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Aging and Senescence of the Leaf Organ

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Leaf senescence is a sequence of biochemical and physiological events comprising the final stage in leaf development. It encompasses the period from a fully expanded mature state up to the death, thereby limiting longevity. The changes occurring during leaf senescence are very complex but highly regulated, and are genetically programmed with actions coordinated at the cellular, tissue, organ, and organism levels. A major breakthrough in our molecular understanding of this phenomenon has been achieved through the characterization of various mutants and senescence-associated genes, including regulatory genes. In particular, a genetic screening and assay system for leaf senescence has been well established in *Arabidopsis*, which led leaf senescence into the realm of genetic subject along with the rich genetic and genomic resources in this model plant. These advances have not only revealed the existence of a complex regulatory network of senescence-associated signaling pathways, but have also allowed us to postulate the molecular mechanisms for signal perception, execution, and regulation. The key regulatory genes identified to date encode a variety of proteins, including transcription regulators and signal-transduction proteins, regulators of protein degradation, proteins associated with phytohormone pathways, and regulators of metabolism. Elucidation of their roles in leaf senescence and analyses of senescence regulatory pathways, including systems-level approaches, will increase our knowledge of the networks involved in senescence activity.

Keywords: developmental aging, leaf senescence, longevity, nutrient salvage, programmed cell death

SENESCENCE, PROGRAMMED CELL DEATH, AND DEVELOPMENTAL AGING IN PLANTS

Senescence is a developmental process that leads to the death of a cell, an organ, or an organism and occurs at the final stage of their development. This phenomenon is ubiquitous characteristics of the biological world. For centuries, senescence and death have been assumed to be an inevitable bad destiny. However, from an ontogenetic perspective, biologists now consider these processes to be evolutionarily acquired and genetically programmed developmental strategies rather than one of simple and passive degeneration.

As with many other organisms, senescence in plants is a type of programmed cell death (PCD) that is dictated by a genetic program (Nam, 1997; van Doorn and Woltering, 2004). PCD in higher plants occurs throughout the development of most organs, and can be triggered by environmental factors such as pathogen infection and physical injury (Jones and Dangl, 1996). Cell death during senescence is observed in the leaves, petals, fruits, and whole plants. In contrast, other types of PCD such as a hypersensitive response (HR), involve rather localized cell death. Senescence also is manifested by a more prolonged period of PCD compared with other types, which are generally associated with acute and rapid cell death. This slower rate is connected, at least in part, with the efficient recycling of nutrients that are released during senescence (Lim et al., 2003).

Although these terms are often used interchangeably when referring to animals, senescence and developmental aging are different concepts. The first term applies to the final stage of development that leads to death. In contrast, developmental aging is addition of timing to a cell, organ, or a whole plant and occurs throughout development (Lim et al., 2003). In this sense, the latter concept would be a major determinant of senescence but is not senescence in itself.

MITOTIC AND POST-MITOTIC SENESCENCE IN PLANTS

Plants exhibit two types of senescence: mitotic and post-mitotic (Gan, 2003). The shoot apical meristem cell can undergo a certain number of mitotic divisions to produce organs such as leaves and flowers. Loss of capacity for further divisions in the meristem upon aging is called mitotic, or proliferative, senescence (Hensel et al., 1993). In contrast, post-mitotic senescence is a degenerative process that occurs after cellular maturation, and leads to the death of cells. It occurs in organs such as leaves and petals. In our review, only leaf senescence-related research will be discussed.

LEAF SENESCENCE

Like other senescence events in plants, leaf senescence is the final stage of development, from maturation to degeneration in leaf life history. In nature, this phenomenon is most typically observed in the autumn leaves of trees and other perennial plants. Green leaves turn yellow or red before death, and are eventually discarded. Annual plants such as grain crops follow a similar process when they turn from green to yellow as the grain ripens before harvest.

During its life span, a leaf undergoes several developmen-

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Abbreviations: HR, hypersensitive reaction; PCD, programmed cell death; SAG, senescence-associated gene

tal phases. Initially it is expanding rapidly, importing carbon and nitrogen and undergoing rapid protein synthesis until it reaches its full capacity for photosynthesis. Then the mature leaf becomes an asset to the plant, contributing to the supply of carbon. During that time, protein turnover is at a consistently low level, which continues until internal or external conditions initiate the onset of senescence. In that final stage, leaf cells undergo sequential disorganization and dramatic alterations in their cellular metabolism (Noodén, 1988; Nam, 1997). These changes include the loss of photosynthetic activity and hydrolysis of macromolecules that have accumulated during the growth phase. Degenerative activities are concomitant with a massive remobilization of hydrolyzed molecules to the growing parts of plants such as young leaves, developing seed, and fruits. Thus, leaf senescence can be considered as a nutrient recycling program at the organismal level, and is an important developmental process for plant fitness (Nam, 1997; Lim et al., 2003). From an agricultural perspective, however, leaf senescence may limit yields and also cause post-harvest yellowing and loss of nutrients in vegetable crops. Thus, the study of this process not only contributes to our knowledge about this fundamental process, but may lead us to devise ways in which to manipulate senescence in order to improve agronomic productivity (Noodén, 1988; Nam, 1997; Buchanan-Wollaston et al., 2003; Lim and Nam, 2005).

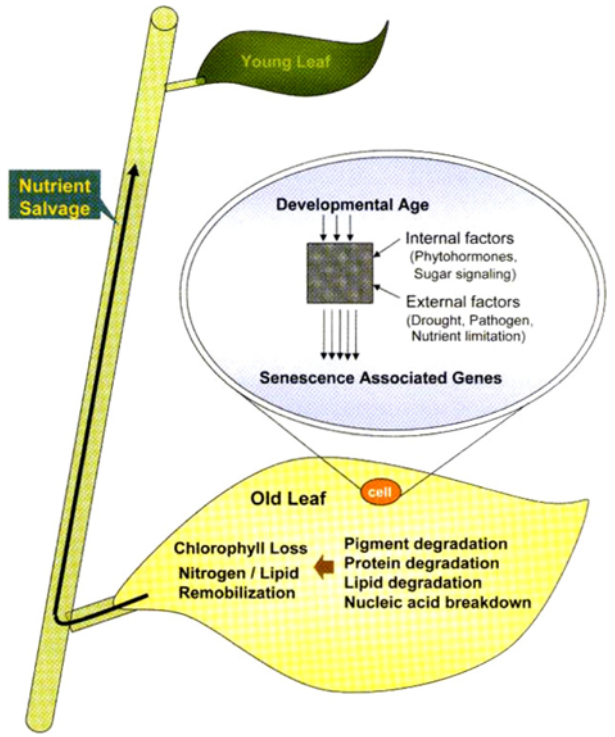

Figure 1. Senescence is the integrated response by a cell, tissue, organ, or organism to age, developmental status, and environment. To maximize fitness, plants incorporate all these factors in executing proper progression of senescence. For the senescence program to proceed, there are likely to be genes that function in various aspects of degeneration, including chlorophyll breakdown, and nitrogen and lipid remobilization.

SYMPTOMS OF LEAF SENESCENCE

Leaf senescence is a degenerative process that involves orderly and sequential changes in cellular physiology and biochemistry. These steps are most easily understood from the standpoint of nutrient salvage, e.g., the hydrolysis of macromolecules and subsequent remobilization that requires the operation of a complex array of metabolic pathways (Fig. 1).

The initial stage of senescence symptoms is a breakdown in membrane structure within the chloroplasts, where >50% of the leaf protein and >70% of its lipids are present (Bleecker and Patterson, 1997; Hörtensteiner and Feller, 2002). Chloroplast degeneration is accompanied by chlorophyll degradation and a progressive loss of chloroplast proteins, e.g., ribulose bisphosphate carboxylase (Rubisco) and chlorophyll *a/b* binding protein (CAB) (Bate et al., 1990; Bleeker and Patterson, 1997). The complete hydrolysis of proteins to free amino acids depends on the actions of several endo- and exopeptidases. Senescence-associated cystein proteases, which are accumulated in the vacuole, also play a role in protein degradation (Hörtensteiner and Feller, 2002; Otegui et al., 2005). Lipid-degrading enzymes, such as phospholipase D, phosphatidic acid phosphatase, lytic acyl hydrolase, and lipooxygenase, appear to be involved in the hydrolysis and metabolism of membrane lipids (Thompson et al., 1998, 2000). The majority of fatty acids is either oxidized to provide energy for the senescence process or processed to a-ketoglutarate via the glyoxylate cycle. This α-ketoglutarate can be converted into phloem-mobile sugars through gluconeogenesis or else used to mobilize the amino acids released during leaf protein degradation (Buchanan-Wollaston, 1997).

A massive decrease in nucleic acids occurs during leaf senescence. Total RNA levels are rapidly reduced along with the progression of senescence (Taylor et al., 1993). Their initial decline is very apparent in the chloroplast rRNAs and cytoplasmic rRNAs. The amount of various rRNA species is likely to be regulated coordinately, although this aspect has not yet been analyzed. This decrease in rRNAs contents is followed by that of the cytoplasmic mRNA and tRNA, and is accompanied by enhanced activity of several RNases. Nonetheless, how exactly each RNase functions during senescence is still unknown.

The earliest structural evidence for senescence appears in the chloroplast, manifested as changes in the grana and the formation of lipid droplets. Polysomes and ribosomes generally decrease fairly early, reflecting diminished protein synthesis. In comparison, the mitochondria and nucleus, both essential to energy production and gene expression, remain intact until the last stages (Quirino et al., 2000). This is because the senescing cells must be functional for progression of senescence until a late stage of senescence, possibly for the efficient re-utilization of cellular materials.

In the final stage of senescence, when the leaves have turned almost completely yellow, typical symptoms of PCD, such as controlled vacuolar collapse, chromatin condensation, and DNA laddering have been reliably detected in naturally senescing leaves from a variety of plants including rice,

tobacco, and five trees (Yen and Yang, 1998; Simeonova et al., 2000; Cao et al., 2003). Eventually, disintegration of the plasma and vacuolar membranes become apparent, and the loss of integrity in the plasma membrane then leads to a disruption in cellular homeostasis, ending the life of a cell.

GLOBAL-SCALE MOLECULAR PICTURES OF LEAF SENESCENCE

Large-scale identification and analysis of senescence-associated genes

Leaf senescence is accompanied by changes in gene expression that should be controlled in a highly coordinated manner. Several approaches, including differential display, differential cDNA library screening, suppression subtractive hybridization, and enhancer trapping, have been taken to identify the so-called "senescence-associated genes" (SAGs) that show enhanced expression during leaf senescence (Buchanan-Wollaston, 1997; John et al., 1997; Park et al., 1998; Quirino et al., 1999; He et al., 2001; Gepstein et al., 2003; Guterman et al., 2003). Recently, use of the DNA microarrays has allowed the identification of SAGs with a genome-wide molecular view of leaf senescence. For example, a DNA microarray with 13490 aspen ESTs has been employed to analyze the transcriptome of aspen leaves during autumn senescence (Andersson et al., 2004). In *Arabidopsis*, Affymetrix GeneChip arrays representing 24000 genes have been utilized to assess the changes in global expression patterns during that process (Guo et al., 2004; Buchanan-Wollaston et al., 2005; van der Graaff et al., 2006). From that analysis, more than 800 SAGs have now been identified, illustrating the dramatic alteration in cellular physiology that underlies the developmental transition to the senescence stage. Although these assays were done on naturally senescing leaves, other researchers have examined the global patterns associated with dark-induced senescence of *Arabidopsis* leaves, as well as sucrose starvation-induced senescence in cell suspension cultures (Swidzinski et al., 2002; Lin and Wu, 2004). Comparisons of these microarray data provide a picture of the similarities and differences among processes, as influenced by various factors. The senescence process in wheat flag leaves has been investigated using cDNA microarrays based on a 9K wheat unigene set. Analysis of genes that are differentially expressed has revealed considerable overlap between those up-regulated in wheat flag yellowing leaves and genes previously observed to be associated with senescence in dicot plant species. These data support the idea that a generic senescence program exists across monocot and dicot plant species (Gregersen and Holm, 2007).

The SAGs identified from these studies include genes for potential regulatory factors as well as those that execute the senescence program. Their spectrum is mostly consistent with known biochemical and physiological symptoms, but also provides many new insights into molecular events and regulation during leaf senescence. However, functioning of the vast majority of SAGs has not been determined biochemically and/or genetically. Their characterization should provide knowledge for understanding leaf senescence and applying it to agricultural purposes.

The mRNA expression is only one aspect of the functional regulation of a gene. Thus, care must be taken to base our descriptions of the regulatory pathways for leaf senescence solely on mRNA expression analyses. Of course, other regulatory mechanisms, such as protein-level expression, protein stability, or localization of regulatory proteins, will certainly be involved.

Comparative transcriptome analysis between developmental and dark/starvation-induced senescence in Arabidopsis

Plant senescence can be influenced by several internal and external factors (Fig. 1), including developmental age, changes in hormone levels, shading, and other environmental stresses. One obvious question regarding leaf senescence is how the cellular states of senescence caused by several senescence-inducing factors differ. To address this, one must compare expression patterns of the SAGs in response to various senescence-inducing treatments. Early studies have shown that, although senescence symptoms in *Arabidopsis* leaves may appear to be similar, the leaf cells, depending on the factors that have induced their senescence, utilize different as well as common sets of genes (Nam, 1997; Park et al., 1998).

Later, microarray experiments have been conducted to compare gene expression patterns related to natural or developmental age-dependent leaf senescence with those found during dark- or sucrose starvation-induced senescence (Buchanan-Wollaston et al., 2005). This analysis revealed not only similarities but also considerable differences in the gene expression patterns among them. For example, pathways for essential metabolic processes such as nitrogen mobilization were utilized variably among the senescence conditions caused by the different factors. Interestingly, the expression pattern seen in the sucrose starvation-induced senescence of suspension cells is more similar to that in dark-induced senescence, indicating that the latter is mostly due to reduced sugar levels in a non-photosynthetic environment.

Ethylene, JA, and SA signals induce well-characterized stress-response pathways, and they also affect leaf senescence. The role and importance of these signals has been investigated by examining the expression of SAGs in mutant plants with lesions in the signaling pathways of those hormones. The analysis revealed that all three pathways participate in regulating many SAGs during developmental age-dependent senescence, thus providing a detailed molecular picture of the role for each. Surprisingly, the SA pathway is not involved in dark- or starvation-induced senescence, whereas the JA and ethylene pathways are active in both of those as well as age-dependent senescence. The importance of the SA pathway in age-dependent leaf senescence is further supported by the finding that age-dependent but not dark-induced leaf senescence is delayed in plants defective in that SA pathway (Buchanan-Wollaston et al., 2005).

Metabolic profiling during leaf senescence

Diaz et al. (2005) have conducted experiments to directly monitor metabolic changes during leaf senescence. Their

initial targets were sugars and nitrogen compounds because these are associated with nutrient allocation and recycling. In *Arabidopsis* leaves, sugar accumulates at an early phase of senescence (Masclaux et al., 2000; Diaz et al., 2005), reaching a peak level before dropping rapidly. Ordinarily, during their growth phase, leaves utilize the sugar they synthesize, but when full expansion or maturation is attained, those sugars begin to accumulate. That elevated sugar level can then repress photosynthesis, which in turn induces senescence. This view is consistent with a previous hypothesis that an age-related decline in photosynthesis may trigger leaf senescence (Masclaux et al., 2000).

Metabolic analysis of amino acid contents has revealed that, whereas the proportions of various amino acids remain relatively stable during the growth phase, major changes are detected as soon as senescence starts. For example, at that stage, the relative levels of leucine (Leu), isoleucine (Ile), tyrosine (Tyr), and arginine (Arg) rise, while those of glutamic acid (Glu), aspartic acid (Asp), and glutamine (Gln) decrease. Glu is the primary donor for the synthesis of all other amino acids, and Asp, a direct product of Glu transamination, is involved in the biosynthesis of Lys, Thr, Met, Ile, and Leu. This observation implies that there is an active interconversion of amino acids in senescing leaves. Further interesting insights have been obtained by comparing the amino acid profiles of five different recombinant inbred lines (RILs) that exhibit a differential senescence phenotype. Late-senescing lines appear to mobilize Gln and Asn, i.e., the major amino acids translocated in the phloem sap, more efficiently than do early-senescing lines, raising the possibility that leaf senescence might be affected by nitrogen-mobilization efficiency. This senescence phenotype is also correlated with a balance between glycine and serine. These two amino acids form during photorespiration, and the Gly/Ser ratio is commonly used as an indicator of photorespiratory activity (Wingler et al., 2000). Early-senescing lines show a higher ratio (even in non-senescing leaves) than do the late-senescing lines, implying that photorespiration influences senescence. If true, the glycine/serine ratio could serve as a new metabolic marker for leaf behavior, although more rigorous tests must be evaluated on various senescence mutants and treatment conditions. Tyrosine is a precursor for vitamin E (α-tocopherol), which is an effective antioxidant. Elevated levels of Tyr during senescence might be associated with the protection of leaf cells from oxidative stress via the increased synthesis of that vitamin.

Although the use of metabolic profiling for leaf senescence is just beginning, transcriptome analyses have clearly demonstrated the possibility of a drastic change in metabolic pathways during senescence. A more thorough picture will certainly provide unique insight that is not easily obtained from other assays. Other approaches are needed for determining changes within a broad spectrum of metabolites, for analysis of various mutants under multiple test conditions, and for better utilizing our current bioinformatics tools.

GENES/PATHWAYS REGULATING THE ONSET AND PROGRESSION OF LEAF SENESCENCE

To understand the molecular mechanisms/pathways involved in the leaf senescence network, genetics strategies have been established to select mutants exhibiting an early- or delayed-senescence phenotype in *Arabidopsis* (Fig. 2). Several corresponding genes have now been isolated. Characterization of transgenic plants that carry potential senescence-controlling genes has also added to the compilation of new regulatory components, although most of those genes have not been characterized in terms of their mechanism of action. Nevertheless, these findings also point toward interplay among those factors. Based on the characteristics of the identified genes, we have grouped them into the following categories:

Developmental-aging factors

Senescence is certainly associated with the process of developmental aging in *Arabidopsis* and thus occurs after a certain time point is reached. Thus, there should be a cellular mechanism(s) that measures the age of a cell, tissue, organ, or entire body for the initiation and/or progression of senescence. However, no reports have been made about genes that alter senescence by controlling developmental aging, nor is it well known how a particular developmental age initiates leaf senescence.

Metabolic rate has been associated with life span in many organisms. Specifically, caloric restriction can extend that span (Ewbank et al., 1997; Guarente, 1997; Kimura et al., 1997). This rate might also regulate developmental aging in plants. The *Arabidopsis* mutant *ore4-1*, which contains a lesion in a plastid ribosomal small subunit 17 protein (PRPS17), displays reduced photosynthetic activity and delayed age-dependent leaf senescence (Woo et al., 2002). This phenotype is likely due to lower metabolism because the chloroplasts, the major energy source for plant growth via photosynthesis, are only partially functional in that mutant. This observation strongly suggests that metabolic rate might be one of the key mechanisms involved in developmental leaf senescence.

Factors regulating developmental processes in addition to leaf senescence

Although leaf senescence occurs in age-dependent manner, it is also finely tuned by other endogenous developmental factors (Fig. 1). Several lines of evidence from mutants or transgenic lines have revealed that factors regulating developmental processes, e.g., phytohormones or sugars, are involved in modulating this senescence. Studies have also unraveled the complex and extensive interactions among these factors, similar to those shown for other developmental processes.

Phytohormones Cytokinins have long been known as anti-senescence agents because they have a dramatic effect on the longevity of various organs in many plants. Exogenous cytokinin treatment results in delayed leaf senescence. Moreover, endogenous levels of cytokinins decline in parallel with the progression of this senescence, thereby illustrating the control exercised by that hormone. A striking example of this suppressive effect has been observed in transgenic tobacco and lettuce plants that express the *IPT* gene, an *Agrobacterium*-originated cytokinin biosynthesis

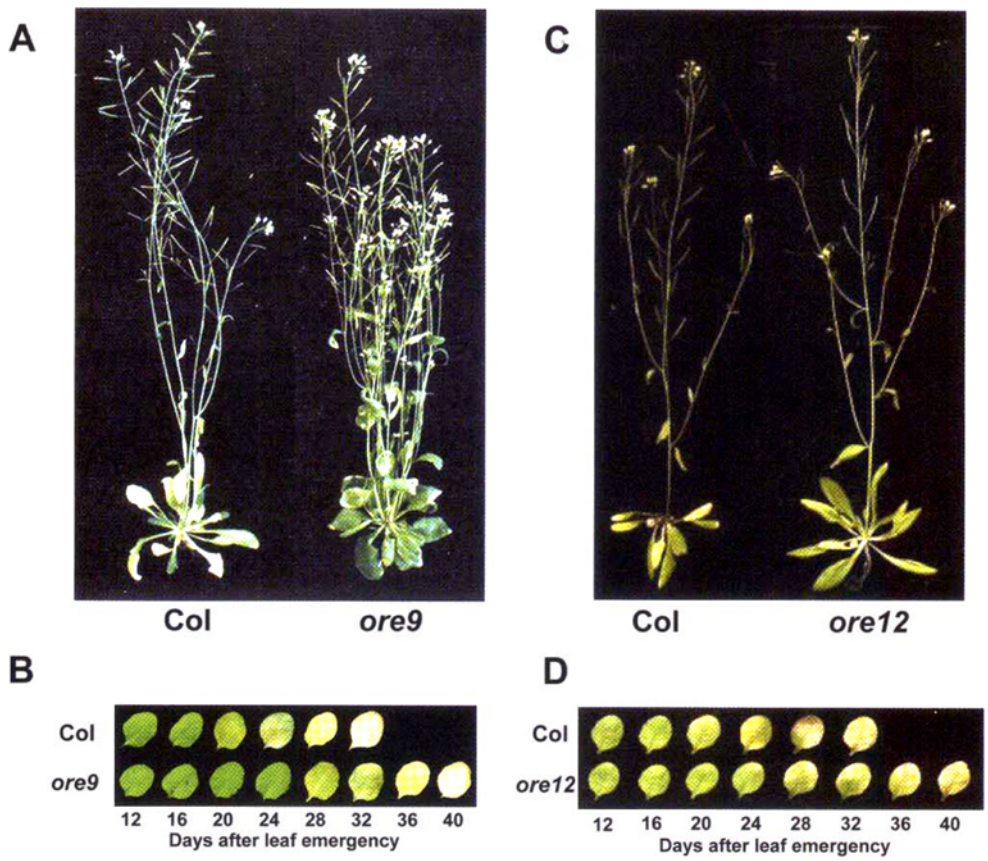

Figure 2. (**A**) Whole-plant phenotypes of wild type (Col) and senescence-delayed mutant (*ore9*) at 43 d after germination. (**B**) Age-dependent senescence phenotypes of fourth rosette leaf from wild type (Col) and *ore9* mutant. (**C**) Whole-plant phenotypes of wild type (Col) and senescence-delayed mutant (*ore12*) at 38 d after germination. (**D**) Age-dependent senescence phenotypes of fourth rosette leaf from wild type (Col) and *ore12* mutant. Photographs show representative leaves at each time point.

gene, under the control of the senescence-specific *SAG12* promoter. Transgenic plants show markedly delayed leaf senescence (Gan and Amasino, 1995; McKenzie et al., 1998; McCabe et al., 2001).

A recent discovery showed that AHK3, one of three cytokinin receptors in *Arabidopsis*, plays a major role in controlling cytokinin-mediated leaf longevity (Kim et al., 2006). This conclusion has been based on characterizations of the gain-of-function *Arabidopsis* mutant, *ore12-1*, which shows delayed leaf senescence due to a missense mutation in the *AHK3* gene (Fig. 2). A loss-of-function mutation of *AHK3*, but not of the other receptors, confers reduced sensitivity to cytokinin in cytokinin-mediated delay of leaf senescence. Kim et al. (2006) have also demonstrated that phosphorylation of the ARR2 response regulator, mediated by AHK3, is essential for the control of leaf longevity. Nonetheless, further investigation is needed into the exact mechanism by which the phosphorylated ARR2 leads to the induction or repression of genes that regulate and/or execute this senescence.

Ethylene, essential for fruit ripening, also has a role in leaf senescence (Grbic and Bleecker, 1995; Gonzalez and Botella, 2003). The importance of endogenous ethylene has been clearly demonstrated in certain *Arabidopsis* mutants, e.g., *etr1-1* and *ein3/ore3*, which are defective in their ethylene perception and signal transduction. These mutations cause measurable delays in the initiation of leaf senescence, but have little effect on its progression (Grbic and Bleecker, 1995; Oh et al., 1997). This suggests that ethylene may assist in coordinating a timely transition of the leaf to the final state, but is not essential to the process itself. Interestingly, leaves of transgenic *Arabidopsis* and tomato plants that constitutively over-produce ethylene do not exhibit early senescence, suggesting that this hormone alone is not sufficient for initiation. It has been indicated that ethylene-mediated pathways leading to leaf senescence in *Arabidopsis* depend on age-dependent factors; thus, ethylene can induce senescence only after the leaves reach a certain developmental stage (Grbic and Bleecker, 1995; Jing et al., 2002).

Salicylic acid (SA) is also involved in the regulation of this senescence. Its levels increase in senescing leaves, possibly accounting for the senescence-enhanced expression of some genes (Morris et al., 2000). Consistent with this observation, *Arabidopsis* plants that manifest a dramatically reduced level of SA, as a result of overexpression of a SA-degrading enzyme encoded by *NahG*, show decreased expression of some *SAG* genes. These transgenics exhibit a time-delayed phenotype during both age-dependent senescence, but not in dark-induced senescence, thus supporting the microarray data. All of these observations indicated that the SA pathway has a very specific role in natural senes-

cence, possibly in the final death phase (Morris et al., 2000; Buchanan-Wollaston et al., 2005).

Exogenous treatment with methyl jasmonate induces leaf-yellowing by activating a subset of *SAGs*, suggesting that MJ is a senescence-promoting hormone (He et al., 2002). This idea is consistent with the observation that *SAG* expression is reduced in a jasmonic acid-insensitive mutant, *coi1*, even though no phenotype may be visible (Xiao et al., 2004). It is possible that other factors might induce leaf senescence in the absence of JA.

Auxin, which regulates many aspects of plant growth and development, may also influence leaf senescence, based on the finding that a mutation in *auxin response factor2* (*arf2*) causes a delay in leaf senescence as well as petal abscission (Ellis et al., 2005; Okushima et al., 2005). However, it is yet to be seen whether the auxin pathways are directly or indirectly involved because auxin and the *arf2* mutants likewise cause a pleiotropic effect on plant development.

Abscisic acid (ABA) is a key hormone that mediates plant responses to environmental stresses. Except for some circumstantial evidence, its role in leaf senescence has not been clearly defined. ABA levels rise in senescing leaves, and exogenously applied ABA induces the expression of several SAGs (Weaver et al., 1998), consistent with an effect on leaf senescence. Environmental stresses, including drought, high salinity, and low temperatures, have positive influences on leaf senescence and, under those conditions, leaf ABA contents rise. Concurrent with this increase (Gepstein and Thimann, 1980), genes encoding the key enzyme in ABA biosynthesis show greater expression (van der Graaff et al., 2006). Nevertheless, no crucial link between ABA and leaf senescence has yet been discovered in genetic analyses.

It should be noted that plant hormones interact or crosstalk with one another, constituting a complex network of regulation. This is likely the case in controlling plant senescence. However, cautions must be taken when interpreting various results because many phytohormone mutants have been characterized at the seedling stage and hormone signaling during that period might not be the same as at the senescing stage. It should be also noted that these plant hormones could be involved in correlative control of senescence, although this does not occur in *Arabidopsis* (Hensel et al., 1993).

Sugar signaling/Photosynthetic activity Sugar signaling has emerged as an important regulator of leaf senescence (Rolland et al., 2002). Several lines of evidence suggest that a high concentration of sugars lowers photosynthetic activity and induces leaf senescence (Jang et al., 1997; Dai et al., 1999; Quirino et al., 2000; Moore et al., 2003). Senescence is then triggered when those concentrations go above an acceptable level. Hexokinases are involved in sugar sensing in higher plants. Studies using their overexpressors have demonstrated that increased hexokinase levels stimulate a rise in sugar content that is associated with reduced photosynthetic activity (Jang et al., 1997; Dai et al., 1999). One notable phenotype found in transgenic plants is accelerated leaf senescence, supporting the idea that lower photosynthetic activity may be related to premature leaf senescence via hexokinase. Moreover, a glucose-insensitive *Arabidopsis* mutant (*gin2*), with a lesion in one of the hexokinases, shows delayed senescence (Moore et al., 2003). However, the senescence phenotypes observed in transgenic and mutant lines have not been thoroughly examined and must be analyzed in greater detail to support this proposal. The *hys1* (*hypersenescence1*) mutant has increased sensitivity to exogenously applied sugars as well as an accelerated leaf senescence phenotype (Yoshida et al., 2002b). Therefore, one might suggest that an enhanced sugar signal in that mutant causes diminished photosynthesis and induces premature senescence, likely via hexokinase.

Sugar-signaling pathways interact intimately with those signaling pathways regulated by hormones, e.g., auxin, cytokinin, or abscisic acid, during plant development. This is likely the case in the regulation of *Arabidopsis* leaf senescence. Such control through sugar signaling probably is also affected by other factors, such as nitrogen status and developmental stage. Integration of these factors into a senescence program might be important to the proper regulation of timing for its onset and progression.

External factors that affect leaf senescence

As with many other plant growth and developmental processes, senescence is highly influenced by environmental conditions, including pathogen infection, nutrient or water stress, or oxidative stresses induced by ozone or UV-B. Thus, senescence should be an integrated response within plants to external environmental factors, as is true for endogenous developmental signals. However, no formal report has yet addressed how these factors interact to coordinate senescence.

Light, perceived by a variety of photoreceptors, affects developmental processes over the entire life span, and may also play a role in leaf senescence (Cherry et al., 1991; Thiele et al., 1999). For example, transgenic plants overexpressing phytochrome A (*PhyA*) or phytochrome B (*PhyB*) exhibit greater longevity. In the case of the *PhyB* overexpressor, the initiation point for leaf senescence is the same as that of the wild type, but the time during which chlorophyll degradation is completed is extended by about three to four weeks in transgenic plants. This suggests that *PhyB* might be involved in controlling the progression rate. Such results may need to be further confirmed using other senescence markers. Although a mechanism for delayed senescence has not yet been proposed, one cause might be higher contents of chlorophyll.

Exposure to extremely high or low temperatures, pathogen attack, or water/nutrient deficiency can also trigger leaf-yellowing. Thus, parts of the signaling pathways that are associated with environmental stresses would be predicted to regulate leaf senescence. Expression profiles of 402 potential stress-related genes that encode known or putative transcription factors from *Arabidopsis* have been monitored in various organs, at different developmental stages, and under several biotic and abiotic stresses (Chen et al., 2002). Among the 43 transcription factor genes that are reportedly induced during senescence, 28 are also induced by stress treatment, suggesting extensive overlapping responses. Downstream genes for senescence-enhanced transcription factors might play a role either in executing leaf senescence

or in protecting the cellular functions required for proper progression or completion of that senescence.

Other regulatory genes

Other regulatory genes for leaf senescence have been identified via genetic screening of senescence mutants and through the functional identification of some SAGs.

Protein degradation has a potentially important role in dictating the life span of many organisms. Recent genetic studies in *Arabidopsis* demonstrated that protein degradation is also involved in controlling leaf senescence (Woo et al., 2001; Yoshida et al., 2002a; Oh et al., 2005) (Fig. 2). The ORE9 encodes an F-box protein, which is a component of the ubiquitin E3 ligase/SCF complex (Woo et al., 2001). SCF complexes are known to ubiquitinate specific target substrates (Patton et al., 1998). ORE9 possibly promotes leaf senescence by targeting a senescence suppressor for degradation. Proteolysis by the N-end rule pathway, one of the ubiquitin pathways, also appears to be a mechanism that regulates *Arabidopsis* leaf senescence. In a *delayed-leaf-senescence 1 (dls1)* mutant that is defective in arginyl tRNA:protein transferase (R-transferase, a component of N-end rule proteolysis pathway), both age-dependent and dark-induced senescence progress more slowly than in wild-type plants. Thus, DLS1 might function in the degradation of proteins that negatively regulate this senescence. Nonetheless, the *ore9* and *dls1* mutations might have different roles. For example, the *dls1* mutation delays not only the initiation of leaf senescence but also its progression, whereas the *ore9* mutation mainly affects initiation.

Many transcription factors identified in *Arabidopsis* are upregulated at least three-fold in senescing leaves. These belong to 20 different families. The largest groups include NAC, WRKY, C2H2 type zinc finger, AP2/EREBP, and MYB family proteins. A few have been further characterized in relation to leaf senescence. *WRKY53* is upregulated at a very early stage but transcripts decrease again over time, implying that the gene plays a regulatory role in early senescence events (Hinderhofer and Zentgraf, 2001). A knockout line of *WRKY53* shows delayed senescence, while inducible *overexpression* causes precocious senescence, evidence that it functions as a positive element (Miao et al., 2004). Identification of the direct target genes for WRKY53 should further reveal the regulatory pathways for WRKY53-mediated senescence. The *WRKY6* gene controls a set of genes, including one that encodes a receptor-like protein kinase, *SIRK*, that is strongly upregulated during leaf senescence (Robatzek and Somssich, 2002). The T-DNA knockout mutant of *AtNAP*, a gene encoding an NAC family transcription factor, exhibits significantly delayed leaf senescence (Guo and Gan, 2006). Thus, *AtNAP* might function as a positive element. *AtNAP* orthologs in kidney bean and rice are also upregulated in that final process. Intriguingly, an *NAC* gene regulating senescence increases the contents of protein, zinc, and iron in wheat grain (Uauy et al., 2006). Therefore, this result direct links the regulation of senescence with nutrient remobilization. The potential functions for the majority of leaf senescence-associated transcription factors remain to be elucidated.

Receptor kinases can potentially act as key components in the perception of senescence signals and in the subsequent phosphorylation cascades involved in a senescence program. In fact, soybean contains a leucine-rich repeat receptor-like kinase gene (*GmSARK*) associated with leaf senescence (Li et al., 2006). The RNA interference (RNAi)-mediated knocking down of GmSARK dramatically retards soybean leaf senescence while its overexpression greatly accelerates that progression. This implies a positive role for that protein.

Another example of SAGs for which *in vivo* functioning has been assayed is the autophagy genes. Autophagy, an intracellular process for vacuolar bulk degradation of cytoplasmic components, is required for nutrient cycling. Mutants carrying a T-DNA insertion within three *Arabidopsis* autophagy genes - *AtAPG7*, *AtAPG9*, and *AtAPG18a* – exhibit premature leaf senescence (Doelling et al., 2002; Hanaoka et al., 2002; Xiong et al., 2005). In these mutants, nutrients may be less efficiently utilized during the execution of senescence or else some of the components necessary for its progression may not be efficiently provided.

CONCLUDING REMARKS

Senescence is the integrated response of a cell, tissue, organ, or organism to age, developmental status, and environment. Thus, to maximize its fitness, a plant must incorporate all of these factors to ensure proper progression. This can be achieved by fine-tuning the mechanisms that involve a delicate regulation of gene expression. In the last decade, genomics and genetics approaches have focused on identifying senescence regulators and describing those regulatory mechanisms. Although those processes are still poorly understood, many new insights have already been gained.

One future challenge will be to elucidate the roles of potential regulatory genes in senescence. Such efforts will enable us to reveal a complex regulatory network that includes signal pathways for various senescence-influencing factors. Utilization of large collection of T-DNA insertion lines or TILLING approach in *Arabidopsis* will allow the functional analysis of the genes which were identified from microarray data. However, a senescence phenotype from these mutants may not be obvious due to either gene or functional redundancy caused by the various pathways that lead to senescence. In this case, it may be necessary to generate double, triple, and even higher-order mutants in those redundant genes or functional pathways. Transgenic approaches that incorporate inducible overexpression, mini-genes, or RNA interference could also overcome the limitations of loss-of-function mutants (Gan and Amasino, 1999; He and Gan, 2002)). Recently introduced genomics technologies, such as microarrays, proteomics, or metabolomics, will also help us to see distinct molecular phenotypes, even when no aberrant phenotype is observed.

Genetics approaches also have led to discoveries of many regulatory components. Considering the nature of senescence, there should be many more mutants that can be identified through well-designed screening schemes. Alternatively, one could exploit the diverse genetics resources

now available in *Arabidopsis*. The chemical mutagenized pool, as well as T-DNA insertional knockout or activation-tagging lines, also will provide novel alleles and prove useful when characterizing the functions of novel senescence regulatory factors. Furthermore, the generation of a T-DNA pool in which inducible promoters are inserted into the genome would be a valuable tool for screening negative elements by inducing a gene at a maturation or senescence stage and then observing the phenotypic effect. Using global gene-expression analyses, e.g., via DNA chips, in combination with proteomics approaches or through altered sub-cellular localization, signaling, metabolic functions, or *in vivo* protein-protein interactions, will likely reveal the molecular mechanism(s) for leaf senescence that is mediated by these regulatory factors. It will also be important to extend these studies to agronomic and other crop plants. Understanding how senescence is controlled in different species will allow for future manipulation of that natural process.

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LITERATURE CITED

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, Lundeberg J, Nilsson O, Sandber G, Strauss S, Sundberg B, Uhlen M, Jansson S, Nilsson P (2004) A transcriptional timetable of autumn senescence. Genome Biol 5: R24

Bate NJ, Rothstein SJ, Thompson JE (1990) Expression of nuclear and chloroplast photosynthesis-specific genes during leaf senescence. J Exp Bot 239: 801-811

Bleecker AB, Patterson SE (1997) Last exit: Senescence, abscission, and meristem arrest in *Arabidopsis*. Plant Cell 9: 1169-1179

Buchanan-Wollaston V (1997) The molecular biology of leaf senescence. J Exp Bot 48: 181-199

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

Cao J, Jiang F, Sodmergen, Cui K (2003) Time-course of programmed cell death during leaf senescence in *Eucommia ulmoides*. J Plant Res 162: 7-12

Chen W, Provart NJ, Glazebrook J, Katagiri F, Chang HS, Eulgem T, Mauch F, Luan S, Zou G, Whitham SA, Budworth PR, Tao Y, Xie Z, Chen X, Lam S, Kreps JA, Harper JF, Si-Ammour A, Mauch-Mani B, Heinlein M, Kobayashi K, Hohn T, Dangl JL, Wang X, Zhu T (2002) Expression profile matrix of *Arabidopsis* transcription factor genes suggests their putative functions in response to environmental stresses. Plant Cell 14: 559-574

Cherry, JR, Hershey HP, Vierstra RD (1991) Characterization of tobacco expressing functional oat phytochrome. Plant Physiol 96: 775-785

Dai N, Schaffer A, Petreikov M, Shahak Y, Giller Y, Ratner K, Levine A, Granot D (1999) Overexpression of *Arabidopsis* hexokinase in tomato plants inhibits growth, reduces photosynthesis, and induces rapid senescence. Plant Cell 11: 1253-1266

Diaz C, Purdy S, Christ A, Morot-Gaudry JF, Wingler A, Masclaux-Daubresse C (2005) Characterization of markers to determine the extent and variability of leaf senescence in *Arabidopsis*: A metabolic profiling approach. Plant Physiol 138: 898-908

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

Ellis CM, Nagpal P, Young JC, Hagen G, Guilfoyle TJ, Reed JW (2005) AUXIN RESPONSE FACTOR1 and AUXIN RESPONSE FACTOR2 regulate senescence and floral organ abscission in *Arabidopsis thaliana*. Development 132: 4563-4574

Ewbank JJ, Barnes TM, Lakowski B, Lussier M, Bussey H, Hekimi S (1997) Structural and functional conservation of the *Caenorhabditis elegans* timing gene clk-1. Science 275: 980-983

Gan S (2003) Mitotic and postmitotic senescence in plants. Sci Aging Knowl Environ 38: 7

Gan S, Amasino RM (1995) Inhibition of leaf senescence by autoregulated production of cytokinin. Science 270: 1986-1988

Gan S, Amasino RM (1999) Developmental targeting of gene expression by the use of a senescence-specific promoter. *In* P Reynolds, ed, Inducible Gene Expression in Plants. CAB International, New York, pp 169-186

Gepstein S, Sabehi G, Carp MJ, Hajouj T, Nesher MF, Yariv I, Dor C, Bassani M (2003) Large-scale identification of leaf senescence-associated genes. Plant J 36: 629-642

Gepstein S, Thimann KV (1980) Changes in the abscisic acid content of oat leaves during senescence. Proc Natl Acad Sci USA 77: 2050-2053

Gonzalez N, Botella JR (2003) Characterization of three ACC synthase gene family members during post-harvest-induced senescence in broccoli (*Brassica oleracea* L. var. italica). J Plant Biol 46: 223-230

Grbic V, Bleecker AB (1995) Ethylene regulates the timing of leaf senescence in *Arabidopsis*. Plant J 8: 595-602

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

Guarente L (1997) Aging: What makes us tick? Science 275: 943-944

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

Guo Y, Gan S (2006) AtNAP, a NAC family transcription factor, has an important role in leaf senescence. Plant J 46: 601-612

Guterman A, Hajouj T, Gepstein S (2003) Senescence-associated mRNAs that may participate in signal transduction and protein trafficking. Physiol Plant 118: 439-446

Hanaoka H, Noda T, Shirano Y, Kato T, Hayashi H, Shibata D, Tabata S, Ohsumi Y (2002) Leaf senescence and starvation-

induced chlorosis are accelerated by the disruption of an *Arabidopsis* autophagy gene. Plant Physiol 129: 1181-1193

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

He Y, Fukushige H, Hildebrand DF, Gan S (2002) Evidence supporting a role of jasmonic acid in *Arabidopsis* leaf senescence. Plant Physiol 128: 876-884

He Y, Tang W, Swain JD, Green AL, Jack TP, Gan S (2001) Networking senescence-regulating pathways by using *Arabidopsis* enhancer trap lines. Plant Physiol 126: 707-716

Hensel L, Grbic V, Baumgarten DA, Bleecker AB (1993) Developmental and age-related processes that influence the longevity and senescence of photosynthetic tissues in *Arabidopsis*. Plant Cell 5: 553-564

Hinderhofer K, Zentgraf U (2001) Identification of a transcription factor specifically expressed at the onset of leaf senescence. Planta 213: 469-473

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

Jang JC, Leon P, Zhou L, Sheen J (1997) Hexokinase as a sugar sensor in higher plants. Plant Cell 9: 5-19

Jing HC, Sturre MJG, Hille J, Dijkwel PP (2002) *Arabidopsis* onset of leaf death mutants identify a regulatory pathway controlling leaf senescence. Plant J 32: 51-63

John I, Hackett R, Cooper W, Drake R, Farrell A, Grierson D (1997) Cloning and characterization of tomato leaf senescence-related cDNAs. Plant Mol Biol 33: 641-651

Jones AM, Dangl JL (1996) Logjam at the Styx: Programmed cell death in plants. Trends Plant Sci 1: 114-119

Kim HJ, Ryu H, Hong SH, Woo HR, Lim PO, Lee IC, Sheen J, Nam HG, Hwang I (2006) Cytokinin-mediated control of leaf longevity by AHK3 through phosphorylation of ARR2 in *Arabidopsis*. Proc Natl Acad Sci USA 103: 814-819

Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G (1997) *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. Science 277: 942-946

Li XP, Gan R, Li PL, Ma YY, Zhang LW, Zhang R, Wang Y, Wang NN (2006) Identification and functional characterization of a leucine-rich repeat receptor-like kinase gene that is involved in regulation of soybean senescence. Plant Mol Biol 61: 829-844

Lim PO, Nam HG (2005) The molecular and genetic control of leaf senescence and longevity in *Arabidopsis*. Curr Top Dev Biol 67: 49-83

Lim PO, Woo HR, Nam HG (2003) Molecular genetics of leaf senescence in *Arabidopsis*. Trends Plant Sci 8: 272-278

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

Masclaux C, Valadier M, Brugière N, Morot-Gaudry J, 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

McCabe MS, Garratt LC, Schepers F, Jordi WJRM, Stoopen GM, Davelaar E, van Rhijk JHA, Power B, Davey MR (2001) Effects of PSAG12-IPT gene expression on development and senescence in transgenic lettuce. Plant Physiol 127: 505-516

McKenzie MJ, Mett V, Reynolds PHS, Jameson PE (1998) Controlled cytokinin production in transgenic tobacco using a copper-inducible promoter. Plant Physiol 116: 969-977

Miao Y, Laun T, Zimmermann P, Zentgraf U (2004) Targets of the WRKY53 transcription factor and its role during leaf senescence in *Arabidopsis*. Plant Mol Biol 55: 853-867

Moore B, Zhou L, Rolland F, Hall Q, Cheng WH, Liu YX, Hwang I, Jones T, Sheen J (2003) Role of the *Arabidopsis* glucose sensor HXK1 in nutrient, light, and hormonal signaling. Science 300: 332-336

Morris KAH, Mackerness S, Page T, John CF, Murphy AM, Carr JP, Buchanan-Wollaston, V (2000) Salicylic acid has a role in regulating gene expression during leaf senescence. Plant J 23: 677-685

Nam HG (1997) Molecular genetic analysis of leaf senescence. Curr Opin Biotech 8: 200-207

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

Oh MH, Kim JH, Zulfugarow IS, Moon YH, Rhew TH, Lee CH (2005) Effects of benzyladenine and abscisic acid on the disassembly process of photosystems in an *Arabidopsis* delayed-senescence mutant, *ore9*. J Plant Biol 48: 170-177

Oh SA, Park JH, Lee GI, Paek KH, Park SK, Nam HG (1997) Identification of three genetic loci controlling leaf senescence in *Arabidopsis thaliana*. Plant J 12: 527-535

Okushima Y, Mitina I, Quach HL, Theologis A (2005) AUXIN RESPONSE FACTOR 2 (ARF2): A pleiotropic developmental regulator. Plant J 43: 29-46

Otegui MS, Noh YS, Martinez DE, Vila Petroff MG, Staehelin LA, Amasino RM, Guiamet JJ (2005) Senescence-associated vacuoles with intense proteolytic activity develop in leaves of *Arabidopsis* and soybean. Plant J 41: 831-844

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

Patton EE, Willems AR, Tyers M (1998) Combinational control in ubiquitin-dependent proteolysis: Don't Skp the F-box hypothesis. Trends Genet 14: 236-243

Quirino BF, Noh YS, Himelblau E, Amasino RM (2000) Molecular aspects of leaf senescence. Trends Plant Sci 5: 278-282

Quirino BF, Normanly J, Amasino RM (1999) Diverse range of gene activity during *Arabidopsis thaliana* leaf senescence includes pathogen-independent induction of defense-related genes. Plant Mol Biol 40: 267-278

Robatzek S, Somssich IE (2002) Targets of AtWRKY6 regulation during plant senescence and pathogen defense. Genes Dev 16: 1139-1149

Rolland F, Moore B, Sheen J (2002) Sugar sensing and signaling in plants. Plant Cell Suppl 14: S185-205

Simeonova E, Sikira S, Charzynska M, Mostowska A (2000) Aspects of programmed cell death during leaf senescence of mono- and dicotyledonous plants. Protoplasma 214: 93-101

Swidzinski JA, Sweetlove LJ, Leaver CJ (2002) A custom microarray analysis of gene expression during programmed cell death in *Arabidopsis thaliana*. Plant J 30: 431-446

Taylor CB, Bariola PA, delCardayre SB, Raines RT, Green PJ (1993) RNS2: A senescence associated RNase of *Arabidopsis* that diverged from the S-RNase before speciation. Proc Natl Acad Sci USA 90: 5118-5122

Thiele A, Herold M, Lenk I, Quail PH, Gatz C (1999) Heterologous expression of *Arabidopsis* phytochrome B in transgenic potato influences photosynthetic performance and tuber development. Plant Physiol 120: 73-81

Thompson J, Taylor C, Wang TW (2000) Altered membrane lipase expression delays leaf senescence. Biochem Soc Trans 28: 775-777

Thompson JE, Froese CD, Madey E, Smith MD, Hong Y (1998) Lipid metabolism during plant senescence. Prog Lipid Res 37: 119-141

Uauy C, Fahima T, Blechl A, Dubcovsky J (2006) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. Science 314: 1298-1301

van der Graaff E, Schwacke R, Schneider A, Desimone M, Flugge UI, Kunze R (2006) Transcription analysis of *Arabidopsis* membrane transporters and hormone pathways during developmental and induced leaf senescence. Plant Physiol 141: 776-792

van Doorn WG, Woltering EJ (2004) Senescence and programmed cell death: Substance or semantics? J Exp Bot 55: 2147-153

Weaver LM, Gan S, Quirino B, Amasino RM (1998) A comparison of the expression patterns of several senescence-associated genes in response to stress and hormone treatment. Plant Mol Biol 37: 455-469

Wingler A, Lea PJ, Quick WP, Leegood RC (2000) Photorespiration: Metabolic pathways and their role in stress protection. Philos Trans R Soc Lond B Biol Sci 355: 1517-1529

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

Woo HR, Goh CH, Park JH, Teyssendier B, Kim JH, Park YI, Nam HG (2002) Extended leaf longevity in the *ore4-1* mutant of *Arabidopsis* with a reduced expression of a plastid ribosomal protein gene. Plant J 31: 331-340

Xiao S, Dai L, Liu F, Wang Z, Peng W, Xie D (2004) COS1: An *Arabidopsis* coronatine insensitive1 suppressor essential for regulation of jasmonate-mediated plant defense and senescence. Plant Cell 16: 1132-1142

Xiong Y, Contento AL, Bassham DC (2005) AtATG18a is required for the formation of autophagosomes during nutrient stress and senescence in *Arabidopsis thaliana*. Plant J 42: 535-546

Yen CH, Yang CH (1998) Evidence for programmed cell death during leaf senescence in plants. Plant Cell Physiol 39: 922-927

Yoshida S, Ito M, Callis J, Nishida I, Watanabe A (2002a) A delayed leaf senescence mutant is defective in arginyl-tRNA:protein arginyltransferase, a component of the N-end rule pathway in *Arabidopsis*. Plant J 32: 129-137

Yoshida S, Ito M, Nishida I, Watanabe A (2002b) Identification of a novel gene HYS1/CPR5 that has a repressive role in the induction of leaf senescence and pathogen-defence responses in *Arabidopsis thaliana*. Plant J 29: 427-437