Trends in Plant Science May 2015, Vol. 20, No. 5

# Chloroplast degradation: one organelle, multiple degradation pathways

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**Degradation of chloroplasts is a hallmark of both natural and stress-induced plant senescence. Autophagy and senescence-associated vacuoles are two established cellular pathways for chloroplast degradation. Recently, a third independent pathway for chloroplast degradation was reported. Here we will discuss this new discovery in relation to the other known pathways.**

Plants have evolved convergent mechanisms to cope with adverse environmental conditions that are generally energetically demanding. Such energetic demands may require the disassembly of organelles or organelle components and the redirection of cellular building blocks. Up to 80% of the leaf nitrogen pool is found in chloroplasts [1]. Thus, in addition to the turnover required to maintain homeostasis and to overcome stress-induced damage to chloroplast components, the degradation of chloroplasts and the recycling of their nutrients plays a major role in coping with stresses and during leaf senescence.

There are two characterized pathways that are involved in the degradation of chloroplast proteins, Senescence Associated Vacuoles (SAVs) and autophagy. SAVs are small proteolytic vacuolar compartments that accumulate in senescing leaves. Interestingly, they were shown to contain the chloroplast stroma proteins Rubisco and glutamine synthetase, but seem to lack thylakoid-associated proteins [2]. Data regarding the origin of SAVs and the manner by which plastid proteins are relocated into their lumen is still unknown. More information is available on autophagy-dependent chloroplast degradation. In this process, cytosolic components are engulfed by a specialized double-membrane structure (autophagosome) that delivers them to the vacuole for recycling [3]. Autophagy was shown to be involved in the delivery of Rubisco-Containing Bodies (RCBs), an autophagic body containing stroma proteins, to the vacuole [1,4]. Although both SAVs and RCBs are induced in senescing leaves and contain Rubisco, but not thylakoid associated proteins [2,4,5], they are apparently distinct structures, because SAVs are not dependent on autophagy [2]. Moreover, it seems that SAVs do not reach the central vacuole but rather exhibit an independent proteolytic activity (Figure 1). Intriguingly, autophagy is also involved in the night-time delivery of starch from plastids to vacuoles via Small Starch Granules like structures (SSGLs), a process which is not necessarily associated with stress [6].

The first example of a protein that is specifically involved in vesicular delivery of chloroplast components to the vacuole (V-Chlorophagy) emerged only recently with the discovery of a new type of autophagy-dependent bodies in *Arabidopsis* (*Arabidopsis thaliana*) [7]. This protein, termed ATG8-Interacting 1 (ATI1), was shown to interact with both the core-autophagy component, ATG8f [8] and with plastid proteins, thus enabling their delivery to the vacuole in novel bodies, termed ATI-PS bodies [7]. ATI-PS bodies and RCBs differ in size, and hence are most likely different entities. Moreover, ATI1 deficiency does not affect Rubisco stability, but increases the stability of the chloroplast localized, ATI1-interacting protein, Peroxiredoxin A [7]. It was suggested that ATI1 might act as a specialized cargo receptor for V-Chlorophagy of its chloroplast localized interacting proteins (Figure 1). Two other proteins that were shown to be involved in autophagy-dependent V-Chlorophagy are the ESCRT-III subunit paralogs Charged Multivesicular Body 1A and 1B (CHMP1A and CHMP1B) [9]. CHMP1A and B were shown to be necessary for phagophore maturation of autophagic plastid bodies, and the degradation of plastid components is greatly reduced in their absence. Furthermore, RCB-like vesicles accumulate in a CHMP1-deficient mutant and it was suggested that CHMP1 participates in their delivery to the vacuole. By contrast, ATI-PS bodies were not observed in the mutant [9].

Interestingly, a recent publication by S. Wang and E. Blumwald suggests a third plastid degradation pathway that is SAV and autophagy independent, and the involvement of another player, the Chloroplast Vesiculation (CV) protein (Figure 1) [10]. The *CV* gene, which has homologs in all sequenced plant species, was initially identified in rice (*Oryza sativa*) as a gene with elevated expression in pSARK-IPT transgenic plants that exhibit increased chloroplast stability. Subsequent work on the *Arabidopsis* homolog of CV suggested that its expression is induced during senescence and following abiotic stress. Over-expression of CV is apparently lethal, but transient or induced over-expression of this protein induces the formation of CV-containing vesicles (CCVs) and ultimately chloroplast degradation. Conversely, knockdown of CV leads to delayed degradation of chloroplasts and enhances the tolerance to stress. In addition to stromal proteins, CCVs also contain thylakoid membrane and lumenal proteins,

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1360-1385/
 http://dx.doi.org/10.1016/j.tplants.2015.03.013

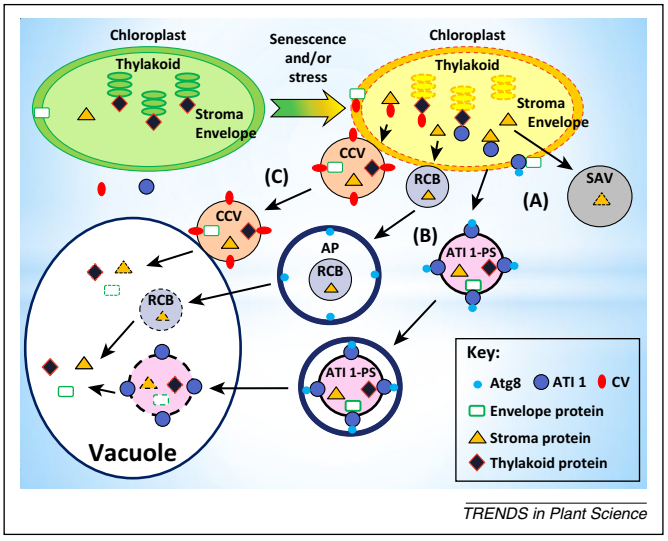


**Figure 1**. Working model of the degradation pathways of chloroplast proteins. Under favorable growth conditions, ATI1 and CV are expressed at a basal level. Under stress conditions or during senescence, ATI1 and CV expression is induced and chloroplast proteins can be degraded through three different pathways **(A, B, C)**. **(A)** stromal proteins are imported into small senescence-associated vacuoles (SAV) for proteolysis. **(B)** ATI1 interacts with chloroplast proteins in the ATI-plastid body (ATI1-PS), or stromal proteins are imported into the Rubisco-containing body (RCB), and then both of these bodies transport their cargo proteins to autophagosomes (AP), which are eventually transported to the vacuole for degradation through the autophagy machinery. **(C)** CV targets chloroplast proteins and induces the formation of CV-containing vesicles (CCVs) directly from the chloroplast membranes, and then the CCV is eventually transported to the vacuole for degradation. What determines which degradation pathway will be activated, and whether these three pathways co-exist in the same cell at the same time still remain elusive. The broken lines indicate the breakdown of the chloroplast membrane and the thylakoid membranes.

suggesting the participation of thylakoid membranes in the formation of CCVs. The CCVs are then mobilized to the vacuole through a pathway independent of autophagy and SAVs [10].

The discovery of a protein involved in a new pathway for chloroplast degradation is very intriguing. ATI1, CHMP1 and CV were all shown to mediate the vacuolar degradation of both soluble (stromal) and membrane (either envelope or thylakoid) proteins [7,9,10]. Both ATI1 and CV were implicated in the response to abiotic stress and during senescence [7,10]. The suggested involvement of CHMP1 in the vacuolar delivery of RCBs might imply a similar role [9]. One important difference between the autophagy-dependent pathway and the CCV pathway lies in their effect on plants response to stresses. Transgenic CV-silenced plants displayed increased chloroplast stability and enhanced tolerance to abiotic stress [10]. By contrast, ATI1 suppression reduced plant resistance to salt stress and enhanced senescence [7]. Indeed, though autophagy is involved in chloroplast degradation, autophagy-defective mutants unexpectedly show early-senescence phenotype, with accelerated loss of chlorophyll and chloroplast proteins [3]. This might suggest a prosurvival recycling role for autophagy-dependent chloroplast degradation, whereas CV-mediated degradation might be more destructive. Furthermore, while CV contains a chloroplast transit signal peptide and seems to be only associated with chloroplasts and CCV, ATI1 and CHMP1 are also involved in other cellular processes. CHMP1 interacts with ESCRT-related proteins and is required for proper endosomal sorting of plasma membrane proteins, whereas ATI1 is also found on ER-associated bodies that are subsequently transported to the vacuole [8,9]. Thus, it will be interesting to see whether there is a regulatory connection between these seemingly different processes, and how do they converge to a physiological response to different stresses and stimuli.

There are still many open questions regarding the different chloroplast degradation pathways. It will be especially important and interesting to decipher the interactions and relationships between the three pathways, and to elucidate the identity and function of other involved proteins. This may aid to answer the question: why do plants require multiple degradation pathways for a single organelle?

## Acknowledgments

The research in our laboratory is supported by grants from The Israel Science Foundation; Bi-national Agriculture Research and Development (BARD); Israeli Ministry of Agriculture; Alternative Energy Research Initiation (AERI) Program; and the ICORE Biofuels Program.

## References

1 Ishida, H. *et al.* (2008) Mobilization of rubisco and stroma-localized fluorescent proteins of chloroplasts to the vacuole by an ATG gene-dependent autophagic process. *Plant Physiol.* 148, 142–155
2 Martinez, D.E. *et al.* (2008) 'Senescence-associated vacuoles' are involved in the degradation of chloroplast proteins in tobacco leaves. *Plant J.* 56, 196–206
3 Liu, Y. and Bassham, D.C. (2012) Autophagy: pathways for self-eating in plant cells. *Annu. Rev. Plant Biol.* 63, 215–237
4 Chiba, A. *et al.* (2003) Exclusion of ribulose-1,5-bisphosphate carboxylase/oxygenase from chloroplasts by specific bodies in naturally senescing leaves of wheat. *Plant Cell Physiol.* 44, 914–921
5 Otegui, M.S. *et al.* (2005) Senescence-associated vacuoles with intense proteolytic activity develop in leaves of *Arabidopsis* and soybean. *Plant J.* 41, 831–844
6 Wang, Y. *et al.* (2013) Autophagy contributes to leaf starch degradation. *Plant Cell* 25, 1383–1399
7 Michaeli, S. *et al.* (2014) *Arabidopsis* ATG8-INTERACTING PROTEIN1 is involved in autophagy-dependent vesicular trafficking of plastid proteins to the vacuole. *Plant Cell* 26, 4084–4101
8 Honig, A. *et al.* (2012) A new type of compartment, defined by plant-specific Atg8-interacting proteins, is induced upon exposure of *Arabidopsis* plants to carbon starvation. *Plant Cell* 24, 288–303
9 Spitzer, C. *et al.* (2015) The endosomal protein CHARGED MULTIVESICULAR BODY PROTEIN1 regulates the autophagic turnover of plastids in *Arabidopsis*. *Plant Cell* 27, 391–402
10 Wang, S. and Blumwald, E. (2014) Stress-induced chloroplast degradation in *Arabidopsis* is regulated via a process independent of autophagy and senescence-associated vacuoles. *Plant Cell* 26, 4875–4888