



## Review

# Programmed senescence of plant organs

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## Abstract

The senescence of plant organs associated with reproductive development has been studied extensively during the past century, and it has long been recognized that this type of death is internally programmed. The regulation of organ senescence as well as its biochemical and genetic determinants has been an historically rich area of research. Certain plant hormones have been implicated as regulators or modulators of organ senescence and many of the biochemical pathways associated with the senescence syndrome have been elucidated. The genetic basis of organ senescence has also been well established by the identification of mutations that impair the senescence program and recently, transgenic plants have been used to critically determine the role of specific enzymes and hormonal signals in mediating programmed senescence of plant organs. Here, we review the current understanding of the processes that regulate leaf, flower and fruit senescence, emphasizing the role that programmed organ senescence plays in the adaptive fitness of plants.

**Keywords:** survivorship; fruit ripening; organ senescence

**Abbreviations:** PCD, programmed cell death; ACC, aminocyclopropane carboxylic acid; *Nr*, *Never ripe*

## Introduction

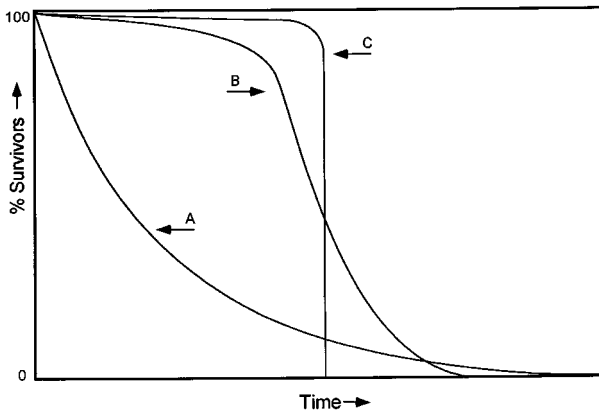
Programmed cell death (PCD) is an integral part of many aspects of plant development including xylogenesis, sex determination, leaf abscission and the hypersensitive response to pathogen infection. In each of these cases, specific groups of cells within a larger population of living cells are triggered to die. This feature of cell-specificity of the death process clearly implicates specific development signals and cell-autonomous biochemical processes targeting the execution of individual cells. There is a great deal of current research attempting to identify common features of these PCD processes, including the role of nucleosomal fragmentation,

cysteine proteases and lipid-based signal molecules (Gilchrist, 1997; Dangl, 1997). It now appears likely that these cases of cell-autonomous PCD may be analogous to apoptotic cell death that has been characterized in many animal cells.

In this article we will address senescence of whole plants or of plant organs which differ fundamentally from the processes listed above in that entire populations of cells die synchronously. In most cases, organ senescence and even whole-plant senescence represents a PCD process because it is genetically determined, under the control of endogenous regulators and requires the ordered activation of specific gene expression. In many cases, the endogenous signals and genetic determinants of organ senescence are well characterized, although the understanding of the biochemical mechanisms that lead to cell death is only fragmentary. The best characterized cases of programmed organ senescence are associated with plant reproductive development, particularly leaf senescence that is triggered by flowering, flower senescence that is triggered by pollination and the ripening and senescence of fruit.

## Leaf senescence

The concept that whole plant and organ senescence is a normal developmental process and under endogenous control is often attributed to Molisch (1938) who theorized that senescence was endogenously induced by depletion of material in assimilatory portions of the plant. This idea of 'exhaustive death' has been largely discredited since leaf senescence is triggered in many plants in the absence of nutrient mobilization to developing fruit (Leopold *et al*, 1959; Thomas and Stoddart, 1980). However, the association of leaf senescence with the transition from vegetative to reproductive development is widely accepted as being integral to the reproductive strategy of plants. Leopold (1961) described plant senescence in terms of survivorship curves (Pearl and Miner, 1935; Figure 1). The first of these curves describes the death of populations of individuals as a simple stochastic process in which death occurs randomly as would be observed in populations that experience death at a constant rate due to predation, stress or other external forces (curve A). Most animals in the wild belong to this group. The second curve describes a population in which the probability of death increases after a certain period of time. This pattern describes the survivorship of many domesticated animals, man, and many perennial plants which exhibit a low mortality rate until about half the maximal age and then rapidly decline (curve B). These populations appear to become more susceptible to external forces as a result of progressive deterioration of endogenous defense systems (aging), with death often resulting from disease. The third curve describes a population in which death of a population occurs catastrophically (curve C). In the absence of an external life-threatening



**Figure 1** Generalized survivorship curves for populations in which (A) death occurs randomly as a result of external forces; (B) the probability of death increases after a certain period of time; and (C) all the members of the population die at once as a result of an endogenous programmed developmental event. [Redrawn from Pearl and Miner (1935)].

force, this pattern of death reflects the most extreme case of senescence in which all the members of the population die at once as a result of an endogenously programmed developmental event. This pattern is typical of annual plant species and does not result from an external catastrophe but from an endogenous signal and program that synchronizes the death of large populations of plants immediately following the reproductive developmental phase. There are many examples of this pattern of monocarpic senescence including most of the annual crops such as wheat and soybean.

The analysis of survivorship curves clearly illustrates that many plant species exhibit a life history strategy in which death and sudden population turnover are programmed events. Leopold (1961) reasoned that programmed senescence in populations that do not experience significant predation may serve as a mechanism to facilitate population turnover and he proposed that programmed plant senescence serves as a catalyst for evolutionary change. In addition to providing fuel for natural selection, programmed plant senescence also provides the basis for nutrient remobilization to seeds and to facilitate seed dispersal through ripening and ultimate deterioration of fruit. Thus, the life history of annual plants differs significantly from that of many animals and programmed senescence plays a central role in plant reproductive strategies and potentially in their long-term evolutionary fitness. The process of whole plant senescence is dominated by programmed death of leaves, and there has been intense research to identify the signals triggering the death response and to identify genetic and biochemical determinants of leaf senescence.

### Endogenous signals regulating leaf senescence

It has long been recognized that development of various plant organs is influenced by other plant parts (Sachs, 1882) and it is generally accepted that this developmental control is mediated by plant hormones. In general terms, ethylene and abscisic acid are commonly thought of as promoters of

senescence while cytokinin, auxin and gibberellins have been considered senescence inhibitors. This is a deceptively simplified view, however, and many cases exist where a senescence promoting or inhibiting hormone can have the opposite effect, depending on the tissue type, stage of development and/or interactions with other hormones. The role of the plant hormone ethylene in regulating leaf senescence has been rigorously tested by the analysis of transgenic plants suppressed for ethylene production and in mutants defective in ethylene perception. Senescence is delayed in both transgenic ethylene deficient plants (Picton *et al*, 1993a; John *et al*, 1995) and ethylene insensitive mutant plants (Bleecker *et al*, 1988; Grbic and Bleecker, 1995). However, plants that constitutively overproduce ethylene did not exhibit premature leaf senescence (Lanahan *et al*, 1994; Guzman and Ecker, 1990) as might be expected from the senescence promoting effect of exogenously applied ethylene. Grbic and Bleecker (1995) resolved this apparent discrepancy by hypothesizing that the development of leaves in the presence of high constitutive levels of ethylene reduces the endogenous sensitivity of the plant to ethylene-induced senescence. The available data convincingly shows that ethylene is not necessary for leaf senescence to occur but that it may act to modulate the timing of this process.

Other plant hormones have been shown to promote senescence in a variety of organs and cell types, such as the yellowing of detached leaves promoted by application of abscisic acid (El-Antably *et al*, 1967) or methyl jasmonate (Ueda and Kato, 1980). The responses to these hormones, however, are often mediated by ethylene, implying a secondary role, in many cases, as promoters of senescence. A recently identified class of plant hormones, the brassinosteroids, appear to be involved in a number of developmental processes and may promote leaf and chloroplast senescence (Li *et al*, 1996), although a direct role remains to be demonstrated.

The retardation of leaf senescence by cytokinin has been well-documented. Richmond and Lang (1957) reported that kinetin reduced the amount of protein lost from detached leaves, Mothes and Engelbrecht (1961) showed that treatment of a region of a senescing leaf with kinetin prevented the loss of chlorophyll and caused the accumulation of amino acids in that region, and Osborne (1962) demonstrated that the application of kinetin to senescing leaves resulted in an increase in the rate of RNA or protein synthesis relative to untreated leaves. Recently, analysis of transgenic plants with increased levels of cytokinins in specific tissues normally undergoing senescence provided overwhelming evidence implicating cytokinin as a senescence-retarding hormone. Expression of a bacterial gene encoding isopentenyl transferase under the control of a senescence-regulated promoter in transgenic tobacco resulted in elevated cytokinin levels and greatly retarded leaf senescence (Gan and Amasino, 1995). In addition, rates of photosynthesis in these transgenic plants were maintained at levels comparable to young non-senescing leaves, demonstrating that endogenous cytokinin can regulate the senescence process. In tomato fruit, the expression of the same bacterial gene under the control of a fruit-specific promoter resulted in

altered ripening properties and the authors concluded that, as in leaves, elevated cytokinin levels prevented the progression of fruit senescence (Martineau *et al.*, 1994).

Taken together, it is clear that a number of hormones influence leaf senescence and that the process can be manipulated by modifying endogenous hormone levels. However, the endogenous signals that initiate leaf senescence or transmit a 'reproductive signal' from the developing ovary and fruit to the vegetative plant organs are unknown and are unlikely to be the hormones described above.

### Genetic and biochemical determinants of leaf senescence

Leaf senescence is genetically determined and delayed or non-senescent mutants of soybean, fescue and sorghum have been identified (Pierce *et al.*, 1984; Thomas, 1987; Duncan *et al.*, 1981). Among these mutants a class of non-senescent sorghum genotypes exhibited altered cytokinin levels, suggesting that one mechanism of genetic control of senescence is altered hormone biosynthesis or transport (Ambler *et al.*, 1987). Other non-senescent mutants appear more limited in their scope, with the fescue non-yellowing (NY) mutant being defective in chlorophyll loss but essentially unchanged in other parameters of senescence (Thomas and Stoddart, 1975; Thomas, 1987).

In addition to the genetic evidence that specific lesions can block the process of senescence it is also well-established that protein synthesis is required for senescence to proceed (Thomas, 1976; Shibaoka and Thimann, 1970; De Vecchi, 1971; Martin and Thimann, 1972; Makovetzki and Goldschmidt, 1976; Yu and Kao, 1981) and recent results have identified the induction of a number of specific mRNAs that are associated with leaf senescence. *In vitro* translation of mRNA from senescing leaves demonstrated that the amount of translatable mRNA encoding the small subunit of RuBPCase and other photosynthetic proteins decline during senescence, while several other mRNAs increase (Speirs and Brady, 1981; Skadsen and Cherry, 1983; Hensel *et al.*, 1993). This result demonstrated that there is both positive and negative regulation of gene expression associated with leaf senescence. Strong evidence that leaf senescence involves an ordered disassembly of cell components has been revealed by the identification of mRNAs whose expression is senescence-regulated. The cDNA clones representing senescence-regulated mRNAs encode a number of proteins with activities targeted towards the breakdown of cellular components, including ribonucleases (Taylor *et al.*, 1993), polyubiquitin (Garbarino *et al.*, 1995), cysteine proteases (Hensel *et al.*, 1993; Lohman *et al.*, 1994; Smart *et al.*, 1995; Buchanan-Wollaston and Ainsworth, 1997) and cell wall hydrolases (King *et al.*, 1995). In addition, mRNA encoding glutamine synthetase is senescence-regulated, suggesting a role of this enzyme in the remobilization of protein nitrogen into a commonly translocated amino acid, glutamine (Kamachi *et al.*, 1992). The identification and cataloguing of a large number of genes whose expression is regulated by senescence

clearly indicates that the program of cell death is highly coordinated and serves a valuable role in the remobilization of scarce nutrients to surviving plant organs, such as seeds. A classification scheme for the entire set of known senescence-regulated genes has been recently proposed and provides a conceptual framework for assembling the complex processes that contribute to programmed senescence of leaves (Buchanan-Wollaston, 1997).

### Flower senescence

The flower is one of the most ephemeral of plant organ systems because it is specialized for the specific functions of pollen dispersal and reception, after which time many individual floral organs senesce while others develop further to form seeds and fruit. This transition in flower function involves the programmed senescence of the petals and sepals. Perianth senescence of some flowers occurs as part of a temporal program with the petals and sepals senescing strictly as a function of age. For example, daylily flowers senesce 12–18 h after flower opening (Lukaszewski and Reid, 1989; Lay-Yee *et al.*, 1992). The endogenous signals that regulate age-dependent petal senescence are completely uncharacterized, although the process is accompanied by the regulated expression of a suite of genes, some of which are functionally related to those associated with leaf senescence. Other flowers, such as petunia, gradually senesce over a period of days after flower opening, but this process is accelerated by pollination. In still other flowers perianth senescence is absolutely dependent on pollination and in these cases the external stimuli and endogenous signals that regulate programmed senescence have been examined in detail (O'Neill *et al.*, 1993; O'Neill and Nadeau, 1997; O'Neill, 1997).

### Endogenous signals regulating pollination-induced flower senescence

In flowers whose senescence is pollination-dependent or pollination-accelerated, including petunia, carnation, cyclamen and orchids, senescence and the pollination event is accompanied by a sudden and rapid increase in endogenous ethylene production (Nichols, 1966, 1968; Bufler *et al.*, 1980; Halevy *et al.*, 1984; Nichols *et al.*, 1983; Whitehead *et al.*, 1983; Hall and Forsyth, 1967; Porat *et al.*, 1994). Indeed, it has been known for over 30 years that senescence of certain orchid flowers was accompanied by ethylene evolution and that senescence could be induced by application of exogenous ethylene (Akamine, 1963). Most importantly, the effects of exogenous ethylene could be mimicked by pollination, which indicated that ethylene played a central role in signaling the onset of programmed flower senescence.

Because the stigma is the initial site of perception of pollination, the initial pollination signal must be transduced and translocated to promote senescence processes in the distal organs of the flower, such as the perianth. A detailed characterization of transduction of the pollination signal in orchid flowers indicates that the initial pollination event promotes the synthesis of the immediate precursor of ethylene, aminocyclopropane-carboxylic acid (ACC), in the stigma by induction of expression of an ACC synthase gene

**Table 1** Genes that are upregulated during fruit ripening and encode proteins of known function or exhibit significant sequence homology to proteins of known function. Proteins are grouped into five general categories based on their proposed cellular function. Multiple references indicate the cloning of a single gene sequence

Homology or function	Plant	Reference
<i>Cell Wall Disassembly</i>		
polygalacturonase	tomato	Slater <i>et al</i> , 1985; DellaPenna <i>et al</i> , 1986; Lincoln <i>et al</i> , 1987; Sheehy <i>et al</i> , 1987
polygalacturonase	avocado	Dopico <i>et al</i> , 1993; Kutsunai <i>et al</i> , 1993
polygalacturonase	peach	Lester <i>et al</i> , 1994
polygalacturonase	apple	Atkinson, 1994
polygalacturonase (MPG1)	melon	Hadfield, Rose and Bennett, unpublished
polygalacturonase (MPG2)	melon	Hadfield, Rose and Bennett, unpublished
polygalacturonase (MPG3)	melon	Hadfield, Rose and Bennett, unpublished
pectin methylesterase	tomato	Hall <i>et al</i> , 1994; Turner <i>et al</i> , 1996
$\beta$ -galactosidase	tomato	Carey <i>et al</i> , 1995
$\beta$ -galactosidase	apple	Ross <i>et al</i> , 1994
endoglucanase	avocado	Christofferson <i>et al</i> , 1984
endoglucanase (TCel 1)	tomato	Lashbrook <i>et al</i> , 1994
endoglucanase (TCel 2)	tomato	Lashbrook <i>et al</i> , 1994
endoglucanase	pepper	Ferrarese <i>et al</i> , 1995; Harpster <i>et al</i> , 1997
expansin	tomato	Rose <i>et al</i> , 1997
expansin	melon	Rose <i>et al</i> , 1997
expansin	strawberry	Rose <i>et al</i> , 1997
xyloglucan endotransglycosylase	tomato	Arrowsmith & de Silva, 1995
xyloglucan endotransglycosylase	melon	Rose and Bennett, unpublished
<i>Ethylene Biosynthesis and Signal Transduction</i>		
ACC <sup>1</sup> synthase	zucchini	Sato and Theologis, 1989
ACC synthase	tomato	Van Der Straeten <i>et al</i> , 1990
ACC synthase	apple	Dong <i>et al</i> , 1991; Lay-Yee and Knighton, 1995
ACC synthase	melon	Miki <i>et al</i> , 1995
ACC oxidase	tomato	Slater <i>et al</i> , 1985
ACC oxidase	avocado	McGarvey <i>et al</i> , 1992
ACC oxidase	apple	Dong <i>et al</i> , 1992; Ross <i>et al</i> , 1992
ACC oxidase	melon	Balague <i>et al</i> , 1993
S-adenosyl-L-methionine synthetase	kiwifruit	Whittaker <i>et al</i> , 1995
oxoglutarate-dependent dioxygenase	tomato	Lincoln <i>et al</i> , 1987
ethylene receptor	tomato	Wilkinson <i>et al</i> , 1995b
<i>Pigmentation</i>		
phytoene synthase (TOM5)	tomato	Slater <i>et al</i> , 1985
phytoene synthase	pepper	Romer <i>et al</i> , 1993
phytoene synthase	melon	Karvouni <i>et al</i> , 1995
phytoene desaturase	tomato	Aracri <i>et al</i> , 1994; Pecker <i>et al</i> , 1992
phytoene desaturase	pepper	Huguenev <i>et al</i> , 1992
GGPP <sup>2</sup> synthase	pepper	Kuntz <i>et al</i> , 1992
chalcone synthase	strawberry	Wilkinson <i>et al</i> , 1995a
<i>Stress and Pathogen Related</i>		
heat shock protein (TOM111)	tomato	Slater <i>et al</i> , 1985
heat shock protein (TOM66)	tomato	Fray <i>et al</i> , 1990
proteinase inhibitor (E17)	tomato	Lincoln <i>et al</i> , 1987
proteinase inhibitor (2A11)	tomato	Pear <i>et al</i> , 1989
cysteine proteinase inhibitor	avocado	Dopico <i>et al</i> , 1993
thaumatin	avocado	Dopico <i>et al</i> , 1993
thaumatin	cherry	Fils-Lycaon <i>et al</i> , 1996
endo-chitinase	avocado	Dopico <i>et al</i> , 1993
plant defensin	pepper	Meyer <i>et al</i> , 1996
major latex protein	pepper	Pozueta-Romero <i>et al</i> , 1995
major latex protein	melon	Aggelis <i>et al</i> , 1997
metallothionenin	kiwifruit	Ledger and Gardner, 1994
pollen allergen	apple	Atkinson <i>et al</i> , 1996
<i>Metabolism</i>		
acid invertase	tomato	Klann <i>et al</i> , 1992; Elliott <i>et al</i> , 1993
short chain alcohol dehydrogenase	tomato	Picton <i>et al</i> , 1993b
alcohol dehydrogenase	tomato	Longhurst <i>et al</i> , 1994
histidine decarboxylase	tomato	Slater <i>et al</i> , 1985
glutamate decarboxylase	tomato	Gallego <i>et al</i> , 1995
arginine decarboxylase	tomato	Rastogi <i>et al</i> , 1993
UDPGT <sup>3</sup>	tomato	Picton <i>et al</i> , 1993b
membrane intrinsic protein	tomato	Slater <i>et al</i> , 1985
lipoxigenase (tomloxA)	tomato	Ferrie <i>et al</i> , 1994

Table continued overleaf

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Homology or function	Plant	Reference
<i>Metabolism</i>		
lipoxygenase (tomloxB, LOX)	tomato	Ferrie <i>et al</i> , 1994, Kausch and Handa, 1995
alternative oxidase	mango	Cruz-Hernandez and Gomez-Lim, 1995
40S ribosomal protein s12 (RNase)	strawberry	Wilkinson <i>et al</i> , 1995a
annexin	strawberry	Wilkinson <i>et al</i> , 1995a
ubiquitin conjugating enzyme	tomato	Picton <i>et al</i> , 1993b
vacuolar processing protease	citrus	Alonso and Granell, 1995
cytochrome P450	avocado	Bozak <i>et al</i> , 1990

<sup>1</sup>l-aminocyclopropane-1-carboxylic acid. <sup>2</sup>geranylgeranyl pyrophosphate. <sup>3</sup>UDP-glucosyl and glucuronosyl transferase

(Bui and O'Neill, submitted). ACC produced in the stigma may be oxidized directly to ethylene or potentially translocated to distal floral organs where it serves as substrate for ACC oxidase, the enzyme responsible for its conversion to ethylene. This model suggests that interorgan transduction of the pollination signal occurs by translocation of a hormone precursor, but that the endogenous signal initiating programmed senescence of flower organs is ethylene. This conclusion is supported by tomato mutants defective in ethylene perception in which the flowers do not senesce (Lanahan *et al*, 1994). In this regard, the regulation of flower senescence differs fundamentally from the regulation of leaf senescence, where ethylene appears to only modify the primary signals that underlie the senescence process.

### Genetic and biochemical determinants of flower senescence

Like leaf senescence, flower organ senescence also serves to remobilize nutrients from the petals to the developing ovary and the process is active, being brought about by changes in gene expression (Lawton *et al*, 1989, 1990; Woodson, 1987; Borochoy and Woodson, 1989). A number of cDNAs have been isolated from senescing carnation petals which were shown to be regulated by ethylene and correlated with the senescence process (Lawton *et al*, 1989, 1990). Two of the senescence-related cDNAs encode a  $\beta$ -galactosidase (SR12) and a glutathione s-transferase (SR5; Meyer *et al*, 1991). The  $\beta$ -galactosidase in senescing flower petals is most likely involved in cell wall disassembly that accompanies many senescence processes and it is speculated that the glutathione s-transferase acts in detoxification of lipid and DNA hydroperoxides associated with senescence-induced oxidative processes (Sylvestre *et al*, 1989). Thus, as with leaf senescence, biochemical events associated with macromolecular disassembly of floral organs are regulated at the level of gene expression in senescing petals. Because the senescence program of floral organs has been primarily investigated in those flowers whose senescence is pollination-induced and ethylene-regulated, it is not yet known what other signals may regulate the programmed senescence of other types of flowers and whether the biochemical processes of macromolecular disassembly are similar.

### Fruit ripening and senescence

Fruit ripening is perhaps the most extensively characterized senescing plant organ. This process is a highly ordered

developmental event that results in significant modification of the tissue composition prior to its ultimate deterioration and death. The ripening process promotes dissemination of seeds and so plays an obvious evolutionary role in expanding the geographic distribution of plant species. While fruit ripening promotes seed dissemination by animal consumption, subsequent senescence of the fruit results in increased pathogen susceptibility and seed dispersal following microbial tissue maceration.

In spite of its ecological role in seed dispersal, ripening has been primarily studied because of its economic significance in regulating the quality and longevity of fruit for human consumption. Like flower senescence, fruit ripening can be subdivided into at least two categories. The best studied category includes fruit such as tomato and bananas, where ripening is ethylene-regulated and is associated with a large increase in respiration. The large increase in respiration was originally called the climacteric and fruit in this category are thus referred to as 'climacteric fruit'. In contrast, ripening of nonclimacteric fruit is ethylene independent and is not associated with increased respiration. While it is clear that both classes of fruit undergo similar processes involving changes in pigmentation and disassembly of macromolecular cell components, very little is known about the endogenous regulators of nonclimacteric fruit ripening while a great deal is known about the regulation of climacteric fruit ripening.

### Endogenous signals regulating fruit ripening

Ripening fruit have historically provided an excellent model system to study the hormonal control of senescence and early experiments showed that ethylene is the major factor contributing to the ripening and senescence of climacteric fruit. Evidence that ethylene was a positive senescence factor included the early observations that senescence of unripe fruit was stimulated by exposure to combustion fumes (Sievers and True, 1912) and that the active component of these fumes was ethylene (Denny, 1924). The possibility that ethylene in combustion fumes mimicked natural regulators of ripening and senescence became clear somewhat later, when ethylene was identified as a natural metabolite produced by some fruit (Gane, 1934) and when it was observed that ethylene depletion by hypobaric storage inhibited fruit ripening (Burg and Burg, 1965). The possible role of ethylene as the primary regulator of fruit ripening and senescence was a powerful concept that motivated a detailed elucidation of the ethylene biosynthetic pathway in plants which culminated in

1978 with the identification of the immediate precursor of ethylene, 1-aminocyclopropane-carboxylic acid (ACC; Adams and Yang, 1979). Shortly thereafter, the enzyme responsible for ACC biosynthesis, ACC synthase, was identified and shown to be a primary site of regulation of the ethylene biosynthetic pathway (Kende and Boller, 1981). The role of ethylene as the primary regulator of fruit ripening was then firmly established by suppression of ethylene production in transgenic fruit expressing antisense ACC-synthase or ACC-oxidase genes which inhibited ripening in a manner that was fully reversible by external application of ethylene (Oeller *et al*, 1991; Hamilton *et al*, 1990).

While it is clear that ethylene is a major determinant of ripening and senescence of climacteric fruit, there appear to be as yet unidentified endogenous signals that are associated with fruit maturation and enhanced sensitivity to ethylene. Because it is now known that expression of the ethylene receptor gene, *Nr*, is developmentally regulated in fruit (Payton *et al*, 1996), it is possible that competence to perceive ethylene and enter the ripening and senescence process may depend on elaboration of components of an ethylene response pathway.

### Genetic and biochemical determinants of fruit ripening

The identification and characterization of tomato mutants impaired in their ability to ripen demonstrated that fruit ripening is genetically determined (Tigchelaar *et al*, 1978). Many of these mutants are altered only in their ability to synthesize carotenoids, with other ripening changes occurring normally. Other mutants, however, are affected in multiple ripening properties and recently, it has been shown that a lesion in the gene encoding the ethylene receptor is the molecular basis of the ethylene-insensitive mutant, *Never-ripe (Nr)* (Wilkinson *et al*, 1995b). The prevention of fruit ripening by inhibitors of protein synthesis further supports the genetic basis of ripening and suggests that it involves an orderly process of disassembly that relies on the completion of a defined set of biochemical reactions (Frenkel *et al*, 1968; Brady *et al*, 1970).

One of the most prominent biochemical processes in ripening and senescing fruit is the disassembly of macromolecules, particularly cell wall polysaccharides (Brady, 1988; Fischer and Bennett, 1991). Changes in translatable mRNA populations were shown to occur in ripening fruit (Speirs *et al*, 1984; Christoffersen *et al*, 1982) and a number of these products were identified, among the first of which were the cell wall hydrolases endo- $\beta$ -1,4-glucanase (Christoffersen *et al*, 1984) and polygalacturonase (DellaPenna *et al*, 1986). In addition, a large number of other ripening-regulated genes have been identified including those that encode ethylene biosynthetic enzymes, lycopene biosynthetic enzymes and proteases. Table 1 summarizes all of the ripening-regulated genes with a putative function that have been identified to date. A large number of ripening-regulated genes of unknown function have also been catalogued and because of the extensive research in this area, the list of ripening-regulated genes grows longer on a regular basis. The precise function of each ripening-regulated gene has only been tested in a few

cases, usually by the production of transgenic plants in which expression of a specific gene is suppressed by the action of antisense or sense transgenes or by its ectopic expression (Sheehy *et al*, 1988; Oeller *et al*, 1991; Smith *et al*, 1988; Giovannoni *et al*, 1989). By this process, the major role of ACC synthase in regulating the induction of fruit ripening and the role of phytoene synthase in regulating production of carotenoids has been demonstrated (Oeller *et al*, 1991; Bramley *et al*, 1992). In contrast, experiments to suppress the expression of single cell wall hydrolase genes have had only minor effects on the overall ripening process, suggesting that the complex biochemical and structural changes that accompany ripening and senescence result from the cooperative action of a potentially large number of gene products (Sheehy *et al*, 1988; Smith *et al*, 1988; Giovannoni *et al*, 1989; Lashbrook and Bennett, unpublished result).

### Conclusions

Many plant organs undergo programmed senescence which is genetically determined, controlled by endogenous regulators and carried out by the ordered activation of specific gene expression. The unique life history strategies of annual plants provides an unusual example of programmed senescence of an entire organism and, indeed, entire plant populations. Although the cellular mechanisms of programmed senescence of entire plant organs are not known, there are a number of commonalities in the endogenous signals that trigger the death process and in the classes of genes that are activated to carry out the 'execution'. This suggests that the cellular mechanisms of programmed organ senescence may also share common features.

Unlike other PCD processes in plants, where cell death is restricted to a few cells involved in a hypersensitive response or to developing tracheary elements, organ senescence proceeds uniformly in large contiguous cell populations. In this regard, the propagation of signals that regulate programmed organ senescence may differ fundamentally from signals that elicit cell death in other developmental responses where death is confined to restricted cell populations. Thus, a fundamental issue for future research will be to understand how endogenous signals of cell death are propagated in contiguous cell populations of senescing organs and how this process differs from cell death processes that become restricted to a few cells within the same organ.

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