed but not demonstrated. Whether the receptors form regulatory disulfide bridges under physiological conditions remains to be established.

Toc12, a subunit of the TOC complex in the chloroplast intermembrane space that associates with Toc64 and Hsp70, might also be sensitive to Cys redox modification [17]. Structural prediction pointed to the proximity of two conserved Cys in the Toc12 family and point mutational analysis suggested the formation of an intramolecular disulfide bridge that regulates the chaperone activity of the protein. The authors suggested a functional relation of Toc12 with the mistargeting and arrest of certain preproteins in the IMS under light conditions [6]. However, biochemical evidence for Cys oxidation in Toc12 is still lacking as is information on redox fluctuation in the IMS as a consequence of light or changing metabolic status. To date, redox machineries equivalent to those catalyzing the oxidation of proteins in the periplasmic space of bacteria or the IMS of mitochondria have not been identified in the chloroplast envelope [18].

Evidence of a role for redox in regulating the preprotein conducting channel of the TIC complex of the inner membrane, Tic110, emerged in biochemical assays [19]. Experiments revealed a redox-active intramolecular disulfide bridge in Tic110, whose activity appears to be regulated by a stromal thioredoxin (Trx). Trx is a ubiquitous small disulfide protein that is well known to mediate redox changes in target proteins of both the chloroplast stroma and thylakoid membrane. A proteomic study suggested that an isoform of Trx is closely associated with the chloroplast inner envelope membrane [20]. Because the active disulfide of the Tic110 channel protein was found to be in the reduced state in chloroplasts isolated from darkened plants, it was concluded that regulation linked to redox status is particularly relevant during periods of oxidative stress as chloroplasts adapt to changes in the environment.

During import, Tic110 recruits the stromal motor complex, composed of soluble Hsp93 and membrane-anchored Tic40, at the envelope. Hsp93 (also known as ClpC) acts as a stromal chaperone in protein import. Interestingly, the activity of the cyanobacterial homolog of Hsp93 has also been linked to Trx [21]. The redox active disulfide(s) is yet to be identified. Earlier genetic studies showed that the expression of cyanobacterial *hsp93* is modulated by the redox state of the cell [22]. By contrast, in plants, transcript levels of the gene increased in short-day *ntrc* rosette leaves of knockout plants lacking chloroplast NADP–thioredoxin reductase with a built-in Trx [23]. Even if the chloroplast and cyanobacterial Hsp93 proteins function in different pathways and are not equally regulated, they are both likely to act as chaperones [24]. Sequence analysis of cyanobacterial Hsp93 revealed a single conserved Cys residue in this chaperone family, suggesting that, if linked to Trx, the regulatory disulfide would be inter- rather than intramolecular [14]. Structural homology modeling of the plant Hsp93 isoform is consistent with the formation of an intramolecular disulfide bridge due to the presence of a second conserved Cys. According to the proposed model, the conserved Cys would be located in the nucleotide-binding motif, suggesting that the disulfide bridge could have either a structural role or function in regulating ATPase activity or in forming the ternary Hsp93–Tic40–Tic110 complex. Further experiments are needed to confirm the formation of a disulfide bridge in chloroplast Hsp93 and to evaluate its functional significance.

It is noteworthy that a mixed disulfide bridge developed between Tic110 and the co-chaperone, Tic40, under oxidizing conditions [25]. Tic40 is a membrane protein with two stroma-exposed hydrophilic domains, one of which preferably interacts with Tic110 and the other with Hsp93 [26]. However, the only conserved Cys in Tic40 capable of oxidative crosslinking with Tic110 is found in the most C-terminal stromal domain, which is indeed predicted to associate with Hsp93 [27]. Refinements in the association analyses in the ternary complex between the import motor

Table 1. Redox properties of proteins involved in chloroplast protein transport (Figure 1)

Subunit	Function	Redox element	Refs
Toc75	Channel-forming protein at the outer envelope membrane.	Participates in disulfide crosslinking with TOC GTPases under oxidizing environment. Contains conserved cysteines at the N-terminal POTRA domain and at the beginning of the so-called motif 3, an active membrane element in transport.	[11,12,14]
Toc159 and Toc34	GTPase receptors of the TOC complex.	Involved in intermolecular disulfide bridge formation. Contain one conserved cysteine in the GTPase domain involved in dimer formation.	[11,12,14,15]
Toc12	DnaJ-like co-chaperone at the IMS.	Contains a CxGxxC motif at the DnaJ domain involved in a functional important intramolecular disulfide bridge.	[17]
Tic110	Channel-forming protein at the inner envelope membrane.	Forms intramolecular disulfide bridge under oxidizing conditions. Activity probably regulated by stromal Trx.	[19]
Hsp93	Chaperone of the TIC protein import motor.	The cyanobacterial ortholog has been linked to Trx. Computational analysis points to conserved cysteine residues in plant Hsp93 with potential for intramolecular disulfide bridge formation.	[14,21]
Tic62	Redox sensor of the TIC complex.	Bimodular protein. The N-terminal domain belongs to the short-chain dehydrogenase family and contains an NADP(H)-binding motif; the C-terminal domain belongs specifically and interacts with FNR. Dynamic binding to the TIC complex according to the metabolic redox state of the chloroplast.	[30,33,34]
Tic32	Redox sensor of the TIC complex.	Bimodular protein. The N-terminal domain belongs to the short-chain dehydrogenase family and contains an NADP(H)-binding motif; the C-terminal tail interacts specifically with stromal calmodulin. Dynamic binding to the TIC complex according to the metabolic redox state of the chloroplast.	[31,33]
Tic55	Redox sensor of the TIC complex.	A membrane protein that exposes to the stroma a Rieske domain, a catalytic domain that coordinates a mononuclear non-heme Fe(II) and a hydrophilic CxxC domain of unknown function. Activity probably regulated by stromal Trx.	[28,29]

Hsp93–Tic40 and the Tic110 protein channel are obviously in order.

Tic55, another member of the TIC complex, has also recently been linked to Trx [28]. Tic55 is a membrane protein with three hydrophilic domains exposed to the stroma: a Rieske domain, a catalytic domain with a mononuclear non-heme Fe(II) active site and a domain of unknown function that carries a conserved CxxC motif [29]. The specific contribution of Tic55 to preprotein import remains elusive. It has been proposed that the protein might regulate import via its function in electron transfer or as a redox sensor. In a recent study, the authors captured Tic55 from an inner envelope preparation on an affinity column using a mutated Trx-*f* as bait [28]. Sequence analysis showed that Tic55 contains several conserved Cys that could form an oxidant-induced disulfide bridge in the regulation of protein import [14,28].

Effects of the relative NADPH/NADPH ratio on protein import: the TIC redox translocon

Molecular studies on the import machinery of the chloroplast envelope revealed that the Tic62 and Tic32 subunits contain binding motifs for redox-active cofactors that possibly affect protein import [30,31] (Table 1). Together with the Rieske protein Tic55, these components have been proposed to form the "redox regulon" of the TIC complex [32].

Tic32 was found to be extrinsically attached to the inner envelope membrane and to contain a functional NAD(P)-binding domain characteristic of the family of short-chain dehydrogenases [33,34]. Biochemical analysis demonstrated that its interaction with the translocon is regulated by the chloroplast NADPH/NADP ratio: more oxidizing conditions (lower NADPH/NADP ratio) favor association of Tic32 with the TIC translocon, whereas more reducing conditions (higher NADPH/NADP ratio) have the opposite effect and lower the affinity of the Tic32 subunits for the core of the TIC complex. Tic32 members also contain a C-terminal extension that specifically binds chloroplast calmodulin (CaM) in a manner that is enhanced under oxidizing conditions. Moreover, *in vitro* protein import experiments have demonstrated that the translocation of certain substrates is impeded by CaM inhibitors or calcium ionophores [35]. Considering that the calcium level in chloroplasts fluctuates in a light/dark-dependent manner, it seems plausible that metabolic factors such as CaM modulate the interaction of Tic32 with the core of the TIC complex.

The interaction of Tic62, another short-chain dehydrogenase family member, with the TIC core complex has also been found to depend on the metabolic NADPH/NADP ratio. Like Tic32, the affinity of Tic62 for the TIC complex is influenced by chloroplast redox status. Association is increased with lower NADPH/NADP ratios, conditions under which Tic62 shows higher affinity for FNR, a second specific interaction partner [33,34]. Furthermore, Tic62 can reversibly shuttle between the envelope membrane and the stroma as a function of the relative NADPH/NADP ratio. Under more reducing conditions, Tic62 is partially released to the stroma.

The molecular features of Tic62 and Tic32 were recently put in the context of protein import as a reflection of their modification by the chloroplast NADPH/NADP ratio [12]. These authors observed that perturbing this ratio altered the import efficiency of some but not all proteins that follow the general import pathway (Box 1). The import of other proteins taken up by import mechanisms apparently unrelated to TOC and TIC was also not affected by this ratio. It was suggested that the interaction of Tic62 and Tic32 with TIC might account for the observed effect. The recruitment of the redox regulated subunits to the translocon might, therefore, result in different TIC subcomplexes in which Tic62 and Tic32 couple the redox state of chloroplasts to nucleotide binding and thereby alter import.

Despite the apparent importance of redox in regulating preprotein import into chloroplasts, genetic studies have revealed that none of the genes encoding proteins of the "redox regulon" seems essential for plant viability under standard conditions. The function of the encoded proteins might, therefore, lie either in fine-tuning of transport of several substrates or in enabling chloroplasts to adjust to a change in environmental conditions [36–38].

Redox control transport and folding in the thylakoidal lumen

Recently, it was found that 40% of the proteins residing in the lumen are linked to Trx or a related redox-active protein, thus underscoring the importance of redox-linked regulation in this compartment [39–42]. It is of interest that lumenal proteins have two options for import, the cpTat and cpSec pathways, both adapted from bacteria [43] (Figure 1). In the cpTat pathway, prefolded proteins are transported into the lumen after entering and achieving their correct conformation in the stroma. By contrast, cpSec translocates unfolded units in an ATP-dependent manner with folding occurring after translocation. The SecA subunit has been recently linked to Trx in cyanobacteria [21]. Although further biochemical experiments are in order, it appears that the disulfide bridge in SecA is redox-active, thus offering a role for redox in regulating thylakoid protein transport from the stromal side in both plants and cyanobacteria [14]. Unlike their stromal counterparts, redox-active proteins of the lumen examined so far are active when oxidized [44]. However, there appears to be no correlation between redox activity of transported proteins and mode of import. For example, although both are redox-active, FKBP13 and OE33 are translocated by different pathways (cpTat and cpSec, respectively) [45,46]. Based on current knowledge, it seems that selection of a particular translocation pathway is determined by chaperone and, possibly, by redox requirements of proteins harboring active Cys residues. The situation is reminiscent of the sulfhydryl oxidation system functional in the IMS of mitochondria and the periplasmic space of bacteria [18]. In both cases, specialized machineries catalyze the oxidative folding of proteins via a disulfide relay system involving coordination between soluble regulatory components and the transmembrane reductant transfer system. However, in contrast to these systems, little is known about the redox properties and regulatory mechanisms active in the lumen and new possibilities are just beginning to emerge [14,47–49].

Looking to the future

Recent observations have added new insight to the complex and highly regulated process of preprotein import into chloroplasts. On one hand, change in the organization of the TOC GTPase receptor isoforms results in different TOC complexes, each apparently with affinity for a particular class of preproteins. On the other hand, the oxidative cross linking of subunits of the outer envelope translocon influences the transport efficiency of a subset of preproteins when *in vitro* protein import experiments are performed in isolated chloroplasts. The TIC machinery is also subjected to dynamic changes in composition and activity through its response to regulatory redox signals from the chloroplast. Thus, although the relative NADPH/NADP ratio typically remains stable in chloroplasts due to photosynthesis during the day and to the oxidative pentose phosphate pathway at night, the equilibrium might be significantly altered as a result of changes in metabolism, particularly during adaptation to oxidative stress conditions. Current evidence suggests that the activity of several TIC subunits is linked to redox by way of Trx, particularly in response to oxidative events.

The unveiling of a role for redox in regulating and coordinating chloroplast protein import raises new questions. For example, biochemical mechanisms that link metabolic redox state of the cell to the reversible association of redox-sensing subunits with the TIC complex are still poorly understood. Further research is also needed to elucidate specific redox modifications in translocon subunits and their relation to import activity. The endogenous substrates for the redox sensor components await identification as do participants that transmit redox information to the translocon machinery. According to present evidence, Trx could communicate a redox signal to Tic110, Hsp93 and Tic55, probably during environmental adaptation. The Cys residues functional in these and other participating proteins need to be identified and characterized. A related unknown concern is the consequence of redox modification on protein activity. Questions similar to these apply to the TOC subunits that contain redox-active Cys, some exposed to the cytosol. Finally, a major area for future development concerns the mechanism by which redox-active proteins are transported and folded following their entrance into the thylakoid lumen via the cpTat and cpSec (Box 2) [43].

Acknowledgements

M.B. acknowledges support from the Spanish National Research Council (200940I204). Support from the Deutsche Forschungsgemeinschaft (SFB 594 and CIPSM) to J.S. is gratefully acknowledged. B.B.B. gratefully acknowledges receipt of a Research Award from the Alexander von Humboldt Foundation and ongoing support from the California Agricultural Experiment Station.

References

1 Balsera, M. *et al.* (2009) Protein import machineries in endosymbiotic organelles. *Cell Mol. Life Sci.* 66, 1903–1923

2 Jarvis, P. (2008) Targeting of nucleus-encoded proteins to chloroplasts in plants. *New Phytol.* 179, 257–285

3 Kessler, F. and Schnell, D. (2009) Chloroplast biogenesis: diversity and regulation of the protein import apparatus. *Curr. Opin. Cell Biol.* 21, 494–500
4 Woodson, J.D. and Chory, J. (2008) Coordination of gene expression between organellar and nuclear genomes. *Nat. Rev. Genet.* 9, 383–395
5 Kakizaki, T. *et al.* (2009) Coordination of plastid protein import and nuclear gene expression by plastid-to-nucleus retrograde signaling. *Plant Physiol.* 151, 1339–1353
6 Hirohashi, T. *et al.* (2001) Maize non-photosynthetic ferredoxin precursor is mis-sorted to the intermembrane space of chloroplasts in the presence of light. *Plant Physiol.* 125, 2154–2163
7 Bolter, B. and Soll, J. (2007) Import of plastid precursor proteins into pea chloroplasts. *Methods Mol. Biol.* 390, 195–206
8 Friedman, A.L. and Keegstra, K. (1989) Chloroplast protein import – quantitative-analysis of precursor binding. *Plant Physiol.* 89, 993–999
9 Row, P.E. and Gray, J.C. (2001) The effect of amino acid-modifying reagents on chloroplast protein import and the formation of early import intermediates. *J. Exp. Bot.* 52, 57–66
10 Pilon, M. *et al.* (1992) New insights into the import mechanism of the ferredoxin precursor into chloroplasts. *J. Biol. Chem.* 267, 2548–2556
11 Seedorf, M. and Soll, J. (1995) Copper chloride, an inhibitor of protein import into chloroplasts. *FEBS Lett.* 367, 19–22
12 Stengel, A. *et al.* (2009) Preprotein import into chloroplasts via the Toc and the Tic complexes is regulated by redox signals in *Pisum sativum*. *Mol. Plant* 2, 1181–1197
13 Schürmann, P. and Buchanan, B.B. (2008) The ferredoxin/thioredoxin system of oxygenic photosynthesis. *Antioxid. Redox Signal.* 10, 1235–1273
14 Balsera, M. *et al.* (2009) Oxidative stress and redox regulation in plants. In *Protein Import in Chloroplasts: An Emerging Regulatory Role for Redox* (Jacquot, J.P., ed.), pp. 277–332, Elsevier
15 Lee, J.H. *et al.* (2009) Toc receptor dimerization participates in the initiation of membrane translocation during protein import into chloroplasts. *J. Biol. Chem.* 284, 31130–31141
16 Rahim, G. *et al.* (2009) *In vivo* interaction between *at*Toc33 and *at*Toc159 GTP-binding domains demonstrated in a plant split-ubiquitin system. *J. Exp. Bot.* 60, 257–267
17 Becker, T. *et al.* (2004) Toc12, a novel subunit of the intermembrane space preprotein translocon of chloroplasts. *Mol. Biol. Cell* 15, 5130–5144
18 Herrmann, J.M. *et al.* (2009) Thiol oxidation in bacteria, mitochondria and chloroplasts: common principles but three unrelated machineries? *Biochim. Biophys. Acta* 1793, 71–77
19 Balsera, M. *et al.* (2009) Characterization of TIC110, a channel-forming protein at the inner envelope membrane of chloroplasts, unveils a response to Ca^{2+} and a stromal regulatory disulfide bridge. *J. Biol. Chem.* 284, 2603–2616
20 Ferro, M. *et al.* (2003) Proteomics of the chloroplast envelope membranes from *Arabidopsis thaliana*. *Mol. Cell Proteomics* 2, 325–345
21 Mata-Cabana, A. *et al.* (2007) Membrane proteins from the cyanobacterium *Synechocystis sp*. PCC 6803 interacting with thioredoxin. *Proteomics* 7, 3953–3963
22 Hihara, Y. *et al.* (2003) DNA microarray analysis of redox-responsive genes in the genome of the cyanobacterium *Synechocystis* sp strain PCC 6803. *J. Bacteriol.* 185, 1719–1725
23 Lepistö, A. *et al.* (2009) Chloroplast NADPH-thioredoxin reductase interacts with photoperiodic development in *Arabidopsis*. *Plant Physiol.* 149, 1261–1276
24 Andersson, F.I. *et al.* (2006) Cyanobacterial ClpC/HSP100 protein displays intrinsic chaperone activity. *J. Biol. Chem.* 281, 5468–5475
25 Stahl, T. *et al.* (1999) Tic40, a new "old" subunit of the chloroplast protein import translocon. *J. Biol. Chem.* 274, 37467–37472
26 Kovacheva, S. *et al.* (2005) *In vivo* studies on the roles of Tic110, Tic40 and Hsp93 during chloroplast protein import. *Plant J.* 41, 412–428
27 Bedard, J. *et al.* (2007) Functional similarity between the chloroplast translocon component, Tic40, and the human co-chaperone, Hsp70-interacting protein (Hip). *J. Biol. Chem.* 282, 21404–21414
28 Bartsch, S. *et al.* (2008) Three thioredoxin targets in the inner envelope membrane of chloroplasts function in protein import and chlorophyll metabolism. *Proc. Natl. Acad. Sci. U. S. A.* 105, 4933–4938
29 Caliebe, A. *et al.* (1997) The chloroplastic protein import machinery contains a Rieske-type iron-sulfur cluster and a mononuclear iron-binding protein. *EMBO J.* 16, 7342–7350
30 Kuchler, M. *et al.* (2002) Protein import into chloroplasts involves redox-regulated proteins. *EMBO J.* 21, 6136–6145
31 Hormann, F. *et al.* (2004) Tic32, an essential component in chloroplast biogenesis. *J. Biol. Chem.* 279, 34756–34762
32 Stengel, A. *et al.* (2007) Protein import into chloroplasts: new aspects of a well-known topic. *Biol. Chem.* 388, 765–772
33 Chigri, F. *et al.* (2006) Calcium regulation of chloroplast protein translocation is mediated by calmodulin binding to Tic32. *Proc. Natl. Acad. Sci. U. S. A.* 103, 16051–16056
34 Stengel, A. *et al.* (2008) TIC62 redox-regulated translocon composition and dynamics. *J. Biol. Chem.* 283, 6656–6667
35 Chigri, F. *et al.* (2005) Calcium regulation of chloroplast protein import. *Plant J.* 42, 821–831
36 Hormann, F. *et al.* (2004) Tic32, an essential component in chloroplast biogenesis. *J. Biol. Chem.* 279, 34756–34762 Q4
37 Boij, P. *et al.* (2009) In vivo studies on the roles of Tic55-related proteins in chloroplast protein import in *Arabidopsis thaliana*. *Mol. Plant* 2, 1397–1409
38 Benz, J.P. *et al.* (2009) *Arabidopsis* Tic62 and ferredoxin-NADP(H) oxidoreductase form light-regulated complexes that are integrated into the chloroplast redox poise. *Plant Cell* 21, 3965–3983
39 Gopalan, G. *et al.* (2004) Structural analysis uncovers a role for redox in regulating FKBP13, an immunophilin of the chloroplast thylakoid lumen. *Proc. Natl. Acad. Sci. U. S. A.* 101, 13945–13950
40 Hall, M. *et al.* (2008) Thioredoxin interactions of the chloroplast lumen of Arabidopsis thaliana indicate a redox regulation of the Xanthophyll cycle. In *Photosynthesis. Energy from the Sun, p* (Allen, J.F. *et al.*, eds), pp. 099–1102, Netherlands, Springer
41 Marchand, C. *et al.* (2006) Comparative proteomic approaches for the isolation of proteins interacting with thioredoxin. *Proteomics* 6, 6528–6537
42 Hall, M. *et al.* (2010) Thioredoxin targets of the plant chloroplast lumen and their implications for plastid function. *Proteomics* 10, 987–1001
43 Aldridge, C. *et al.* (2009) Protein transport in organelles: protein transport into and across the thylakoid membrane. *FEBS J.* 276, 1177–1186
44 Buchanan, B.B. and Luan, S. (2005) Redox regulation in the chloroplast thylakoid lumen: a new frontier in photosynthesis research. *J. Exp. Bot.* 56, 1439–1447
45 Cline, K. *et al.* (1992) Protein-specific energy-requirements for protein-transport across or into thylakoid membranes – 2 lumenal proteins are transported in the absence of ATP. *J. Biol. Chem.* 267, 2688–2696
46 Gupta, R. *et al.* (2002) A chloroplast FKBP interacts with and affects the accumulation of Rieske subunit of cytochrome bf complex. *Proc. Natl. Acad. Sci. U. S. A.* 99, 15806–15811
47 Motohashi, K. and Hisabori, T. (2006) HCF164 receives reducing equivalents from stromal thioredoxin across the thylakoid membrane and mediates reduction of target proteins in the thylakoid lumen. *J. Biol. Chem.* 281, 35039–35047
48 Marcaida, M.J. *et al.* (2006) Structure of cytochrome c(6A), a novel dithio-cytochrome of *Arabidopsis thaliana*, and its reactivity with plastocyanin: implications for function. *J. Mol. Biol.* 360, 968–977
49 Singh, A.K. *et al.* (2008) Identification of an atypical membrane protein involved in the formation of protein disulfide bonds in oxygenic photosynthetic organisms. *J. Biol. Chem.* 283, 15762–15770
50 Chen, M.H. *et al.* (2004) Signal peptide-dependent targeting of a rice alpha-amylase and cargo proteins to plastids and extracellular compartments of plant cells. *Plant Physiol.* 135, 1367–1377
51 Villarejo, A. *et al.* (2005) Evidence for a protein transported through the secretory pathway en route to the higher plant chloroplast. *Nat. Cell Biol.* 7, 1224–1231
52 Nanjo, Y. *et al.* (2006) Rice plastidial N-glycosylated nucleotide pyrophosphatase/phosphodiesterase is transported from the ER-Golgi to the chloroplast through the secretory pathway. *Plant Cell* 18, 2582–2592
53 Miras, S. *et al.* (2002) Non-canonical transit peptide for import into the chloroplast. *J. Biol. Chem.* 277, 47770–47778
54 Nada, A. and Soll, J. (2004) Inner envelope protein 32 is imported into chloroplasts by a novel pathway. *J. Cell Sci.* 117, 3975–3982

55 Hofmann, N.R. and Theg, S.M. (2005) Chloroplast outer membrane protein targeting and insertion. *Trends Plant Sci.* 10, 450–457
Q5 56 Inoue, K. (2007) The chloroplast outer envelope membrane: the edge of light and excitement. *J. Integr. Plant Biol.* 49, 1100–1111
57 May, T. and Soll, J. (2000) 14-3-3 proteins form a guidance complex with chloroplast precursor proteins in plants. *Plant Cell* 12, 53–63
58 Qbadou, S. *et al.* (2006) The molecular chaperone Hsp90 delivers precursor proteins to the chloroplast import receptor Toc64. *EMBO J.* 25, 1836–1847
59 Hinnah, S.C. *et al.* (2002) The chloroplast protein import channel Toc75: pore properties and interaction with transit peptides. *Biophys. J.* 83, 899–911
60 Qbadou, S. *et al.* (2007) Toc64 – a preprotein-receptor at the outer membrane with bipartide function. *J. Mol. Biol.* 367, 1330–1346
61 Heins, L. *et al.* (2002) The preprotein conducting channel at the inner envelope membrane of plastids. *EMBO J.* 21, 2616–2625
62 Shi, L.X. and Theg, S.M. (2010) A stromal heat shock protein 70 system functions in protein import into chloroplasts in the moss *Physcomitrella patens*. *Plant Cell* 22, 205–220
63 van Dooren, G.G. *et al.* (2008) *Toxoplasma gondii* Tic20 is essential for apicoplast protein import. *Proc. Natl. Acad. Sci. U. S. A.* 105, 13574–13579
64 Teng, Y.S. *et al.* (2006) Tic21 is an essential translocon component for protein translocation across the chloroplast inner envelope membrane. *Plant Cell* 18, 2247–2257
65 Duy, D. *et al.* (2007) PIC1, an ancient permease in *Arabidopsis* chloroplasts, mediates iron transport. *Plant Cell* 19, 986–1006
66 Reddick, L.E. *et al.* (2007) In vitro comparative kinetic analysis of the chloroplast Toc GTPases. *J. Biol. Chem.* 282, 11410–11426
67 Bauer, J. *et al.* (2000) The major protein import receptor of plastids is essential for chloroplast biogenesis. *Nature* 403, 203–207
68 Kubis, S. *et al.* (2003) The Arabidopsis *ppi1* mutant is specifically defective in the expression, chloroplast import, and accumulation of photosynthetic proteins. *Plant Cell* 15, 1859–1871
69 Ivanova, Y. *et al.* (2004) Members of the Toc159 import receptor family represent distinct pathways for protein targeting to plastids. *Mol. Biol. Cell* 15, 3379–3392
70 Jarvis, P. *et al.* (1998) An Arabidopsis mutant defective in the plastid general protein import apparatus. *Science* 282, 100–103
71 Brautigam, A. *et al.* (2008) Comparative proteomics of chloroplast envelopes from C-3 and C-4 plants reveals specific adaptations of the plastid envelope to C-4 photosynthesis and candidate proteins required for maintaining C-4 metabolite fluxes. *Plant Physiol.* 148, 568–579