Minireview

Leaf senescence - not just a 'wear and tear' phenomenon

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Abstract

A recent, genome-wide study shows that the transcriptional program underlying leaf senescence is active and complex, reflecting the activation of more than 2,000 genes in *Arabidopsis*, with gene products involved in a broad spectrum of regulatory, biochemical and cellular events.

Aging and senescence in plants

Senescence, aging and death - conceived of in the past as inevitable, negative processes - are now considered an integral part of differentiation and development. Leaf senescence is one of the most conspicuous processes to have been studied in the context of plant aging and senescence, and has an important impact on agriculture, affecting crop yield and the shelf life of leafy vegetables [1,2]. This terminal phase of leaf development cannot be described simply as a collection of passive and deteriorative processes during which a gradual decline in vital systems takes place. Extensive physiological and biochemical studies on leaf senescence in the last three decades have suggested that it is a highly regulated and active process, which is characterized by differential and sequential changes in almost every subcellular compartment. Leaf senescence involves diverse metabolic changes associated with multiple biochemical pathways [1-3]. Guo *et al.* [4] now report a comprehensive study on the transcriptional program of *Arabidopsis* leaf senescence. This study not only provides genetic confirmation for many previous biochemical and physiological findings, but also adds significant new information indicating that leaf senescence, like other developmental processes, is a very dynamic, complex and active program that requires the activation of many genes.

In both plants and animals, programmed cell death (PCD) plays a crucial role, mainly during development and differentiation. Although there is insufficient information, at this stage, to postulate that animals and plants share common and basic regulatory mechanisms of PCD, similar biochemical and cellular changes are often displayed in both systems. An example of a process involving PCD in plants is the formation of the xylem elements that die and lose their content in order to conduct water and solutes. The autolysis of cells in roots, cell death during pollination, embryo development and seed maturation are also referred to as depending on PCD. Leaf senescence displays the three criteria suggested by Barlow [5] to be necessary to define it as a plant PCD process: first, cells die at a predictable time and location; second, death has some beneficial effect on plant development; and third, cell death is encoded in the hereditary material. This definition excludes necrotic cell death due to accidental damage, or injury as a result of exposure to a toxic environment.

Gene expression during leaf senescence

Although leaf senescence can generally be defined as a late developmental process leading to death, the primary molecular pathway of the senescence program is not known. Leaf yellowing due to chlorophyll degradation is often considered to be the main marker for leaf senescence. Chlorophyll breakdown may also indicate the early disintegration of the photosynthetic machinery localized in the chloroplast. The drastic biochemical transition characterizing the onset of leaf senescence, however, requires more than 'just' deterioration of the activity of existing proteins or downregulation of gene expression. Indeed, recent genomic and molecular studies support the notion that the onset of senescence involves *de novo* synthesis of proteins and the expression of a complex array of genes whose products are involved in, and are responsible for, the multitude of senescence-related biochemical and cellular changes [4,6-8].

The comprehensive transcriptome study by Guo *et al.* [4] provides the largest available list (6,200) of senescence-associated expressed sequence tags (ESTs), representing approximately 2,500 genes, in *Arabidopsis* leaves. It has been hypothesized that the ESTs found in senescing tissues represent genes that are expressed in the fully senescent leaf, when all transcripts present in non-senescent leaves have already been degraded. Given that the current database of *Arabidopsis* ESTs does not represent senescing tissues, it is perhaps not surprising that an additional 100 new genes, not found in the public databases, were identified in this study.

Functional classification of senescence-associated genes

As in many other genomic studies, the biological relevance of the senescence-associated transcriptome study of Guo *et al.* [4] depends on our ability to predict the function of individual genes. Functional assignment of the genes represented in their senescent-leaf EST collection was carried out using the sequence annotation and classification of genes in the *Arabidopsis* databases. The general picture emerging from this study is that leaf senescence is indeed, like any other developmental process, a very dynamic and complex phenomenon that involves the activation of a large number of genes representing a broad spectrum of functional categories. The relative abundance of transcripts represented by the various categories during senescence differs substantially compared to those represented at other developmental stages, however. For example, massive degradation of cellular components, a distinct feature of leaf senescence, is reflected by the high ratio of the number of genes for primary catabolism over those for anabolism found in the senescence EST database (1.84) as compared to this ratio for the entire *Arabidopsis* genome (0.57).

The main functional categories of the senescence-related ESTs as reported by Guo *et al.* [4] are summarized in Table 1. These data provide important clues to the regulatory and metabolic processes associated with leaf senescence. Macromolecule degradation is reflected by the upregulation of several groups of genes whose products are responsible for the intensive degradation of proteins, lipids, nucleotides and polysaccharides during senescence. Seven percent of the ESTs are from genes involved in proteolysis; among these, those encoding cysteine and other types of proteases are prominent. Upregulation of the ubiquitin/polyubiquitin genes and genes whose products are associated with the activation and ligation reactions of the ubiquitination pathway is evidence for proteolysis via the ubiquitin pathway [4,6,7]. Genes encoding the components of the proteosome have also been demonstrated to be actively upregulated during leaf senescence [7]. These results support the suggestion that senescence may be regulated by the ubiquitin pathway through the breakdown of negative regulatory molecules [9].

The dramatic biochemical shift from a photosynthetically active organ into a senescing leaf is induced by an array of endogenous factors, such as age and hormonal regulation, as well as by external factors, such as biotic and abiotic stresses. It is not known how these signals are perceived by the plant, but the study of Guo *et al.* [4] identified 182 genes

Table 1

Major functional categories of senescence-associated genes*

Functional category	Associated processes	Abundant genes
Macromolecule degradation	Breakdown of proteins, nucleic acids, lipids and polysaccharides	Cysteine proteases, ubiquitin-related genes, RING finger proteins, nucleases, lipases/acylhydrolases, phospholipases, glucanases, β-glucosidase, pectinesterases, and polygalacturonase
Nutrient recycling	Transport of peptides, amino acids, sugars, purines, pyrimidines and ions	Oligopeptide transporters, ammonium transporter purine and pyrimidine transporters, glutamine synthetase and glutamate synthase, sugar transporters (MFSs), and ABC transporters
Defense and cell rescue mechanisms	Abiotic and biotic stress, and oxidative stress	Metallothionein, glutathione *S*-transferase, protein similar to jasmonate-inducible protein, glutathione peroxidase, and cold-regulated protein COR6.6
Transcriptional regulation	Transcription factors	Zinc finger proteins, basic helix-loop-helix proteins, bZIP proteins, HMG-box proteins and transcription factors of the WRKY, NAC, AP2, MYB, HB, TCP and GRAS families
Signal transduction	Protein phosphorylation and dephosphorylation	Receptor-like kinases, components of MAP kinase signal cascades, phosphatases and phospholipases, calcium-binding EF-hand protein RD20, calcium-dependent protein kinases, and cytoskeleton-associated proteins

*Determined by the abundance of senescence-associated ESTs, as described by Guo *et al.* [4]. bZIP, basic leucine zipper; HB, homeobox protein; HMG, high mobility group; MAP, mitogen-activated kinase; MSF, major facilitator superfamily; NAC, no apical meristem (NAM) proteins.

encoding apparent components of signal perception and transduction pathways. The putative senescence-induced signals are, as in other developmental processes, likely to be perceived by signaling molecules belonging to various classes. Candidates include plant receptor kinases - transmembrane kinases that have been implicated in ligand perception [10]. Genes encoding senescence-associated receptor-like kinases (SARK and SIRK) have also been identified in bean and *Arabidopsis* [11,12]. Among the 610 genes encoding receptor-like proteins found in the *Arabidopsis* genome, 44 are expressed in the senescing leaf [4].

Signal-transduction pathways linked to the components that perceive the senescence signals are predicted to trigger a cascade of events inside the cells. Signaling cascades frequently involve the addition and removal of phosphate groups (phosphorylation and dephosphorylation) from cellular proteins. Indeed, a limited number of genes encoding components of protein-phosphorylation cascades have been identified in the senescing leaf, and prominent among these are genes encoding members of the mitogen-activated protein (MAP) kinase cascade [4]. MAP kinase cascades are known to link extracellular stimuli to a wide range of cellular responses in animal cells and yeast, and may be involved in the senescence program as well.

Following the signaling pathways downstream, the targets are likely to be transcription factors that can act as switches to initiate differential gene expression upon binding to specific *cis*-elements of target-gene promoters. The 134 genes encoding transcription factors identified by Guo *et al*. [4] represent 5.4% of the total number of senescence-associated genes. These genes provide a key to the understanding of the regulatory pathways of the senescence program. Furthermore, subsets of target genes are regulated by specific transcription factors, helping, in turn, to identify the genes expressed as the final output of the pathway. The two largest groups of senescence-related transcription factors are NAC transcription factors, which exist exclusively in plants and have previously been shown to control organ development and response to pathogens, and the WRKY transcription-factor group, which are also known to control the response to pathogens and are elicited by salicylic acid. The involvement of WRKY6, WRKY18, WRKY22/29 and WRKY53 in leaf senescence has already been demonstrated, and the expression of members of this group is upregulated both in defense responses and during leaf senescence [12]. Interestingly, zinc-finger proteins and other transcription-factor families have been identified in the senescence EST collection of Guo *et al*. [4], whereas none of the MADS-box transcription factors known to participate in flower development were found to be expressed during senescence.

Some orthologs of the senescence proteases found by Guo *et al*. [4] have also been identified in autumn leaves of the *Populus* tree [6]. A recent genomic study employing EST sequencing and microarrays of gene expression during autumn leaf senescence of *Populus* trees suggests that, as during leaf senescence of annual plants, there is a dramatic shift in gene expression reflecting the transition from anabolic to catabolic processes, chlorophyll degradation, oxidation of fatty acids and nutrient mobilization [8].

In addition to the significant information regarding the existence and nature of the biochemical pathways and regulatory mechanisms involved in leaf senescence, the vast collection of genes described in these recent transcriptome studies [4,6-8] also provides the basis for future reverse-genetic studies of the senescence program. We can look forward to insights into the molecular basis for leaf senescence and, ultimately, elucidation of the complex pathways involved.

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