Communicative & Integrative Biology 6:2, e23001; March/April 2013; © 2013 Landes Bioscience

The ubiquitin-proteasome system regulates chloroplast biogenesis

Weihua Huang, Qihua Ling and Paul Jarvis*
Department of Biology; University of Leicester; Leicester, UK

In a recently published work, we identified the first chloroplast-localized E3 ligase, and showed that it degrades TOC translocon proteins by the ubiquitin-proteasome system (UPS). This regulation was found to be critical for plastid developmental transitions, such as the conversion of etioplasts to chloroplasts. Here we discuss the importance of SP1 for plants under different circumstances, and speculate about SP1 function and its possible application in agriculture. We anticipate that further work in this area may reveal additional roles of the UPS system, through action on other chloroplast OEPs with different roles in plastid biogenesis and function.

Keywords: *Arabidopsis thaliana*, ubiquitin-proteasome system (UPS), chloroplast, TOC translocon, SP1, E3 ligase, outer envelope protein (OEP)

Submitted: 11/23/12

Accepted: 11/26/12

http://dx.doi.org/10.4161/cib.23001

*Correspondence to: Paul Jarvis;
Email: rpj3@le.ac.uk

Addendum to: Ling Q, Huang W, Baldwin A, Jarvis P. Chloroplast biogenesis is regulated by direct action of the ubiquitin-proteasome system. Science 2012; 338:655–9; PMID:23118188; http://dx.doi.org/10.1126/science.1225053.

In eukaryotic organisms, selective protein breakdown by the ubiquitin-proteasome system (UPS) is widely used to regulate development and other processes. In general, the function of the UPS pathway is to conjugate ubiquitin (a 76 amino acid protein) to Lys residues within substrate proteins by the consecutive action of ubiquitin activating enzyme (E1), ubiquitin conjugating enzyme (E2) and ubiquitin protein ligase (E3), thus targeting them for degradation by the 26S proteasome. In the model plant *Arabidopsis thaliana*, nearly 6% of proteins are related to the UPS system, and they are found to contribute significantly to a wide range of processes, including embryogenesis, morphogenesis, hormone signaling and responses to environmental challenges. Approximately 90% of these UPS proteins are E3 ubiquitin ligases, which confer substrate specificity to the pathway.[1,2] The principal sites of action of the UPS are the cytosol and nucleus.

Chloroplasts in plant cells originate from the endosymbiosis of a cyanobacteria-like prokaryote.[3] During the ensuing evolution, the host cell and the endosymbiont became well integrated, with many endosymbiont genes being transferred to the nuclear genome.[4] Thus, many chloroplast proteins are synthesized in the cytosol as preproteins, which are then imported into the chloroplast through translocons at the outer and inner envelope membranes of chloroplasts (TOC and TIC, respectively). The TOC complex is composed of the Toc75 channel and the Toc159 and Toc34 receptor subunits that recognize the preproteins. In Arabidopsis (and other species), there are multiple isoforms of the receptors which recognize different sets of preproteins: atToc159 and atToc33 are the major receptor isoforms in chloroplasts, and these mediate the import of the highly-abundant, photosynthesis-related proteins; by contrast, atToc132/atToc120 and atToc34 are minor isoforms that mediate the import of non-photosynthetic, housekeeping proteins.[4]

It is very interesting that the first chloroplast-localized E3 ligase to be identified is a regulator of the TOC translocon.[5] Using genetic screening for second-site suppressors of *plastid protein import1* (*ppi1*; an Arabidopsis atToc33 mutant), we identified SUPPRESSOR OF PPI1 LOCUS1 (SP1), a RING-type E3 ligase protein. Using in vitro and in vivo assays, the SP1 protein was shown to ubiquitinate all tested TOC proteins, but not other chloroplast outer envelope proteins (OEPs). Moreover, abundance of ubiquitinated TOC proteins depended on proteasomal activity, indicating that TOC proteins are targeted by SP1 for UPS-mediated degradation. Interestingly, *sp1* single mutant

plants are defective in greening (de-etiolation) and leaf senescence. Further analysis showed that SP1 selectively regulates TOC protein levels in response to developmental and environmental cues. During de-etiolation, atToc132/atToc120 (which are specialized for non-photosynthetic protein import) are degraded by SP1 through the UPS pathway. This may allow the released Toc75 channels to associate with atToc159 and atToc33, which are specialized for the import of photosynthetic proteins.

By analyzing public microarray data (Genevestigator[6]), we found that in Arabidopsis the mRNA expression levels of the *atTOC132*, *atTOC120* and *atTOC159* genes are quite stable during de-etiolation. This further supports the importance of the UPS regulation of TOC proteins in this process. Compared with transcriptional regulation of gene expression, such post-transcriptional regulation can control protein amounts more promptly and precisely in response to different cues, especially when the proteins are unwanted. Post-transcriptional regulation of the import machinery by the UPS enables the rapid development of etioplasts into chloroplasts in response to a dark-to-light shift. It should be noted that de-etiolation and photomorphogenesis are very important processes for plants, and several key regulators such as COP1, HY5 and PIF1/PIF3 have been well studied.[7-11] COP1, another RING-type E3 ligase, together with the SPA complex, functions as a negative regulator for photomorphogenesis by destabilizing HY5 (a nuclear transcription factor that activates downstream light-inducible genes) through the UPS system in the dark. PIF1 and PIF3 negatively regulate chlorophyll synthesis and chloroplast development when seedlings are in the dark. These regulatory events prevent the premature development of chloroplasts in darkness.

Given that the *sp1* single mutant displays no obvious abnormal phenotypes under optimal, controlled growth conditions, the SP1-mediated TOC reorganization perhaps allows fine-tuning in response to more challenging conditions, which are common in nature. For example, as we have shown, SP1 aids seedling survival after prolonged dark stress upon germination.[5] Besides, TOC reorganization may also occur during other plastid transition processes, such as during senescence when chloroplasts transform into gerontoplasts,[5] and perhaps also fruit ripening when the green chloroplasts convert into carotenoid-pigmented chromoplasts. Whether SP1 can control fruit ripening will be particularly interesting. The answer to this question will have major implications for food quality and shelf life, and many aspects of agricultural food production.

Since the 26S proteasome only exists in the cytosol and nucleus, most likely only the chloroplasts' OEPs are potential targets of the UPS. Our discovery opens an interesting new door to the regulatory mechanisms that govern chloroplast OEPs. So far, besides the TOC proteins, only a small number of OEPs have been identified, and even fewer have had their functions elucidated.[12,13] Examples include: the DGD1/2, MGD2/3 and LACS9 proteins that are involved in lipid metabolism, and SFR2 which is a galactolipid remodeling enzyme and is essential for freezing tolerance in Arabidopsis;[12-15] PDV1/2 which are related to chloroplast division;[12,13] CHUP1 which is responsible for chloroplast redistribution in different light regimes;[12] and a chloroplast outer envelope-bound PHD transcription factor, PTM, that mediates chloroplast retrograde signals to the nucleus.[16] Other OEPs include OEP16, OEP21, OEP24 and OEP37, which are probably cation-selective channels.[13]

In our study, we identified homologs of *SP1* in the Arabidopsis genome, and data showed that the encoded proteins are also located in the chloroplast envelope.[5] Intriguingly, SP1's closest relative does not share redundancy with SP1. Perhaps these other E3 ligases target other OEPs, which act in different aspects of chloroplast biogenesis. An important challenge to be addressed in future experiments is the identification of the substrates of these, and perhaps other, E3 ligases in chloroplasts; this will not be trivial, as it cannot be done in silico, and so multiple experimental assays will be necessary to identify their functions. In addition, other basic questions concerning the function of SP1 itself need to be answered. Although most TOC proteins are substrates of SP1, how they are selectively degraded under different circumstances remains unclear. Since SP1 itself can be ubiquitinated, and degraded by the UPS, this is one possible mechanism;[5] but there may also be other mechanisms controlling SP1 dynamics, to be identified in future experiments.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Moon J, Parry G, Estelle M. The ubiquitin-proteasome pathway and plant development. Plant Cell 2004; 16:3181-95; PMID:15579807; http://dx.doi.org/10.1105/tpc.104.161220.
2. Vierstra RD. The ubiquitin-26S proteasome system at the nexus of plant biology. Nat Rev Mol Cell Biol 2009; 10:385-97; PMID:19424292; http://dx.doi.org/10.1038/nrm2688.
3. Jarvis P. Organellar proteomics: chloroplasts in the spotlight. Curr Biol 2004; 14:R317-9; PMID:15084303; http://dx.doi.org/10.1016/j.cub.2004.03.054.
4. Jarvis P. Targeting of nucleus-encoded proteins to chloroplasts in plants. New Phytol 2008; 179:257-85; PMID:19086173; http://dx.doi.org/10.1111/j.1469-8137.2008.02452.x.
5. Ling Q, Huang W, Baldwin A, Jarvis P. Chloroplast biogenesis is regulated by direct action of the ubiquitin-proteasome system. Science 2012; 338:655-9; PMID:23118188; http://dx.doi.org/10.1126/science.1225053.
6. Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, Oertle L, et al. Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. Adv Bioinformatics 2008; 2008:420747; PMID:19956698; http://dx.doi.org/10.1155/2008/420747.
7. Osterlund MT, Hardtke CS, Wei N, Deng XW. Targeted destabilization of HY5 during light-regulated development of Arabidopsis. Nature 2000; 405:462-6; PMID:10839542; http://dx.doi.org/10.1038/35013076.
8. von Arnim AG, Deng XW. Light inactivation of Arabidopsis photomorphogenic repressor COP1 involves a cell-specific regulation of its nucleocytoplasmic partitioning. Cell 1994; 79:1035-45; PMID:8001131; http://dx.doi.org/10.1016/0092-8674(94)90034-5.
9. Huq E, Al-Sady B, Hudson M, Kim C, Apel K, Quail PH. Phytochrome-interacting factor 1 is a critical bHLH regulator of chlorophyll biosynthesis. Science 2004; 305:1937-41; PMID:15448264; http://dx.doi.org/10.1126/science.1099728.
10. Stephenson PG, Fankhauser C, Terry MJ. PIF3 is a repressor of chloroplast development. Proc Natl Acad Sci U S A 2009; 106:7654-9; PMID:19380736; http://dx.doi.org/10.1073/pnas.0811684106.
11. Zhong S, Zhao M, Shi T, Shi H, An F, Zhao Q, et al. *EIN3/EIL1* cooperate with *PIF1* to prevent photo-oxidation and to promote greening of Arabidopsis seedlings. Proc Natl Acad Sci U S A 2009; 106:21431-6; PMID:19948955; http://dx.doi.org/10.1073/pnas.0907670106.
12. Inoue K. Emerging roles of the chloroplast outer envelope membrane. Trends Plant Sci 2011; 16:550-7; PMID:21775189; http://dx.doi.org/10.1016/j.tplants.2011.06.005.

13. Inoue K. The chloroplast outer envelope membrane: the edge of light and excitement. J Integr Plant Biol 2007; 49:1100-11; http://dx.doi.org/10.1111/j.1672-9072.2007.00543.x.
14. Moellering ER, Muthan B, Benning C. Freezing tolerance in plants requires lipid remodeling at the outer chloroplast membrane. Science 2010; 330:226-8; PMID:20798281; http://dx.doi.org/10.1126/science.1191803.
15. Thorlby G, Fourrier N, Warren G. *The SENSITIVE TO FREEZING2* gene, required for freezing tolerance in *Arabidopsis thaliana*, encodes a beta-glucosidase. Plant Cell 2004; 16:2192-203; PMID:15258268; http://dx.doi.org/10.1105/tpc.104.024018.
16. Sun X, Feng P, Xu X, Guo H, Ma J, Chi W, et al. A chloroplast envelope-bound PHD transcription factor mediates chloroplast signals to the nucleus. Nat Commun 2011; 2:477; PMID:21934661; http://dx.doi.org/10.1038/ncomms1486.