Biochimica et Biophysica Acta 1847 (2015) 949–956

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Review

Redox meets protein trafficking☆

Bettina Bölter [a,b,1], Jürgen Soll [a,b,*], Serena Schwenkert [a,b]

[a] Department Biologie I-Botanik, Ludwig-Maximilians-Universität, Großhadernerstr. 2-4, D-82152 Planegg-Martinsried, Germany
[b] Munich Center for Integrated Protein Science CiPSM, Ludwig-Maximilians-Universität, Feodor-Lynen-Strasse 25, D-81377 Munich, Germany

ARTICLE INFO

Article history:
Received 25 November 2014
Received in revised form 16 January 2015
Accepted 19 January 2015
Available online 24 January 2015

Keywords:
Chloroplasts
Protein transport
Redox regulation

ABSTRACT

After the engulfment of two prokaryotic organisms, the thus emerged eukaryotic cell needed to establish means of communication and signaling to properly integrate the acquired organelles into its metabolism. Regulatory mechanisms had to evolve to ensure that chloroplasts and mitochondria smoothly function in accordance with all other cellular processes. One essential process is the post-translational import of nuclear encoded organellar proteins, which needs to be adapted according to the requirements of the plant. The demand for protein import is constantly changing depending on varying environmental conditions, as well as external and internal stimuli or different developmental stages. Apart from long-term regulatory mechanisms such as transcriptional/translation control, possibilities for short-term acclimation are mandatory. To this end, protein import is integrated into the cellular redox network, utilizing the recognition of signals from within the organelles and modifying the efficiency of the translocon complexes. Thereby, cellular requirements can be communicated throughout the whole organism. This article is part of a Special Issue entitled: Chloroplast Biogenesis

1. Introduction

Plant cells harbor two types of essential organelles, chloroplasts and mitochondria, which both have been acquired by endosymbiotic engulfment of once free living prokaryotes [1]. Integration of these organelles into the cell metabolism required the establishment of communication and transport pathways between host cell and endosymbionts. Only few of the genes derived from the bacterial ancestors are still transcribed and translated within the organelles [2–4]. During evolution most genes have been translocated to the nucleus and consequently the majority of proteins have to be directed, sorted and imported post-translationally into their destined cellular compartments [5]. To facilitate translocation of cytosolic synthesized preproteins across the organellar double membranes, both, plastids and mitochondria, were equipped with multi-protein translocon complexes, providing energy consuming but efficient and adaptable translocation pathways. To this end, the cell partly made use of existing mechanisms from the prokaryotes, partly new pathways evolved over time. Thus, the cellular complex communication and signaling network represents a mosaic of prokaryotic and eukaryotic features. One of the adopted ancient mechanisms is the communication and regulation by reduction–oxidation (redox) reactions, which was already present in bacteria at the occurrence of the endosymbiotic events [6,7]. Consequently, a plethora of processes and pathways is regulated by this chemical switch between reduced and oxidized states of metabolites and proteins. This includes the photosynthetic machinery of chloroplasts as well as the respiratory chain in mitochondria [8]. Chloroplast photosynthesis produces reduction equivalents in the form of NADPH as well as – potentially toxic – reactive oxygen species, which are directly involved in signaling processes that lead to acclimation of the whole organism in response to environmental conditions such as light intensity or temperature. NADPH itself in relation to its oxidized counterpart $NADP^+$ can act as a signaling means for communicating the stromal redox state to the surrounding cell [9], e.g. by the malate valve [10]. By providing electrons to various enzymes via specific transfer proteins like FNR (Ferredoxin-NADP(H)-oxidoreductase) or FTR (Ferredoxin–thioredoxin-reductase) NADPH influences other regulatory pathways such as the thioredoxin system [11]. This small thiol protein de-/activates key enzymes of many essential metabolic pathways by transferring or accepting electrons and thereby forming or dissolving disulfide bridges within its target proteins [12]. Very prominent examples for this manner of regulation are the Calvin–Benson-Cycle and the oxidative pentose phosphate cycle [13]. Another "pool" for electrons besides the Trx system set free by the oxygen evolving complex by splitting water at the start of the photosynthetic electron transport chain is the plastoquinone pool (PQ), in which the reduction state itself is a critical regulator for adapting photosynthetic activity [14,15]. To ensure proper functioning of all organellar processes under varying conditions it is of utmost importance to coordinate the post-translational import of preproteins in response to the plastidial and mitochondrial requirements, respectively. Therefore, it is not only feasible but rather inevitable to integrate the regulation of organellar protein import into the cellular redox network. In this review we are aiming to summarize the current knowledge of the

☆ This article is part of a Special Issue entitled: Chloroplast Biogenesis.

* Corresponding author. Tel.: +49 089 2180 74750
E-mail address: soll@lmu.de (J. Soll).
[1] Fax: +49 089 218074752.

http://dx.doi.org/10.1016/j.bbabio.2015.01.010
0005-2728/

impact of redox-mediated processes on protein import into chloroplasts and mitochondria. We will highlight differences and similarities in these systems and extend our view to oxidative protein folding in bacteria.

2. Redox-mediated processes in bacteria and thylakoid lumen

The bacterial disulfide bond (Dsb) system ensures proper protein folding in the periplasm and the involved factors have been well characterized over the past decades. The initially identified and central component of this system is DsbA, a soluble protein with thioredoxin-like features. DsbA contains a highly redox-active CXXC motif and can bind to its substrates during their translocation into the periplasm [16,17]. In turn, DsbA is re-oxidized by DsbB, which is located in the inner cytoplasmic membrane exposing two loops with cysteine pairs into the periplasm. DsbB binds a ubiquinone molecule, thereby shuttling electrons to the cytochrome c oxidase and to oxygen [18,19]. During the folding process many proteins require more than one disulfide bond to attain their native conformation. However, DsbA connects the cysteines during translocation and thus the formation of non-native bonds is a frequent occurrence. Consequently, the disulfides must be isomerized until the protein adopts its native conformation. The isomerization is initiated by DsbC, a thioredoxin-like protein related to DsbA, also containing a CXXC redox-active motif and promoting mixed disulfide bonds with the falsely paired substrate thiols [20,21]. DsbG, another thioredoxin related protein, can also function as an oxidoreductase, but mainly seems to be involved in protecting formed thiol bonds from oxidation [22,23]. A fourth protein, DsbD, comes into play to oxidize DsbC. DsbD is an integral membrane protein with two exposed periplasmic domains. Redox-active cysteines seem to be able to accept electrons directly from thioredoxin in the cytoplasm and transfer them to the periplasmic domain [24,25]. This reveals a sophisticated mechanism of shuttling electrons between membranes, which is yet not fully elucidated (see Fig. 1, left panel).

Surprisingly, no homologue for DsbB is found in some bacterial groups including Actinobacteria and Cyanobacteria, as well as in higher plant chloroplasts. Yet, it was found that these bacteria seem to contain a homologue of the eukaryotic vitamin K epoxide reductase (VKOR) fused to DsbA or a thioredoxin. In eukaryotes VKOR is localized to the endoplasmic reticulum and is required to maintain the quinone vitamin K in a reduced state [26]. The cyanobacterial homologue of VKOR, SynDsaAB, is vital for optimal photoautotrophic growth and can complement *Escherichia coli* strains deficient in disulfide bond formation [27].

Recently, several examples have shown that an oxidative folding mechanism also exists in the lumen of thylakoid membranes, a chloroplast sub-compartment which is derived from the bacterial periplasm [28]. Disulfide bonds have been analyzed in the luminal protein FKBP13 [29], a peptidyl-prolyl cis-trans isomerase, which is known to catalyze protein folding by activating isomerization of proline residues [30]. Interestingly, the enzymatic activity of FKBP was shown to be activated by oxidation, in contrast to stromal FKBP isoforms which are activated upon reduction [31]. Moreover, FKBP20-2, a luminal photosystem II assembly factor, contains a disulfide bridge, which is however not required for activity and may represent a structural feature [32]. Intriguingly, a lumen localized protein with thiol-oxidizing properties has been identified recently. The protein, LTO1 (Lumen Thiol Oxidoreductase 1) or AtVKOR-DsbA, is a homologue of the cyanobacterial fusion protein SynDsaAB and likewise has properties of both enzymes, DsbA and DsbB. As expected, it can complement for both proteins in *E. coli*. Although LTO1 is dispensable for photoautotrophic growth, mutant plants are strongly retarded in growth and especially the accumulation of photosystem II (PSII) was found to be reduced [33,34]. The thioredoxin like domain of LTO1 was shown to interact with PsbO, a PSII subunit of the water-splitting manganese cluster, and to introduce a disulfide bond in PsbO in vitro [33]. Recently, more potential substrates of LTO1 have been identified linking LTO1 to ROS homeostasis [35] and to ABA-mediated stress response [36]. Thiol-oxidation in the lumen may be especially important considering the reducing environment due to high ascorbate levels [37] (see Fig. 1, right panel).

In addition to the thiol-bond forming enzymes the chloroplast lumen also comprises thiol-reducing enzymes, with the ability to transfer reducing power from the stroma into the lumen, which are related to the bacterial DsbD-family [38]. Two such proteins in *Arabidopsis* are CCDA, a thiol-disulfide oxidoreductase, and HCF164, a membrane anchored thioredoxin-like protein. Mutants of both are deficient in cytochrome b_6f assembly, indicating their functioning along the same pathway [39–41]. Studies with the *Chlamydomonas* protein CCS5, which is functionally equivalent to Hcf164, suggest that CCS5/HCF164 function in the conversion of apo- to holocytochrome *f* [42]. CCDA and HCF164 may therefore cooperate in transferring electrons across the thylakoid membrane to reduce luminal substrate proteins (see Fig. 1, right panel).

3. Organellar import

As plants are sessile organisms they have to adapt their protein homeostasis rapidly – not only in response to successive developmental stages, but also to external, constantly changing environmental stimuli. To allow the regulation of organellar functioning, the import of proteins can be fine-tuned on various levels, including redox-mediated effects inside and outside chloroplasts and mitochondria, phosphorylation events or alteration of the composition of the translocon complexes.

4. The disulfide relay in the intermembrane space of mitochondria

A number of conserved pathways are operating to target proteins into the mitochondrial matrix, the intermembrane space (IMS) or directly to the outer and inner membranes. The general import pathway is conserved in all eukaryotes and uses the translocase of the outer mitochondrial membrane (TOM) and the translocase of the inner mitochondrial membrane (TIM), including Tim17 and Tim23, in the inner membrane to facilitate translocation of proteins into the matrix. Proteins which are retained in the IMS require a cooperation of the TOM and the mitochondrial IMS assembly (MIA) machinery, in which import and oxidative protein folding act in concert. Carrier proteins, residing in the inner membrane, utilize the TOM complex and the inner membrane translocase TIM22, whereas outer membrane proteins are inserted with the help of TOM and the sorting and assembly machinery (SAM) as well as small TIM proteins [43]. Although several components such as the channel protein Tom40 are highly conserved among various eukaryotic lineages, other components have evolved in a convergent manner, an example being the receptor protein Tom20, the function of which is similar, but the plant and yeast or mammalian counterparts are not phylogenetically related [44]. Approximately 50–100 proteins are localized to the IMS in mitochondria, which have various essential functions ranging from metabolite and lipid transport to communication across the membranes and apoptosis [45]. Most soluble IMS proteins lack typical N-terminal, cleavable signal peptides and their transport mechanism is clearly distinguished from matrix proteins [46,47]. Efficient import and retention of many of these proteins relies on the so-called 'disulfide relay system' [48]. Interestingly, well-studied IMS proteins such as Ccs1, Sco1 [49], Cox17 [50] and the small Tim proteins [51–53] share a helix–loop–helix structure and harbor conserved cysteine residues appearing as either twin-CX_3C or twin-CX_9C motifs.

The two central and essential components of the disulfide relay system in mitochondria of mammals and yeast are the oxidoreductase Mia40 [54,55], which acts as a redox-activated intramitochondrial receptor, and the sulfhydryl oxidase Erv1 [56,57]. Reduced and unfolded IMS proteins are translocated across the outer mitochondrial membrane via the TOM complex and are subsequently recognized by Mia40. Similar to its substrates, Mia40 harbors a helix–loop–helix structure towards the C-terminus, the helices of which are connected by two stabilizing

disulfide bonds [58–60]. These two helices provide a hydrophobic binding groove for the substrate proteins, which are recognized via MISS or ITS (mitochondrial IMS-sorting signal or IMS-targeting signal) sequences [61,62]. The N-terminal part of Mia40 comprises the redox-active CPS motif, whereby a mixed thiol-bond with the substrate protein can be formed. Subsequently, an intramolecular disulfide bond is formed in the substrate protein upon release of Mia40. Neither the substrate protein bound to Mia40 nor the released, but oxidized and folded protein, is able to travel back into the cytosol. Mia40 therefore not only acts in oxidative protein folding but also assists the translocation process. The second important component of the disulfide relay system, Erv1 serves to re-oxidize the CPC motif in Mia40. Erv1 acts as dimer and contains an N-terminal shuttle domain and a C-terminal FAD domain, both comprising a conserved CXXC motif [63–65]. To re-oxidize Mia40, the unstructured shuttle domain specifically interacts with Mia40, initiating an electron transfer chain. After reduction of the N-domain, electrons are transferred to the CXXC motif within the FAD binding domain, which can then be passed on to the FAD cofactor from where the electrons are further transferred to cytochrome c and the respiratory chain or directly to oxygen as a final acceptor [66–68] (see Fig. 1, middle panel).

Although all mitochondria originate from the same endosymbiotic event, several components of the import machinery have evolved differently and are unique to plants, such as Tom20 and Om64, as mentioned above [44]. Two single genes encoding Mia40 and Erv1 are present in the genome of *A. thaliana*. As expected, *erv1* T-DNA insertion mutants displayed a lethal phenotype, although the cysteine motif arrangement differs from the yeast counterpart [69]. Interestingly, a putative Erv1 homologue was identified in the outer chloroplast envelope membrane in a proteomics approach, however its actual presence and function remains to be verified [70]. Surprisingly, *Arabidopsis mia40* null-mutants did not show any obvious growth phenotype. Although the levels of several mitochondrial proteins were found to be reduced in *mia40*, plant mitochondria appear to be able to run the disulfide relay system without Mia40, since established substrates such as the Tim proteins were found to import normally. Moreover, Mia40 seems to have acquired novel functions, since it is not only targeted to plant mitochondria, but also to peroxisomes [69].

Despite the fact that the mitochondrial IMS is derived from the bacterial periplasm, the oxidative folding systems are not directly evolutionary related, although the underlying mechanisms are comparable. In contrast to the numerous proteins with assigned functions identified in the mitochondrial IMS, only a few proteins have been identified in the chloroplast IMS presumably acting in protein translocation. Additionally, up to date no analogous disulfide system to the mitochondrial IMS or the bacterial periplasm has been identified.

5. Preprotein recognition and transport across the outer chloroplast membrane

Cytosolic events can post-translationally modulate the efficiency of protein import. Thereby, some preproteins are phosphorylated by the cytosolic kinases STY8, STY17 and STY46 within their presequences, which leads to the association with 14-3-3 proteins and enhances the import rate, especially during chloroplast biogenesis [71–73]. Initial recognition of preproteins at the chloroplast surface is predominantly mediated by the receptor proteins Toc159 and Toc34, which are part of the translocon at the outer membrane of chloroplasts (TOC). Both comprise a cytosolic GTP binding domain (G-domain), a hydrophobic membrane anchor (M-domain) [74] and build a complex with the channel forming β-barrel protein Toc75 [75,76]. Toc75 and its homologues are part of the Omp85 family, also found in gram-negative bacteria and mitochondria [77]. These Toc proteins are frequently represented by gene families in higher plants and the individual isoforms are differentially expressed and are thought to form distinct sub-complexes, which may be involved in the modulation of protein import [78]. For example, Toc34 is represented by AtToc34 and AtToc33 in *A. thaliana*, with AtToc33 being expressed in young photosynthetic tissues and AtToc34 at constant levels in all tissues. In line with this, analyses of knock-out mutants revealed a pale phenotype for a*ttoc33*, whereas a*ttoc34* leaves are normally developed, but show a slight defect in root growth [79,80]. Moreover, phosphorylation of pea Toc34 and AtToc33 have been implicated to play a role in regulating Toc complex assembly – specifically by affecting Toc34 homodimerization and Toc34–Toc159 heterodimerization, as well as by changing their affinity to preproteins [81,82]. Although studies involving complementation analyses with mutated AtToc33 and AtToc34 proteins have been performed, the in vivo function of Toc33/34 phosphorylation is still controversially discussed and the exact physiological conditions triggering the modification as well as the responsible kinases remain to be unraveled [81–84]. In addition to the GTPase domain, Toc159 and its paralogues (Toc120, Toc132, Toc90) contain an acidic extension N-terminal (A-domain), which significantly differs in length in the paralogues and may have a supportive role in specific preprotein recognition [80,85,86]. The idea of classifying preproteins according to their function, i.e. acting in photosynthesis or as 'house-keeping' proteins, and the specific recognition by differentially composed Toc-complexes seems tempting at first sight. However, only a few substrates have been identified and the effect of transcriptional down regulation by retrograde signaling has to be taken into account [78,87,88]. Issues addressing specific Toc complexes, which either display preprotein specificity or tissue and plastid-type dependent variances will undoubtedly be further investigated in the future.

Next to the Toc core complex proteins a fourth protein, Toc64, was found to be loosely associated. It comprises a tetra-tricopeptide repeat (TPR) domain, similar to translocon components in other organelles, such as Tom70 in mitochondria of yeast and humans or Sec72 in the yeast endoplasmic reticulum (ER) [89–92]. Via their TPR domain these proteins interact with the cytosolic chaperones HSP90 and HSP70, which in turn bind to preproteins and thus allow an initial recognition mechanism [93]. Tom70 is lacking in plant mitochondria, however, it may be functionally replaced by Om64, a close homologue of Toc64 [94,95]. Additionally, the plant specific TPR domain containing protein AtTPR7 has been recently identified [96]. Although AtTPR7 was initially detected in chloroplast outer envelopes (designated as OEP61), a large portion, if not all, is ER resident, where it is associated with the Sec translocon and plays a role in post-translational translocation [96,97]. Chaperone-mediated recognition can be circumvented in vivo, since single knockouts of these TPR-domain-containing docking proteins do not show clear growth phenotypes [98]. Nevertheless, knock-out of Om64 and the three Tom20 isoforms is lethal in *Arabidopsis*, although a Tom20 triple knockout likewise has only a minor phenotype [99].

6. Redox-regulation at the TOC translocon

Interestingly, all of the TOC proteins contain conserved cysteine residues. Sequence comparisons and structural analyses revealed seven conserved cysteines in Toc75 of flowering (*A. thaliana*, *O. sativa*, *P. sativum*) and non-vascular plants (*Physcomitrella patens*), four of which are located in the cytosolic POTRA domain. Of the five cysteines found in AtToc159, two are conserved in vascular and non-vascular plants. Both Toc159 cysteines are located within the GTPase domain and one of them is also conserved in Toc34. The crystal structure of Toc34 demonstrates that this cysteine is located in the longest loop of the GTPase domain in an exposed position [100]. AtToc64 comprises 10 cysteine residues, six of them conserved across the lineages and one of them is located within the TPR domain. A role for these cysteine residues in redox-regulation is highly feasible considering that conservation of cysteines during evolution increases their likelihood of being involved in the formation of inter- or intra-protein thiol-bonds [101] (see Fig. 2).

First indications of redox-regulation in the import process were obtained by analyzing the import of ferredoxin under reducing conditions. It was found that the addition of DTT strongly stimulated the import process [102]. In contrast, $CuCl_2$, which acts as an oxidizing agent, could prevent precursor binding and thus inhibit protein import. A hetero-oligomeric complex was detected consisting of Toc75, Toc159 and Toc34 upon $CuCl_2$ treatment [103]. Moreover, other reducing agents, in addition to DTT, such as TCEP, GSH and β-ME showed the same effect as DTT. In line with the evolutionary conservation of the cysteine residues, a similar effect was observed using the moss *P. patens* and the green alga *Chlamydomonas* for import experiments [104]. Two dimensional redox SDS-PAGE revealed a redox-dependent oligomerization of the TOC proteins, forming a hetero-oligomer consisting of Toc75, Toc34 and Toc159 and additionally a Toc64 homo-oligomer. These oligomers could be envisioned to simply block the import pore or – alternatively or additionally – the oxidized stated of the receptor proteins could prevent efficient preprotein binding [105].

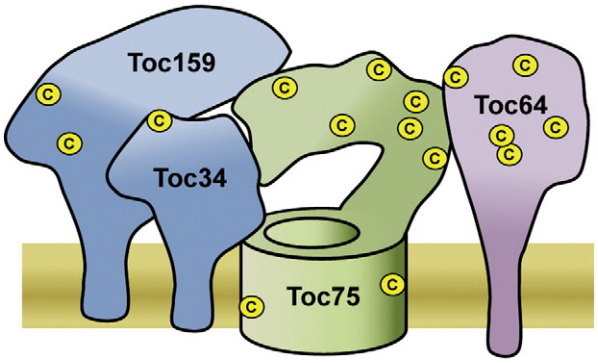

Fig. 2. Conserved cysteine residues in the TOC complex components. Cysteine residues (yellow circles) conserved among vascular and non-vascular plants are highlighted. Alignments for the cysteine residue identification were performed with *A. thaliana*, *O. sativa*, *P. sativum* and *P. patens* using CLUSTAL W.

7. Translocation across the inner chloroplast membrane

Passage through the inner envelope membrane is mediated by the TIC complex. A single protein in the intermembrane space, Tic22, has been assigned to this translocon and supposedly keeps the incoming precursor in an import competent state by shielding exposed hydrophobic residues in a chaperone-like manner [106,107]. In *Arabidopsis* Tic22 exists as two isoforms (Tic22-III and Tic22-IV) and a double mutant of both isoforms displayed a pale phenotype and a reduction in protein import [108]. Moreover, the presence of a HSP70-type chaperone in the IMS was proposed but conclusive molecular evidence is still lacking [109,110].

Several components of the TIC complex have been identified in recent years [111] though their molecular functions are still ambiguous. Tic110 was the first protein to be assigned to the inner envelope translocon [110] and is its most abundant member. The topology of Tic110 was controversially discussed for a long time due to seemingly contradictory experimental results from different groups. Some years ago we could build a topological model which does justice to nearly all published data except for the results obtained from protease treatments [112]. According to this model, Tic110 is anchored in the inner envelope membrane by two undisputed hydrophobic alpha helices located in the very N-terminus of the mature protein (Fig. 3). While others postulate that the C-terminus forms a huge soluble domain in the stroma [113] we detected four amphipathic helices that transverse the membrane and contribute to channel formation [114]. This model is supported by cysteine labeling and the fact that the soluble domain of Tic110 lacking the N-terminal hydrophobic helices can spontaneously insert into liposomes and lipid bilayers, where it forms a cation selective channel. The same channel activity can be observed upon isolation of native Tic110 from inner envelope membranes (Bölter and Götze, unpublished data). Consequently, Tic110 exposes domains not only to the stroma but also into the intermembrane space. In agreement with all functional studies the C-terminus is located in the stroma where it interacts with chaperones such as Hsp93 and Hsp70 [111]. In that regard it is supported by Tic40, which by means of two conserved Sti1-domains acts as a co-chaperone [115,116]. Tic20 is a small integral membrane protein, which was also shown to have channel activity [117] and is indeed discussed as an additional protein translocation pore [118] in concert with Tic21. The role of the latter putative TIC subunit is quite unclear, since the same protein was allocated a function in iron transport [119,120] and named Pic1. Regulatory subunits are dynamically associated with the core translocon: Tic32, Tic55 and Tic62 [105,121–123]. All of these proteins have been found in tight association with Tic110 and seem to be involved in redox regulation of the import process. Tic32 was detected by direct interaction with the N-terminal part of Tic110 [124], Tic55 in a complex with Tic110 isolated by BN-PAGE [122] and Tic62 in a similarly prepared complex [125].

Quite recently, a different translocation complex was proposed [126] consisting of Tic20 and three newly identified proteins found in close

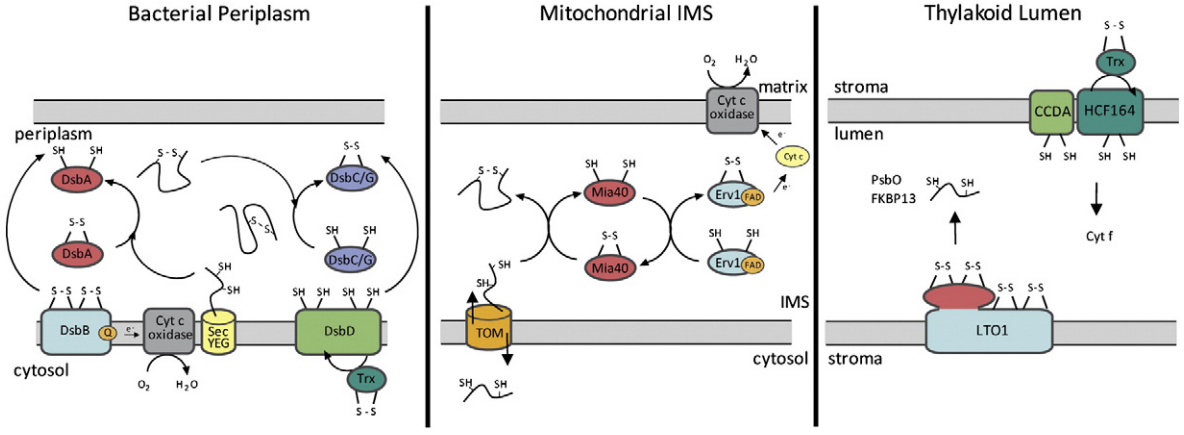

Fig. 1. Comparison of the thiol-oxidation systems in bacteria, mitochondria and the thylakoid lumen. Proteins exported to the bacterial periplasm by the SecYEG translocon are folded with the assistance of the DsbA/B oxidoreductases (left panel). Non-native formed disulfide bonds are isomerized further by DsbC and DsbG, which are re-oxidized by DsbD using electrons from cytosolic thioredoxin (left panel). Mitochondrial import of preproteins into the IMS is coupled to thiol-bond formation assisted by Mia40. Oxidized proteins cannot slide back into the Tom40 channel and are retained in the IMS. Erv1, which contains a FAD cofactor, re-oxidizes Mia40 and transfers the electrons to cytochrome c, the cytochrome c oxidase and eventually oxygen (middle panel). In the thylakoid membrane a fusion protein with DsbA and DsbB like features, LTO1, is found. Its targets are luminal proteins such as the PSII subunit PsbO and the immunophilin FKBP13. CCDA and HCF164 are functioning in reducing luminal proteins and both seem to be involved in cytochrome b_6f assembly (right panel).

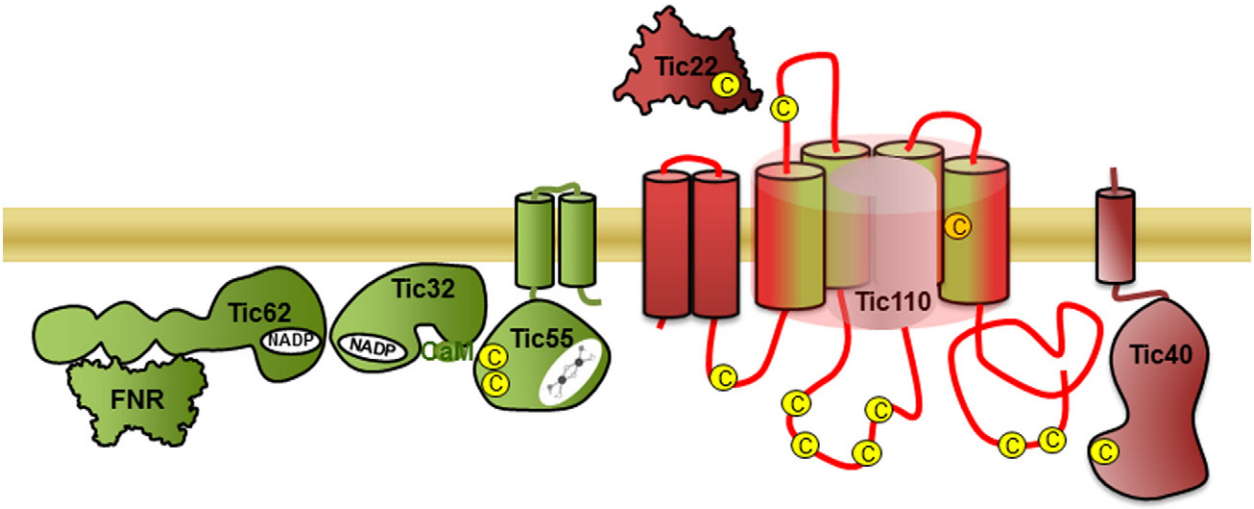

Fig. 3. Model of the classic general TIC complex. Please note that from the central channel Tic110 only one monomer is depicted for clarity. Yellow circles represent conserved cysteines within the sequences. The surface structure of Tic22 was approximated from the PfTic22 crystal structure (PDB 4E6Z), that from the FNR dimer from the pea isoform (PDB 3MHP).

interaction with Tic20. A ProtA/TEV-tagged version of Tic20 was used to complement a *tic20* T-DNA insertion line and to pull out associated proteins by Protein G sepharose after membrane solubilization. Elution was achieved by specific cleavage with the TEV protease, thereby avoiding elution of unspecifically bound proteins from the matrix. A similar complex was pulled out by using a tagged precursor as bait. Whereas this complex also contained the core TOC components, no traces of Tic110 and Tic40 were reported to be present. This could indicate that incoming preproteins might engage different complexes upon entry into chloroplasts. Surprisingly, however, the same precursor construct (lacking the TEV cleavage site) was used two decades earlier by other researchers to pull down Tic110, Toc75 and Toc34 from pea chloroplasts in close association with the preprotein [75] which raises the question why the same chimeric precursor upon exit from the TOC complex should travel via different TIC machineries across the inner envelope membrane.

Setting this discrepancy aside, Tic20/Tic100/Tic56/Tic214 (= Ycf1) were postulated to build the "true" TIC translocon (see Fig. 4). Support for this notion was taken from the fact that all proteins seem essential for plant viability and that the reconstituted complex showed channel activity in a black lipid bilayer system. As an additional argument in favor of the new TIC complex the evolutionary conservation of Tic20 and Ycf1 was used. Oddly enough, though the evolutionary conservation of Tic20 can be well documented, the same does not apply for Ycf1. This chloroplast encoded gene is not only missing in an important branch of monocots, namely all grasses such as rice, maize, millet and other crops, but likewise in several dicots [127]. It might be imaginable that grasses eventually developed an alternative translocation system functioning only with Tic20 and maybe yet unidentified components, but it is absolutely implausible that within the dicots different TIC complexes evolved independently many times. Thus, while Ycf1 certainly plays an important role in chloroplast biogenesis, it might have other functions than being a central member of the general import translocon.

8. Redox-regulation at the Tic translocon

In view of the observed thiol dependent activity of the TOC complex it can be expected for this signal to be also effective at the TIC complex, since the two translocation machineries must act in concert. A thiol-dependent interaction between Tic110 and Tic40 has been observed

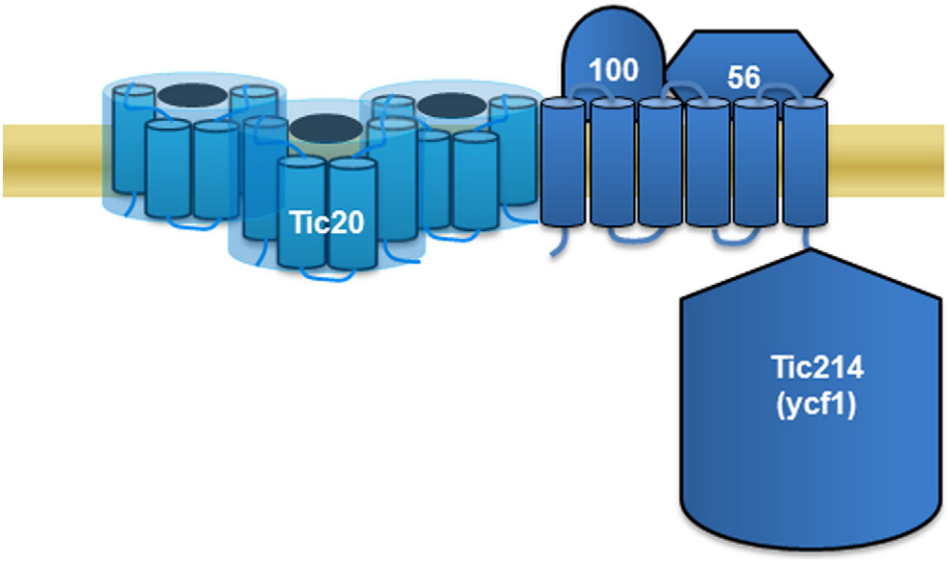

Fig. 4. Alternatively proposed Tic complex. Tic20 forms an oligomeric complex and interacts with newly identified inner envelope proteins, which have been named Tic56, Tic100 and Tic214. The latter is identical to ycf1, a chloroplast encoded open reading frame with yet unknown function.

(X-link by copper chloride) though it is not clear yet which role this dynamics might play in vivo [116]. Tic110 itself has been found to contain one or two regulatory disulfide bridges [112]. Depending on the cysteines involved the formation/dissolving of such intramolecular bonds could have immense influence on the structure and function of this central TIC component. Hypothetically, the induced molecular movements could lead to opening/closure of the channel and thereby regulate the rate of incoming preproteins. Whereas the reduction/oxidation of cysteines within TOC components needs to be mediated from the cytosolic side, thiol formation in Tic110 must be conveyed by stromal proteins. Perfect candidates for this job have been identified in the stromal thioredoxin family, which has been demonstrated to act on disulfide bonds in Tic110 [112]. Since the redox state of thioredoxins is directly coupled not only to photosynthetic activity but also many other redox-dependent processes in chloroplasts it is quite feasible to imagine that a signal transported within this important redox system eventually reaches the import machineries.

Though for the intermembrane space component Tic22 no redox-mediated modulation has been reported, it contains a well conserved cysteine (amino acid 100 in the apicoplast crystal structure, [107]), which could participate in intermolecular disulfide bonds with itself, other not yet identified IMS residents or with Tic110 which exposes one cysteine into the IMS (Fig. 3). Presently, however, this scenario is a mere speculation and needs to be experimentally addressed.

Apart from the redox state of the thiol system the ratio of $NADP^+$ to NADPH is a direct measure for the total stromal redox state (see above). It critically influences many biosynthetic pathways within chloroplasts which depend on the provision of electrons, e.g. the Calvin Benson Cycle. For these pathways to run smoothly all the required enzymes have to be imported at a specific rate depending on the actual need within the organelles. Therefore, protein import activity must be adapted according to these requirements. We and others have shown that the stromal redox state influences the import efficiency of a certain class of precursor proteins [104,128]. Consequently, some regulatory components of the Tic translocon are dynamically associated with the core complex in response to the stromal redox state [129]. Tic62 was shown to shuttle from a membrane associated state at the inner envelope as well as at the thylakoids to the stroma in response to changing $NADP^+$/NADPH ratios [129]. Since one important function of Tic62 in vascular plants is the tethering of FNR to these membranes via specific C-terminal motifs [130] its shuttling could critically influence electron transfer processes from this photosynthetic enzyme to different acceptor proteins which in itself could constitute a signal transduction chain. Especially the triple location at the inner envelope, stroma and the thylakoids makes Tic62 a perfect candidate for mediating signal transfer from the photosynthetically active thylakoids to the import machinery. A second function of this protein could be a direct electron transfer onto yet unknown acceptor proteins since Tic62 comprises an NADPH binding site and is, at least in vitro, active as a dehydrogenase [129]. Thereby, a signal cascade consisting of electron transfer might be established from thylakoids through the stroma to the inner envelope membrane. Lately, another binding partner of FNR named Trol has been described [131]. This intrinsic thylakoid protein comprises a similar single C-terminal extension as is found in repetitive manner in Tic62 and was demonstrated to interact with FNR [131,132]. Since a small fraction of Trol was reported to be localized to the inner envelope it might also participate in the signal transduction chain involving Tic62/FNR. Remarkably, the FNR binding C-terminal motif is restricted to vascular plants, indicating that this regulatory mechanism has evolved only after plants executed the step onto land and developed their sophisticated vascular system. This might suggest that algae and plants still living in or close to water such as mosses do not need to adapt their protein import activity in response to changing stromal redox conditions.

A second protein possibly involved in redox regulation is Tic32 – another member of a dehydrogenase family capable of transferring electrons, which as Tic62 dissociates from the TIC complex under reduced conditions. Interestingly, Tic32 is also subject to calmodulin/Ca^{2+} dependent regulation. It could be shown that calmodulin (CaM) directly binds to Tic32 and that application of specific CaM inhibitors decreased import efficiency [123]. Thus, two very different modes of regulation convene at this TIC component. The third member of the so-called redox regulon is Tic55, a Rieske protein found in close vicinity of Tic110. It is anchored to the inner envelope by two alpha helices and exposes its bulk into the stroma. Recently, Tic55 was identified as a potential thioredoxin target by affinity chromatography on a Trx-column [70]. While further biochemical evidence for this is still missing, it is in line with the presence of a CXXC motif found in the stromal domain. Tic55 is an ancient protein with orthologues found in cyanobacteria and it shows homology to the LLS1 (lethal leaf spot 1) family of oxygenases [133].The molecular function of Tic55 is still enigmatic, though the Rieske center should be able to accept and transfer electrons and thus could be included in a possible electron transfer chain at the inner envelope. With a glance at mitochondria where the electrons from the IMS relay system eventually end up on the Rieske protein cytochrome c, which then transfers them onto molecular oxygen, it is tempting to speculate that Tic55 might act in a similar manner. Though this scenario is by now still hypothetic it represents an elegant possibility of how to integrate the import process into the redox regulatory network of chloroplasts and the surrounding cell. This type of import regulation is clearly involved in fine-tuning of the process rather than representing a molecular on/off-switch since single knock out mutants of the redox regulon components have no reported defects in protein import (our own unpublished data and [134,135]).

Acknowledgments

Financial support from the German Research Council (DFG, SFB1035, project A4, to J.S. and S.S.) and the Munich Center for Integrated Protein Science (CiPSM) to B.B., J.S. and S.S. is acknowledged.

References

[1] S.B. Gould, R.F. Waller, G.I. McFadden, Plastid evolution, Annu. Rev. Plant Biol. 59 (2008) 491–517.
[2] W. Martin, B. Stoebe, V. Goremykin, S. Hapsmann, M. Hasegawa, K.V. Kowallik, Gene transfer to the nucleus and the evolution of chloroplasts, Nature 393 (1998) 162–165.
[3] J.N. Timmis, M.A. Ayliffe, C.Y. Huang, W. Martin, Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes, Nat. Rev. Genet. 5 (2004) 123–135.
[4] L. Margulis, Origin of Eukaryotic Cells, 1970.
[5] D. Leister, Chloroplast research in the genomic age, Trends Genet. 19 (2003) 47–56.
[6] N. Fedoroff, Redox regulatory mechanisms in cellular stress responses, Ann. Bot. 98 (2006) 289–300.
[7] T. Zeller, G. Klug, Thioredoxins in bacteria: functions in oxidative stress response and regulation of thioredoxin genes, Naturwissenschaften 93 (2006) 259–266.
[8] D. Rosso, A.G. Ivanov, A. Fu, J. Geisler-Lee, L. Hendrickson, M. Geisler, G. Stewart, M. Krol, V. Hurry, S.R. Rodermel, D.P. Maxwell, N.P.A. Hüner, IMMUTANS does not act as a stress-induced safety valve in the protection of the photosynthetic apparatus of *Arabidopsis* during steady-state photosynthesis, Plant Physiol. 142 (2006) 574–585.
[9] M. Taniguchi, H. Miyake, Redox-shuttling between chloroplast and cytosol: integration of intra-chloroplast and extra-chloroplast metabolism, Curr. Opin. Plant Biol. 15 (2012) 252–260.
[10] L.E. Fridlyand, J.E. Backhausen, R. Scheibe, Flux control of the malate valve in leaf cells, Arch. Biochem. Biophys. 349 (1998) 290–298.
[11] P. Schurmann, B.B. Buchanan, The ferredoxin/thioredoxin system of oxygenic photosynthesis, Antioxid. Redox Signal. 10 (2008) 1235–1274.
[12] B.B. Buchanan, P. Schurmann, R.A. Wolosiuk, J.P. Jacquot, The ferredoxin/thioredoxin system: from discovery to molecular structures and beyond, Photosynth. Res. 73 (2002) 215–222.
[13] B. Buchanan, Thioredoxin: an unexpected meeting place, Photosynth. Res. 92 (2007) 145–148.
[14] W. Haehnel, Photosynthetic electron transport in higher plants, Annu. Rev. Plant Physiol. 35 (1984) 659–693.
[15] N. Huner, K. Dahal, L. Hollis, R. Bode, D. Rosso, M. Krol, A.G. Ivanov, Chloroplast redox imbalance governs phenotypic plasticity: the "grand design of photosynthesis" revisited, Front. Plant Sci. 3 (2012).

[16] L.W. Guddat, J.C. Bardwell, J.L. Martin, Crystal structures of reduced and oxidized DsbA: investigation of domain motion and thiolate stabilization, Structure 6 (1998) 757–767.
[17] H. Kadokura, J. Beckwith, Detecting folding intermediates of a protein as it passes through the bacterial translocation channel, Cell 138 (2009) 1164–1173.
[18] J.C. Bardwell, J.O. Lee, G. Jander, N. Martin, D. Belin, J. Beckwith, A pathway for disulfide bond formation in vivo, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 1038–1042.
[19] D. Missiakas, C. Georgopoulos, S. Raina, Identification and characterization of the *Escherichia coli* gene dsbB, whose product is involved in the formation of disulfide bonds in vivo, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 7084–7088.
[20] A.A. McCarthy, P.W. Haebel, A. Torronen, V. Rybin, E.N. Baker, P. Metcalf, Crystal structure of the protein disulfide bond isomerase, DsbC, from *Escherichia coli*, Nat. Struct. Biol. 7 (2000) 196–199.
[21] A. Zapun, D. Missiakas, S. Raina, T.E. Creighton, Structural and functional characterization of DsbC, a protein involved in disulfide bond formation in *Escherichia coli*, Biochemistry 34 (1995) 5075–5089.
[22] P.H. Bessette, J.J. Cotto, H.F. Gilbert, G. Georgiou, In vivo and in vitro function of the *Escherichia coli* periplasmic cysteine oxidoreductase DsbG, J. Biol. Chem. 274 (1999) 7784–7792.
[23] M. Depuydt, S.E. Leonard, D. Vertommen, K. Denoncin, P. Morsomme, K. Wahni, J. Messens, K.S. Carroll, J.F. Collet, A periplasmic reducing system protects single cysteine residues from oxidation, Science 326 (2009) 1109–1111.
[24] E.J. Stewart, F. Katzen, J. Beckwith, Six conserved cysteines of the membrane protein DsbD are required for the transfer of electrons from the cytoplasm to the periplasm of *Escherichia coli*, EMBO J. 18 (1999) 5963–5971.
[25] F. Katzen, J. Beckwith, Transmembrane electron transfer by the membrane protein DsbD occurs via a disulfide bond cascade, Cell 103 (2000) 769–779.
[26] R.J. Dutton, D. Boyd, M. Berkmen, J. Beckwith, Bacterial species exhibit diversity in their mechanisms and capacity for protein disulfide bond formation, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 11933–11938.
[27] A.K. Singh, M. Bhattacharyya-Pakrasi, H.B. Pakrasi, Identification of an atypical membrane protein involved in the formation of protein disulfide bonds in oxygenic photosynthetic organisms, J. Biol. Chem. 283 (2008) 15762–15770.
[28] J.M. Herrmann, F. Kauff, H.E. Neuhaus, Thiol oxidation in bacteria, mitochondria and chloroplasts: common principles but three unrelated machineries? Biochim. Biophys. Acta (BBA) Mol. Cell Res. 1793 (2009) 71–77.
[29] G. Gopalan, Z. He, Y. Balmer, P. Romano, R. Gupta, A. Heroux, B.B. Buchanan, K. Swaminathan, S. Luan, 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 (2004) 13945–13950.
[30] J. Fanghanel, G. Fischer, Insights into the catalytic mechanism of peptidyl prolyl cis/trans isomerases, Front. Biosci. 9 (2004) 3453–3478.
[31] K. Motohashi, F. Koyama, Y. Nakanishi, H. Ueoka-Nakanishi, T. Hisabori, Chloroplast cyclophilin is a target protein of thioredoxin. Thiol modulation of the peptidyl-prolyl cis-trans isomerase activity, J. Biol. Chem. 278 (2003) 31848–31852.
[32] A. Lima, S. Lima, J.H. Wong, R.S. Phillips, B.B. Buchanan, S. Luan, A redox-active FKBP-type immunophilin functions in accumulation of the photosystem II supercomplex in *Arabidopsis thaliana*, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 12631–12636.
[33] M. Karamoko, S. Cline, K. Redding, N. Ruiz, P.P. Hamel, Lumen thiol oxidoreductase1, a disulfide bond-forming catalyst, is required for the assembly of photosystem II in *Arabidopsis*, Plant Cell 23 (2011) 4462–4475.
[34] W.K. Feng, L. Wang, Y. Lu, X.Y. Wang, A protein oxidase catalysing disulfide bond formation is localized to the chloroplast thylakoids, FEBS J. 278 (2011) 3419–3430.
[35] Y. Lu, H.R. Wang, H. Li, H.R. Cui, Y.G. Feng, X.Y. Wang, A chloroplast membrane protein LTO1/AtVKOR involving in redox regulation and ROS homeostasis, Plant Cell Rep. 32 (2013) 1427–1440.
[36] Y. Lu, J.J. Peng, Z.B. Yu, J.J. Du, J.N. Xu, X.Y. Wang, Thylakoid membrane oxidoreductase LTO1/AtVKOR is involved in ABA-mediated response to osmotic stress in *Arabidopsis*, Physiol. Plant. (2014). http://dx.doi.org/10.1111/ppl.12268.
[37] S.Z. Toth, G. Schansker, G. Garab, The physiological roles and metabolism of ascorbate in chloroplasts, Physiol. Plant. 148 (2013) 161–175.
[38] M. Karamoko, S.T. Gabilly, P.P. Hamel, Operation of trans-thylakoid thiol-metabolizing pathways in photosynthesis, Front. Plant Sci. 4 (2013) 476.
[39] K. Lennartz, H. Plucken, A. Seidler, P. Westhoff, N. Bechtold, K. Meierhoff, HCF164 encodes a thioredoxin-like protein involved in the biogenesis of the cytochrome b(6)f complex in *Arabidopsis*, Plant Cell 13 (2001) 2539–2551.
[40] K. Motohashi, T. Hisabori, 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 (2006) 35039–35047.
[41] M.L. Page, P.P. Hamel, S.T. Gabilly, H. Zegzouti, J.V. Perea, J.M. Alonso, J.R. Ecker, S.M. Theg, S.K. Christensen, S. Merchant, A homolog of prokaryotic thiol disulfide transporter CcdA is required for the assembly of the cytochrome b6f complex in *Arabidopsis* chloroplasts, J. Biol. Chem. 279 (2004) 32474–32482.
[42] S.T. Gabilly, B.W. Dreyfuss, M. Karamoko, V. Corvest, J. Kropat, M.D. Page, S.S. Merchant, P.P. Hamel, CCS5, a thioredoxin-like protein involved in the assembly of plastid c-type cytochromes, J. Biol. Chem. 285 (2010) 29738–29749.
[43] M.W. Murcha, B. Kmiec, S. Kubiszewski-Jakubiak, P.F. Teixeira, E. Glaser, J. Whelan, Protein import into plant mitochondria: signals, machinery, processing, and regulation, J. Exp. Bot. 65 (2014) 6301–6335.
[44] C. Carrie, M.W. Murcha, J. Whelan, An in silico analysis of the mitochondrial protein import apparatus of plants, BMC Plant Biol. 10 (2010) 249.
[45] F.N. Vogtle, J.M. Burkhart, S. Rao, C. Gerbeth, J. Hinrichs, J.C. Martinou, A. Chacinska, A. Sickmann, R.P. Zahedi, C. Meisinger, Intermembrane space proteome of yeast mitochondria, Mol. Cell. Proteomics 11 (2012) 1840–1852.
[46] C.M. Koehler, New developments in mitochondrial assembly, Annu. Rev. Cell Dev. Biol. 20 (2004) 309–335.
[47] J.M. Herrmann, K. Hell, Chopped, trapped or tacked–protein translocation into the IMS of mitochondria, Trends Biochem. Sci. 30 (2005) 205–211.
[48] N. Mesecke, N. Terziyska, C. Kozany, F. Baumann, W. Neupert, K. Hell, J.M. Herrmann, A disulfide relay system in the intermembrane space of mitochondria that mediates protein import, Cell 121 (2005) 1059–1069.
[49] L.S. Field, Y. Furukawa, T.V. O'Halloran, V.C. Culotta, Factors controlling the uptake of yeast copper/zinc superoxide dismutase into mitochondria, J. Biol. Chem. 278 (2003) 28052–28059.
[50] F. Arnesano, E. Balatri, L. Banci, I. Bertini, D.R. Winge, Folding studies of Cox17 reveal an important interplay of cysteine oxidation and copper binding, Structure 13 (2005) 713–722.
[51] H. Lu, S. Allen, L. Wardleworth, P. Savory, K. Tokatlidis, Functional TIM10 chaperone assembly is redox-regulated in vivo, J. Biol. Chem. 279 (2004) 18952–18958.
[52] S.P. Curran, D. Leuenberger, E. Schmidt, C.M. Koehler, The role of the Tim8p–Tim13p complex in a conserved import pathway for mitochondrial polytopic inner membrane proteins, J. Cell Biol. 158 (2002) 1017–1027.
[53] S.P. Curran, D. Leuenberger, W. Oppliger, C.M. Koehler, The Tim9p–Tim10p complex binds to the transmembrane domains of the ADP/ATP carrier, EMBO J. 21 (2002) 942–953.
[54] A. Chacinska, S. Pfannschmidt, N. Wiedemann, V. Kozjak, L.K. Sanjuan Szklarz, A. Schulze-Specking, K.N. Truscott, B. Guiard, C. Meisinger, N. Pfanner, Essential role of Mia40 in import and assembly of mitochondrial intermembrane space proteins, EMBO J. 23 (2004) 3735–3746.
[55] N. Terziyska, T. Lutz, C. Kozany, D. Mokranjac, N. Mesecke, W. Neupert, J.M. Herrmann, K. Hell, Mia40, a novel factor for protein import into the intermembrane space of mitochondria is able to bind metal ions, FEBS Lett. 579 (2005) 179–184.
[56] M. Hagiya, A. Francavilla, L. Polimeno, I. Ihara, H. Sakai, T. Seki, M. Shimonishi, K.A. Porter, T.E. Starzl, Cloning and sequence analysis of the rat augmenter of liver regeneration (ALR) gene: expression of biologically active recombinant ALR and demonstration of tissue distribution, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 8142–8146.
[57] D. Fass, The Erv family of sulfhydryl oxidases, Biochim. Biophys. Acta 1783 (2008) 557–566.
[58] L. Banci, I. Bertini, C. Cefaro, S. Ciofi-Baffoni, A. Gallo, M. Martinelli, D.P. Sideris, N. Katrakili, K. Tokatlidis, MIA40 is an oxidoreductase that catalyzes oxidative protein folding in mitochondria, Nat. Struct. Mol. Biol. 16 (2009) 198–206.
[59] L. Banci, I. Bertini, C. Cefaro, L. Cenacchi, S. Ciofi-Baffoni, I.C. Felli, A. Gallo, L. Gonnelli, E. Luchinat, D. Sideris, K. Tokatlidis, Molecular chaperone function of Mia40 triggers consecutive induced folding steps of the substrate in mitochondrial protein import, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 20190–20195.
[60] S. Kawano, K. Yamano, M. Naoe, T. Momose, K. Terao, S. Nishikawa, N. Watanabe, T. Endo, Structural basis of yeast Tim40/Mia40 as an oxidative translocator in the mitochondrial intermembrane space, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 14403–14407.
[61] D. Milenkovic, T. Ramming, J.M. Muller, L.S. Wenz, N. Gebert, A. Schulze-Specking, D. Stojanovski, S. Rospert, A. Chacinska, Identification of the signal directing Tim9 and Tim10 into the intermembrane space of mitochondria, Mol. Biol. Cell 20 (2009) 2530–2539.
[62] D.P. Sideris, N. Petrakis, N. Katrakili, D. Mikropoulou, A. Gallo, S. Ciofi-Baffoni, L. Banci, I. Bertini, K. Tokatlidis, A novel intermembrane space-targeting signal docks cysteines onto Mia40 during mitochondrial oxidative folding, J. Cell Biol. 187 (2009) 1007–1022.
[63] J. Lee, G. Hofhaus, T. Lisowsky, Erv1p from *Saccharomyces cerevisiae* is a FAD-linked sulfhydryl oxidase, FEBS Lett. 477 (2000) 62–66.
[64] D.L. Coppock, C. Thorpe, Multidomain flavin-dependent sulfhydryl oxidases, Antioxid. Redox Signal. 8 (2006) 300–311.
[65] C.K. Wu, T.A. Dailey, H.A. Dailey, B.C. Wang, J.P. Rose, The crystal structure of augmenter of liver regeneration: a mammalian FAD-dependent sulfhydryl oxidase, Protein Sci. 12 (2003) 1109–1118.
[66] M. Bien, S. Longen, N. Wagener, I. Chwalla, J.M. Herrmann, J. Riemer, Mitochondrial disulfide bond formation is driven by intersubunit electron transfer in Erv1 and proofread by glutathione, Mol. Cell 37 (2010) 516–528.
[67] E. Lionaki, M. Aivaliotis, C. Pozidis, K. Tokatlidis, The N-terminal shuttle domain of Erv1 determines the affinity for Mia40 and mediates electron transfer to the catalytic Erv1 core in yeast mitochondria, Antioxid. Redox Signal. 13 (2010) 1327–1339.
[68] L. Banci, I. Bertini, V. Calderone, C. Cefaro, S. Ciofi-Baffoni, A. Gallo, E. Kallergi, E. Lionaki, C. Pozidis, K. Tokatlidis, Molecular recognition and substrate mimicry drive the electron-transfer process between MIA40 and ALR, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 4811–4816.
[69] C. Carrie, E. Giraud, O. Duncan, L. Xu, Y. Wang, S. Huang, R. Clifton, M. Murcha, A. Filipovska, O. Rackham, A. Vrielink, J. Whelan, Conserved and novel functions for *Arabidopsis thaliana* MIA40 in assembly of proteins in mitochondria and peroxisomes, J. Biol. Chem. 285 (2010) 36138–36148.
[70] S. Bartsch, J. Monnet, K. Selbach, F. Quigley, J. Gray, D. von Wettstein, S. Reinbothe, C. Reinbothe, 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 (2008) 4933–4938.
[71] T. Martin, R. Sharma, C. Sippel, K. Waegemann, J. Soll, U.C. Vothknecht, A protein kinase family in *Arabidopsis* phosphorylates chloroplast precursor proteins, J. Biol. Chem. 281 (2006) 40216–40223.
[72] T. May, J. Soll, 14-3-3 proteins form a guidance complex with chloroplast precursor proteins in plants, Plant Cell 12 (2000) 53–64.
[73] G. Lamberti, C. Drurey, J. Soll, S. Schwenkert, The phosphorylation state of chloroplast transit peptides regulates preprotein import, Plant Signal. Behav. 6 (2011) 1918–1920.

[74] W.L. Chang, J. Soll, B. Bolter, The gateway to chloroplast: re-defining the function of chloroplast receptor proteins, Biol. Chem. 393 (2012) 1263–1277.
[75] D.J. Schnell, F. Kessler, G. Blobel, Isolation of components of the chloroplast protein import machinery, Science 266 (1994) 1007–1012.
[76] K. Keegstra, K. Cline, Protein import and routing systems of chloroplasts, Plant Cell 11 (1999) 557–570.
[77] I.E. Gentle, L. Burri, T. Lithgow, Molecular architecture and function of the Omp85 family of proteins, Mol. Microbiol. 58 (2005) 1216–1225.
[78] E. Demarsy, A.M. Lakshmanan, F. Kessler, Border control: selectivity of chloroplast protein import and regulation at the TOC-complex, Front. Plant Sci. 5 (2014) 483.
[79] M. Gutensohn, B. Schulz, P. Nicolay, U.-I. Flügge, Functional analysis of the two *Arabidopsis* homologues of Toc34, a component of the chloroplast protein import apparatus†, Plant J. 23 (2000) 771–783.
[80] S. Kubis, R. Patel, J. Combe, J. Bedard, S. Kovacheva, K. Lilley, A. Biehl, D. Leister, G. Rios, C. Koncz, P. Jarvis, Functional specialization amongst the *Arabidopsis* Toc159 family of chloroplast protein import receptors, Plant Cell 16 (2004) 2059–2077.
[81] H. Aronsson, J. Combe, R. Patel, P. Jarvis, In vivo assessment of the significance of phosphorylation of the *Arabidopsis* chloroplast protein import receptor, atToc33, FEBS Lett. 580 (2006) 649–655.
[82] M. Oreb, M. Zoryan, A. Vojta, U.G. Maier, L.A. Eichacker, E. Schleiff, Phosphomimicry mutant of atToc33 affects early development of *Arabidopsis thaliana*, FEBS Lett. 581 (2007) 5945–5951.
[83] M. Jelic, J. Soll, E. Schleiff, Two Toc34 homologues with different properties, Biochemistry 42 (2003) 5906–5916.
[84] M. Oreb, A. Hofle, P. Koenig, M.S. Sommer, I. Sinning, F. Wang, I. Tews, D.J. Schnell, E. Schleiff, Substrate binding disrupts dimerization and induces nucleotide exchange of the chloroplast GTPase Toc33, Biochem. J. 436 (2011) 313–319.
[85] S. Infanger, S. Bischof, A. Hiltbrunner, B. Agne, S. Baginsky, F. Kessler, The chloroplast import receptor Toc90 partially restores the accumulation of Toc159 client proteins in the *Arabidopsis thaliana* pp i2 mutant, Mol. Plant 4 (2011) 252–263.
[86] H. Inoue, C. Rounds, D.J. Schnell, The molecular basis for distinct pathways for protein import into *Arabidopsis* chloroplasts, Plant Cell 6 (2010) 1947–1960.
[87] S. Bischof, K. Baerenfaller, T. Wildhaber, R. Troesch, P.A. Vidi, B. Roschitzki, M. Hirsch-Hoffmann, L. Hennig, F. Kessler, W. Gruissem, S. Baginsky, Plastid proteome assembly without Toc159: photosynthetic protein import and accumulation of N-acetylated plastid precursor proteins, Plant Cell 23 (2011) 3911–3928.
[88] S. Dutta, H.J. Teresinski, M.D. Smith, A split-ubiquitin yeast two-hybrid screen to examine the substrate specificity of atToc159 and atToc132, two *Arabidopsis* chloroplast preprotein import receptors, PLoS One 9 (2014) e95026.
[89] S. Qbadou, T. Becker, O. Mirus, I. Tews, J. Soll, E. Schleiff, The molecular chaperone Hsp90 delivers precursor proteins to the chloroplast import receptor Toc64, EMBO J. 25 (2006) 1836–1847.
[90] K. Sohrt, J. Soll, Toc64, a new component of the protein translocon of chloroplasts, J. Cell Biol. 148 (2000) 1213–1221.
[91] J.C. Young, N.J. Hoogenraad, F.U. Hartl, Molecular chaperones Hsp90 and Hsp70 deliver preproteins to the mitochondrial import receptor Tom70, Cell 112 (2003) 41–50.
[92] D. Feldheim, R. Schekman, Sec72p contributes to the selective recognition of signal peptides by the secretory polypeptide translocation complex, J. Cell Biol. 126 (1994) 935–943.
[93] T. Schlegel, O. Mirus, A. von Haeseler, E. Schleiff, The tetratricopeptide repeats of receptors involved in protein translocation across membranes, Mol. Biol. Evol. 24 (2007) 2763–2774.
[94] O. Chew, R. Lister, S. Qbadou, J.L. Heazlewood, J. Soll, E. Schleiff, A.H. Millar, J. Whelan, A plant outer mitochondrial membrane protein with high amino acid sequence identity to a chloroplast protein import receptor, FEBS Lett. 557 (2004) 109–114.
[95] R. Lister, C. Carrie, O. Duncan, L.H. Ho, K.A. Howell, M.W. Murcha, J. Whelan, Functional definition of outer membrane proteins involved in preprotein import into mitochondria, Plant Cell 19 (2007) 3739–3759.
[96] R. Schweiger, N.C. Muller, M.J. Schmitt, J. Soll, S. Schwenkert, AtTPR7 is a chaperone docking protein of the Sec translocon in Arabidopsis, J. Cell Sci. 125 (2012) 5196–5207.
[97] O. von Loeffelholz, V. Kriechbaumer, R.A. Ewan, R. Jonczyk, S. Lehmann, J.C. Young, B.M. Abell, OEP61 is a chaperone receptor at the plastid outer envelope, Biochem. J. 438 (2011) 143–153.
[98] H. Aronsson, P. Boij, R. Patel, A. Wardle, M. Topel, P. Jarvis, Toc64/OEP64 is not essential for the efficient import of proteins into chloroplasts in Arabidopsis thaliana, Plant J. 52 (2007) 53–68.
[99] O. Duncan, M.W. Murcha, J. Whelan, Unique components of the plant mitochondrial protein import apparatus, Biochim. Biophys. Acta 1833 (2013) 304–313.
[100] Y.J. Sun, F. Forouhar, H.M. Li Hm, S.L. Tu, Y.H. Yeh, S. Kao, H.L. Shr, C.C. Chou, C. Chen, C.D. Hsiao, Crystal structure of pea Toc34, a novel GTPase of the chloroplast protein translocon, Nat. Struct. Biol. 9 (2002) 95–100.
[101] M. Balsera, E. Uberegui, D. Susanti, R. Schmitz, B. Mukhopadhyay, P. Schürmann, B. Buchanan, Ferredoxin:thioredoxin reductase (FTR) links the regulation of oxygenic photosynthesis to deeply rooted bacteria, Planta 237 (2013) 619–635.
[102] M. Pilon, B. de Kruijff, P.J. Weisbeek, New insights into the import mechanism of the ferredoxin precursor into chloroplasts, J. Biol. Chem. 267 (1992) 2548–2556.
[103] M. Seedorf, J. Soll, Copper chloride, an inhibitor of protein import into chloroplasts, FEBS Lett. 367 (1995) 19–22.
[104] A. Stengel, J.P. Benz, B.B. Buchanan, J. Soll, B. Bolter, Preprotein import into chloroplasts via the Toc and Tic complexes is regulated by redox signals in *Pisum sativum*, Mol. Plant 2 (2009) 1181–1197.
[105] A. Stengel, J.P. Benz, J. Soll, B. Bolter, Redox-regulation of protein import into chloroplasts and mitochondria: similarities and differences, Plant Signal. Behav. 5 (2010) 105–109.
[106] M. Rudolf, A.B. Machettira, L.E. Groß, K.L. Weber, K. Bolte, T. Bionda, M.S. Sommer, U.G. Maier, A.P.M. Weber, E. Schleiff, J. Tripp, In vivo function of Tic22, a protein import component of the intermembrane space of chloroplasts, Mol. Plant 6 (2013) 817–829.
[107] S. Glaser, G.G. van Dooren, S. Agrawal, C.F. Brooks, G.I. McFadden, B. Striepen, M.K. Higgins, Tic22 is an essential chaperone required for protein import into the apicoplast, J. Biol. Chem. 287 (2012) 39505–39512.
[108] M. Rudolf, A.B. Machettira, L.E. Gross, K.L. Weber, K. Bolte, T. Bionda, M.S. Sommer, U.G. Maier, A.P. Weber, E. Schleiff, J. Tripp, In vivo function of Tic22, a protein import component of the intermembrane space of chloroplasts, Mol. Plant 6 (2013) 817–829.
[109] R.M. Ratnayake, H. Inoue, H. Nonami, M. Akita, Alternative processing of *Arabidopsis* Hsp70 precursors during protein import into chloroplasts, Biosci. Biotechnol. Biochem. 72 (2008) 2926–2935.
[110] F. Kessler, G. Blobel, Interaction of the protein import and folding machineries of the chloroplast, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 7684–7689.
[111] S. Schwenkert, J. Soll, B. Bolter, Protein import into chloroplasts—how chaperones feature into the game, Biochim. Biophys. Acta 1808 (2011) 901–911.
[112] M. Balsera, T.A. Goetze, E. Kovacs-Bogdan, P. Schurmann, R. Wagner, B.B. Buchanan, J. Soll, B. Bolter, 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 (2009) 2603–2616.
[113] Y.D. Paila, L.G.L. Richardson, D.J. Schnell, New Insights into the Mechanism of Chloroplast Protein Import and Its Integration with Protein Quality Control, Organelle Biogenesis and Development, J. Mol. Biol. (2014).
[114] L. Heins, A. Mehrle, R. Hemmler, R. Wagner, M. Kuchler, F. Hormann, D. Sveshnikov, J. Soll, The preprotein conducting channel at the inner envelope membrane of plastids, EMBO J. 21 (2002) 2616–2625.
[115] J. Bedard, S. Kubis, S. Bimanadham, P. Jarvis, Functional similarity between the chloroplast translocon component, Tic40, and the human co-chaperone, Hsp70-interacting protein (Hip), J. Biol. Chem. 282 (2007) 21404–21414.
[116] T. Stahl, C. Glockmann, J. Soll, L. Heins, Tic40, a new "old" subunit of the chloroplast protein import translocon, J. Biol. Chem. 274 (1999) 37467–37472.
[117] E. Kovacs-Bogdan, J.P. Benz, J. Soll, B. Bolter, Tic20 forms a channel independent of Tic110 in chloroplasts, BMC Plant Biol. 11 (2011) 133.
[118] S. Kikuchi, M. Oishi, Y. Hirabayashi, D.W. Lee, I. Hwang, M. Nakai, A 1-megadalton translocation complex containing Tic20 and Tic21 mediates chloroplast protein import at the inner envelope membrane, Plant Cell 21 (2009) 1781–1797.
[119] D. Duy, R. Stübe, G. Wanner, K. Philippar, The chloroplast permease PIC1 regulates plant growth and development by directing homeostasis and transport of iron, Plant Physiol. 155 (2011) 1709–1722.
[120] D. Duy, G. Wanner, A.R. Meda, N. von Wiren, J. Soll, K. Philippar, PIC1, an ancient permease in *Arabidopsis* chloroplasts, mediates iron transport, Plant Cell 19 (2007) 986–1006.
[121] M. Küchler, S. Decker, F. Hormann, J. Soll, L. Heins, Protein import into chloroplasts involves redox-regulated proteins, EMBO J. 21 (2002) 6136–6145.
[122] A. Caliebe, R. Grimm, G. Kaiser, J. Lubeck, J. Soll, L. Heins, The chloroplastic protein import machinery contains a Rieske-type iron–sulfur cluster and a mononuclear iron-binding protein, EMBO J. 16 (1997) 7342–7350.
[123] F. Chigri, F. Hormann, A. Stamp, D.K. Stammers, B. Bolter, J. Soll, U.C. Vothknecht, Calcium regulation of chloroplast protein translocation is mediated by calmodulin binding to Tic32, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 16051–16056.
[124] F. Hoermann, M. Küchler, D. Sveshnikov, U. Oppermann, Y. Li, J. Soll, Tic32, an essential component in chloroplast biogenesis, J. Biol. Chem. 279 (2004) 34756–34762.
[125] M. Kuchler, S. Decker, F. Hormann, J. Soll, L. Heins, Protein import into chloroplasts involves redox-regulated proteins, EMBO J. 21 (2002) 6136–6145.
[126] S. Kikuchi, J. Bédard, M. Hirano, Y. Hirabayashi, M. Oishi, M. Imai, M. Takase, T. Ide, M. Nakai, Uncovering the protein translocon at the chloroplast inner envelope membrane, Science 339 (2013) 571–574.
[127] N.W. Parker, Y. Wang, D. Meinke, Natural variation in sensitivity to a loss of chloroplast translation in *Arabidopsis*, Plant Physiol. 166 (2014) 2013–2027.
[128] D.-W. Zhang, S.H.U. Yuan, F.E.I. Xu, F. Zhu, M. Yuan, H.-X. Ye, H.-Q. Guo, X.I.N. Lv, Y. Yin, H.-H. Lin, Light intensity affects chlorophyll synthesis during greening process by metabolite signal from mitochondrial alternative oxidase in *Arabidopsis*, Plant Cell Environ. (2014). http://dx.doi.org/10.1111/pce.12438 (n/a-n/a).
[129] A. Stengel, P. Benz, M. Balsera, J. Soll, B. Bölter, TIC62 redox-regulated translocon composition and dynamics, J. Biol. Chem. 283 (2008) 6656–6667.
[130] F. Alte, A. Stengel, J.P. Benz, E. Petersen, J. Soll, M. Groll, B. Bolter, Ferredoxin:NADPH oxidoreductase is recruited to thylakoids by binding to a polyproline type II helix in a pH-dependent manner, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 19260–19265.
[131] S. Juric, K. Hazler-Pilepic, A. Tomasic, H. Lepedus, B. Jelicic, S. Puthiyaveetil, T. Bionda, L. Vojta, J.F. Allen, E. Schleiff, H. Fulgosi, Tethering of ferredoxin:NADP+ oxidoreductase to thylakoid membranes is mediated by novel chloroplast protein TROL, Plant J. 60 (2009) 783–794.
[132] M. Lintala, N. Schuck, I. Thormählen, A. Jungfer, K.L. Weber, A.P.M. Weber, P. Geigenberger, J. Soll, B. Bölter, P. Mulo, Arabidopsis tic62 trol mutant lacking thylakoid-bound ferredoxin–NADP+ oxidoreductase shows distinct metabolic phenotype, Mol. Plant 7 (2014) 45–57.
[133] S. Reumann, K. Keegstra, The endosymbiotic origin of the protein import machinery of chloroplastic envelope membranes, Trends Plant Sci. 4 (1999) 302–307.
[134] P. Boij, R. Patel, C. Garcia, P. Jarvis, H. Aronsson, In vivo studies on the roles of Tic55-related proteins in chloroplast protein import in *Arabidopsis thaliana*, Mol. Plant 2 (2009) 1397–1409.
[135] J.P. Benz, A. Stengel, M. Lintala, Y.H. Lee, A. Weber, K. Philippar, I.L. Gugel, S. Kaieda, T. Ikegami, P. Mulo, J. Soll, B. Bolter, *Arabidopsis* Tic62 and Ferredoxin-NADP(H) oxidoreductase form light-regulated complexes that are integrated into the chloroplast redox poise, Plant Cell 21 (2009) 3965-3683.