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REVIEW

Protein Degradation and Nitrogen Remobilization during Leaf Senescence

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Leaf senescence, a type of programmed cell death, is a complex and highly regulated process that involves the degradation of macromolecules, including proteins, nucleic acids, and lipids. Nutrients, especially nitrogen, are re-mobilized from senescing leaves to newly developing tissues or reserve organs. Our review focuses on three pathways for protein breakdown and the resorption of N during this process: the ubiquitin/proteosome system, the chloroplast degradation pathway, and the vacuolar and autophagic pathway. We propose that two relative biochemical cycles exist for amino acid recycling and N-export -- the GS/GOGAT cycle and the PPDK-GS/GOGAT cycle.

Keywords: GS/GOGAT pathway, leaf senescence, nitrogen (N) resorption, PPDK-GS/GOGAT pathway, programmed cell death (PCD), protein degradation

As the final stage of plant development, leaf senescence is a complex and highly regulated process that involves a decline in photosynthesis, the dismantling of chloroplasts, and the degradation of macromolecules, such as proteins, nucleic acids, and lipids. Nutrients are re-mobilized from the senescing leaves to other plant parts, e.g., newly developed vegetative tissue, developing seeds, or storage organs (Buchanan-Wollaston and Ainsworth, 1997). Because resorption reduces the need for nutrient uptake from the environment, this strategy can be of particular importance to plants growing under nutrient-poor conditions (Aerts, 1990). Senescence has attracted attention, especially in monocarpic plants, such as cereals, because efficient nutrient use is critical to high crop yields. Therefore, the improvement of nutrient mobilization from senescing leaves has been a major objective of plant breeding programs.

Leaf senescence is a type of programmed cell death (PCD) that involves the controlled disassembly of cells (Buchanan-Wollaston et al., 2003). This process, which entails the entire realm of metabolism, culminates in tissue death and, often, in the nearly complete removal of all cellular components except the walls. To identify and characterize the central players in the regulatory network, as well as individual steps and catabolic activities during senescence, 100 senescence-associated genes (SAGs) have already been cloned (Buchanan-Wollaston et al., 2003) from various plant species, including maize (*Zea mays* L.; Smart et al., 1995), sweet potato (*Ipomoea batatas*; Drake et al., 1996), barley (*Hordeum vulgare* L.; Hajouj et al., 2000), and *Arabidopsis thaliana* L. (Lohman et al., 1994; He and Gan, 2002). Likewise, genome-wide analysis of SAG expression using microarrays has recently been implemented, notably in *Arabidopsis* (Buchanan-Wollaston et al., 2003, 2005; Lin and Wu, 2004), various *Populus* species (Andersson et al., 2004), aspen (*Populus tremula* L.; Bhalerao et al., 2003), and wheat (*Triticum aestivum* L.; Gregersen and Holm, 2007). Sequence and/or functional analyses have revealed that SAG-encoded proteins include proteases; nucleases; lipid-, carbohydrate-, and nitrogen-metabolizing enzymes; stress responsive proteins; and transcriptional regulators. The major functional category for SAGs is the metabolization of macromolecules.

In *Arabidopsis*, levels of Cu, Cr, Fe, K, Mo, N, P, S, and Zn drop dramatically during senescence, suggesting that these nutrients are being transported from aging leaves. Nitrogen resorption is close to 90%; that of phosphorus and sulfur is 40% (Himelblau and Amasino, 2001). The considerable amount of internal N-mobilization is astonishingly efficient, as demonstrated in N-starved oat (*Avena sativa* L.) plants that are able to complete a life cycle based on the initial supply of nitrogen in the seeds and the eventual production of viable grain (Mei and Thimann, 1984). Such resorption is a complicated process, involving the metabolization of almost all macromolecule substances, e.g., proteins, nucleotides, lipids, and polysaccharides. Nitrogen exists mainly in chlorophyll and proteins; that which is contained in the former is not exported from senescing leaves, but remains within the cells in the form of linear tetrapyrrolic catabolites, which are ultimately lost when those leaves are shed (see reviews by Hörtensteiner and Feller, 2002; and Eckhardt et al., 2004). Protein degradation is the primary role of N-resorption, where the majority of that remobilization is due to the hydrolysis of proteins to amino acids, which are probably inter-converted, hydrolyzed, catabolized, or exported without any modifications (Quirino et al., 2000; Brouquisse et al., 2001; Hörtensteiner and Feller, 2002; Feller, 2004; Krupinska, 2006). Because little is known about this process, our review focuses on the pathways involved in protein breakdown, amino acid recycling, and N-export during leaf senescence.

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†Abbreviations: APG, autophagic; AS, asparagine synthetase; AspAT, aspartate aminotransferase; Clp, chloroplast; ClpP, chloroplast protein; Cys, cysteine; GDH, glutamate dehydrogenase; GOGAT, glutamate synthase; GS, glutamine synthetase; N, nitrogen; OAA, oxaloacetic acid; PCD, programmed cell death; PEP, phosphoenol pyruvate; PEPC, PEP carboxykinase; PPDK, pyruvate orthophosphate dikinase; RCB, Rubisco-containing bodies; ROS, reactive oxygen species; SAG, senescence-associated gene

THE PATHWAY OF PROTEIN DEGRADATION

Functional and homologous analyses of SAGs have revealed three major protein degradation pathways during leaf senescence -- the ubiquitin/proteosome system, the chloroplast degradation pathway, and the vacuolar and autophagic (APG) pathway.

The Ubiquitin/proteosome Pathway

The ubiquitin-26S proteasome pathway is important for targeted protein degradation both during normal development and in response to environmental factors (Sullivan et al., 2003). It is critical to the elimination of abnormal cytoplasmic proteins and the rapid turnover of short-lived proteins. In this pathway, covalent attachment of the 76-amino acid ubiquitins is used as a signal to target specific proteins for degradation by the 26S proteasome (Smalle and Vierstra, 2004). Three enzymes -- ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin protein ligase (E3) -- are involved. E1s, encoded by a single gene or a small family of related genes, can initiate this pathway. All E1s form thiolester bonds with the C-terminal glycine of ubiquitin through a conserved cysteine (Cys) residue. E2 accepts ubiquitin from an E1 through a thioester linkage via a conserved UBC domain with a cysteinyl sulfhydryl group (Kraft et al., 2005). Finally, ubiquitin is attached to the target protein by E3 recognition. In all components of the ubiquitin/26S proteasome pathway, approximately 90% of the genes encode elements of E3 ubiquitin ligases. It has been proposed that the large number of E3s determines substrate specificity. Once the protein substrate is assembled by a polyubiquitin chain, it is sufficient for degradation by a multi-subunit complex, the 26S proteasome (Zhang and Xie, 2007).

Many genes associated with this pathway are senescence-induced, which indicates that ubiquitin-dependent proteolysis may be an important aspect of non-chloroplast protein degradation during leaf senescence (Park et al., 1998). Moreover, senescence in *Arabidopsis* is delayed for mutations of Ore9, which encodes an F-box protein and interacts with a component of the plant SCF complex that controls selective ubiquitination and, subsequently, proteolysis of target proteins (Woo et al., 2001). Expression profiles for the involvement of the ubiquitin-26S proteasome pathway in senescing *Arabidopsis* leaves have been systemically surveyed, and have demonstrated that UBQ3 and UBQ4 are the predominant polyubiquitin genes showing upregulation, while other ubiquitin-related protein genes are also highly represented (Lin and Wu, 2004). Genes encoding polyubiquitin, the ubiquitin-protein ligase E3, and a ubiquitin-conjugating enzyme, are senescence-induced in the aging cotyledons of cotton, and expression of some genes encoding proteasome constituent proteins, such as the 26S proteasome ATPase subunit and 26S ATP/ubiquitin-dependent proteinase chain S4, also are up-regulated in those tissues (Shen et al., 2006). These results imply the involvement of ubiquitin proteasome-dependent proteolysis. Transcriptome analysis of the senescence of wheat flag leaves has detected some genes associated with the ubiquitin pathway, which suggests the existence of the ubiquitin/proteosome pathway. These genes include the 20S proteasome â5 subunit, kelch repeat F-box protein, ubiquitin-conjugating enzyme E2, and ubiquitin-like protein 5 (Gregersen and Holm, 2007).

The Chloroplast Degradation Pathway

During leaf senescence, the breakdown of proteins by proteinases provides a large pool of cellular N for recycling. Chloroplast (Clp) proteins, which account for >70% of all leaf proteins, are thought to be a major source of N for mobilization (Hörtensteiner and Feller, 2002). Hence, their breakdown is the first step in N-resorption. Proteolysis in Clp has been extensively reviewed in past years (Adam, 2001; Nair and Ramaswamy, 2004; Krupinska, 2006). Much of the protein catabolism in Clp occurs either in the early phase, during its transition from proplastid to plastid, or during senescence (Adam, 1996). Several plant proteases have been characterized by identifying bacterial homologues within the Clp (Lindahl et al., 1996). Three types of proteases (ClpP) have now been categorized according to their cellular compartments: stroma, thylakoid membrane, and lumina (Adam, 2001). Studies of these proteases were initially prompted by an investigation of leaf senescence (Buetow, 1997). In those tissues, a proteolytic system is proposed to be newly synthesized and some Clp proteins are degraded within the Clps before their breakdown (Hörtensteiner and Feller, 2002). These proteases include the ATP-dependent Clp proteases (stromal proteases), FtsH and Lon (both thylakoid-membrane proteases), and the ATP-independent Deg (thylakoid-membrane and lumenal proteases) (Guo et al., 2004; Lin and Wu, 2004; Shen et al., 2006; Gregersen and Holm, 2007), all of which have now been extensively characterized in senescing leaves (Gottesman, 1996; Adam et al., 2001; 2006; Adam and Clarke, 2002; Sakamoto, 2006).

Clp protease consists of a Ser-type endopeptidase, which relies on the ATP-dependent unfolding activity from an Hsp100 molecular chaperone to initiate protein degradation in bacteria (Gottesman, 1996; Adam et al., 2006; Sakamoto, 2006). Although Clp proteases are most diverse in higher plants, knowledge of their functioning is limited. The *Arabidopsis thaliana* genome contains at least 23 genes predicted to encode various Clp proteases -- 10 are Hsp100 chaperones (4 ClpB, 2 ClpC, 1 ClpD, and 3 ClpX), 6 are proteolytic subunits (ClpP), and 4 are ClpP-like (ClpR) (Adam et al., 2006; Sakamoto, 2006). ClpRs, which are unique to photosynthetic organisms, are similar in sequence and size to the ClpP proteins but lack the conserved catalytic triad (Ser-His-Asp) typical of Ser-type proteases (Adam et al., 2006; Sakamoto, 2006). Whereas ClpS appears to be specific to higher plants (Peltier et al., 2001), ClpT is the plant version of a bacterial Clp modulator (Peltier et al., 2004). The plant ClpS should not be confused with the bacterial modulator of the same name (Dougan et al., 2002), which, in plants, has been termed ClpT. Finally, ClpB3, the other Hsp100 localized in Clp, does not contain a ClpP recognition site in its sequence, but likely acts as a chaperone (Keeler et al., 2000). Figure 1 presents this chloroplast degradation pathway.

Most of the genes encoding Clp proteases have been

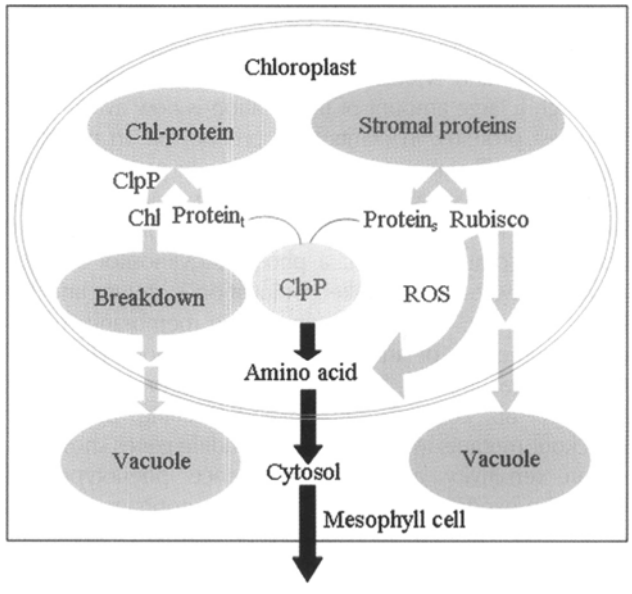

Figure 1. Chloroplast degradation pathway of leaf proteins. ClpP, chloroplast protease; ROS, reactive oxygen species; Protein$_t$, protein released from Chl-protein; Protein$_s$, stromal protein except for Rubisco.

found in senescing *Arabidopsis* leaves (Lin and Wu, 2004), with some in the Clp protease complex (i.e., ClpP1-3, ClpP5-6) being expressed constitutively (Nakabayashi et al., 1999; Zheng et al., 2002). Two other Clp protease subunits, ClpR3 and ClpR4, which have not been characterized previously, also show constitutive expression patterns in those tissues. This observation coincides with the prediction that Clp proteases are housekeeping proteases to turn over Clp proteins, thereby maintaining appropriate stoichiometry and removing damaged or mis-targeted proteins (Adam and Clarke, 2002). Only two Clp protease members are down-regulated: ClpS1 (Peltier et al., 2001) and ClpP4 (Nakabayashi et al., 1999). However, strong dark-induction of ClpD/ERD1 (Nakashima et al., 1997; Nakabayashi et al., 1999) and upregulation of ClpC1 (Lin and Wu, 2004) also have been found, implying that they play regulatory roles in the functioning of the Clp protease complex in senescing *Arabidopsis* leaves. Both ClpC1 and ClpD encode proteins with sequence similarity to HSP100 chaperones. Upregulation of these two transcripts might reflect a need for the recruitment of unfolded proteins for degradation by Clp proteases during senescence.

In contrast to the expression patterns of stromal-localized Clp proteases, the thylakoid-associated protease family members -- FtsH, DegP, and Lon – are poorly characterized in senescing *Arabidopsis* leaves (Lin and Wu, 2004). FtsH protease, a metalloprotease, is responsible for quality control of photosynthetic proteins and the biogenesis of Clp (Chen et al., 2000; Takechi et al., 2000; Haussuhl et al., 2001). DegP1, 5, and 8 are localized on the lumen side of the thylakoid membrane and, thus, are considered good candidates for the degradation of lumen proteins (Adam et al., 2001: Adam and Clarke, 2002). Another DegP protease, DegP2, is localized on the stromal side of the thylakoid membrane, and is responsible for the initial cleavage of damaged D1, i.e., the reaction center of PS II, before the complete degradation of D1 by FtsH (Haussuhl et al., 2001). Surprisingly, in senescing *Arabidopsis* leaves, all four members of the DegP proteases and two of the eight Clp FtsH proteases seem to be down-regulated whereas one Lon protease is marginally up-regulated. For these membrane-associated proteases to participate in degrading the Clp proteins of senescing leaves, FtsH, DegP and Lon must be regulated at the translational and/or post-translational level(s) (Lin and Wu, 2004).

In thylakoid membranes, proteins exist in the form of pigment-protein complexes (Chl-protein), accounting for more than 30% of the total protein of Clps (Matile, 1992). Mae et al. (1993) have examined shaded and unshaded leaves of *Lolium tremulentum* and have reported that the senescence-related decrease in Rubisco is hardly affected by light intensity, whereas a senescence-associated decline in the levels of other proteins in the photosynthetic apparatus (e.g., LHCII and cytochrome *f*) is clearly retarded under low-light conditions. These results indicate that stromal and thylakoid-membrane proteins are degraded by different pathways. For example, the breakdown of thylakoid proteins, such as LHCP II, requires parallel detoxification of the released chlorophyll; this has been shown in a stay-green *Festuca* mutant, where Chl catabolism is blocked so that the LHCP protein is stabilized and not degraded (Thomas and Donnison, 2000). Therefore, the first step in the degradation of proteins (such as LHCP) may be the removal of chlorophyll catabolites, thereby destabilizing the protein complex and allowing degradation by Chl proteases.

Rubisco, which represents about 50% of the total protein content in leaves from C3 plants, is the dominant stromal enzyme in Clps. However, no genes involved in the degradation of stromal proteins, e.g., Rubisco, have been found in the Clps from their senescing leaves (Guo et al., 2004; Lin and Wu, 2004; Shen et al., 2006; Gregersen and Holm, 2007). These results suggest that Clp protease is not involved in the degradation of Rubisco, making the role of Clp protease and its possible substrates in higher plants unclear (Adam and Clarke, 2002). Stromal enzymes, such as Rubisco, may be degraded by two other pathways: 1) ROS initiation or 2) vacuolar degradation. In the first, Rubisco degradation can be non-enzymatically initiated by reactive oxygen species when Clps are incubated under photo-oxidative stress conditions (Ishida et al., 2002). However, it is not clear whether increased ROS could initiate this early degradation during senescence. Although ROS levels do rise during that phase, this is likely a result of macromolecule degradation processes, thus occurring after protein and lipid degradation is initiated. In support of the second pathway, at least a fraction of the Rubisco is degraded by vacuolar proteases following the engulfment of part of the Clp population (Minamikawa et al., 2001).

The Vacuolar and Autophagic (APG) Pathway

Autophagy, a Greek word meaning to 'eat oneself', is needed for non-specific protein and organelle turnover. It can be induced by leaf senescence or multiple stress factors, including cellular damage, nutrient deficiency, or pathogen attack (Levine and Klionsky, 2004). Several types of autophagy

exist: 1) biosynthetic autophagy, as seen with yeast in the trafficking of the vacuolar protein aminopeptidase during cytoplasm-to-vacuole transport (CVT); 2) microautophagy; and 3) macroautophagy (Klionsky, 2005). In microautophagy, material is engulfed directly by the vacuole via invagination of the tonoplast, followed by a pinching off of the membrane to release a vesicle containing the cytoplasmic constituents inside the vacuole lumen. Microautophagy-like processes have been observed during the deposition of storage proteins in germinating seeds, when starch granules and storage proteins are degraded in the vacuoles (van der Wilden et al., 1980; Toyooka et al., 2001). By contrast, macroautophagy is initiated in the cytoplasm by the formation of cup-shaped membranes of unknown source that enclose the material to be degraded. These membranes elongate and eventually fuse together to produce a double-membrane autophagosome containing cytoplasmic material. The outer membrane fuses with the tonoplast, releasing an autophagic body (consisting of the inner membrane and contents) into the vacuole. Vacuolar acid hydrolases then degrade the autophagic body, and the degradation products are presumably transported back to the cytosol (Levanony et al., 1992). In addition to this pathway, autophagosomes can fuse with small lysosomes or endosomes, in which the contents can be degraded before eventual fusion with the vacuole (Rose et al., 2006; Toyooka et al., 2006). When the cysteine protease inhibitor E-64 is used to block autophagosomal degradation in *Arabidopsis* and barley, cytoplasmic inclusions accumulate as autophagic bodies within the central vacuole, whereas, in tobacco (*Nicotiana tabacum* L.), they accumulate in smaller organelles, the autolysosomes, outside the central vacuole (Inoue et al., 2006; also see review by Bassham, 2007).

In plants, macroautophagy is associated with leaf senescence (Matile and Winkenbach, 1971). For wheat, vesicles in the cytoplasm that include Rubisco and/or Rubisco degradation products (Rubisco-containing bodies; RCB) and other stromal proteins have been detected by ultrastructural analyses of leaves either senescing naturally or that are induced to senesce by darkness (Chiba et al., 2003). These RCBs are surrounded by a double membrane and other membranous structures in the cytoplasm. In contrast to the colored globules observed during soybean leaf senescence (Guiamet et al., 1999) and broccoli (*Brassica oleracea* L.) floret senescence (Terai et al., 2000), these wheat vesicles appear very early in the process, when Rubisco degradation begins. Therefore, RCBs are postulated to be involved in the degradation of stromal proteins at the onset of senescence. Further ultrastructural analysis has indicated that these bodies may be degraded by autophagocytosis similar to autophagy in yeast, which is a process of vacuolar or lysosomal degradation of cytoplasmic components, including organelles, under nutrient-deficient conditions (Klionsky and Oshumi, 1999). Autophagocytosis has been proposed as one of several mechanisms for plant cell death (Noodén, 2004). During ultrastructural analyses, membrane whorls (typical for macroautophagy) are indeed often observed around the vesicles found in senescing cells (Noodén, 1988). Based on their sequence similarity to the yeast proteins required for macroautophagy (Nair and Klionsky, 2005), a number of *Arabidopsis* proteins are now predicted to function in macroautophagy, primarily in the formation of autophagosomes (Thompson and Vierstra, 2005; Bassham et al., 2006). Although a large amount of information is now available on the yeast proteins, in plants their precise role in macroautophagy is still unknown. Most autophagy proteins can be classified into several major complexes or processes, including protein kinases involved in the initiation or regulation of autophagosome formation, a phosphatidylinositol 3-kinase complex, two ubiquitin-like conjugation systems, and the ATG9 complex, which may function in membrane recruitment to the site of autophagosome formation. These systems are required in *Arabidopsis* (Yoshimoto et al., 2004; Suzuki et al., 2005; Thompson and Doelling, 2005), and knockout mutants in several corresponding genes show starvation sensitivity and early-senescence phenotypes, as would be expected for a disruption in autophagy (Doelling et al., 2002; Yoshimoto et al., 2004; Suzuki et al., 2005; Thompson and Doelling, 2005). Besides *Arabidopsis*, autophagic proteins have been found in the senescing leaves of other species. These include Hsp70-related proteins that help transport individual proteins directly into the vacuole without the use of a vesiculated intermediate (Cuervo et al., 2000; Shen et al., 2006), and the AUT1-like autophagocytosis proteins (Gregersen and Holm, 2007). Such results suggest that autophagic proteins are required for maintaining cellular activities during leaf senescence, possibly by removing and degrading damaged proteins. Figure 2 depicts the vacuolar and autophagic (APG) pathway for protein degradation.

After transport into the vacuoles through macroautophagy, substrate proteins are degraded by vacuolar exo- and endoproteases, such as cysteine proteinase (SAG12), aspartic proteinase, serine proteinase, cathepsin B-like Cys proteinase, papain-like Cys proteinase, peptidases, endopeptidase, and aminopeptidase. Activation of these vacuolar proteases in senescing plants has been well documented in *Arabidop-*

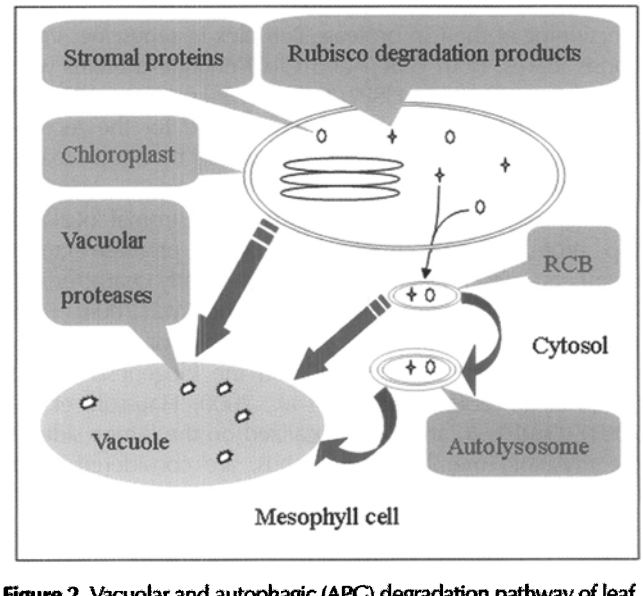

Figure 2. Vacuolar and autophagic (APG) degradation pathway of leaf proteins. RCB, Rubisco-containing bodies.

sis (Buchanan-Wollaston et al., 2003, 2005; Lin and Wu, 2004), various *Populus* species (Andersson et al., 2004), aspen (Bhalerao et al., 2003), cotton (Shen et al., 2006), and wheat (Gregersen and Holm, 2007). Although their activity during leaf senescence may be induced by different factors, those factors may then converge in a single degradation pathway (Martínez et al., 2007).

REMOBILIZATION OF NITROGEN

Senescence is not just a simple degradation of macromolecules, but is a controlled process involving the initiation of remobilization for nutrients, notably nitrogen, via the metabolism of carboxylic and amino acids. In the aboveground portions of rice plants, approximately 80% of the total N in the panicle is re-mobilized through the phloem from senescing organs (Tabuchi et al., 2007). Most nitrogen-remobilization from the leaves is due to the hydrolysis of proteins to amino acids, which are then probably inter-converted, hydrolyzed, catabolized, or exported without any alterations (e.g., the production of amides from other amino acids). The amino acids derived from this protein degradation may be exported via the phloem with or without prior modification. In the main axes of tobacco plants, the total amino acid content in phloem exudates is 1.6-fold higher from old leaves than from young leaves, suggesting that the former exports more amino acids in their phloem sap, thereby serving as source leaves. The phloem also transports high levels of glutamine, asparagine, serine, and proline, especially within mature leaves found at the intermediate position between young and old leaves (Masclaux-Daubresse et al., 2006). Levels of glutamine, the most abundant amino acid in the phloem, remains constant during leaf development, making it the major N-transporting form. Likewise, asparagine proportions are higher in the phloem sap than in the leaf blade, suggesting that this amino acid is also dedicated for export. The most abundant amino acids (glutamine, asparagine, serine, and proline) present in the senescing leaf-blade phloem are all derived from glutamate. According to the recent identification and characterization of SAGs (Buchanan-Wollaston et al., 2003, 2005; Guo et al., 2004; Lin and Wu, 2004; Shen et al., 2006; Gregersen and Holm, 2007), two relative biochemical cycles of amino acid-recycling and N-export -- the GS/GOGAT pathway and the PPDK-GS/GOGAT pathway – have been proposed.

The GS/GOGAT Pathway

The amino acids released by protein degradation may be further transformed by the action of amino transferase, generating free ammonia. Another source of free ammonia may be nucleotide degradation. This can be confirmed by analyzing the leaf senescence-induced expression of genes encoding enzymes, such as urease and cytidine deaminase (Guo et al., 2004). Upregulation of genes encoding an ammonium transporter have been found in senescing leaves of *Arabidopsis* (Guo et al., 2004). Therefore, it is essential that toxic ammonium be immediately re-assimilated into the amino acids to avoid detrimental effects and to provide

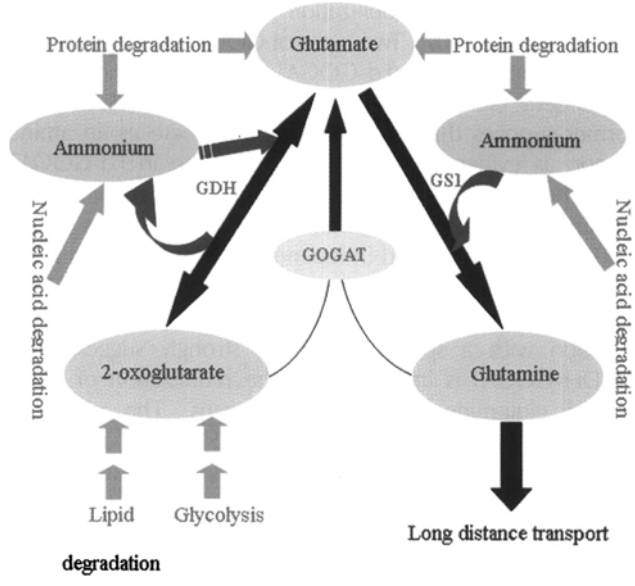

Figure 3. GS/GOGAT pathway of nitrogen-resorption. GDH, glutamate dehydrogenase; GOGAT, glutamate synthase; GS1, cytosolic glutamine synthetase.

nitrogenous forms suitable for N-cycling. It has been proposed that ammonium is directly incorporated into glutamate by the amination of 2-oxoglutarate via glutamate dehydrogenase (NADH-GDH; EC 1.4.1.2) and, subsequently, into glutamine by cytosolic glutamine synthetase (GS1; EC 6.3.1.2), another key enzyme in the assimilation of ammonia. GDH and cytosolic GS1 play major roles in the synthesis and reallocation of amino acids in senescing leaves (Miflin and Habash, 2002). Studies of source-sink relationships have shown that GDH is induced in old leaves when N-remobilization is maximal (Srivastava and Singh, 1987; Masclaux et al., 2000). This has led to the proposal that the physiological role of GDH is to synthesize glutamate for translocation (Miflin and Habash, 2002). However, the rate of glutamate formation can be as low as 0.2% of the total ammonium release (Yamaya et al., 1986) or 1.2% of the oxidative deamination rate of glutamate (Aubert et al., 2001). Therefore, GDH must catalyze the reversible oxidative deamination of glutamate to give 2-oxoglutarate and ammonium (Aubert et al., 2001). It is conceivable that GDH supplies 2-oxoglutarate by glutamate oxidation for the nitrogen and carbon cycles in old leaves. Figure 3 illustrates this GS-GOGAT cycle.

The GS-GOGAT cycle is the primary route for ammonium assimilation in senescing leaves. Glutamate is formed by the action of glutamate synthase (GOGAT), utilizing glutamine and 2-oxoglutarate; subsequently, glutamine is synthesized from ammonia, catalyzed by glutamine synthetase (GS). The major form of nitrogen in the phloem sap is glutamine. GSs are often divided into cytosolic (GS1) and chloroplastic (GS2) types, according to their cellular distribution. GS1 plays a major role in the synthesis of glutamine for transport and remobilization of leaf organic N (Tercé-Laforgue et al., 2004; Martin et al., 2005), whereas GS2 participates in the reassimilation of ammonia from photorespiration in photosynthetic tissues (Kamachi et al., 1992). Recent work with a knock-out mutant of rice has clearly shown that GS1 is

responsible for N-remobilization in the senescing organs (Tabuchi et al., 2007). Two types of GOGATs occur in plants: ferredox-independent (Fd-GOGAT; EC 1.4.7.1) and NADH-dependent GOGAT (NADH- GOGAT; EC 1.4.1.14). The former supplies the only source of glutamate as an amino donor, as evidenced by mutants defective in Fd-GOGAT that show reversible lethal phenotypes (Somerville and Ogren, 1980; Blackwell et al., 1988; Ferrario-Méry et al., 2002). In contrast to Fd-independent GOGAT, which functions in concert with GS2, expression of NADH-GOGAT is often coupled with GS1 (Coschigano et al., 1998). Overexpression with a specific promoter strongly suggests that NADH-GOGAT is important for the reutilization of transported glutamine in developing organs. The increased expression of NADH-GOGAT and GS1 have also been identified during leaf senescence in various plant species, including *Arabidopsis* (Buchanan-Wollaston et al., 2003, 2005; Lin and Wu, 2004),various *Populus* species (Andersson et al., 2004), aspen (Bhalerao et al., 2003), potato (*Solanum tuberosum* L.; Teixeira et al., 2005), cotton (Shen et al., 2006), and wheat (Kichey et al., 2005; Gregersen and Holm, 2007).

An integration has been proposed for the degradation of carbohydrates, fatty acids, and proteins, leading to the remobilization of N. As a key compound, 2-oxoglutarate participates in a range of reactions in the context of N-recycling, notably in trans-aminating reactions to replenish glutamate removed by nitrogen assimilation reactions and translocation to sink organs. Thus, 2-oxoglutarate for the assimilation of N via GS could be provided by citrate synthase and by aconitate hydratase and isocitrate dehydrogenase (NADP). The input for these reactions might come from acetyl-coenzyme A via the degradation of fatty acids (Baker et al., 2006), oxaloacetate generated by phosphoenol pyruvate carboxylase, and, further upstream, from glycolysis. The transcriptional upregulation of genes for a number of enzymatic processes in this model has been reported in various senescing plant leaves, such as from *Arabidopsis* (Buchanan-Wollaston et al., 2003, 2005; Lin and Wu, 2004), aspen (Bhalerao et al., 2003), several *Populus* species (Andersson et al., 2004), potato (Teixeira et al., 2005), cotton (Shen et al., 2006), and wheat (Kichey et al., 2005; Gregersen and Holm, 2007). It is noteworthy that, for example, transcriptional upregulation for NADP has not been reported in senescing *Arabidopsis* leaves (Buchanan-Wollaston et al., 2005), although the peroxisomal citrate synthase transcript is listed as being up-regulated.

The PPDK-GS/GOGAT Pathway

In the PPDK-GS/GOGAT pathway (Fig. 4), phosphoenol pyruvate (PEP) is formed by the action of pyruvate orthophosphate dikinase (PPDK; E.C.2.7.9.1), utilizing ATP and pyruvate. PPDK is an enzyme commonly found in organisms, from bacteria (Cooper and Kornberg, 1967; Pocalyko et al., 1990) and protozoa (Bruderer et al., 1996) to plants (Hatch and Slack, 1968). Although first isolated in C4 plants, PPDK is also present in C3 species. Microarray analysis of transcript abundance in dark-induced senescing *Arabidopsis* leaves has shown that cytosolic PPDK is significantly up-regulated (Lin and Wu, 2004). PEP is then transferred to oxaloacetic acid (OAA) by the action of PEP carboxykinase (PEPC; EC 4.1.1.31), providing carbon skeletons for the refurnishment of amino acids. Glutamate, rather than free ammonia, contributes to the amination of OAA as the amino donor; this reaction is catalyzed by aspartate aminotransferase (AspAT; E.C. 2.6.1.1), generating aspartate for further reactions. As one of the fusions between the GS/GOGAT and PPDK-GS/GOGAT cycles, 2-oxoglutarate is another outgrowth of this amination process. Glutamine, generated from the amination of glutamate, is another fusion between these cycles. As shown in the GS/GOGAT cycle, glutamine will reversibly integrate with 2-oxoglutarate, giving glutamate by the action of GOGAT. Subsequently, glutamate is put into the next cycle for N-resorption. Pyruvate for the reassimilation of N via PPDK could be provided mainly by the process of glycolysis and the degradation of fatty acids. Asparagine, which is formed by the action of asparagine synthase, has been proposed as the main N carrier for nitrogen-remobilization (Hayashi and Chino, 1990). Indeed, the amount of free asparagines and the activity of asparagine synthetase (AS; EC:6.3.5.4) increases significantly during leaf senescence (Guo et al., 2004; Lin and Wu, 2004; Shen et al., 2006; Gregersen and Holm, 2007).

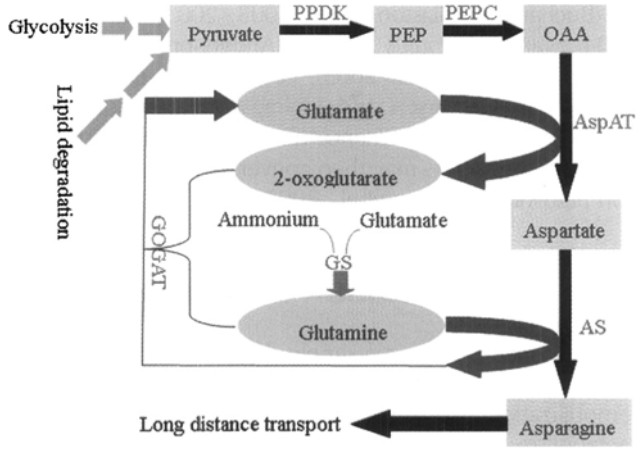

Figure 4. PPDK-GS/GOGAT pathway of nitrogen-resorption. PPDK, pyruvate orthophosphate dikinase; PEP, phosphoenol pyruvate; PEPC, PEP carboxykinase; OAA, oxaloacetic acid; AspAT, aspartate aminotransferase; AS, asparagine synthetase.

In conclusion, the involvement of degradative processes for protein breakdown and N-translocation have been well identified and characterized, most notably in *Arabidopsis*. Such efforts have accelerated our acquisition of knowledge. Nevertheless, much useful information on genes and gene regulation in other plant species should still be gained to confirm the conclusions drawn from the analysis of N-resorption during leaf senescence in *Arabidopsis*. Technologies are rapidly improving for the functional analysis of potential resorption and regulatory genes, and applying these to the study of N-metabolization is a challenge for the next decade. An extension of such studies, including the exploitation of genetic variation, toward crop plants will also be essential. Understanding how N-resorption is controlled in different species will allow for future applications of this

process, through either genetic improvement or the manipulation of key environmental triggers. This will result in great potential benefits throughout the supply chain, from producer to consumer.

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LITERATURE CITED

Adam Z (1996) Protein stability and degradation in chloroplasts. Plant Mol Biol 32: 773-783

Adam Z (2001) Chloroplast proteases and their role in photosynthesis regulation, *In* EM Aro, B Andersson, eds, Regulation of Photosynthesis. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 265-276

Adam Z, Clarke AK (2002) Cutting edge of chloroplast proteolysis. Trends Plant Sci 7: 451-456

Adam Z, Adamska I, Nakabayashi K, Ostersetzer O, Haussuhl K, Manuell A, Zheng B, Vallon O, Rodermel SR, Shinozaki K, Clarke AK (2001) Chloroplast and mitochondrial proteases in *Arabidopsis*. A proposed nomenclature. Plant Physiol 125: 1912-1918

Adam Z, Rudella A, van Wijk KJ (2006) Recent advances in the study of Clp, FtsH and other proteases located in chloroplasts. Curr Opin Plant Biol 9: 234-240

Aerts R (1990) Nutrient use efficiency in evergreen and deciduous species from heathlands. Oecologia 84: 391-397

Andersson A, Keskitalo J, Sjodin A, Bhalerao R, Sterky F, Wissel K, Tandre K, Aspeborg H, Moyle R, Ohmiya Y, Bhalerao R, Brunner A, Gustafsson P, Karlsson J (2004) A transcriptional timetable of autumn senescence. Plant J 38: 38-48

Aubert S, Bligny R, Douce R, Gout E, Ratcliffe RG, Roberts JK (2001) Contribution of glutamate dehydrogenase to mitochondrial glutamate metabolism studied by 13C and 31P nuclear magnetic resonance. J Exp Bot 52: 37-45

Baker A, Graham IA, Holdsworth M, Smith SM, Theodolou FL (2006) Chewing the fat: Beta-oxidation in signalling and development. Trends Plant Sci 11: 124-132

Bassham DC (2007) Plant autophagy - More than a starvation response. Curr Opin Plant Biol 10: 1-7

Bassham DC, Laporte M, Marty F, Moriyasu Y, Ohsumi Y, Olsen LJ, Yoshimoto K (2006) Autophagy in development and stress responses of plants. Autophagy 2: 2-11

Bhalerao R, Keskitalo J, Sterky F (2003) Gene expression in autumn leaves. Plant Physiol 131: 430-442

Blackwell RD, Murray AJS, Lea PJ, Joy KW (1988) Photorespiratory amino donors, sucrose synthesis and the induction of CO_2 fixation in barley deficient in glutamine synthetase and/or glutamate synthase. J Exp Bot 39: 845-858

Brouquisse R, Masclaux C, Feller U, Raymond P (2001) Protein hydrolysis and nitrogen remobilisation in plant life and senescence, *In* PJ Lea, JF Morot-Gaudry, eds, Plant Nitrogen. Springer-Verlag, Berlin, pp 275-293

Bruderer T, Wehrli C, Köhler P (1996) Cloning and characterization of the gene encoding pyruvate phosphate dikinase from Giardia duodenalis. Mol Biochem Parasitol 77: 225-233

Buchanan-Wollaston V, Ainsworth C (1997) Leaf senescence in *Brassica napus*: Cloning of senescence related genes by subtractive hybridisation. Plant Mol Biol 33: 821-834

Buchanan-Wollaston V, Earl S, Harrison E, Mathas E, Navabpour S, Page T, Pink D (2003) The molecular analysis of leaf senescence – A genomics approach. Plant Biotechnol J 1: 3-22

Buchanan-Wollaston V, Page T, Harrison E, Breeze E, Lim PO, Nam HG, Lin JF, Wu SH, Swidzinski J, Ishizaki K, Leaver CJ (2005) Comparative transcriptome analysis reveals significant differences in gene expression and signalling pathways between developmental and dark/starvation-induced senescence in *Arabidopsis*. Plant J 42: 567-585

Buetow DE (1997) Plastid proteases, *In* M Pessarakli, ed, Handbook of Photosynthesis. Marcel Dekker, New York, pp 315-330

Chen M, Choi Y, Voytas DF, Rodermel S (2000) Mutations in the *Arabidopsis* VAR2 locus cause leaf variegation due to the loss of a chloroplast FtsH protease. Plant J 22: 303-313

Chiba A, Ishida H, Nishizawa NK, Makino A, Mae T (2003) Extrusion of ribulose 1,5-bisphosphate carboxylase/oxygenase from chloroplasts by specific bodies in naturally senescing leaves of wheat. Plant Cell Physiol 44: 914-921

Cooper RA, Kornberg HL (1967) The direct synthesis of phosphoenolpyruvate from pyruvate by *Escherichia coli*. Proc Royal Soc Lond B Biol Sci 168: 263-280

Coschigano KT, Melo-Oliveira R, Lim J, Coruzzi GM (1998) *Arabidopsis* gls mutants and distinct Fd-GOGAT genes. Implications for photorespiration and primary nitrogen assimilation. Plant Cell 10: 741-752

Cuervo AM, Gomes AV, Barnes JA (2000) Selective degradation of annexins by chaperone-mediated autophagy. J Biol Chem 275: 33329-33335

Doelling JH, Walker JM, Friedman EM, Thompson AR, Vierstra RD (2002) The APG8/12-activating enzyme APG7 is required for proper nutrient recycling and senescence in *Arabidopsis thaliana*. J Biol Chem 277: 33105-33114

Dougan DA, Reid BG, Horwich AL, Bukau B (2002). ClpS, a substrate modulator of the ClpAP machine. Mol Cell 9: 673-683

Drake R, John I, Farrell A, Cooper W, Schuch W, Grierson D (1996) Isolation and analysis of cDNAs encoding tomato cysteine proteases expressed during leaf senescence. Plant Mol Biol 30: 755-767

Eckhardt U, Grimm B, Hörtensteiner S (2004) Recent advances in chlorophyll biosynthesis and breakdown in higher plants. Plant Mol Biol 56: 1-14

Feller U (2004) Proteolysis, *In* LD Noodén, ed, Plant Cell Death Processes. Elsevier Academic Press, San Diego, pp 107-123

Ferrario-Méry S, Valadier MH, Godfroy N, Miallier D, Hirel B, Foyer CH, Suzuki A (2002) Diurnal changes in ammonia assimilation in transformed tobacco plants expressing ferredoxin-dependent glutamate synthase mRNA in the antisense orientation. Plant Sci 112: 524-530

Gottesman S (1996) Proteases and their targets in *Escherichia coli*. Annu Rev Genet 30: 465-506

Gregersen PL, Holm PB (2007) Transcriptome analysis of senescence in the flag leaf of wheat (*Triticum aestivum* L.). Plant Biotechnol J 5: 192-206

Guiamet JJ, Pichersky E, Noodén LD (1999) Mass exodus from senescing soybean chloroplasts. Plant Cell Physiol 40: 986-992

Guo Y, Cai Z, Gan S (2004) Transcriptome of *Arabidopsis* leaf senescence. Plant Cell Environ 27: 521-549

Hajouj T, Michelis R, Gepstein S (2000) Cloning and characterization of a receptor-like protein kinase gene associated with senescence. Plant Physiol 124: 1305-1314

Hatch MD, Slack CR (1968) A new enzyme for the interconversion of pyruvate and phosphopyruvate and its role in the C4 dicar-

boxylic acid pathway of photosynthesis. Biochem J 106: 141-146

Haussuhl K, Andersson B, Adamska I (2001) A chloroplast DegP2 protease performs the primary cleavage of the photodamaged D1 protein in plant photosystem II. Embo J 20: 713-722

Hayashi H, Chino M (1990) Chemical composition of phloem sap from the upper most internode of the rice plant. Plant Cell Physiol 31: 247-251

He Y, Gan S (2002) A gene encoding an acyl hydrolase is involved in leaf senescence in *Arabidopsis*. Plant Cell 14: 805-815

Himelblau E, Amasino M (2001) Nutrients mobilized from leaves of *Arabidopsis thaliana* during leaf senescence. J Plant Physiol 158: 1317-1323

Hörtensteiner S, Feller U (2002) Nitrogen metabolism and remobilization during senescence. J Exp Bot 53: 927-937

Inoue Y, Suzuki T, Hattori M, Yoshimoto K, Ohsumi Y, Moriyasu Y (2006) AtATG genes, homologs of yeast autophagy genes, are involved in constitutive autophagy in *Arabidopsis* root tip cells. Plant Cell Physiol 47: 1641-1652

Ishida H, Anzawa D, Kokubun N, Makino A, Mae T (2002) Direct evidence for non-enzymatic fragmentation of chloroplastic glutamine synthetase by a reactive oxygen species. Plant Cell Environ 25: 625-631

Kamachi K, Yamaya T, Hayakawa T, Mae T, Ojima K (1992) Vascular bundle-specific localization of cytosolic glutamine synthetase in rice leaves. Plant Physiol 99: 1481-1486

Keeler SJ, Boettger CM, Haynes JG, Kuches KA, Johnson MM, Thureen DL, Keeler CL Jr, Kitto SL (2000) Acquired thermotolerance and expression of the HSP100/ClpB genes of lima bean. Plant Physiol 123: 1121-1132

Kichey T, Le Gouis J, Sangwan B, Hirel B, Dubois F (2005) Changes in the cellular and subcellular localization of glutamine synthetase and glutamate dehydrogenase during flag leaf senescence in wheat (*Triticum aestivum* L.). Plant Cell Physiol 46: 964-974

Klionsky DJ (2005) The molecular machinery of autophagy: Unanswered questions. J Cell Sci 118: 7-18

Klionsky DJ, Oshumi Y (1999) Vacuolar import of proteins and organelles from the cytoplasm. Annu Rev Cell Dev Biol 15: 1-32

Kraft E, Stone SL, Ma L, Su N, Gao Y, Lau OS, Deng XW, Callis J (2005) Genome analysis and functional characterization of the E2 and RING-type E3 ligase ubiquitination enzymes of *Arabidopsis*. Plant Physiol 139: 1597-1611

Krupinska K (2006) Fate and activities of plastids During leaf senescence, *In* RR Wise, JK Hoober, eds, The Structure and Function of Plastids. Springer, Dordrecht, The Netherlands, pp 433-449

Levanony H, Rubin R, Altschuler Y, Galili G (1992) Evidence for a novel route of wheat storage proteins to vacuoles. J Cell Biol 119: 1117-1128

Levine B, Klionsky DJ (2004) Development by self-digestion: Molecular mechanisms and biological functions of autophagy. Dev Cell 6: 463-477

Lin JF, Wu SH (2004) Molecular events in senescing *Arabidopsis* leaves. Plant J 39: 612-628

Lindahl M, Tabak S, Cseke L, Pichersky E, Andersson B, Adam Z (1996) Identification, characterization, and molecular cloning of a homologue of the bacterial FtsH protease in chloroplasts of higher plants. J Biol Chem 271: 29329-29334

Lohman KN, Gan S, John MC, Amasino RM (1994) Molecular analysis of natural leaf senescence in *Arabidopsis thaliana*. Physiol Plant 92: 322-328

Mae T, Thomas H, Gay AP, Makino A, Hidema J (1993) Leaf development in *Lolium tremulentum*: Photosynthesis and photosynthetic proteins in leaves senescing under different irradiances. Plant Cell Physiol 34: 391-399

Masclaux C, Valadier MH, Brugière N, Morot-Gaudry JF, Hirel B (2000) Characterization of the sink/source transition in tobacco (*Nicotiana tabacum* L.) shoots in relation to nitrogen management and leaf senescence. Planta 211: 510-518

Masclaux-Daubresse C, Reisdorf-Cren M, Pageau K, Lelandais M, Grandjean O, Kronenberger J, Valadier MH, Feraud M, Jouglet T, Suzuki A (2006) Glutamine synthetase-glutamate synthase pathway and glutamate dehydrogenase play distinct roles in the sink-source nitrogen cycle in tobacco (*Nicotiana tabacum* L.). Plant Physiol 140: 444-456

Martin A, Belastegiu-Macadam X, Quillere I, Floriot M, Valadier MH, Pommel B, Andrie B, Donnison I, Hirel B (2005) Nitrogen management and senescence in two maize hybrids differing in the persistence of leaf greenness -- Agronomic, physiological and molecular aspects. New Phytol 167: 483-492

Martínez DE, Bartoli CG, Grbic V, Guiamet JJ (2007) Vacuolar cysteine proteases of wheat (*Triticum aestivum* L.) are common to leaf senescence induced by different factors. J Exp Bot 58: 1099-1107

Matile P, Winkenbach F (1971) Function of lysosomes and lysosomal enzymes in the senescing corolla of the morning glory. J Exp Bot 22: 759-771

Matile P (1992) Chloroplast senescence. *In* NR Baker, H Thomas, eds, Crop Photosynthesis: Spatial and Temporal Determinants. Elsevier Science Publishers, Amsterdam, pp 423-440

Mei HS, Thimann KV (1984) The relation between nitrogen deficiency and leaf senescence. Physiol Plant 62: 157-161

Miflin BJ, Habash DZ (2002) The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops. J Exp Bot 53: 979-987

Minamikawa T, Toyooka K, Okamoto T, Hara-Nishimura I, Nishimura M (2001) Degradation of ribulose-bisphosphate carboxylase by vacuolar enzymes of senescing French bean leaves: Immunocytochemical and ultrastructural observations. Protoplasma 218: 144-153

Nair JS, Ramaswamy NK (2004) Chloroplast proteases. Biol Plant 48: 321-326

Nair U, Klionsky DJ (2005) Molecular mechanisms and regulation of specific and nonspecific autophagy pathways in yeast. J Biol Chem 280: 41785-41788

Nakabayashi K, Ito M, Kiyosue T, Shinozaki K, Watanabe A (1999) Identification of clp genes expressed in senescing *Arabidopsis* leaves. Plant Cell Physiol 40: 504-514

Nakashima K, Kiyosue T, Yamaguchi-Shinozaki K, Shinozaki K (1997) A nuclear gene, erd1, encoding a chloroplast targeted Clp protease regulatory subunit homolog is not only induced by water stress but also developmentally up-regulated during senescence in *Arabidopsis thaliana*. Plant J 12: 851-861

Noodén LD (2004) Introduction, *In* LD Noodén, ed, Plant Cell Death Processes. Elsevier Academic Press, San Diego, pp 1-18

Noodén LD (1988) The phenomena of senescence and aging, *In* LD Noodén, AC Leopold, eds, Senescence and Aging in Plants. Elsevier Academic Press, San Diego, pp 1-50

Park JH, Oh SA, Kim YH, Woo HR, Nam HG (1998) Differential expression of senescence-associated mRNAs during leaf senescence induced by different senescence-inducing factors in *Arabidopsis*. Plant Mol Biol 37: 445-454

Peltier JB, Ytterberg J, Liberles DA, Roepstorff P, van Wijk KJ (2001) Identification of a 350-kDa ClpP protease complex with 10 different Clp isoforms in chloroplasts of *Arabidopsis thaliana*. J Biol Chem 276: 16318-16327

Peltier JB, Ripoll DR, Friso G, Rudella A, Cai Y, Ytterberg J, Giacomelli L, Pillardy J, van Wijk KJ (2004) Clp protease complexes from photosynthetic and non-photosynthetic plastids and mitochondria of plants, their predicted three-dimensional structures, and functional implications. J Biol Chem 279:

4768-4781
Pocalyko DJ, Carroll LJ, Martin BM, Babbit PC, Dunaway-Mariano D (1990) Analysis of sequence homologies in plant and bacterial pyruvate phosphate dikinase, enzyme I of the bacterial phosphoenolpyruvate: sugar phosphotransferase system and other PEP-utilising enzymes. Identification of potential catalytic and regulatory motifs. Biochemistry 29: 10757-10765
Quirino BF, Noh YS, Himelblau E, Amasino RM (2000) Molecular aspects of leaf senescence. Trends Plant Sci 5: 278-282
Rose TL, Bonneau L, Der C, Marty-Mazars D, Marty F (2006) Starvation-induced expression of autophagy-related genes in *Arabidopsis*. Biol Cell 98: 53-67
Sakamoto W (2006) Protein degradation machineries in plastids. Annu Rev Plant Biol 57: 599-621
Shen FF, Yu SX, Xie QE, Han XL, Fan SL (2006) Identification of genes associated with cotyledon senescence in upland cotton. Chinese Sci Bull 51: 1085-1094
Smalle J, Vierstra RD (2004) The ubiquitin 26S proteasome proteolytic pathway. Annu Rev Plant Biol 55: 555-590
Smart CM, Hosken SE, Thomas H, Greaves JA, Blair BG, Schuch W (1995) The timing of maize leaf senescence and characterisation of senescence-related cDNAs. Physiol Plant 93: 673-682
Somerville CR, Ogren WL (1980) Inhibition of photosynthesis in *Arabidopsis* mutants lacking leaf glutamate synthase activity. Nature 286: 257-259
Srivastava HS, Singh RP (1987) Role and regulation of L-glutamate dehydrogenase activity in higher plants. Phytochemistry 26: 597-610
Sullivan JA, Shirasu K, Deng XW (2003) The diverse roles of ubiquitin and the 26S proteasome in the life of plants. Nat Rev Genet 4: 948-958
Suzuki NN, Yoshimoto K, Fujioka Y, Ohsumi Y, Inagaki F (2005) The crystal structure of plant ATG12 and its biological implication in autophagy. Autophagy 1: 119-126
Tabuchi M, Abiko T, Yamaya T (2007) Assimilation of ammonium ions and reutilization of nitrogen in rice (*Oryza sativa* L.). J Exp Bot 58: 2319-2327
Takechi K, Sodmergen, Murata M, Motoyoshi F, Sakamoto W (2000) The YELLOW VARIEGATED (VAR2) locus encodes a homologue of FtsH, an ATP-dependent protease in Arabidopsis. Plant Cell Physiol 41: 1334-1346
Teixeira J, Pereira S, Cánovas F, Salema R (2005) Glutamine synthetase of potato (*Solanum tuberosum* L. cv. Désirée) plants: Cell- and organ-specific expression and differential developmental regulation reveal specific roles in nitrogen assimilation and mobilization. J Exp Bot 56: 663-671
Terai M, Watada A, Murphy C, Wergin W (2000) Scanning electron microscopic study of modified chloroplasts in senescing broccoli florets. HortScience 35: 99-103
Tercé-Laforgue T, Dubois F, Ferrario-Méry S, Poude Crecenzo MA, Sangwan R, Hirel B (2004) Glutamate dehydrogenase of tobacco is mainly induced in the cytosol of phloem companion cells when ammonia is provided either externally or released during photorespiration. Plant Physiol 136: 4308-4317
Thomas H, Donnison I (2000) Back from the brink: Plant senescence and its reversibility, *In* JA Bryant, SG Hughes, JM Garland, eds, Programmed Cell Death in Animals and Plants. BIOS Scientific Publishers, Oxford, pp 149-162
Thompson AR, Doelling JH, Suttangkakul A, Vierstra RD (2005) Autophagic nutrient recycling in *Arabidopsis* directed by the ATG8 and ATG12 conjugation pathways. Plant Physiol 138: 2097-2110
Toyooka K, Okamoto T, Minamikawa T (2001) Cotyledon cells of *Vigna mungo* seedlings use at least two distinct autophagic machineries for degradation of starch granules and cellular components. J Cell Biol 154: 973-982
Toyooka K, Moriyasu Y, Goto Y, Takeuchi M, Fukuda H, Matsuoka K (2006) Protein aggregates are transported to vacuoles by a macroautophagic mechanism in nutrient-starved plant cells. Autophagy 2: 96-106
van der Wilden W, Herman EM, Chrispeels MJ (1980) Protein bodies of mung bean cotyledons as autophagic organelles. Proc Natl Acad Sci USA 77: 428-432
Woo HR, Chung KM, Park JH, Oh SA, Ahn T, Hong SH, Jang SK, Nam HG (2001) ORE9, an F-box protein that regulates leaf senescence in *Arabidopsis*. Plant Cell 13: 1779-1790
Yamaya T, Oaks A, Rhodes D, Matsumoto H (1986) Synthesis of [15N] glutamate from [15N]H4+ and [15N] glycine by mitochondria isolated from pea and corn shoots. Plant Physiol 81: 754-757
Yoshimoto K, Hanaoka H, Sato S, Kato T, Tabata S, Noda T, Ohsumi Y (2004) Processing of ATG8s, ubiquitin-like proteins, and their deconjugation by ATG4s are essential for plant autophagy. Plant Cell 16: 2967-2983
Zhang YY, Xie Q (2007) Ubiquitination in abscisic acid-related pathway. Plant Biol 49: 87-93
Zheng B, Halperin T, Hruskova-Heidingsfeldova O, Adam Z, Clarke AK (2002) Characterization of chloroplast Clp proteins in *Arabidopsis*: Localization, tissue specificity and stress responses. Physiol Plant 114: 92-101