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ABSTRACT

Chloroplasts depend on the nucleus for much of their proteome. Consequently, strong transcriptional coordination exists between the genomes, which is attuned to the developmental and physiological needs of the organelle. Recent studies highlight that the post-translational modifier ubiquitin adds another layer to plastid homeostasis and even helps eliminate damaged chloroplasts.

The household we call a plant cell is actually a complex amalgam of compartments and compartmentalized genomes that arose from the primordial soup during the endosymbiosis of several free-living bacteria to form a single-celled eukaryotic photoautotroph. The signature of this relationship is the chloroplast (and the collection of other plastid types), a double membrane-bound compartment that arose from an engulfed cyanobacterium over 1 billion years ago and contains the photosynthetic machinery and other essential metabolic pathways, including those devoted to amino acid, tetrapyrrole, and lipid biosynthesis [1]. The plastid progenitor presumably began with its full complement of cyanobacterial genes, but as plastids evolved in the shadows of the nucleus, much of their coding capacity was transferred such that the nuclear genome now encodes for over 90% of the several thousand resident plastid proteins. These nuclear-encoded precursors are translated in the cytoplasm and then delivered to the organelle via partially conserved N-terminal transit sequences, which are recognized by specialized import machineries (i.e., translocons) on the inner and outer envelope membranes. Following precursor uptake, the transit peptide is removed proteolytically.

To help coordinate expression of the nuclear-encoded pool with their plastid brethren, especially those that assemble into complexes, plants also evolved retrograde signaling pathways that relay information from plastids back to the nucleus to ensure that the nucleus is in tune with the developmental and metabolic needs of the plastid. While not completely understood, several plastid-derived signaling intermediates are involved, including tetrapyrroles, small metabolites, and reactive oxygen species (ROS) [2]. The ultimate targets are numerous nuclear genes that are needed to develop plastids appropriate for the tissue or cell type, developmental state, and environment of the plant, such as etioplasts in dark-grown seedlings, root amyloplasts for starch storage, carotenoid-rich chromoplasts found in flowers and fruits, chloroplasts in photosynthetic tissues, and gerontoplasts in senescing organs.

While many studies have focused on the transcriptional steps, emerging data surprisingly indicate that plants also evolved post-translational mechanisms to maintain plastid homeostasis, synchronize organelle development, and control lifespan. These mechanisms engage selective turnover of chloroplast proteins or even whole chloroplasts via ubiquitin-mediated events. Ubiquitin functions in turnover by first becoming covalently attached to proteins destined for degradation often as chains of polyubiquitin [3]. This conjugation is mediated by an ATP-dependent E1-E2-E3 reaction cascade, with a myriad of ubiquitin ligases (or E3s) choosing the correct substrates. Once ubiquitylated, the proteins are degraded, most often by the 26S proteasome, a multisubunit self-compartmentalized protease bearing receptors that recognize bound ubiquitin moieties. For protein complexes, insoluble protein aggregates, or even whole organelles, all of which are too large for the 26S proteasome, autophagy

becomes engaged [4]. These bulky ubiquitylated species are engulfed by cytoplasmic autophagosomes, which are then deposited into the vacuole (lysosome in animals) for breakdown.

While first thought to be restricted to nuclear and cytoplasmic events, the first clues that ubiquitin controls chloroplast fate came from genetics studies on the *Arabidopsis* E3 CHIP (Carboxy-terminus of Hsc-70-Interacting Protein), which when overexpressed generates defective chloroplasts with increased ROS production [5]. Subsequent studies revealed that CHIP in combination with the heat shock protein cognate HSC70-4 assist in degrading unfolded precursors of various nuclear-encoded chloroplast proteins that have a propensity to aggregate in the cytosol. HSC70-4 binds to CHIP and recognizes the transit sequence of these defective precursors [5], thus promoting precursor ubiquitylation and 26S proteasome-mediated degradation before they accumulate to toxic levels or interfere with the import of properly folded proteins (Figure 1).

A more direct connection between ubiquitin and plastids was revealed after screening for mutants with defective chloroplast biogenesis [6]. The E3 SP1 (Suppressor of Plastid Protein Import-1) was discovered to be a suppressor of mutations compromising one plastid import translocon. SP1 associates with the cytosolic face of the outer membrane through two transmembrane spanning domains where it employs the rest of the ubiquitylation machinery to target the specificity components TOC33 and TOC159 of the import machinery [6]. Via ubiquitylation and subsequent 26S proteasome turnover, SP1 is uniquely positioned to control which nuclear-encoded proteins pass through the translocon (Figure 1). Accordingly, *sp1* mutants inefficiently transition from one plastid developmental phase to another and are hypersensitive to abiotic stress. Stress also triggers the SP1-mediated turnover of the import translocon [7], which could represent a rapid method to adjust the plastid proteome to the prevailing environment.

In a recent study, Woodson *et al.* [8] intimately linked ubiquitin to plastid quality control and longevity. Initial studies showed that *Arabidopsis ferrochetalase-2* mutants (*fc-2*), which accumulate excess of the heme precursor protoporphyrin IXα in chloroplasts, produce abnormally high levels of ROS that damage the photosynthetic capacity and ultrastructure of the organelle. In chloroplast populations, the *fc-2* mutants degrade their chloroplasts 4-5 times faster than wild type, implying that plants can selectively remove damaged plastids. A suppressor screen for factors that would impair this breakdown identified the E3 PUB4 (Plant U-Box-4) [8]. When placed in the *fc-2* background, *pub4* mutants retained high levels of chlorophyll and concomitantly blocked defective chloroplast turnover. Interestingly, *fc-2* chloroplasts became heavily ubiquitylated under light stress, but this modification was

substantially dampened in the *fc-2 pub4* mutant, thus leading to the proposal that ROS stress triggers the PUB4-mediated ubiquitylation of damaged chloroplasts, which then promotes turnover.

Currently, the chloroplast targets of PUB4 are unknown, as are the mechanism(s) that eliminate compromised organelles. Unlike the smaller ubiquitylation targets of CHIP and SP1 [5, 6], a chloroplast is much too large for the 26S proteasome. One likely alternative is autophagy, which is known to remove chloroplasts by a process not yet connected to ubiquitin (Figure 1). Assuming this connection, it is possible that PUB4-mediated quality control removes damage plastids much like the autophagic mechanism that clears defective mitochondria. Here, the mitochondrial surface is first ubiquitylated by the E3 Parkin, which then promotes the autophagic delivery of mitochondria by the receptors optineurin/NDP52, which have affinity for polyubiquitin chains [9]. Unexpectedly, phosphorylated ubiquitin is central to the process. It is reasonable to assume that similar receptor(s) exist for ubiquitylated chloroplasts given the rapidly expanding arsenal now linked to various facets of autophagy [4].

Beyond the above studies, new connections between ubiquitin and chloroplasts are likely. For example, a deep proteomic analysis of ubiquitin conjugates in *Arabidopsis* identified a number of chloroplast proteins that become ubiquitylated [10]. Whereas some of these conjugates likely represent nuclear-encoded precursors prone to CHIP-mediated modification, others could be normal plastid surface proteins regulated by ubiquitin addition. Moreover, recent studies on the E3 component DIF revealed that it modulates chloroplast development by targeting the nuclear-encoded, plastid lumenal protein DAL for ubiquitylation and 26S proteasome turnover before its import. Interestingly, DIF-dependent control of DAL is also intimately connected to plastid ROS production. Consequently, despite the prevailing notion that ubiquitin should be restricted to regulating cytoplasmic and nuclear events, important controls on plant organelle functions should not be overlooked. Stay tuned as the greening of ubiquitin continues.

REFERENCES

1. Jarvis, P. and Lopez-Juez, E. (2013) Biogenesis and homeostasis of chloroplasts and other plastids. *Nat. Rev. Mol. Cell. Biol.* 14, 787-802
2. Chi, W.*, et al.* (2013) Intracellular signaling from plastid to nucleus. *Annu. Rev. Plant Biol.* 64, 559-582
3. Vierstra, R.D. (2009) The ubiquitin-26S proteasome system at the nexus of plant biology. *Nat. Rev. Mol. Cell. Biol.* 10, 385-397

4. Khaminets, A.*, et al.* (2015) Ubiquitin-dependent and independent signals in selective autophagy. *Trends Cell. Biol.* (in press)
5. Lee, S.*, et al.* (2009) 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* 21, 3984-4001
6. Ling, Q.*, et al.* (2012) Chloroplast biogenesis is regulated by direct action of the ubiquitin-proteasome system. *Science* 338, 655-659
7. Ling, Q. and Jarvis, P. (2015) Regulation of chloroplast protein import by the ubiquitin E3 ligase SP1 Is important for stress tolerance in plants. *Curr. Biol.* 25, 2527-2534
8. Woodson, J.D.*, et al.* (2015) Ubiquitin facilitates a quality-control pathway that removes damaged chloroplasts. *Science* 350, 450-454
9. Lazarou, M.*, et al.* (2015) The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. *Nature* 524, 309-314
10 Kim, D.Y.*, et al.* (2013) Advanced proteomic analyses yield a deep catalog of ubiquitylation targets in *Arabidopsis*. *Plant Cell* 25, 1523-1540

Figure 1. Hua and Vierstra

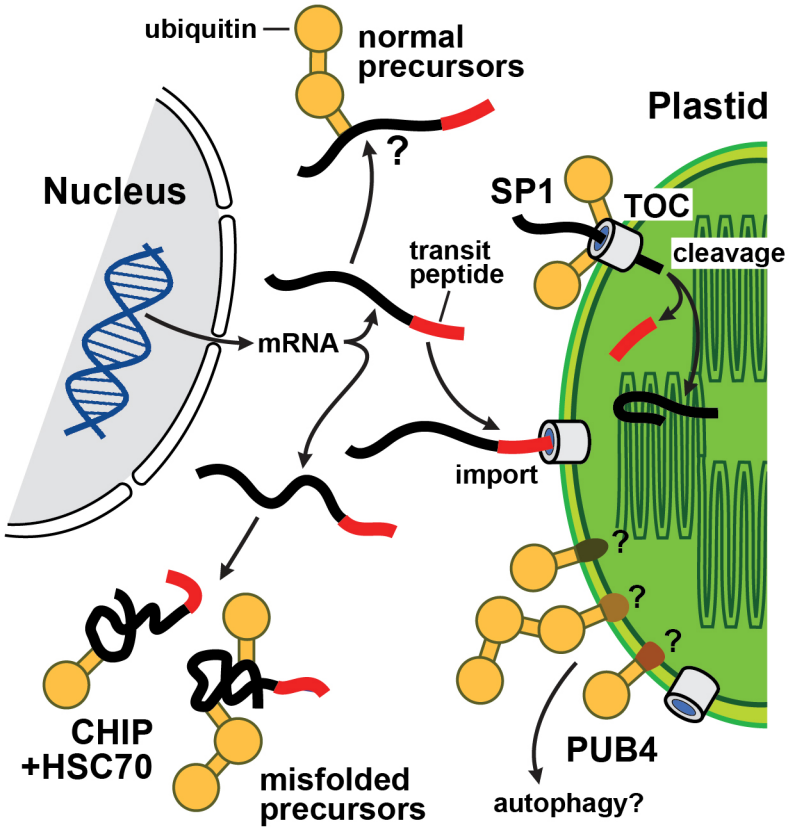

Figure 1. Ubiquitylation Mediated Regulation and Quality Control of Chloroplast.

Many plastid proteins are expressed from nuclear genes and imported into the chloroplast via transit peptide signals. Ubiquitin (orange color) controls this process and eliminates damaged chloroplasts by: (i) removing misfolded precursors by CHIP-mediated ubiquitylation; (ii) removing normal precursors before import through E3s such as DIF, (iii) controls plastid protein import by SP1-mediated ubiquitylation and subsequent 26S proteasome-directed degradation of components of the TOC import translocon, and (iv) eliminates damaged chloroplasts by ubiquitylation of unknown plastid outer membrane proteins by PUB4, which is possibly followed by autophagic breakdown in the vacuole (chlorophagy).