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Autophagy and macro- and micro-nutrient management in plants

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1 **Abstract**

2 Nutrient recycling and mobilization from organ to organ all along the plant lifespan is essential for plant survival
3 under changing environment. Nutrient remobilization to the seeds is also essential for good seed production. In
4 this review we summarise the recent advances made to understand how plant manage nutrient remobilization from
5 senescing organs to sink tissues, and what is the contribution of autophagy in this process. Plant engineering
6 manipulating autophagy for better yield and plant tolerance to stresses will be presented.
7

8 **Introduction**

9 Intracellular recycling plays an essential role in the proper control of cellular events, such as modulating
10 the levels of key regulators, and more importantly, as the main housekeeper that removes cellular debris and
11 replenishes essential nutrients to support new growth [1,2]. The best studied and understood recycling system in
12 plants is the ubiquitin–proteasome pathway in which proteins are ligated with a poly-ubiquitin chain to serve as
13 effective substrates for cleavage by the 26S proteasome [3]. However, the selective degradation of this system is
14 limited to some individual damaged or short-lived (regulatory) proteins, and seems insufficient in bulk protein
15 degradation during leaf senescence. Then, plants employ autophagy pathway for vacuolar bulk turnover of
16 cytoplasmic components. Autophagy entails encapsulation of unwanted cytosolic materials within specialized
17 autophagic vesicles, which are subsequently delivered to the vacuole for proteolysis or hydrolysis [4].

18 Three distinct types of autophagy, micro-, macro- and mega-autophagy, have been reported in plant.
19 Micro-autophagy proceeds by the invagination of tonoplast to trap cytoplasmic material congregated at the vacuole
20 surface to create autophagic bodies within the vacuole (Figure 1). Such micro-autophagy process is poorly
21 described in plants. It was found that the transport of cytoplasmic anthocyanin aggregates into the vacuole is
22 mediated by a process reminiscent of micro-autophagy [5,6,7]. Conversely, macro-autophagy is much better
23 described. It involves double membrane vesicles, named autophagosomes, that sequester cytosolic components
24 [8]. After trafficking to the lytic vacuoles, their outer membrane fuses with tonoplast to release their contents (inner
25 membrane plus cargoes) into the vacuolar lumen. Released bodies are then called autophagic bodies. Autophagic
26 bodies containing luminal constituents are broken down by resident vacuolar hydrolases, and the products are
27 exported back to the cytosol for reuse. While micro-autophagy decreases tonoplast membrane area, macro-
28 autophagy provides new lipid material to the tonoplast. Thus, it is likely that these two types of autophagy may
29 play opposite roles in tonoplast membrane homeostasis. Mega-autophagy involves the massive degradation of the
30 cell at the final phase of developmental programmed cell death (PCD) [9]. During this process, the
31 permeabilization or rupture of tonoplast results in the release of large amounts of hydrolases into the cytoplasm,
32 which completely degrade the cytoplasm and even the cell walls, leading ultimately to cell death. Mega-autophagy
33 has been mainly described in the case of xylem formation in plants [10].

34 Of these three types, macro-autophagy is the best characterized process and is considered as the major
35 form and has received most attention [11]. During leaf senescence, expression of several AuTophagy-related
36 (*ATG*) genes encoding key components for autophagosome formation is increased [12]. The suppression of these
37 genes disrupts the normal development of autophagosomes and results in hypersensitivity to starvations as well as
38 premature leaf senescence. This suggests that autophagy plays a key role in leaf senescence and nutrient recycling
39 [13,14,15,16].
40

41 **Molecular machinery of macro-autophagy in plants**

42 Professor Yoshinori Ohsumi was awarded the Nobel Prize for Physiology or Medicine in 2016 for his
43 discovery of the molecular basis of macro-autophagy (hereafter referred to as autophagy). Together with
44 colleagues, he identified several *ATG* genes that participate in autophagic processes by yeast forward genetic
45 experiments [17]. To date, more than 40 *ATG* genes have been identified in yeast, and the orthologs for most of
46 them have been found in different plant species such as Arabidopsis, rice, wheat, maize, tobacco, barley, foxtail
47 millet, and apple [13,18,19,20,21,22,23]. Functional analysis of these proteins reveals a canonical route for
48 autophagy. Basically, the process of autophagy consists in the induction of the nucleation of pre-autophagosomal
49 structures, membrane elongation, phagophore expansion and then closure, trafficking and delivery of the
50 autophagosome to the vacuole, and finally breakdown of the autophagic membrane and its contents by hydrolases
51 into the vacuole (Figure 1) [2]. The ATG1 and ATG13 proteins, together with two accessory proteins, ATG11 and
52 ATG101, assemble into an active ATG1-ATG13 complex [24] that promotes the nucleation and expansion of a
53 cup-shaped double-membrane (phagophore), which is thought to originate from the endoplasmic reticulum (ER)
54 [25,26,27]. The transmembrane protein ATG9 recruits lipids for phagophore elongation, ATG2 and ATG18
55 proteins facilitate ATG9 cycling [16,26]. Another step involves phagophore decoration with phosphatidylinositol-
56 3-phosphate (PI3P) generated by a class III complex containing the phosphatidylinositol-3-kinase (PI3K) encoded
57 by VACUOLAR PROTEIN SORTING 34 (VPS34), along with three core accessory subunits, ATG6, VPS38 or
58 ATG14, and VPS15 [28,29]. Expansion and closure of the phagophore membranes require two ubiquitination-like
59 systems. Ubiquitin-fold protein ATG8 is initially processed by a cysteine protease ATG4 to expose a C-terminal
60 glycine [30,31], then conjugated to the lipid phosphatidyl ethanolamine (PE) by the conjugating enzyme ATG3
61 [15]. Another ubiquitin-fold protein ATG12 is conjugated to ATG5 by the conjugating enzyme ATG10 [32]. Both
62 of the conjugation systems share a single ATP-dependent activating enzyme ATG7 [13]. The ATG12-ATG5
63 conjugate promotes the lipidation of ATG8 with PE and its anchorage into the phagophore membrane [33]. ATG8
64 decoration of the phagophore membrane facilitates the recruitment and seal of the cargoes inside the
65 autophagosome. ATG4 is also needed to remove and recycle ATG8 from ATG8-PE lining the outer membrane
66 [30], while the ATG8-PE adducts trapped on the autophagosome inner membrane are digested in the vacuole. The
67 autophagosome then transports the cargoes to the vacuole by fusing the outer membrane with tonoplast, and the
68 remaining single-membrane structure (autophagic body) is released inside the vacuole for degradation by proteases
69 and hydrolases. The digested products are then exported from the vacuole for recycling.

70 The TARGET OF RAPAMYCIN (TOR) kinase is a master player in sensing nutrient status of eukaryotic cells.
71 TOR orchestrates cell homeostasis in a fine crosstalk with several other players among which its LST8 and
72 RapTOR partners and SnRK1 kinase [34]. TOR is a well-known positive regulator of ribosome protein synthesis
73 and of translation and a negative post-translation regulator of autophagy under nutrient-rich conditions. It dampens
74 autophagy at post-translational level by hyper-phosphorylating ATG13, which prevents its association with ATG1.
75 Under nutrient-limiting conditions, inactivation of TOR leads to rapid de-phosphorylation of ATG13, allowing it
76 to bind ATG1. The TOR kinase also play a role in regulating the transcription of genes. It activates genes involved
77 in anabolic processes that are essential for rapid growth like amino acid, lipid and nucleotide synthesis and the
78 oxidative pentose phosphate pathway, and represses genes mediating the degradation of proteins, amino acids,
79 lipids and xenobiotic, and autophagy regulation [35]. On the contrary, SnRK1 is a positive regulator of autophagy
80 in Arabidopsis. The KIN10 SnRK1 alpha catalytic subunit is necessary for the activation of autophagy under
81 energy depleted conditions and in response to many other abiotic stresses. SnRK1 can control autophagy through

82 both TOR-independent and TOR-dependent pathways depending on stresses [36]. Autophagy genes and
83 autophagic activity are then strongly induced by nitrogen and carbon limitation in several plant species, as well as
84 by many other stresses as reviewed by Tang and Bassham [37].

85

86 **Selective macro-autophagy**

87 Although autophagy was originally considered as an unrestricted bulk degradation of cytoplasm compounds,
88 recent studies reveal that various routes for selective autophagy exist. Selective autophagy can specifically degrade
89 appropriate cargoes by engaging a wide array of receptors or adaptor proteins that tether the cargoes and also
90 interact with ATG8 [38,39,40]. The interaction between autophagic receptors and ATG8 is mediated by the
91 presence of ATG8-interacting motif (AIM) in each receptor [41]. Recently, a new binding site for autophagy
92 adaptors and receptors was discovered on ATG8. This site engages ubiquitin-interacting motif (UIM)-like
93 sequences rather than the canonical AIM for high-affinity binding to a new class of ATG8 interactors [42]. As
94 ATG8 decorates and controls phagophore membrane expansion, its abundance determines the size of
95 autophagosome [43]. In this way, autophagosomes may undergo drastic membrane expansion and develop into
96 multiple sizes to efficiently and selectively sequester specific cargoes, including protein aggregates, mitochondria,
97 peroxisomes, chloroplasts, proteasome, ribosomes, endoplasmic reticulum, invading pathogens, and other
98 components in plant cells under specific conditions.

99 Several forms of selective autophagy have been reported in plants as chlorophagy (degradation of chloroplasts),
100 reticulophagy (degradation of endoplasmic reticulum), mitophagy (degradation of mitochondria), pexophagy
101 (degradation of peroxisomes), proteaphagy (degradation of proteasomes), ribophagy (degradation of ribosomes),
102 aggregophagy (degradation of intracellular protein aggregates), xenophagy (degradation of intracellular pathogens),
103 degradation of the pre-autophagosomal structure, degradation of TRYPTOPHAN-RICH SENSORY PROTEIN
104 (TSPO) and degradation of brassinosteroid-responsive transcription factor BES1 [2,44,45]. Selective autophagy
105 certainly allows fine organelle quality control and also to the removal of specific cellular waste. At the same time
106 specific autophagy provides cargoes to degradation pathways performing hydrolysis and proteolysis inside the
107 vacuole lumen, it facilitates the release of metabolites that contribute to nutrient recycling. Whether some specific
108 autophagy pathways could be more related to the recycling of specific nutrient, macro- or micro-elements is an
109 interesting question that remains to be investigated.

110 In plants, the first selective autophagy receptor, named Joka2, was identified in tobacco. This NBR1 (NEIGHBOR
111 OF BRCA1 GENE 1) homologue was identified in yeast two-hybrid screen carried out to look for partners of the
112 coiled-coil protein UP9C of unknown function that strongly over-accumulates under sulphur-deficiency [46].
113 Afterwards, the Arabidopsis NBR1 homologue was characterised and shown to target ubiquitinated protein
114 aggregates formed under stress conditions through a C-terminal ubiquitin-associated (UBA) domain [46,47]. Like
115 ATG genes, it was shown that NBR1/Joka2 expression is enhanced under several nutrient starvations as C, N and
116 S limitations. Functional analyses using two *nbr1* knockout mutants revealed that (i) NBR1 is important for plant
117 tolerance to a large spectrum of abiotic stresses, like heat, oxidative, salt, and drought stresses, and (ii) there is an
118 increased accumulation of ubiquitinated insoluble proteins in *nbr1* mutants under heat stress [48,49]. However,
119 unlike *atg5* and *atg7* mutants, *nbr1* is not sensitive to darkness stress or necrotrophic pathogen attack, suggesting
120 that NBR1 is involved in the selective degradation of denatured or damaged non-native proteins generated under
121 high temperature conditions, but not in other “bulk” autophagy. Therefore, autophagy operates through distinct

122 cargo recognition and delivery systems according to biological processes. NBR1 is involved in the selective
123 degradation of denatured or damaged non-native proteins generated under high temperature conditions, but is not
124 involved in other “bulk” autophagy. Interestingly, it was recently reported that NBR1 also specifically binds viral
125 capsid protein and particles of the cauliflower mosaic virus (CaMV) in xenophagy to mediate their autophagic
126 degradation, and thereby restricting the establishment of CaMV infection [50]. Similarly, Joka2/NBR1 mediated
127 selective autophagy pathway contributes to defence against *Phytophthora infestans*. The *Phytophthora infestans*
128 effector protein PexRD54 recognizes potato ATG8CL through an AIM (Maqbool et al., 2016). PexRD54
129 outcompetes binding of ATG8CL with the Joka2/NBR1 to counteract defence-related selective autophagy, thus
130 possibly attenuating autophagic clearance for pathogen or plant proteins that negatively impact plant immunity
131 [51,52]. Upon infection, ATG8CL/Joka2 labelled defence-related autophagosomes are diverted to the host-
132 pathogen interface to focally restrict pathogen growth [52].

133 Subsequently, the ATI1/ATI2 ATG8-binding proteins were also characterized as autophagy receptors. ATI1 is
134 located in ER-bodies and plastid-associated bodies in dark induced leaves [53,54]. The plastid localised ATI1-
135 bodies were also detected in senescing cells and shown to contain stroma proteins. While they likely play a role in
136 chlorophagy, their role in N remobilization during senescence has not been reported so far.

137 Another example of specific autophagy adaptor is RPN10. The proteasome subunit RPN10 was shown to mediate
138 the autophagic degradation of the ubiquitinated 26S proteasomes, known as proteaphagy [55]. Upon stimulation
139 by chemical or genetic inhibition of the proteasome, RPN10 simultaneously binds the ubiquitinated proteasome,
140 via an ubiquitin-interacting motif (UIM), and to ATG8 through another UIM-related sequence that is distinct from
141 the canonical AIM motif. In Arabidopsis, the inhibitor-induced proteaphagy was blocked in mutant expressing an
142 RPN10 truncation that removed the C-terminal region containing these UIMs.

143 In addition to specifically eliminating macromolecular complexes, organelles, and pathogens, selective autophagy
144 can also scavenge individual proteins. For example, TRYPTOPHAN-RICH SENSORY PROTEIN (TSPO) is
145 involved in binding and eliminating highly reactive porphyrin molecules through autophagy by interacting with
146 ATG8 proteins via a conserved AIM motif [56]. A more recent study proposed another role for TSPO to control
147 water transport activity by interacting with and facilitating the autophagic degradation of a variety of aquaporins
148 present in the tonoplast and the plasma membrane during abiotic stress conditions [57].

149

150 **Nutrient remobilization after organelle and protein degradations in senescing leaves**

151 Nitrogen is quantitatively the most important mineral nutrient for plant growth. The use of nitrogen by
152 plants involves several steps, including uptake, assimilation, translocation, recycling and remobilization [58].
153 Plants are static and cannot escape from the multitude of abiotic and biotic stress conditions occurring during their
154 growth period. To deal with these environmental stresses and survive in the fluctuating environment, plants
155 senesce leaves to massively remobilize phloem-mobile nutrients and energy from senescing leaves to developing
156 tissues and storage organs. This way, plants can save and efficiently utilize the limited nutrients and energy for
157 defence, growth, and reproduction [59]. Efficient nitrogen remobilization thus increases the competitiveness of
158 plants especially under nitrogen limiting conditions. For agriculture, high nitrogen remobilization efficiency is
159 interesting as it can reduce the need of nitrogen (N) fertilization, which represents a substantial cost of agricultural
160 production and often causes environmental pollution. In crops, post-anthesis nitrogen remobilization during seed
161 maturation is highly correlated to grain yield and quality [60]. In small-grained cereals like wheat and rice, up to

162 90% of the grain nitrogen content is remobilized from the vegetative plant parts, while the proportion in maize is
163 approximately 35-55% [61].

164 Once senescence is initiated, carbon and nitrogen primary assimilations are progressively replaced by
165 recycling from the catabolism of macromolecules such as proteins and nucleic acids. Up to 75% of the total
166 mesophyll cellular nitrogen is localized in the chloroplasts [62]. The breakdown and recycling of these
167 considerable nitrogen resources depend on three distinct chloroplast degradation pathways that rely on macro-
168 autophagy, senescence-associated-vacuoles and Chloroplast Vesiculation (CV) pathways (see [63] and [2], for
169 reviews). Up to now, although detailed knowledge concerning interactions and relationships between these three
170 chloroplast degradation pathways remains insufficient, the cysteine proteases localized in the vacuole appear to
171 play a particularly important role in all these processes as they proceed during the last steps of the macromolecule
172 break-down in the vacuole. Cysteine proteases as SAG12, Cathepsin B3 (CATHB3), Responsive-to-desiccation
173 21A (RD21A), Arabidopsis aleurain-like protease (AALP) and Vacuolar Processing Enzymes (VPEs) are amongst
174 the most highly overexpressed senescence-related proteases [64,65].

175 Efforts made to understand nitrogen remobilization during leaf senescence have mainly focused on the
176 biochemistry of the degradation of plastidial proteins (Figure 2). Originally observed by immuno-electron
177 microscopy in the cytoplasm and vacuole of naturally senescing wheat leaf cells, the RuBisCo-containing bodies
178 (RCBs) were proposed to be involved in RuBisCo degradation process outside of the chloroplasts [66]. These
179 RCBs contained the large and small subunit of RuBisCo and other stromal proteins as the chloroplastic glutamine
180 synthetase. However, RCB lacked chloroplast envelope or thylakoid components. Sometime, RCBs were found
181 to be surrounded by double membranes, which seem to be derived from the chloroplast envelope. Interestingly
182 several observations presented RCBs in the cytoplasm closely bordered by kinds of bean shaped vesicles that
183 might be isolation membranes characteristic of the intermediate structures of autophagosomes (phagophores)
184 [66,67]. RCBs were frequently visible at the early stages of leaf senescence when RuBisCo starts to decrease
185 without prior chloroplast destruction or chlorophyll degradation and it was proposed that the budding of RCBs
186 from chloroplast stromules was a way to bring chloroplast material out of the organelle [68,69,70]. This material
187 release may explain why during senescence the size of chloroplasts (c.a. 10 μm) decreases to form gerontoplasts
188 (c.a. 4 μm).

189 The demonstration that autophagy plays a prominent role in RCB trafficking to the vacuole was provided using
190 confocal microscopy to visualize stromal and ATG8 proteins tagged with different fluorescent probes. Authors
191 showed that the release of RCBs inside the vacuole required functional autophagy and was absent in autophagy
192 mutants such as *atg5* and *atg7*. Co-localization of autophagosomes and RCBs was also demonstrated and moreover
193 it was also shown that shrunk gerontoplasts could be released inside the vacuole in an ATG4-dependent micro-
194 autophagy pathway [71,72]. The elimination of membrane damaged chloroplast via micro-autophagy was further
195 confirmed and the role of macro-autophagy related membranes harbouring GFP-ATG8 decorations in this process
196 suggested [73].

197

198 **Role of autophagy in nitrogen recycling**

199 As stated earlier, under normal conditions autophagy operates at a basal level that constitutes housekeeping
200 machinery and participates in cell homeostasis. Under nutrient starvation and during leaf senescence autophagy
201 activity is enhanced and its role in nutrient recycling and remobilization at the whole plant level was suspected.

202 The demonstration of the role of autophagy in nutrient recycling and mobilization from source to sinks was
203 provided by Guiboileau et al. [74] (Figure 3). Monitoring ¹⁵N fluxes to the seeds after labelling Arabidopsis rosettes
204 at the vegetative stage, Guiboileau et al. (2012) showed that N remobilization was markedly decreased in *atg*
205 mutants (*atg18a* RNAi, *atg5*, and *atg9*) compared to wild type plants (WT). The decrease was more moderate
206 when plants were grown under high nitrate than under low nitrate conditions, but still significant. Accordingly,
207 authors further found that *atg* mutants accumulated more ammonium, amino acids (AA), proteins, and RNA in
208 their rosette leaves than WT [75]. N remobilization was further evaluated using similar ¹⁵N-labelling procedure in
209 the *atg12* maize mutants which revealed that N remobilization to the kernels was also impaired in autophagy
210 deficient mutants [76].

211 The growth of *atg12* mutants was most often arrested at seedling stage, and adult plants showed enhanced leaf
212 senescence and stunted ear development under nitrogen-starved conditions but not under high-N. Under nutrient-
213 rich conditions, the seed yield of *atg12* plants was much lower, and ¹⁵N reallocation into the seeds was twice less
214 in *atg12* was half of that in WT. The investigation conducted during the vegetative growth period on the rice
215 autophagy-deficient mutant *Osatg7-1* suggested that N remobilization from senescent leaves to young leaves was
216 suppressed [77]. Higher nitrogen content was retained in senescent leaves of *Osatg7-1* mutants as soluble protein
217 and RuBisCo concentrations were higher than that of WT. The reduction of nitrogen available for newly
218 developing tissues in *Osatg7-1* likely led to its reduced leaf area, tillers, and photosynthetic capacity.
219 Unfortunately, the male sterile phenotype of *Osatg7-1* mutants prevented authors from examining the contribution
220 of autophagy-mediated nitrogen remobilization from leaves to seeds during the reproductive growth period.

221 Recently over-expression of autophagy genes was assessed in several plant species. Overexpressing *AtATG5* and
222 *AtATG7* in Arabidopsis delayed senescence, improved seed production and yield under certain conditions [78].
223 Similarly, several reports showed that overexpressing different *ATG8* genes from soybean or millet in Arabidopsis
224 or rice was beneficial to plant performances, increasing tolerance to nitrogen starvations and to drought
225 [79,80,81,82,83].

226 Using the same ¹⁵N labelling procedure as Guiboileau [74], Chen et al. [84] then showed that N remobilization of
227 nitrogen from the rosette leaves to the seeds was improved in Arabidopsis plants overexpressing the *AtATG8a* or
228 the *AtATG8g* gene. In these plants, N seed filling was increased and the amount of nitrogen lost in dry remains
229 decreased. Interestingly the N-remobilization performances of the *AtATG8a* and *AtATG8g* over-expressors were
230 improved only when plants were grown under abundant nitrate supply but not when grown under N limited
231 conditions. This can be explained by the fact that under N-limitation, autophagy activity is already enhanced,
232 which cancels the benefit of stimulating *ATG8* expression through genetic transformation.

233 This demonstration of the beneficial effect on NUE of over-expressing *ATG8* was confirmed by Yu et al. [85].
234 The authors over-expressed the *OsATG8a* gene in rice and found that N% in seeds was increased while N% in dry
235 remains was decreased attesting better N remobilization to the seeds. Interestingly, like in Arabidopsis, the positive
236 effect on plant performances was only observed under sufficient N supply but not under N-limitation.

237

238 **Cross-talk between autophagy and senescence-related cysteine proteases**

239 Although both autophagy and cysteine proteases are key players during leaf senescence, protein proteolysis, and
240 nutrient recycling as shown by recent publications from James et al. [86,87], the relationship between them remains
241 largely unknown. It is admitted that proteins are not degraded inside the autophagosomes but rather transported

242 by them to the lytic vacuoles where proteases and hydrolases operate. As said before, autophagy mutants are
243 impaired in N remobilization and they accumulate large amounts of proteins and amino acids in their rosette leaves.
244 They also present significantly higher protease activities in their rosette leaves than wild type, which supports the
245 hypothesis that proteases and substrates cannot meet each other in autophagy mutants. In order to investigate the
246 nature of the protease activities enhanced in autophagy mutants, Havé et al. [88] used shotgun proteomics to
247 identify these proteases and specific probes to monitor their activity. Results showed that cysteine proteases
248 accounted for the largest proportion (38%) of the over-abundant proteases in autophagy-deficient lines. Activity-
249 based protein profiling (ABPP) analysis with DCG-04 revealed that activities of papain like cysteine proteases
250 (PLCPs) were higher in autophagy-defective plants grown under low-nitrate conditions. Further pull-down
251 experiments using the DCG-04 biotinylated inhibitor of papain like cysteine protease (PLCP), showed that the
252 active PLCPs accumulated in autophagy mutants in low-nitrate condition were mainly SAG12, RD21A, CATHB3,
253 and AALP. The western blots using RD21A, CATHB3, and SAG12 antibodies confirmed that both the mature
254 and immature protease forms were accumulated in the mutant lines, suggesting that there was no defect in protease
255 maturation or trafficking in the autophagy mutants. The specific over-accumulation of these PLCPs under low
256 nitrate but not under high nitrate in autophagy mutants strongly suggested that they are involved in N
257 remobilization, and possibly provide alternative remobilization pathways to autophagy. Such hypotheses need to
258 be confirmed by further investigations and biochemical studies using protease and autophagy double mutants.
259 Havé et al. (2018) also found that the CND41-like aspartate protease AED1 (APOPLASTIC ENHANCED
260 DISEASE SUSCEPTIBILITY-DEPENDENT 1) that have been described by Kato et al. [89] as one of the potential
261 protease involved in RuBisCo degradation was also increased in autophagy mutant. Interestingly AED1 that was
262 up-regulated in the senescing leaves of the *sag12* mutants was also proposed to compensate the absence of SAG12
263 activity for N remobilization [87].

264

265 **Autophagy and other nutrients**

266 Autophagy is likely involved in the recycling not only of proteins but also of membranes and other cell
267 components that certainly contain micro-elements. It is well known that iron in the cell is mainly linked to ferritin
268 and photosystem I, which are located in plants into the plastids in plants. In mammals, ferritin is degraded by
269 NCOA4-mediated autophagy (ferritinophagy) which participates to control ferroptosis and erythropoiesis
270 [90,91]. Although the role of autophagy in the degradation of ferritin has not been demonstrated in plant, it was
271 found that the efficiency of iron (Fe) translocation from vegetative organs to the seeds is severely decreased in
272 several autophagy mutants compared to wild type [92] (Figure 3). The authors confirmed the defect of iron
273 translocation to the seeds in autophagy mutant using ⁵⁷Fe labelling and tracing experiment. This study also showed
274 that not only iron but also manganese (Mn) and zinc (Zn) are sequestered into the rosette leaves. Consistently the
275 lower amounts of Zn and Mn in the seeds of autophagy mutants also suggest that their translocation is dependent
276 of autophagy. This observation is consistent with the study of Eguchi et al. [93] that showed that autophagy is
277 induced under Zn limitation conditions, and that autophagy-deficient mutants (*atg5-4*, *atg10-1*) exhibit early
278 senescence phenotype under Zn limitation and limited growth recovery after Zn resupply.

279 More recently, Shinozaki et al. (unpublished data) confirmed the hypersensitivity of autophagy mutants to zinc
280 limitation. While Zinc limitation induced autophagy in wild-type it triggered accumulation of proteins in
281 autophagy mutants as a mark of autophagy defect. Interestingly, Zn-deficiency symptoms in *atg* mutants

282 recovered under low-light and iron-limited conditions, pointing out the role of Fenton-related oxidative stress in
283 the response of plants to zinc deficiency. This also suggests that the induction of autophagy by zinc deficiency
284 could be mediated by Fenton-generated hydroxy radicals.

285 Inorganic phosphate like nitrogen is one of the major macro-elements needed for plant growth. The recent
286 paper from Naumann et al. [94] reveals that phosphate limitation stimulates autophagy in the root tips of
287 Arabidopsis. Stimulation of autophagy by Pi deprivation was exacerbated in the *pdr2* mutants which is
288 hypersensitive to Pi deficiency. PDR2 protein is located at the endoplasmic reticulum and was hypothesized to
289 play a role in ER-quality control. Blocking ER stress in *pdr2* mutant introducing *ire1a* mutation, or providing ER-
290 stress inhibitors reduced autophagosome formation in response to Pi deprivation. This indicates that the ER-stress
291 induced by low-Pi triggers autophagy in roots under low phosphate. Root growth of autophagy mutants was
292 strongly reduced by Pi deprivation due to early root apical meristem differentiation that lowered meristem activity.
293 When suppressing locally Pi sensing using phosphite application, meristem activity was restored in autophagy
294 mutants. Decreasing iron concentration in the low Pi culture medium also restored apical meristem activity in
295 autophagy mutant, suggesting by the way that iron would also play a role in the ER stress response to Pi deficiency,
296 possibly through the production of reactive oxygen species.

297

298 **Conclusion**

299 The results obtained from the studies of autophagy-defective mutants grown under various starvations clearly
300 indicate the involvement of autophagy in the recycling and remobilization of nutrients at the whole plant level.
301 The studies that increased autophagic activity through the over-expression of some *ATG* genes demonstrated that
302 it could be a powerful approach to improve plant tolerance to starvations and nutrient remobilization from source
303 to sinks. However, recent results enlighten the strong link between nutrient deprivation (N, S, Zn and Pi), and
304 oxidative stress and ER-stress [95, 94]. This questions whether the ER, which is the source of lipid for
305 autophagosome formation [27], could be a sensor of plant environmental stresses and an intermediate in autophagy
306 induction.

307

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Legend of Figures

Figure 1: Schematic representation of macro and micro-autophagy pathways in plants. Nutrient availability controls the TOR kinase activity that in turn regulates post-transcriptionally macro-autophagy through the phosphorylation of ATG1 and ATG13. After nucleation of the pre-autophagosomal structures, the ATG9, ATG18 and ATG2 proteins (in blue) are involved in the expansion of the membrane of the autophagosome. Several ATG proteins (in orange) involved in the conjugation of ATG8 to phosphatidyl-ethanolamine, facilitate ATG8 anchorage to the membrane of the pre-autophagosome and *per se* autophagosome formation and enclosure. The ATG8 interacting motifs facilitate the capture of cargoes to be driven to the central vacuole for degradation. Micro-autophagy consists in the invagination of the tonoplast and participates to the formation of anthocyanin vacuole inclusions (AVI).

Figure 2: Schematic representation of the different steps of nutrient recycling in plant cells. Chloroplast material and unwanted cytoplasmic material are driven to the central vacuole for degradation through the macro-autophagy pathway. Once delivered to the vacuole lumen, autophagic bodies (inner membrane of autophagosome and cargoes) are degraded by the resident proteases and hydrolases. The nutrients released are exported to the cytosol and using transporters or canals. Once inside the cytosol, nutrients are either used for cell metabolism or released out of the cell for source to sink translocation. Interconversions of amino-acids occur in the cytosol to produce the glutamine and asparagine forms that are preferentially used for long distance transport in the phloem. Many black boxes remain to be explored, especially regarding the docking of autophagosomes to the tonoplast and the transport of nutrients out of the vacuole and further out of the cell (question marks).

Figure 3: Modification of macro- and micro- nutrient fluxes in autophagy mutants and over-expressors in Arabidopsis. The green and blue arrows indicate the lack (as percentages) of micro and macro-nutrient allocation to the seeds in the *atg5*-KO mutant relative to wild type [74,92]. The red arrows indicate the extra nitrogen remobilization measured in *ATG8* Arabidopsis over-expressors by comparison to control line under plethoric nitrate conditions [84].

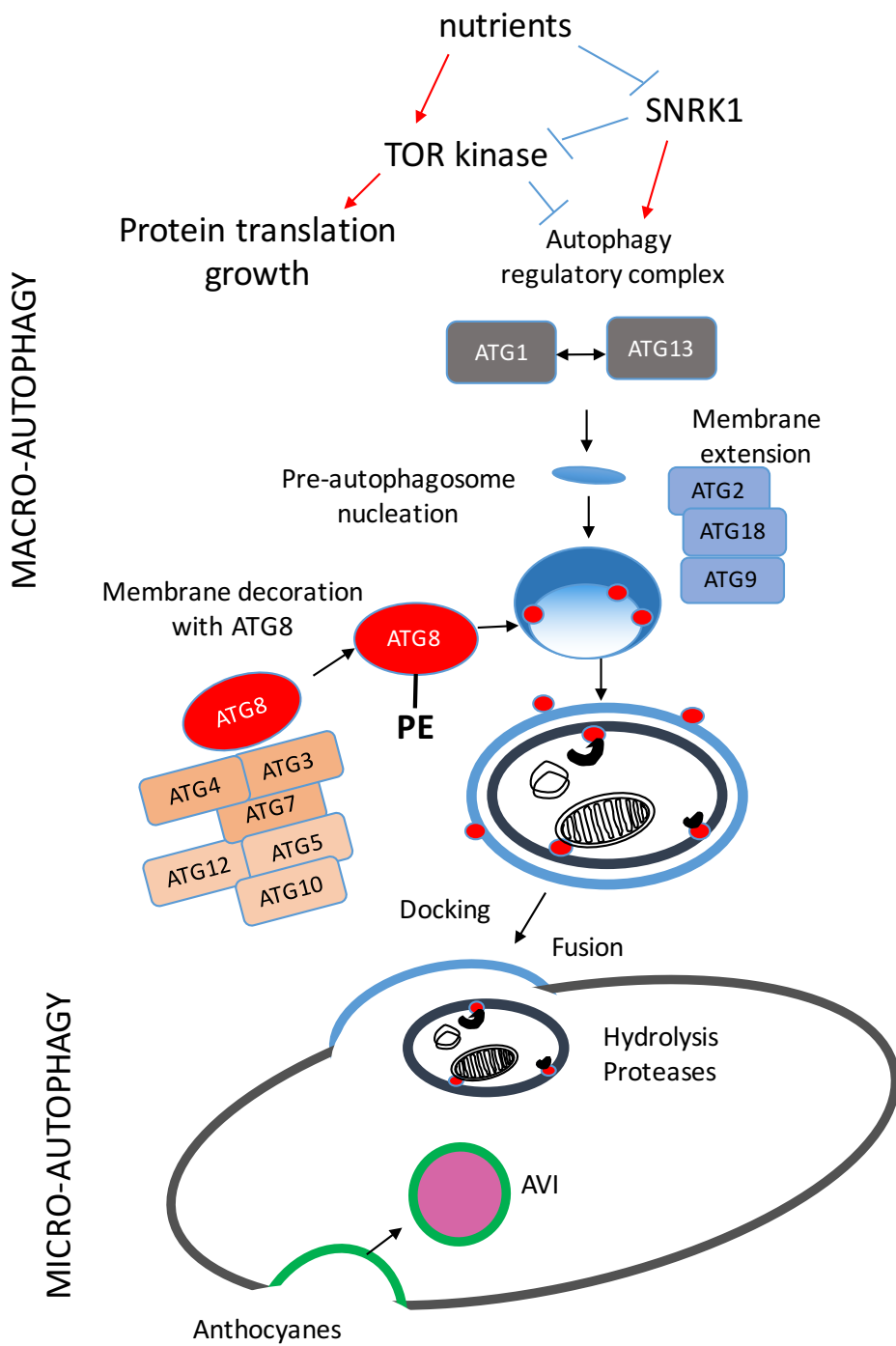


Figure 2

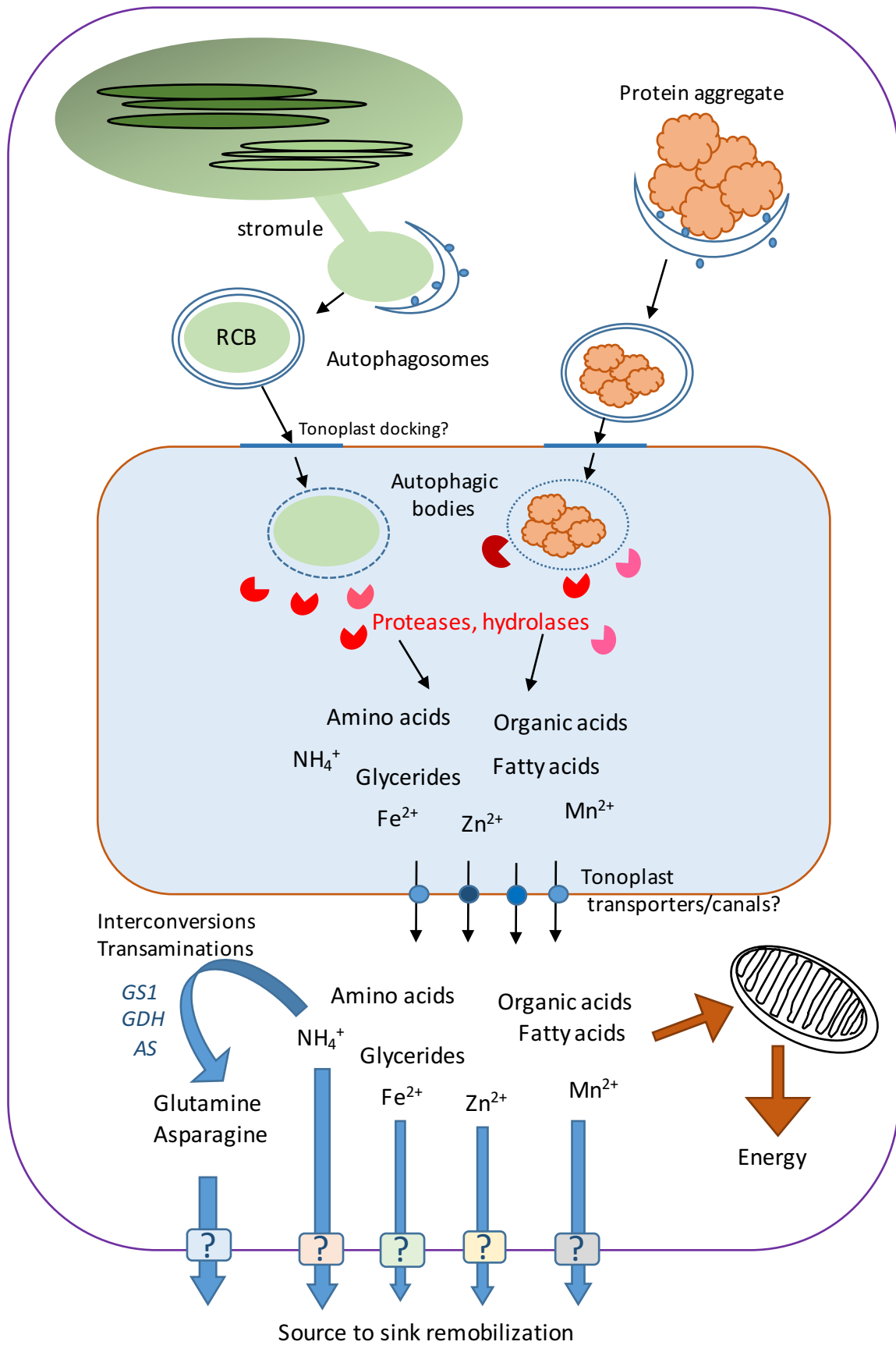
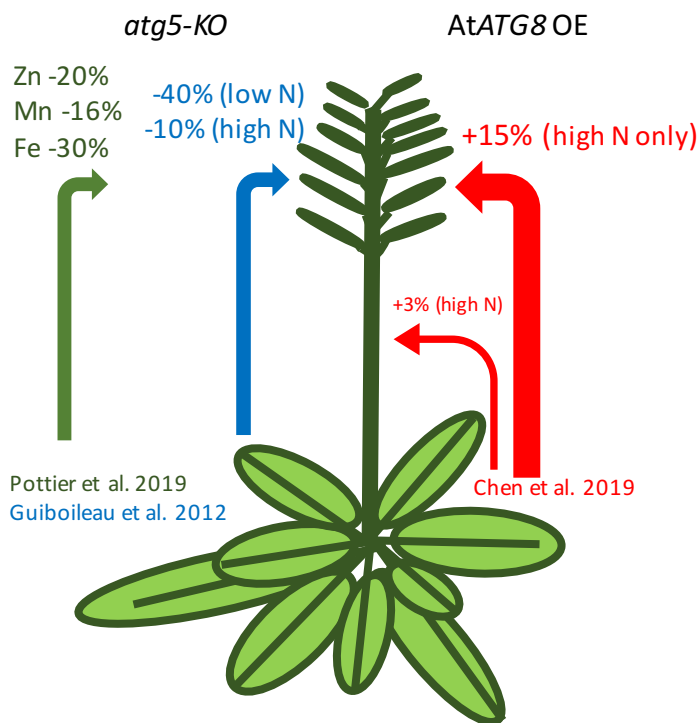


Figure 3

Modification of N, Fe, Zn and Mn fluxes by autophagy



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