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Review

The Role of E3 Ubiquitin Ligases in Chloroplast Function

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Abstract

Chloroplasts are ancient organelles responsible for photosynthesis and various biosynthetic functions essential to most life on Earth. Many of these functions require tightly controlled regulatory processes to maintain homeostasis at the protein level. One such regulatory mechanism is the ubiquitin-proteasome system whose fundamental role is increasingly emerging in chloroplasts. In particular, the role of E3 ubiquitin ligases as determinants in the ubiquitination and degradation of specific intra-chloroplast proteins. Here, we highlight recent advances in understanding the roles of plant E3 ubiquitin ligases in chloroplast function.

Keywords: ubiquitin; E3 ligase; chloroplast; stress; photosynthesis; homeostasis; enzymes

Introduction

As essential and ancient organelles originating from the endosymbiosis of a cyanobacterial organism, chloroplasts are a defining feature of plants responsible for growth and development[1-3]. They are bound by a double membrane envelope surrounding a stromal matrix containing a unique thylakoid membrane home to photosynthesis, a process of transforming CO2 into carbohydrates[2,4]. Chloroplasts also perform an array of important biosynthetic processes such as nitrate assimilation and the synthesis of fatty acids, amino acids, and terpenes[5,6]. To maintain these functions, the chloroplast's diverse proteome must be dynamically controlled.

The chloroplast proteome is known to contain approximately 3,000 proteins. More than 95% of the proteome is nuclear encoded and the remaining ~100 proteins are encoded by the chloroplast genome[5,7,8]. Import of nuclear encoded chloroplast proteins relies on translocation through translocons known as TOC (translocon at the outer envelope membrane of chloroplasts) and TIC (translocon at the inner envelope membrane of chloroplasts)[9-11]. Regulation of the content and quality of the chloroplast proteome is established by several homeostatic mechanisms, including

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proteolysis, transcriptional control, and translation[9,12]. The presence of damaged or incorrectly sorted proteins can be detrimental and lead to the formation of aggregates or malfunction of important cellular pathways[9,13-15]. For instance, several abiotic stresses (i.e., extreme temperature, strong light, and oxidative conditions) can damage properly folded and sorted chloroplast proteins, consequently compromising the integrity of the chloroplast proteome and affecting plant growth[16,17]. Thus, it is imperative that chloroplasts maintain proteome homeostasis.

Recent findings demonstrate a link between the ubiquitin-proteasome system (UPS) and chloroplast function. Notably, plants heavily utilize the UPS to rapidly respond to the ever-changing environment [18-22]. The destruction of a protein by the UPS involves two successive steps: first, conjugation of the protein substrate through covalent attachment of ubiquitin molecules in a process called ubiquitination, and second, degradation of the ubiquitin-conjugated substrate by the 26S proteasome[23,24]. The mechanism of ubiquitination involves the collaborative action of three main enzymes. First, a ubiquitin-activating enzyme (E1) activates ubiquitin through ATP hydrolysis and delivers the activated ubiquitin to a ubiquitin-conjugating enzyme (E2), forming a thioester bond in its cysteine active site with the C-terminus glycine of the activated ubiquitin. Thereafter, a ubiquitin ligase (E3) strictly mediates the transfer of the activated ubiquitin from E2 to the target substrate[13,25]. In a typical scenario, additional ubiquitin molecules can attach via ubiquitin's Lys-48 or Lys-63 residue to form elongated chains in a process called polyubiquitination, required for recognition by proteasomal specific subunits[13,26-28].

Remarkably, the Arabidopsis genome contains thousands of proteins (~6% of the total genome) involved in the ubiquitin system, approximately 90% of which are E3 ubiquitin ligases whose functions are highly dynamic and tightly controlled[1,13,27,29]. Given their large abundance and versatility, E3 ubiquitin ligases are generally classified into 4 different groups: RING (Really Interesting New Gene), HECT (Homologous to the E6AP Carboxyl Terminus), PUB (Plant U-box), and RBR (RING-in-Between-RING)[13,27,30,31]. Within the last decade, advances have been made in illuminating how the UPS and particularly E3 ubiquitin ligases regulate chloroplast function. In this review, we focus on recent progress in understanding the role of E3 ubiquitin ligases in the degradation of chloroplast proteins, which is required for the proper maintenance of the chloroplast proteome.

1.1 SP1, a chloroplast-localized RING-type E3 ligase

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To maintain proper chloroplast function, >95% of chloroplast proteins are nuclear-encoded and rely on chloroplast import machinery to enter the organelle[5,7,8]. This import machinery is comprised of TOC and TIC protein complexes [9-11]. TOC complexes participate in both protein recognition and import pathways, and as such, can exist in various isoforms to mediate substrate specificity[4,32-34]. Studies have demonstrated the role of an E3 ubiquitin ligase, suppressor of plastid protein import 1 (*ppi1)* locus 1 (SP1), in alternating between different compositions of TOC isoforms to fine tune substrate-specific import [35].

SP1, a chloroplast-localized RING-type E3 ligase and outer membrane protein (Omp), was first identified in a mutagenesis screen investigating suppressors of *ppi1*, a Toc33 mutant shown to reduce chloroplast protein import and produce chlorosis[4,36]. The screen displayed defects in Toc33 and Toc75 genes impacting the proper function of protein import and plant growth[36]. Additional SP1 overexpression and mutant analysis confirmed its role in reconfiguring TOC machinery by ubiquitination and subsequent degradation by the 26S proteasome[4,36]. For instance, decreased accumulation of photosynthetic proteins and disproportionate TOC receptor levels were observed in *sp1* single mutants which led to poor photosynthetic performances affecting leaf senescence and de-etiolation[36]. In the same study, SP1 was revealed to interact with and ubiquitinate several TOC proteins, including the *Arabidopsis thaliana* (At) AtToc159, AtToc132, AtToc120, AtToc75, AtToc34, and AtToc33 (**Figure 1A**)[36]. Recent studies propose additional roles for SP1 in chloroplast development and protein import involving plastid interconversion events and tolerance to various stresses (i.e., salt, osmosis, abiotic, and/or ROS production)[36,37].

Furthermore, a chloroplast-associated protein degradation pathway (CHLORAD) was shown to target damaged TOC machinery[7]. In this pathway, SP1 interacts with Omp85-type β-barrel channel protein suppressor of *ppi1* locus2 (SP2) and cell division control protein 48 (CDC48), a conserved AAA+ (ATPases associated with diverse cellular activities) chaperone, to form a complex that mediates TOC protein degradation via protein retrotranslocation and the UPS (**Figure 1A**)[7,38]. While SP1 is involved in mediating the process of ubiquitination in the presence of stress or developmental cues, the exact mode of target substrate recognition by SP1 remains largely elusive [7,36]. Interestingly, it has been shown that SP1 can be auto-ubiquitinated, leading to its self-degradation by the 26S proteasome through CHLORAD[7]. However, the processes regulating SP1 and the existence of other chloroplast-localized E3 ligases remain to be revealed[35].

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Notably, a crucial role for SP1 and the CHLORAD pathway has been discovered in the regulation of fruit ripening in tomato plants[32]. In view of this, two evolutionarily conserved chloroplast SP1 homologues have been studied: SPL1 and SPL2, whose roles are still largely unknown[36,39]. Knockdown expression analysis of SP1 and SPL2 demonstrated delays in leaf senescence and fruit ripening while the opposite effect was apparent in the overexpression analysis of SP1. In addition, SP1 knockdown and RNA analyses on tomato fruit development exhibited changes in color, firmness, and ripening-related gene levels[32]. This indicated that SP1 indirectly induces the retrograde signals required for ripening, influences gene expression, and directly reconfigures TOC complexes to promote the import of ripening-related proteins (i.e., ethylene synthesis, carotenoid synthesis, cell wall modification, lipid metabolism, and chlorophyll catabolism proteins).

1.2 PUB4, a cytosolic E3 ubiquitin ligase

Chloroplasts accumulate oxidative damage in the presence of reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), superoxide anion radicals, and hydrogen peroxide produced during photosynthesis[4,16,40,41]. This occurs when the organelle's electron transport chain exceeds its capacity to transfer electrons under stressful environmental conditions resulting in electron leakage[41-43]. Thus, quality control mechanisms are needed to respond to these injuries and enable chloroplast turnover for the redistribution of nutrients[44-46]. To do so, chloroplasts can communicate information about their condition with the nucleus by retrograde signaling pathways to influence gene expression as well as utilize regulatory mechanisms such as the ubiquitin system or autophagy[42,44,47-51].

While investigating the mechanisms underlying these processes, genetic screens investigating plastid ferrochelatase enzyme mutants and $^1O_2$-induced chloroplast degradation in Arabidopsis revealed Plant U-Box 4 (PUB4), a cytosolic E3 ubiquitin ligase that targets unknown plastid proteins and chloroplasts for vacuolar-dependent degradation[42,52]. Here, Woodson et al. focused on ferrochelatase 1 and 2 (FC1 and FC2) which are conserved plastid enzymes, at the heme-chlorophyll branch point in the tetrapyrrole biosynthetic pathway that function by converting protoporphyrin IX to heme; a conversion involved in retrograde signaling[42,53,54]. In this study, mutant plant lines *fc2-1* and *fc2-2* promoted photosynthetic cell death in the cotyledons and an overproduction of both $^1O_2$ and a photosensitizing intermediate, Proto, within chloroplasts[42]. Notably, elevated levels of nuclear stress-associated genes, ROS, and

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damaged chloroplasts were reported in the *fc2-1/pub4-6* double mutants. Most noteworthy was the observation that ROS-damaged chloroplasts were specifically targeted by PUB4 and required a $^1O_2$-generated signal involving PUB4 (**Figure 1B**)[42].

Recently, a study analyzed a variety of mutants in conjunction with an Arabidopsis *gun1-102 ftsh5-3* double mutant known to promote the degradation of damaged chloroplasts and the formation of a variegated phenotype in the cotyledons and leaves. Chloroplast-localized Genomes Uncoupled-1 (GUN1) protein mediates chloroplast-to-nucleus signals whereas Filamentous temperature sensitive H5 (FtsH5) protein is involved in chloroplast development and photosystem repair. Importantly, the *gun1-102 ftsh5-3 pub4-7* triple mutant prevented the degradation of damaged chloroplasts and did not produce the variegated phenotype[55]. These findings also demonstrate PUB4 as a key player in chloroplast degradation to maintain plastid protein homeostasis. Nonetheless, its target substrates and the precise mechanisms by which it operates remain unknown[44].

Although various systems of protein degradation may indeed exist in chloroplasts, it remains unclear how the organelle is maintained by different degradation systems simultaneously. An investigation into two major eukaryotic degradation pathways, autophagy and the UPS, analyzed *pub4-6 atg5-1* and *pub4-6 atg7-2* double mutants[56]. Both double mutants displayed higher levels of ROS, nitrogen and carbon starvation susceptibility, early cell death, and lower seed production in comparison to wild-type or single mutants[52,56]. Cleavage assays investigating the activity of chloroplast autophagy displayed similar levels of autophagy activity in both wild-type and *pub4-6* mutants under high light treatment[56]. These findings revealed that PUB4-mediated degradation and autophagy act separately, but in parallel to maintain proper control of the proteome[52,56]. Interestingly, several recent studies have further revealed additional roles of PUB4 in plant growth and development, including shoot and root meristem regulation, cytokinin signaling, and microbe-associated molecular pattern-triggered immunity[57-60]. Given the versatility of its function, future studies may shed light on how exactly PUB4 is able to regulate both plant and chloroplast pathways to maintain homeostasis.

1.3 RING-type E3 ligase, COP1

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Light is essential for all life and serves as an important signal not only for plant flowering but also the expression of genes required to construct photosynthetically competent chloroplasts[6,61-63]. In view of this, chloroplast populations are reliant upon light signals in regulating multiple phytohormones, such as ethylene, brassinosteroid, cytokinin, abscisic acid (ABA), auxin, and gibberellin, which play fundamental roles in modulating photosynthetic processes and chloroplast development at the transcriptional level[64-66]. These phytohormone pathways are directly or indirectly controlled by the UPS, and specifically some ubiquitin ligases participate in hormone perception and signaling mechanisms [22,67]. Here, we highlight yet another role for the UPS-phytohormones crosstalk in chloroplast function and development.

Importantly, the construction of photosynthetically competent chloroplasts also requires the import of nuclear-encoded precursor proteins, chlorophyll biosynthesis, and formation of the thylakoid membranes[6,68]. These activities rely on retrograde signaling to manage the expression of photosynthesis-associated nuclear genes (PhANGs)[48-51]. One essential process controlled by the combination of light and phytohormone pathways is seedling de-etiolation, a process of developing functional chloroplasts during the transition from heterotrophic to photoautotrophic growth in plants[62,69-71]. To maintain properly scheduled seedling de-etiolation and photomorphogenesis, Constitutive Photomorphogenesis 1 (COP1), a RING-type E3 ligase, regulates these processes during the dark-to-light shift sensed by photoreceptors (i.e., phytochromes and cryptochromes)[72-75]. The activation of COP1 occurs in the dark when it accumulates in the nucleus and a complex with suppressor of phyA-105 1 (SPA1) is formed to polyubiquitinate activated photoreceptors and positive regulators of light signaling[74,76-79]. Conversely, COP1-mediated degradation of these positive regulators is inhibited upon light exposure[72,75,76]. In addition, the coordinated efforts involved in chloroplast development also rely on the repressive abilities of Phytochrome Interacting Factors (PIFs) (i.e., PIF1 and PIF3) and Ethylene-Insensitive 3 (EIN3) in repressing genes associated with chlorophyll biosynthesis and etiolation[80-82].

Recently, several studies have illuminated an intriguing connection linking hormone, light, and retrograde signaling pathways to the fundamental role of COP1-mediated degradation in chloroplast biogenesis[64,69,83]. ABA is a plant hormone participating in stress signaling and functions antagonistic to light signaling to control developmental processes such as seed germination and stomatal movement[84-86]. A feedback mechanism regulating transcription

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factors ABA Insensitive 4 (ABI4) and Elongated Hypocotyl 5 (HY5), involved in mediating retrograde signaling/plant development and promoting de-etiolation, respectively, revealed that COP1 serves as a convergence point for the integration of light and chloroplast signaling pathways[69,87]. Here, a chloroplast signal mediated by GUN1 and the N-terminus of chloroplast envelope-bound plant homeodomain type transcription factor with transmembrane domains activates ABI4. Accordingly, ABI4 and HY5 are shown to regulate COP1 expression while COP1 targets ABI4 in the light and HY5 in the dark for proteasomal degradation (**Figure 1C**). Regulating the functions of ABI4, HY5, and COP1 ensures the establishment of functional chloroplasts; however, the exact mechanisms underlying chloroplast retrograde signals remain unknown[69]. Similarly, increased COP1 activity was observed under long-term ABA treatments which also displayed elevated levels of Golden2-Like1 (GLK1) degradation[83]. Importantly, the degradation of GLK1, a key transcription factor in regulating *PhANG* expression encoding chlorophyll biosynthesis enzymes, led to varying levels of leaf yellowing indicative of the suppression of chloroplast development in a light-dependent manner under both long-term ABA and different light intensity treatments[83,88].

Furthermore, COP1 also participates in managing several negative regulators in chloroplast biogenesis. For instance, Brassinazole-Resistant 1 (BZR1), a negative regulator of photomorphogenesis and chloroplast development, is indicated as a target of COP1. In the light, active (dephosphorylated) BZR1 is inhibited by HY5 to enable the progression of chloroplast development whereas COP1 targeted inactive forms of BZR1 in the dark for degradation[64,89,90]. Moreover, COP1 has also been shown to interact non-proteolytically with negative regulators of *PhANG*s, such as PIF3 and EIN3. In doing so, COP1 stabilizes PIF3, by preventing its interaction with a kinase, and EIN3, by targeting E3 ubiquitin ligases EIN3-Binding F-box protein 1 and 2 (EBF1 and EBF2) specific to EIN3; thus, inhibiting the subsequent degradation of PIF3 and EIN3[91]. Interestingly, COP1 engages with cytoplasmic processing bodies (p-bodies), granules that can function to store, degrade, and translate mRNA. *Dcp5-1*, a defective p-body mutant in Arabidopsis, coupled with *cop1-6* mutants demonstrated the need for COP1 in promoting the formation of p-bodies in the dark that attenuate the translation of specific mRNAs for chlorophyll biosynthesis[92]. Taken together, these recent studies have revealed that the role of COP1 extends far beyond its involvement in light signaling. COP1 is a crucial component mediating substrate-specific protein levels as well as light, hormone, and retrograde signaling pathways, all of which are essential in chloroplast development and function.

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1.4 Chaperone-dependent cytosolic E3 ligase, CHIP

Owing to the large number of nuclear-encoded proteins comprising the chloroplast proteome, coordinated transport following synthesis in the cytosol to the stroma is critical for the proper function of the organelle[5,12,93]. Thereafter, these precursor proteins are subjected to proteolytic processing for the cleavage of their transit peptides and undergo folding, and/or sorting with the assistance of molecular chaperones to other compartments, such as the thylakoids[5,94-96]. Importantly, chloroplast precursor proteins must be unfolded for translocation[97,98]. Left unfolded and unregulated, they tend to form non-specific protein aggregates in the cytosol[96,99,100]. To date, plants contain >20 chloroplast proteases, including chloroplast caseinolytic proteases (Clps) in the stroma and FtsH on the thylakoid membranes which are both involved in the removal of imported and chloroplast-encoded proteins[101-105]. Remarkably, a cytosolic E3 ubiquitin ligase was discovered to regulate these chloroplast proteases.

Carboxyl Terminus of the Hsp70-Interacting Proteins (CHIP), a highly conserved chaperone-dependent and U-box containing cytosolic E3 ligase, targets chloroplast protease precursor proteins to prevent their accumulation and aggregation in the cytosol[95,106-110]. Targeting these substrates requires the help of heat shock protein 70 or 90 (Hsp70 or Hsp90), molecular chaperones who monitor misfolded proteins[95,111]. A study investigating Arabidopsis *ppi2* mutant plants, shown to impair protein import into chloroplasts, demonstrated an accumulation of precursor proteins in the cytosol and an increase in *Hsc70-4* (an Hsp70 isoform) and *CHIP* expression[108]. The Hsc70-4 protein plays a critical role in interacting with the transit peptides of target substrates[108]. Thenceforth, a complex formation with CHIP and substrate bound Hsp70 or 90 will lead to an interaction with an E2 to facilitate the ubiquitination and degradation of target substrates via the 26S proteasome (**Figure 1D**)[106,108,112].

FtsH and Clp proteases consist of multi-subunit complexes, whereby any alteration to the production of these subunits may result in a disruption of the function and structure of the protease[106,109,113]. AtCHIP can indirectly regulate chloroplast proteases Clp and FtsH by interacting with their cytosolic precursors, including FtsH1, FtsH2, ClpP3, ClpP4, and ClpP5 [106,109,110,112]. For instance, *AtCHIP* overexpression analyses demonstrated a reduction in subunit precursor steady-state levels under high-intensity light conditions [106,110]. Importantly, the overexpression of *AtCHIP* was also observed to prevent the chlorotic phenotypes present in the suppression or overexpression of ClpP4 by

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restoring proper subunit stoichiometry. These findings have been detected in transgenic tobacco plants as well[106]. Therefore, suggesting that CHIP plays a conserved role in maintaining chloroplast protease homeostasis and establishing functional protease core complexes[95,106,110].

Recently, CHIP was reported to be strongly affected by ABA and play a crucial role in stress and heat tolerance in Arabidopsis and tomato plants[114,115]. Notably, increased temperature and heat stress has been attributed to a rise in misfolded chloroplast precursor proteins which can also result in decreased protein import into chloroplasts[95,116]. In the absence of CHIP, increased temperature sensitivity, reduced photosynthetic activity, and elevated levels of photosynthetic protein aggregates were observed[95,115]. Interestingly, these aggregates were still ubiquitinated, implying the existence of other unknown E3 ligases involved in chloroplast function[115]. Strikingly, CHIP is also able to interact with two nuclear-encoded proteins important in chloroplast function. This includes the ribulose-biphosphate carboxylases (Rubisco) small subunit (RbcS) and light harvesting complex photosystem II subunit 6 (Lhcb6), which imply an additional role of CHIP in maintaining the chloroplast proteome[117]. Taken together, these findings suggest the need for a rapid response in preventing the accumulation of unimported chloroplast precursor proteins and balancing protease activity under stressful conditions[95,115].

1.5 TT3.1, a plasma membrane-localized RING-type E3 ligase

Plants are constantly exposed to changing temperatures[19,118,119]. High temperatures may damage many metabolic, photosynthetic, and other various molecular pathways; thus, inhibiting the growth and development of plants as a result of heat stress[118-121]. For that reason, the ability to sense temperature fluctuations is vital to quickly respond and adapt to the environment[118,122,123]. An important advancement in understanding various resistance mechanisms in plants led to the discovery of Thermo-Tolerance 3.1 (TT3.1). TT3.1 is a plasma membrane-localized RING-type E3 ligase necessary for the ubiquitination and vacuolar degradation of TT3.2, a chloroplast precursor protein and “potential thermosensor” protecting the thylakoids under increased temperatures[124].

In this study, overexpression analysis of TT3.1 or knockdown of TT3.2 both showed a notable increase in grain yield under heat stress[124]. Strikingly, heat stress-induced accumulation of TT3.2 has been shown to cause chloroplast damage, implicating the need for high TT3.1 activity for its rapid degradation. TT3.1-mediated degradation of TT3.2

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observed under heat treatment and in transient expression and immunogold-labelling assays revealed that TT3.1 will translocate to the endosomes to intercept and target chloroplast-destined TT3.2 proteins to the endosomes for degradation via vacuoles (**Figure 1E**). Additionally, both TT3.1 and TT3.2 have been detected in valuable crops, such as rice, maize, and wheat[124]. Thus, suggesting a likely conserved mechanism of thermosensing and highlighting new possible strategies in engineering heat tolerant plants. Despite these findings, the mechanisms underlying plasma membrane-to-chloroplast communication to initiate the process of thermotolerance and how the accumulation of TT3.2 causes damage remains unknown[124].

2.1 Role of CDC48 in intra-chloroplast protein degradation

CDC48 is a highly conserved eukaryotic protein and ubiquitin-dependent segregase belonging to the AAA+ family[7,38]. Located in the cytosol and nucleus, CDC48 plays an important role in the CHLORAD pathway. Degradation via CHLORAD is achieved by the coordinated actions of SP1, which directly ubiquitinates target substrates, and retrotranslocation of the target substrates to the cytosol by SP2 and CDC48 proteins for degradation via the 26S proteasome (**Figure 1F**). More specifically, CDC48 provides the proper ATP-powered machinery needed to surpass the physical barriers presented by the chloroplast envelope membranes upon protein extraction[7]. To function, CDC48 requires a complex formation with Ubiquitin Fusion Degradation1 (UFD1)-Nuclear Protein Localization4 (NPL4), a heterodimeric cofactor that binds ubiquitin and small ubiquitin-like modifier proteins[38].

Prior investigation of the CHLORAD pathway suggested that its ubiquitination targets are those of the TOC apparatus[7]. Beyond chloroplast protein import machinery, extensive ubiquitination was recently found in chloroplast fractions, and in greater abundance under UPS or retrotranslocation inhibition. Among these ubiquitinated proteins were the Rubisco large subunit (RbcL) and ATP synthase subunit beta (AtpB), both of which are chloroplast encoded and degraded through the CHLORAD pathway[38]. Most recently, an unpublished study by Sun et al. demonstrated additional targets of the CHLORAD pathway involved in photosynthesis (i.e., electron transport, energy transduction, and carbon fixation), gene expression, and fatty acid/lipid metabolism[125]. One important target substrate of interest is fatty acid export 1 (FAX1), an inner chloroplast envelope protein and key player in fatty acid (FA) export. In the presence of defective CHLORAD-mediated degradation of FAX1, disturbances in cellular metabolic homeostasis (i.e.,

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a notable decrease in cellular lipid species and chloroplast-produced FAs) were observed[125]. Altogether, these findings raise questions about how intra-chloroplast proteins are selected by the CHLORAD pathway and if intra-chloroplast proteins are marked by ubiquitin inside the organelle[38,126]. Further understanding how the CDC48 complex functions under a myriad of stresses and elucidating internal chloroplast ubiquitination processes will be significant in developing new ways for plants to adapt to the ever-changing environmental conditions.

Outlook

Within the last decade, the discovery of several connections between the UPS and chloroplasts has largely altered our current views about the extent to which E3 ubiquitin ligases can maintain their regulatory activities in plants. The study of E3s in chloroplast function, which has led to the identification of ubiquitinated chloroplast proteins, is valuable for future agricultural applications. For instance, E3 ligases such as TT3.1 can potentially be exploited and re-engineered to control the degradation of TT3.2 for chloroplast survival under high temperatures[124]. Uncovering the diverse mechanisms underlying the interactions of E3 ligases with chloroplast associated proteins will be significant to unveiling deeper insights into how the chloroplast proteome can be manipulated to promote adaptation in the ever-changing environments. Perhaps intra-chloroplast target substrates demonstrate an evolved regulatory system, suggesting a large, interconnected network influencing many of the pathways that existed prior to the integration of chloroplasts.

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Author Contribution

K.H. and N.S conceived, wrote, and revised the manuscript.

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Conflict of interest

N.S. has an equity interest in OerthBio LLC and serves on the company's Scientific Advisory Board. The work and data submitted here have no competing interests, or other interests that might be perceived to influence this review article.

References

1. Huang, W.; Ling, Q.; Jarvis, P. The ubiquitin-proteasome system regulates chloroplast biogenesis. *Commun Integr Biol* **2013**, *6*, e23001, doi:10.4161/cib.23001.

2. Zimorski, V.; Ku, C.; Martin, W.F.; Gould, S.B. Endosymbiotic theory for organelle origins. *Curr Opin Microbiol* **2014**, *22*, 38-48, doi:10.1016/j.mib.2014.09.008.

3. Jarvis, P. Organellar proteomics: chloroplasts in the spotlight. *Curr Biol* **2004**, *14*, R317-319, doi:10.1016/j.cub.2004.03.054.

4. Ling, Q.; Jarvis, P. Plant Signaling: Ubiquitin Pulls the Trigger on Chloroplast Degradation. *Curr Biol* **2016**, *26*, R38-40, doi:10.1016/j.cub.2015.11.022.

5. Shi, L.X.; Theg, S.M. The chloroplast protein import system: from algae to trees. *Biochim Biophys Acta* **2013**, *1833*, 314-331, doi:10.1016/j.bbamcr.2012.10.002.

6. Waters, M.T.; Langdale, J.A. The making of a chloroplast. *EMBO J* **2009**, *28*, 2861-2873, doi:10.1038/emboj.2009.264.

7. Ling, Q.; Broad, W.; Trosch, R.; Topel, M.; Demiral Sert, T.; Lymperopoulos, P.; Baldwin, A.; Jarvis, R.P. Ubiquitin-dependent chloroplast-associated protein degradation in plants. *Science* **2019**, *363*, doi:10.1126/science.aav4467.

8. Leister, D. Chloroplast research in the genomic age. *Trends Genet* **2003**, *19*, 47-56, doi:10.1016/s0168-9525(02)00003-3.

9. Nishimura, K.; Kato, Y.; Sakamoto, W. Essentials of Proteolytic Machineries in Chloroplasts. *Mol Plant* **2017**, *10*, 4-19, doi:10.1016/j.molp.2016.08.005.

10. Zhu, D.; Xiong, H.; Wu, J.; Zheng, C.; Lu, D.; Zhang, L.; Xu, X. Protein Targeting Into the Thylakoid Membrane Through Different Pathways. *Front Physiol* **2021**, *12*, 802057, doi:10.3389/fphys.2021.802057.

11. Richardson, L.G.L.; Schnell, D.J. Origins, function, and regulation of the TOC-TIC general protein import machinery of plastids. *J Exp Bot* **2020**, *71*, 1226-1238, doi:10.1093/jxb/erz517.

12. Thomson, S.M.; Pulido, P.; Jarvis, R.P. Protein import into chloroplasts and its regulation by the ubiquitin-proteasome system. *Biochem Soc Trans* **2020**, *48*, 71-82, doi:10.1042/BST20190274.

13. Zheng, N.; Shabek, N. Ubiquitin Ligases: Structure, Function, and Regulation. *Annu Rev Biochem* **2017**, *86*, 129-157, doi:10.1146/annurev-biochem-060815-014922.

14. Tyedmers, J.; Mogk, A.; Bukau, B. Cellular strategies for controlling protein aggregation. *Nat Rev Mol Cell Biol* **2010**, *11*, 777-788, doi:10.1038/nrm2993.

15. Dobson, C.M. Protein folding and misfolding. *Nature* **2003**, *426*, 884-890, doi:10.1038/nature02261.

16. Dietz, K.J.; Turkan, I.; Krieger-Liszkay, A. Redox- and Reactive Oxygen Species-Dependent Signaling into and out of the Photosynthesizing Chloroplast. *Plant Physiol* **2016**, *171*, 1541-1550, doi:10.1104/pp.16.00375.

17. Yang, X.; Li, Y.; Qi, M.; Liu, Y.; Li, T. Targeted Control of Chloroplast Quality to Improve Plant Acclimation: From Protein Import to Degradation. *Front Plant Sci* **2019**, *10*, 958, doi:10.3389/fpls.2019.00958.

18. Trenner, J.; Monaghan, J.; Saeed, B.; Quint, M.; Shabek, N.; Trujillo, M. Evolution and Functions of Plant U-Box Proteins: From Protein Quality Control to Signaling. *Annu Rev Plant Biol* **2022**, *73*, 93-121, doi:10.1146/annurev-arplant-102720-012310.

19. Wang, S.; Lv, X.; Zhang, J.; Chen, D.; Chen, S.; Fan, G.; Ma, C.; Wang, Y. Roles of E3 Ubiquitin Ligases in Plant Responses to Abiotic Stresses. *Int J Mol Sci* **2022**, *23*, doi:10.3390/ijms23042308.

20. Holdsworth, M.J.; Gibbs, D.J. Comparative Biology of Oxygen Sensing in Plants and Animals. *Curr Biol* **2020**, *30*, R362-R369, doi:10.1016/j.cub.2020.03.021.

21. Xu, F.Q.; Xue, H.W. The ubiquitin-proteasome system in plant responses to environments. *Plant Cell Environ* **2019**, *42*, 2931-2944, doi:10.1111/pce.13633.

22. Shabek, N.; Zheng, N. Plant ubiquitin ligases as signaling hubs. *Nat Struct Mol Biol* **2014**, *21*, 293-296, doi:10.1038/nsmb.2804.

23. Shabek, N.; Ciechanover, A. Degradation of ubiquitin: the fate of the cellular reaper. *Cell Cycle* **2010**, *9*, 523-530, doi:10.4161/cc.9.3.11152.

24. Hershko, A.; Ciechanover, A. The ubiquitin system. *Annual review of biochemistry* **1998**, *67*, 425-479, doi:10.1146/annurev.biochem.67.1.425.

25. Sharma, B.; Joshi, D.; Yadav, P.K.; Gupta, A.K.; Bhatt, T.K. Role of Ubiquitin-Mediated Degradation System in Plant Biology. *Front Plant Sci* **2016**, *7*, 806, doi:10.3389/fpls.2016.00806.

26. Saeki, Y. Ubiquitin recognition by the proteasome. *J Biochem* **2017**, *161*, 113-124, doi:10.1093/jb/mvw091.

27. Vierstra, R.D. The ubiquitin-26S proteasome system at the nexus of plant biology. *Nat Rev Mol Cell Biol* **2009**, *10*, 385-397, doi:10.1038/nrm2688.

28. Trempe, J.F. Reading the ubiquitin postal code. *Curr Opin Struct Biol* **2011**, *21*, 792-801, doi:10.1016/j.sbi.2011.09.009.

29. Moon, J.; Parry, G.; Estelle, M. The ubiquitin-proteasome pathway and plant development. *Plant Cell* **2004**, *16*, 3181-3195, doi:10.1105/tpc.104.161220.

30. Linden, K.J.; Callis, J. The ubiquitin system affects agronomic plant traits. *J Biol Chem* **2020**, *295*, 13940-13955, doi:10.1074/jbc.REV120.011303.

31. Trujillo, M. News from the PUB: plant U-box type E3 ubiquitin ligases. *J Exp Bot* **2018**, *69*, 371-384, doi:10.1093/jxb/erx411.

32. Ling, Q.; Sadali, N.M.; Soufi, Z.; Zhou, Y.; Huang, B.; Zeng, Y.; Rodriguez-Concepcion, M.; Jarvis, R.P. The chloroplast-associated protein degradation pathway controls chromoplast development and fruit ripening in tomato. *Nat Plants* **2021**, *7*, 655-666, doi:10.1038/s41477-021-00916-y.

33. Jarvis, P. Targeting of nucleus-encoded proteins to chloroplasts in plants. *New Phytol* **2008**, *179*, 257-285, doi:10.1111/j.1469-8137.2008.02452.x.

34. Demarsy, E.; Lakshmanan, A.M.; Kessler, F. Border control: selectivity of chloroplast protein import and regulation at the TOC-complex. *Front Plant Sci* **2014**, *5*, 483, doi:10.3389/fpls.2014.00483.

35. Ling, Q.; Jarvis, P. Functions of plastid protein import and the ubiquitin-proteasome system in plastid development. *Biochim Biophys Acta* **2015**, *1847*, 939-948, doi:10.1016/j.bbabio.2015.02.017.

36. Ling, Q.; Huang, W.; Baldwin, A.; Jarvis, P. Chloroplast biogenesis is regulated by direct action of the ubiquitin-proteasome system. *Science* **2012**, *338*, 655-659, doi:10.1126/science.1225053.

37. Ling, Q.; Jarvis, P. Regulation of Chloroplast Protein Import by the Ubiquitin E3 Ligase SP1 Is Important for Stress Tolerance in Plants. *Curr Biol* **2015**, *25*, 2527-2534, doi:10.1016/j.cub.2015.08.015.

38. Li, J.; Yuan, J.; Li, Y.; Sun, H.; Ma, T.; Huai, J.; Yang, W.; Zhang, W.; Lin, R. The CDC48 complex mediates ubiquitin-dependent degradation of intra-chloroplast proteins in plants. *Cell Rep* **2022**, *39*, 110664, doi:10.1016/j.celrep.2022.110664.

39. Tracz, M.; Gorniak, I.; Szczepaniak, A.; Bialek, W. E3 Ubiquitin Ligase SPL2 Is a Lanthanide-Binding Protein. *Int J Mol Sci* **2021**, *22*, doi:10.3390/ijms22115712.

40. Foyer, C.H.; Hanke, G. ROS production and signalling in chloroplasts: cornerstones and evolving concepts. *Plant J* **2022**, doi:10.1111/tpj.15856.

41. Asada, K. Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiol* **2006**, *141*, 391-396, doi:10.1104/pp.106.082040.

42. Woodson, J.D.; Joens, M.S.; Sinson, A.B.; Gilkerson, J.; Salome, P.A.; Weigel, D.; Fitzpatrick, J.A.; Chory, J. Ubiquitin facilitates a quality-control pathway that removes damaged chloroplasts. *Science* **2015**, *350*, 450-454, doi:10.1126/science.aac7444.

43. Foyer, C.H.; Noctor, G. Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiologia Plantarum* **2003**, *119*, 355-364, doi:https://doi.org/10.1034/j.1399-3054.2003.00223.x.

44. Woodson, J.D. Control of chloroplast degradation and cell death in response to stress. *Trends Biochem Sci* **2022**, doi:10.1016/j.tibs.2022.03.010.

45. Woodson, J.D. Chloroplast quality control - balancing energy production and stress. *New Phytol* **2016**, *212*, 36-41, doi:10.1111/nph.14134.

46. Rochaix, J.D.; Ramundo, S. Chloroplast signaling and quality control. *Essays Biochem* **2018**, *62*, 13-20, doi:10.1042/EBC20170048.

47. Izumi, M.; Nakamura, S. Chloroplast Protein Turnover: The Influence of Extraplastidic Processes, Including Autophagy. *Int J Mol Sci* **2018**, *19*, doi:10.3390/ijms19030828.

48. Chan, K.X.; Phua, S.Y.; Crisp, P.; McQuinn, R.; Pogson, B.J. Learning the Languages of the Chloroplast: Retrograde Signaling and Beyond. *Annu Rev Plant Biol* **2016**, *67*, 25-53, doi:10.1146/annurev-arplant-043015-111854.

49. de Souza, A.; Wang, J.Z.; Dehesh, K. Retrograde Signals: Integrators of Interorganellar Communication and Orchestrators of Plant Development. *Annu Rev Plant Biol* **2017**, *68*, 85-108, doi:10.1146/annurev-arplant-042916-041007.

50. Hernandez-Verdeja, T.; Strand, A. Retrograde Signals Navigate the Path to Chloroplast Development. *Plant Physiol* **2018**, *176*, 967-976, doi:10.1104/pp.17.01299.

51. Strand, A. Plastid-to-nucleus signalling. *Curr Opin Plant Biol* **2004**, *7*, 621-625, doi:10.1016/j.pbi.2004.09.004.

52. Nakamura, S.; Izumi, M. Chlorophagy does not require PLANT U-BOX4-mediated ubiquitination. *Plant Signal Behav* **2021**, *16*, 1861769, doi:10.1080/15592324.2020.1861769.

53. Scharfenberg, M.; Mittermayr, L.; E, V.O.N.R.-L.; Schlicke, H.; Grimm, B.; Leister, D.; Kleine, T. Functional characterization of the two ferrochelatases in Arabidopsis thaliana. *Plant Cell Environ* **2015**, *38*, 280-298, doi:10.1111/pce.12248.

54. Woodson, J.D.; Perez-Ruiz, J.M.; Schmitz, R.J.; Ecker, J.R.; Chory, J. Sigma factor-mediated plastid retrograde signals control nuclear gene expression. *Plant J* **2013**, *73*, 1-13, doi:10.1111/tpj.12011.

55. Jeran, N.; Rotasperti, L.; Frabetti, G.; Calabritto, A.; Pesaresi, P.; Tadini, L. The PUB4 E3 Ubiquitin Ligase Is Responsible for the Variegated Phenotype Observed upon Alteration of Chloroplast Protein Homeostasis in Arabidopsis Cotyledons. *Genes (Basel)* **2021**, *12*, doi:10.3390/genes12091387.

56. Kikuchi, Y.; Nakamura, S.; Woodson, J.D.; Ishida, H.; Ling, Q.; Hidema, J.; Jarvis, R.P.; Hagihara, S.; Izumi, M. Chloroplast Autophagy and Ubiquitination Combine to Manage Oxidative Damage and Starvation Responses. *Plant Physiol* **2020**, *183*, 1531-1544, doi:10.1104/pp.20.00237.

57. Kinoshita, A.; Seo, M.; Kamiya, Y.; Sawa, S. Mystery in genetics: PUB4 gives a clue to the complex mechanism of CLV signaling pathway in the shoot apical meristem. *Plant Signal Behav* **2015**, *10*, e1028707, doi:10.1080/15592324.2015.1028707.

58. Kinoshita, A.; ten Hove, C.A.; Tabata, R.; Yamada, M.; Shimizu, N.; Ishida, T.; Yamaguchi, K.; Shigenobu, S.; Takebayashi, Y.; Iuchi, S.; et al. A plant U-box protein, PUB4, regulates asymmetric cell division and cell proliferation in the root meristem. *Development* **2015**, *142*, 444-453, doi:10.1242/dev.113167.

59. Wang, Y.; Wu, Y.; Yu, B.; Yin, Z.; Xia, Y. EXTRA-LARGE G PROTEINs Interact with E3 Ligases PUB4 and PUB2 and Function in Cytokinin and Developmental Processes. *Plant Physiol* **2017**, *173*, 1235-1246, doi:10.1104/pp.16.00816.

60. Desaki, Y.; Takahashi, S.; Sato, K.; Maeda, K.; Matsui, S.; Yoshimi, I.; Miura, T.; Jumonji, J.I.; Takeda, J.; Yashima, K.; et al. PUB4, a CERK1-Interacting Ubiquitin Ligase, Positively Regulates MAMP-Triggered Immunity in Arabidopsis. *Plant Cell Physiol* **2019**, *60*, 2573-2583, doi:10.1093/pcp/pcz151.

61. Hills, A.C.; Khan, S.; Lopez-Juez, E. Chloroplast Biogenesis-Associated Nuclear Genes: Control by Plastid Signals Evolved Prior to Their Regulation as Part of Photomorphogenesis. *Front Plant Sci* **2015**, *6*, 1078, doi:10.3389/fpls.2015.01078.

62. Jiao, Y.; Lau, O.S.; Deng, X.W. Light-regulated transcriptional networks in higher plants. *Nat Rev Genet* **2007**, *8*, 217-230, doi:10.1038/nrg2049.

63. Feng, P.; Guo, H.; Chi, W.; Chai, X.; Sun, X.; Xu, X.; Ma, J.; Rochaix, J.D.; Leister, D.; Wang, H.; et al. Chloroplast retrograde signal regulates flowering. *Proc Natl Acad Sci U S A* **2016**, *113*, 10708-10713, doi:10.1073/pnas.1521599113.

64. Cackett, L.; Luginbuehl, L.H.; Schreier, T.B.; Lopez-Juez, E.; Hibberd, J.M. Chloroplast development in green plant tissues: the interplay between light, hormone, and transcriptional regulation. *New Phytol* **2022**, *233*, 2000-2016, doi:10.1111/nph.17839.

65. Liu, X.; Li, Y.; Zhong, S. Interplay between Light and Plant Hormones in the Control of Arabidopsis Seedling Chlorophyll Biosynthesis. *Front Plant Sci* **2017**, *8*, 1433, doi:10.3389/fpls.2017.01433.

66. Muller, M.; Munne-Bosch, S. Hormonal impact on photosynthesis and photoprotection in plants. *Plant Physiol* **2021**, *185*, 1500-1522, doi:10.1093/plphys/kiaa119.

67. Tal, L.; Gil, M.X.A.; Guercio, A.M.; Shabek, N. Structural Aspects of Plant Hormone Signal Perception and Regulation by Ubiquitin Ligases. *Plant Physiol* **2020**, *182*, 1537-1544, doi:10.1104/pp.19.01282.

68. Jarvis, P.; Lopez-Juez, E. Biogenesis and homeostasis of chloroplasts and other plastids. *Nat Rev Mol Cell Biol* **2013**, *14*, 787-802, doi:10.1038/nrm3702.

69. Xu, X.; Chi, W.; Sun, X.; Feng, P.; Guo, H.; Li, J.; Lin, R.; Lu, C.; Wang, H.; Leister, D.; et al. Convergence of light and chloroplast signals for de-etiolation through ABI4-HY5 and COP1. *Nat Plants* **2016**, *2*, 16066, doi:10.1038/nplants.2016.66.

70. Feng, S.; Martinez, C.; Gusmaroli, G.; Wang, Y.; Zhou, J.; Wang, F.; Chen, L.; Yu, L.; Iglesias-Pedraz, J.M.; Kircher, S.; et al. Coordinated regulation of Arabidopsis thaliana development by light and gibberellins. *Nature* **2008**, *451*, 475-479, doi:10.1038/nature06448.

71. Bai, M.Y.; Shang, J.X.; Oh, E.; Fan, M.; Bai, Y.; Zentella, R.; Sun, T.P.; Wang, Z.Y. Brassinosteroid, gibberellin and phytochrome impinge on a common transcription module in Arabidopsis. *Nat Cell Biol* **2012**, *14*, 810-817, doi:10.1038/ncb2546.

72. Xu, X.; Paik, I.; Zhu, L.; Huq, E. Illuminating Progress in Phytochrome-Mediated Light Signaling Pathways. *Trends Plant Sci* **2015**, *20*, 641-650, doi:10.1016/j.tplants.2015.06.010.

73. Galvao, V.C.; Fankhauser, C. Sensing the light environment in plants: photoreceptors and early signaling steps. *Curr Opin Neurobiol* **2015**, *34*, 46-53, doi:10.1016/j.conb.2015.01.013.

74. Deng, X.W.; Caspar, T.; Quail, P.H. cop1: a regulatory locus involved in light-controlled development and gene expression in Arabidopsis. *Genes Dev* **1991**, *5*, 1172-1182, doi:10.1101/gad.5.7.1172.

75. McNellis, T.W.; von Arnim, A.G.; Deng, X.W. Overexpression of Arabidopsis COP1 results in partial suppression of light-mediated development: evidence for a light-inactivable repressor of photomorphogenesis. *Plant Cell* **1994**, *6*, 1391-1400, doi:10.1105/tpc.6.10.1391.

76. Hoecker, U. The activities of the E3 ubiquitin ligase COP1/SPA, a key repressor in light signaling. *Curr Opin Plant Biol* **2017**, *37*, 63-69, doi:10.1016/j.pbi.2017.03.015.

77. Seluzicki, A.; Burko, Y.; Chory, J. Dancing in the dark: darkness as a signal in plants. *Plant Cell Environ* **2017**, *40*, 2487-2501, doi:10.1111/pce.12900.

78. Kim, T.H.; Kim, B.H.; von Arnim, A.G. Repressors of photomorphogenesis. *Int Rev Cytol* **2002**, *220*, 185-223, doi:10.1016/s0074-7696(02)20006-6.

79. Hoecker, U.; Quail, P.H. The phytochrome A-specific signaling intermediate SPA1 interacts directly with COP1, a constitutive repressor of light signaling in Arabidopsis. *J Biol Chem* **2001**, *276*, 38173-38178, doi:10.1074/jbc.M103140200.

80. Gommers, C.M.M.; Monte, E. Seedling Establishment: A Dimmer Switch-Regulated Process between Dark and Light Signaling. *Plant Physiol* **2018**, *176*, 1061-1074, doi:10.1104/pp.17.01460.

81. Zhong, S.; Zhao, M.; Shi, T.; Shi, H.; An, F.; Zhao, Q.; Guo, H. EIN3/EIL1 cooperate with PIF1 to prevent photo-oxidation and to promote greening of Arabidopsis seedlings. *Proc Natl Acad Sci U S A* **2009**, *106*, 21431-21436, doi:10.1073/pnas.0907670106.

82. Liu, X.; Liu, R.; Li, Y.; Shen, X.; Zhong, S.; Shi, H. EIN3 and PIF3 Form an Interdependent Module That Represses Chloroplast Development in Buried Seedlings. *Plant Cell* **2017**, *29*, 3051-3067, doi:10.1105/tpc.17.00508.

83. Lee, J.; Choi, B.; Yun, A.; Son, N.; Ahn, G.; Cha, J.Y.; Kim, W.Y.; Hwang, I. Long-term abscisic acid promotes golden2-like1 degradation through constitutive photomorphogenic 1 in a light intensity-dependent manner to suppress chloroplast development. *Plant Cell Environ* **2021**, *44*, 3034-3048, doi:10.1111/pce.14130.

84. Tuteja, N. Abscisic Acid and abiotic stress signaling. *Plant Signal Behav* **2007**, *2*, 135-138, doi:10.4161/psb.2.3.4156.

85. Yadukrishnan, P.; Datta, S. Light and abscisic acid interplay in early seedling development. *New Phytol* **2021**, *229*, 763-769, doi:10.1111/nph.16963.

86. Eckstein, A.; Krzeszowiec, W.; Banas, A.K.; Janowiak, F.; Gabrys, H. Abscisic acid and blue light signaling pathways in chloroplast movements in Arabidopsis mesophyll. *Acta Biochim Pol* **2016**, *63*, 449-458, doi:10.18388/abp.2016_1382.

87. Leon, P.; Gregorio, J.; Cordoba, E. ABI4 and its role in chloroplast retrograde communication. *Front Plant Sci* **2012**, *3*, 304, doi:10.3389/fpls.2012.00304.

88. Waters, M.T.; Wang, P.; Korkaric, M.; Capper, R.G.; Saunders, N.J.; Langdale, J.A. GLK transcription factors coordinate expression of the photosynthetic apparatus in Arabidopsis. *Plant Cell* **2009**, *21*, 1109-1128, doi:10.1105/tpc.108.065250.

89. Li, Q.F.; He, J.X. BZR1 Interacts with HY5 to Mediate Brassinosteroid- and Light-Regulated Cotyledon Opening in Arabidopsis in Darkness. *Mol Plant* **2016**, *9*, 113-125, doi:10.1016/j.molp.2015.08.014.

90. Kim, B.; Jeong, Y.J.; Corvalan, C.; Fujioka, S.; Cho, S.; Park, T.; Choe, S. Darkness and gulliver2/phyB mutation decrease the abundance of phosphorylated BZR1 to activate brassinosteroid signaling in Arabidopsis. *Plant J* **2014**, *77*, 737-747, doi:10.1111/tpj.12423.

91. Pan, Y.; Shi, H. Stabilizing the Transcription Factors by E3 Ligase COP1. *Trends Plant Sci* **2017**, *22*, 999-1001, doi:10.1016/j.tplants.2017.09.012.

92. Jang, G.J.; Yang, J.Y.; Hsieh, H.L.; Wu, S.H. Processing bodies control the selective translation for optimal development of Arabidopsis young seedlings. *Proc Natl Acad Sci U S A* **2019**, *116*, 6451-6456, doi:10.1073/pnas.1900084116.

93. Kakizaki, T.; Matsumura, H.; Nakayama, K.; Che, F.S.; Terauchi, R.; Inaba, T. Coordination of plastid protein import and nuclear gene expression by plastid-to-nucleus retrograde signaling. *Plant Physiol* **2009**, *151*, 1339-1353, doi:10.1104/pp.109.145987.

94. Chen, B.; Retzlaff, M.; Roos, T.; Frydman, J. Cellular strategies of protein quality control. *Cold Spring Harb Perspect Biol* **2011**, *3*, a004374, doi:10.1101/cshperspect.a004374.

95. Zhang, Y.; Xia, G.; Zhu, Q. Conserved and Unique Roles of Chaperone-Dependent E3 Ubiquitin Ligase CHIP in Plants. *Front Plant Sci* **2021**, *12*, 699756, doi:10.3389/fpls.2021.699756.

96. Esser, C.; Alberti, S.; Hohfeld, J. Cooperation of molecular chaperones with the ubiquitin/proteasome system. *Biochim Biophys Acta* **2004**, *1695*, 171-188, doi:10.1016/j.bbamcr.2004.09.020.

97. Ruprecht, M.; Bionda, T.; Sato, T.; Sommer, M.S.; Endo, T.; Schleiff, E. On the impact of precursor unfolding during protein import into chloroplasts. *Mol Plant* **2010**, *3*, 499-508, doi:10.1093/mp/ssp116.

98. Walker, D.; Chaddock, A.M.; Chaddock, J.A.; Roberts, L.M.; Lord, J.M.; Robinson, C. Ricin A chain fused to a chloroplast-targeting signal is unfolded on the chloroplast surface prior to import across the envelope membranes. *J Biol Chem* **1996**, *271*, 4082-4085, doi:10.1074/jbc.271.8.4082.

99. Lee, D.W.; Jung, C.; Hwang, I. Cytosolic events involved in chloroplast protein targeting. *Biochim Biophys Acta* **2013**, *1833*, 245-252, doi:10.1016/j.bbamcr.2012.03.006.

100. Vabulas, R.M.; Raychaudhuri, S.; Hayer-Hartl, M.; Hartl, F.U. Protein folding in the cytoplasm and the heat shock response. *Cold Spring Harb Perspect Biol* **2010**, *2*, a004390, doi:10.1101/cshperspect.a004390.

101. Nishimura, K.; Kato, Y.; Sakamoto, W. Chloroplast Proteases: Updates on Proteolysis within and across Suborganellar Compartments. *Plant Physiol* **2016**, *171*, 2280-2293, doi:10.1104/pp.16.00330.

102. Majsec, K.; Bhuiyan, N.H.; Sun, Q.; Kumari, S.; Kumar, V.; Ware, D.; van Wijk, K.J. The Plastid and Mitochondrial Peptidase Network in Arabidopsis thaliana: A Foundation for Testing Genetic Interactions and Functions in Organellar Proteostasis. *Plant Cell* **2017**, *29*, 2687-2710, doi:10.1105/tpc.17.00481.

103. van Wijk, K.J. Protein maturation and proteolysis in plant plastids, mitochondria, and peroxisomes. *Annu Rev Plant Biol* **2015**, *66*, 75-111, doi:10.1146/annurev-arplant-043014-115547.

104. Nishimura, K.; van Wijk, K.J. Organization, function and substrates of the essential Clp protease system in plastids. *Biochim Biophys Acta* **2015**, *1847*, 915-930, doi:10.1016/j.bbabio.2014.11.012.

105. Ito, K.; Akiyama, Y. Cellular functions, mechanism of action, and regulation of FtsH protease. *Annu Rev Microbiol* **2005**, *59*, 211-231, doi:10.1146/annurev.micro.59.030804.121316.

106. Wei, J.; Qiu, X.; Chen, L.; Hu, W.; Hu, R.; Chen, J.; Sun, L.; Li, L.; Zhang, H.; Lv, Z.; et al. The E3 ligase AtCHIP positively regulates Clp proteolytic subunit homeostasis. *J Exp Bot* **2015**, *66*, 5809-5820, doi:10.1093/jxb/erv286.

107. Yan, J.; Wang, J.; Li, Q.; Hwang, J.R.; Patterson, C.; Zhang, H. AtCHIP, a U-box-containing E3 ubiquitin ligase, plays a critical role in temperature stress tolerance in Arabidopsis. *Plant Physiol* **2003**, *132*, 861-869, doi:10.1104/pp.103.020800.

108. Lee, S.; Lee, D.W.; Lee, Y.; Mayer, U.; Stierhof, Y.D.; Lee, S.; Jurgens, G.; Hwang, I. Heat shock protein cognate 70-4 and an E3 ubiquitin ligase, CHIP, mediate plastid-destined precursor degradation through the ubiquitin-26S proteasome system in Arabidopsis. *Plant Cell* **2009**, *21*, 3984-4001, doi:10.1105/tpc.109.071548.

109. Shen, G.; Yan, J.; Pasapula, V.; Luo, J.; He, C.; Clarke, A.K.; Zhang, H. The chloroplast protease subunit ClpP4 is a substrate of the E3 ligase AtCHIP and plays an important role in chloroplast function. *Plant J* **2007**, *49*, 228-237, doi:10.1111/j.1365-313X.2006.02963.x.

110. Shen, G.; Adam, Z.; Zhang, H. The E3 ligase AtCHIP ubiquitylates FtsH1, a component of the chloroplast FtsH protease, and affects protein degradation in chloroplasts. *Plant J* **2007**, *52*, 309-321, doi:10.1111/j.1365-313X.2007.03239.x.

111. Genest, O.; Wickner, S.; Doyle, S.M. Hsp90 and Hsp70 chaperones: Collaborators in protein remodeling. *J Biol Chem* **2019**, *294*, 2109-2120, doi:10.1074/jbc.REV118.002806.

112. Yee, D.; Goring, D.R. The diversity of plant U-box E3 ubiquitin ligases: from upstream activators to downstream target substrates. *J Exp Bot* **2009**, *60*, 1109-1121, doi:10.1093/jxb/ern369.

113. Adam, Z.; Rudella, A.; van Wijk, K.J. Recent advances in the study of Clp, FtsH and other proteases located in chloroplasts. *Curr Opin Plant Biol* **2006**, *9*, 234-240, doi:10.1016/j.pbi.2006.03.010.

114. Zhou, J.; Zhang, Y.; Qi, J.; Chi, Y.; Fan, B.; Yu, J.Q.; Chen, Z. E3 ubiquitin ligase CHIP and NBR1-mediated selective autophagy protect additively against proteotoxicity in plant stress responses. *PLoS Genet* **2014**, *10*, e1004116, doi:10.1371/journal.pgen.1004116.

115. Zhang, Y.; Lai, X.; Yang, S.; Ren, H.; Yuan, J.; Jin, H.; Shi, C.; Lai, Z.; Xia, G. Functional analysis of tomato CHIP ubiquitin E3 ligase in heat tolerance. *Sci Rep* **2021**, *11*, 1713, doi:10.1038/s41598-021-81372-8.

116. Dutta, S.; Mohanty, S.; Tripathy, B.C. Role of temperature stress on chloroplast biogenesis and protein import in pea. *Plant Physiol* **2009**, *150*, 1050-1061, doi:10.1104/pp.109.137265.

117. Luo, J.; Shen, G.; Yan, J.; He, C.; Zhang, H. AtCHIP functions as an E3 ubiquitin ligase of protein phosphatase 2A subunits and alters plant response to abscisic acid treatment. *Plant J* **2006**, *46*, 649-657, doi:10.1111/j.1365-313X.2006.02730.x.

118. Hayes, S.; Schachtschabel, J.; Mishkind, M.; Munnik, T.; Arisz, S.A. Hot topic: Thermosensing in plants. *Plant Cell Environ* **2021**, *44*, 2018-2033, doi:10.1111/pce.13979.

119. Wigge, P.A. Ambient temperature signalling in plants. *Curr Opin Plant Biol* **2013**, *16*, 661-666, doi:10.1016/j.pbi.2013.08.004.

120. Battisti, D.S.; Naylor, R.L. Historical warnings of future food insecurity with unprecedented seasonal heat. *Science* **2009**, *323*, 240-244, doi:10.1126/science.1164363.

121. Howarth, C.J.; Ougham, H.J. Gene expression under temperature stress. *New Phytol* **1993**, *125*, 1-26, doi:10.1111/j.1469-8137.1993.tb03862.x.

122. Penfield, S. Temperature perception and signal transduction in plants. *New Phytol* **2008**, *179*, 615-628, doi:10.1111/j.1469-8137.2008.02478.x.

123. Bita, C.E.; Gerats, T. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Front Plant Sci* **2013**, *4*, 273, doi:10.3389/fpls.2013.00273.

124. Zhang, H.; Zhou, J.F.; Kan, Y.; Shan, J.X.; Ye, W.W.; Dong, N.Q.; Guo, T.; Xiang, Y.H.; Yang, Y.B.; Li, Y.C.; et al. A genetic module at one locus in rice protects chloroplasts to enhance thermotolerance. *Science* **2022**, *376*, 1293-1300, doi:10.1126/science.abo5721.

125. Sun, Y.; Yao, Z.; Chen, H.; Ye, Y.; Lyu, Y.; Broad, W.; Fournier, M.; Chen, G.; Hu, Y.; Mohammed, S.; et al. Ubiquitin-based pathway acts inside chloroplasts to regulate photosynthesis. *bioRxiv* **2022**, 2022.2006.2006.494369, doi:10.1101/2022.06.06.494369.

126. Trosch, R. Ubiquitination of intra-chloroplast proteins. *Nat Plants* **2022**, *8*, 453, doi:10.1038/s41477-022-01162-6.

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Figure Legend

Figure 1. Schematic representation of the mechanisms of E3 ubiquitin ligases in regulating chloroplast function. (**A**) SP1-mediated degradation of TOC machinery through the CHLORAD pathway. The specific subunits targeted (Toc33/34/75/120/132 and Toc159) are represented as TOC (detailed in section 1.1). (**B**) Light and dark pathways of the feedback mechanism involving COP1, HY5, and ABI4 in chloroplast development (detailed in section 1.2). (**C**) Role of PUB4 in chloroplast degradation (detailed in section 1.3). (**D**) CHIP-mediated degradation of chloroplast protease precursor proteins in the cytosol (detailed in section 1.4). (**E**) Simplified schematic of TT3.1-mediated degradation of TT3.2 under heat stress (detailed in section 1.5). (**F**) CHLORAD pathway involving SP1 and CDC48 (detailed in section 2.1). Ub, ubiquitin; OM, outer membrane; IM, inner membrane; PTM, plant homeodomain type transcription factor with transmembrane domains. All images were created with BioRender.com.

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