Senescence Processes in Plants

Edited by

SUSHENG GAN
Department of Horticulture
Cornell University
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Editorial Offices:
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Contributors

Dr Ian Armstead Plant Genetics and Breeding Department, Institute of Grassland & Environmental Research, Plas Gogerddan, Aberystwyth, Ceredigion SY23 3EB, Wales, UK
Dr Marie-Jeanne Carp Department of Biology, Technion – Israel Institute of Technology, Haifa 32000, Israel
Dr Jen-Chih Chen Department of Plant Sciences, University of California, One Shields Drive, Davis, CA 95616, USA
Dr Paul P. Dijkwel Department of Molecular Biology of Plants, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Kerklaan 30, 9751 NN, Haren, The Netherlands
Dr Iain Donnison Plant Genetics and Breeding Department, Institute of Grassland & Environmental Research, Plas Gogerddan, Aberystwyth, Ceredigion SY23 3EB, Wales, UK
Dr Andreas M. Fischer Department of Plant Sciences, 210 AgBioScience Facility, Montana State University, Bozeman, MT 59717, USA
Dr Isaac Galyuon Department of Molecular Biology and Biotechnology, School of Biological Sciences, University of Cape Coast, Cape Coast, Ghana
Dr Susheng Gan Department of Horticulture, G51 Emerson Hall, Cornell University, Ithaca, NY 14853, USA
Dr Shimon Gepstein Department of Biology, Technion – Israel Institute of Technology, Haifa 32000, Israel
Dr James J. Giovannoni USDA-ARS Plant, Soil and Nutrition Lab and Boyce Thompson Institute for Plant Research, Cornell University Campus, Tower Road, Ithaca, NY 14853, USA
Dr Yongfeng Guo Department of Horticulture, G51 Emerson Hall, Cornell University, Ithaca, NY 14853, USA
Dr Jacques Hille Department of Molecular Biology of Plants, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Kerklaan 30, 9751 NN, Haren, The Netherlands
Marianne Hopkins Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1
Dr Stefan Hörtensteiner Institute of Plant Sciences, University of Bern, Altenbergrain 21, CH-3013 Bern, Switzerland
Dr Catherine Howarth Plant Genetics and Breeding Department, Institute of Grassland & Environmental Research, Plas Gogerddan, Aberystwyth, Ceredigion SY23 3EB, Wales, UK

Dr Hai-Chun Jing Wheat Pathogenesis Programme, Plant-Pathogen Interactions Division, Rothamsted Research, Harpenden AL5 2JQ, UK
Dr Hyo Jung Kim Division of Molecular Life Sciences, Pohang University of Science and Technology (POSTECH), Pohang, Kyungbuk 790-784, South Korea
Dr David W. Lee Department of Biological Sciences, Florida International University, Miami, FL 33199, USA
Dr Amnon Lers Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, The Volcani Center, PO Box 6, Bet Dagan 50250, Israel
Dr Pyung Ok Lim Department of Science Education, Cheju National University, Cheju 690-756, Korea
Dr Hong Gil Nam Division of Molecular Life Sciences, Pohang University of Science and Technology (POSTECH), Pohang, Kyungbuk 790-784, South Korea
Linda McNamara Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1
Dr Helen Ougham Plant Genetics and Breeding Department, Institute of Grassland & Environmental Research, Plas Gogerddan, Aberystwyth, Ceredigion SY23 3EB, Wales, UK
Professor Michael S. Reid Department of Plant Sciences, University of California, One Shields Drive, Davis, CA 95616, USA
Jos H.M. Schippers Department of Molecular Biology of Plants, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Kerklaan 30, 9751 NN, Haren, The Netherlands
Catherine Taylor Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1
Professor Dr Howard Thomas Plant Genetics and Breeding Department, Institute of Grassland & Environmental Research, Plas Gogerddan, Aberystwyth, Ceredigion SY23 3EB, Wales, UK
Dr John Thompson Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1
Tzann-Wei Wang Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1
Dr Ulrike Zentgraf ZMBP, General Genetics, University of Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany

Preface

The importance of research into plant senescence cannot be overemphasized. Senescence processes are unique developmental programs that involve unique mechanisms. For example, unlike many other developmental processes in plants that involve cell division, cell differentiation, and/or cell growth (enlargement), leaf senescence is achieved by a massive operation of programmed cell death and nutrient recycling. It is known that new gene expression is required in order for leaf cells to destroy themselves and to recycle nutrients. The cell has to maintain its machinery necessary for new gene expression and nutrient transport while its subcellular structure and macromolecules are being dismantled by some of the new gene products. How gene expression is regulated and how this complex process operates are currently among the most significant biological questions.

Senescence has a tremendous impact on agriculture. Leaves are the primary organs that absorb light energy from the sun and convert it to chemical energy in the form of sugars via photosynthesis. With the onset of senescence, the photosynthetic capability of a leaf declines sharply. Therefore, leaf senescence limits crop yield and biomass production, and contributes substantially to postharvest loss in vegetable and ornamental crops during transportation, storage and on shelves. In addition, proteins, antioxidants and other nutritional compounds are degraded during senescence. Senescing tissues also become more susceptible to pathogen infection, and some of the pathogens may produce toxins, rendering food unsafe. Mitotic senescence may also determine sizes of leaves, fruits and whole plants.

This scientific and economic significance means that much effort has been made to understand the senescence processes in plants and to devise means of manipulating them agriculturally. During the past few years there has been significant progress in this regard, especially in the molecular, genetic and genomic aspects.

This volume summarizes recent progress in the physiology, biochemistry, cell biology, molecular biology, genomics, proteomics, and biotechnology of plant senescence. The term *senescence* has been used by both plant and animal biologists, but it may describe completely different processes. Beginning with senescence-related terminology and our current knowledge of mitotic senescence in plants (a less well-studied area, Chapter 1), the book focuses on post-mitotic senescence, including senescence of leaves (Chapters 2 through 10), flowers (Chapter 11), and fruits (Chapter 12). This research has led to the development of various new biotechnologies for manipulating the senescence processes of fruit (Chapter 12) and leaves (Chapter 13), some of which are approaching commercialization.

Senescence Processes in Plants will be a very useful reference book for senescence researchers based in academia and industry. It may also serve as a textbook for advanced undergraduate students and graduate students.

I would like to thank all the authors for their excellently written chapters and the publishers for their enthusiasm.

Susheng Gan

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1 Mitotic senescence in plants

Susheng Gan

1.1 Introduction

The word *senescence* derives from two Latin words: *senex* and *senescere*. *Senex* means 'old'; this Latin root is shared by 'senile', 'senior', and even 'senate'. In ancient Rome the 'Senatus' was a 'council of elders' that was composed of the heads of patrician families. *Senescere* means 'to grow old'. The Merriam-Webster online dictionary defines *senescence* as 'the state of being old or the process of becoming old'. Aging is also the process of getting older. Therefore, aging has been regarded as a synonym of senescence, and the two words have often been used interchangeably, which, in some cases, is fine but in some other cases causes confusion. This chapter will first briefly discuss the terminology of senescence, and then will review the literature related to mitotic senescence, a topic that has not been well discussed in the plant senescence research area.

1.2 Terminology and types of senescence

Senescence is a universal phenomenon in living organisms, and the word *senescence* has been used by scientists working on a variety of systems, such as yeast, fruit fly, worm, human being and plants. However, the meaning of the word *senescence* to scientists working on different organisms can be different, and the difference can be subtle in some cases and very obvious in some other cases. Here I try to clarify the term at cellular and organismal levels to avoid possible confusion.

At the cellular level, as shown in the cartoon in Figure 1.1, a cell's life history consists of mitotic and postmitotic processes (Gan, 2003). A cell may undergo a certain number of mitotic divisions to produce daughter cells. After a limited number of divisions (e.g. about 40 divisions in human fibroblasts), the cell can no longer divide mitotically. Once a cell ceases mitotic division permanently, it is called mitotic senescence. In the literature concerning yeast, germline cells and mammalian cells in culture, this type of senescence is often referred to as cellular senescence, replicative senescence, proliferative senescence or, sometimes, replicative aging (Sedivy, 1998; Takahashi *et al.*, 2000; Ben-Porath and Weinberg, 2005; Patil *et al.*, 2005). If a cell keeps dividing and fails to undergo mitotic senescence (e.g. cancer cells), it is said to be 'immortalized'. Therefore, mitotic senescence is a mechanism to suppress cancer development. If a cell stops mitosis temporarily due to unfavorable conditions but retains its mitotic capacity and can re-enter mitotic cycles to produce

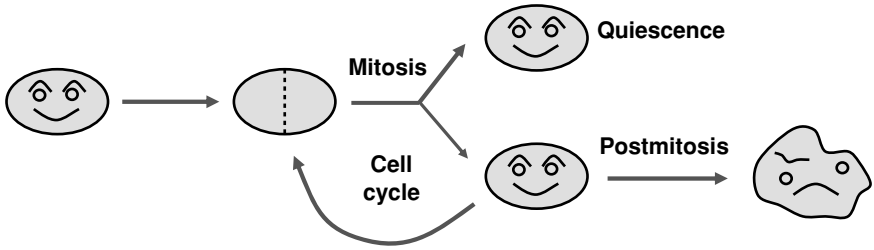

Figure 1.1 Illustration of a cell's life history consisting of both mitotic and postmitotic processes. When the cell stops dividing, it is called mitotic senescence or replicative senescence or proliferative senescence. The active degenerative and attrition process of the cell that can no longer undergo cell division is postmitotic senescence. If a cell stops dividing due to, for example, adverse conditions, but will resume division, the status of the cell is called cell quiescence.

more daughter cells, the temporarily undividing or resting status or process is called cell quiescence (Stuart and Brown, 2006). Although a mitotically senescent cell is not dead it may undergo degenerative process leading to death. If the degeneration is solely a function of age, 'aging' is the right word to describe it. In animal literature, the term 'cell(ular) aging' or 'postmitotic aging', or 'postmitotic senescence' is used for this process. If the degeneration is an active yet quick process, it is a form of 'apoptosis' or 'programmed cell death'. It however should be noted that mitotically senescent mammalian cells in culture are resistant to 'apoptosis'. Most of the postmitotic cells are somatic in nature (e.g. brain, neuron, and muscle cells); the term *somatic senescence* is therefore also used in literature concerning animals. As will be discussed below, postmitotic senescence also occurs in plant somatic tissues such as leaves, flowers and fruits. Compared with postmitotic senescence in animals, leaf/flower/fruit senescence (that involves an active but slow degenerative process) and hypersensitive response (involving an active yet very quick degenerative process) are typical postmitotic senescence processes in plants.

At the organismal level, when an organism's ability to respond to stress declines, its homeostasis becomes increasingly imbalanced, and its risk of disease increases with age, which leads to the ultimate death of the whole organism. This is the aging of the whole organism, and is often referred to as organismal senescence. Although cellular senescence may contribute to organismal senescence (Ben-Porath and Weinberg, 2005), the latter is much more inclusive, for example many age-related diseases, such as Alzheimer's disease, are parts of organismal aging. In literature concerning plants, organismal senescence is senescence of the whole plant. Among the most studied whole plant senescence processes is monocarpic senescence. Annuals (e.g. *Arabidopsis*), biennials (e.g. wheat) and some perennials (e.g. bamboo) possess a monocarpic life pattern, which is characterized by only a single reproductive event in the life cycle. After flowering (and setting seeds or fruits), the whole plant will senesce and die. Monocarpic senescence includes three coordinated processes: (a) senescence of somatic organs and tissues such as leaves (a form of postmitotic senescence, see below), (b) arrest of shoot apical meristems (SAM),

a form of mitotic senescence or proliferative senescence (see below), and (c) permanent suppression of axillary buds to prevent formation of new shoots/branches. This third aspect of whole plant senescence has not received enough attention in the senescence research community.

1.3 Plants exhibit mitotic senescence, postmitotic senescence and cell quiescence

Plants exhibit both types of senescence (Figure 1.2). An example of mitotic senescence in plants is the arrest of apical meristem; the meristem consists of nondifferentiated, germline-like cells that can divide finite times to produce cells that will be then differentiated to form new organs such as leaves and flowers. The arrest of apical meristem is also called proliferative senescence in plant literature (Hensel *et al.*, 1994). This is similar to replicative senescence in yeast and animal cells in culture, as discussed above. Another example of mitotic senescence is the arrest of mitotic cell division at early stages of fruit development. Fruit size is a function of cell number, cell size and intercellular space, and cell number is the major factor. Cell number is determined at the very early stage of fruit development and remains unchanged thereafter (Tanksley, 2004). Postmitotic senescence occurs in some plant organs, such as leaves and floral petals. Once formed, cells in these organs rarely undergo cell division; their growth is mainly contributed by cell expansion; thus, their senescence, unlike mitotic senescence, is not due to an inability to divide. This type of senescence involving predominantly somatic tissues is very similar to that

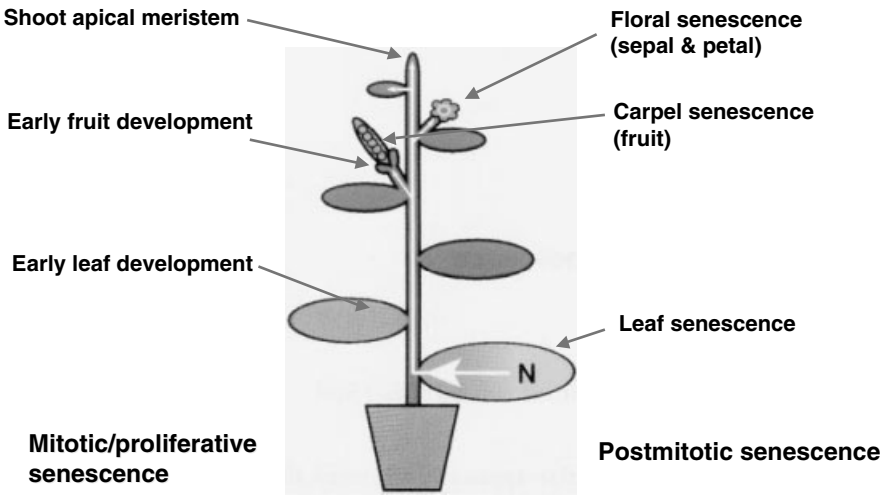

Figure 1.2 Mitotic and postmitotic senescence in plants. Mitotic senescence occurs in SAM, in fruits and leaves that are at very early stages of development. In contrast, postmitotic senescence occurs in leaves, flowers and fruits that are at late stages of development (thus leaf senescence, flower senescence and fruit senescence, respectively).

of such animal model systems as *Drosophila* and *Caenorhabditis elegans* whose adult bodies, with exception of germline, are postmitotic (Gan, 2003).

Cell quiescence also occurs in plants. Cells of apical meristems will stop dividing under unfavorable conditions. For example, the apical meristems of many trees will stop proliferative process when they perceive the short-day photoperiod signal; short day often means that the winter season is coming. These meristem cells retain their division capability during winters and will resume division activity when spring is coming. Therefore, the short-day-induced cell quiescence is an evolutionary fitness strategy. A recent study shows that ethylene and abscisic acid may play a role in regulating the temporary 'arrest' of tree meristem (Ruonala *et al.*, 2006).

1.4 Mitotic senescence: arrest of SAM

1.4.1 Initiation of SAM

SAM is a dome-shaped structure at the tip of a stem that consists of small isodiametric cells with thin-wall and dense protoplasm. It is formed at the globular stage during embryogenesis, and at least three genes, *SHOOT MERISTEM LESS* (*STM*), *CUP-SHAPED COTYLEDONS* (*CUC*)*1* and *CUC2*, are required for SAM initiation, because mutation in *STM* or in both *CUC1* and *CUC2* results in no formation of SAM (Bowman and Eshed, 2000). *STM* encodes a homeodomain transcription factor and *CUC1* and *CUC2* encode duplicated NAC family transcription factors.

1.4.2 Maintenance of SAM

SAM is responsible for generating above-ground postembryonic organs such as leaves and flowers. The SAM cells keep dividing mitotically, and some of their daughter cells undergo differentiation to form various aerial organs while others remain as stem cells that can divide further (Bowman and Eshed, 2000). The balance between the numbers of daughter cells that remain as meristem cells and that undergo differentiation is precisely controlled; if too many daughter cells enter differentiation, the pool of meristem cells will be depleted. Several genes have been shown to regulate this balance. In *Arabidopsis, STM* and *WUSCHEL (WUS,* a gene that also encodes a homeodomain transcription factor) are necessary to keep cells undifferentiated and dividing. Specifically, WUS produces a noncell autonomous signal that activates cell division in combination with STM (Gallois *et al.*, 2002). On the other hand, combined *WUS/STM* functions can initiate the progression from stem cells to organ initiation (Gallois *et al.*, 2002). The balance is also regulated by *CLAVATA* (*CLV*)*1*, *2* and *3*, because mutations in these genes lead to too many cells in the SAM (thus a too big SAM). Therefore, these three genes may inhibit cell division or promote cell differentiation in the SAM. *CLV1* encodes a receptor kinase and *CLV2* a receptor-like protein. *CLV3* encodes a small protein that may act as a ligand for the CLV1/2 receptor heterodimer complex. *STM* and *CLV* may function independently in regulating *SAM*, and *WUS* may act downstream of the

CLV pathway. Recent studies show that a transcription factor complex consisting of C-, D-, and E-type MADS-box proteins controls the stem cell population in the floral meristem (Ferrario *et al.*, 2006). In addition, the homeodomain/leucine zipper transcription factor REVOLUTA (Otsuga *et al.*, 2001) may also control the relative growth of apical (and nonapical) meristems in *Arabidopsis* (Talbert *et al.*, 1995).

1.4.3 Arrest of SAM: a mitotic senescence in nature

After producing certain number of organs (leaves and flowers), the SAM cells cease dividing. The loss of cell division capability of SAM is called the arrest of SAM. The arrest is a proliferative senescence process (Hensel *et al.*, 1994). Figure 1.3 shows an arrested primary inflorescence apex compared with a proliferating one in *Arabidopsis*.

1.4.3.1 Physiological regulation

Reproductive development appears to play an important role in regulating proliferative senescence in plants, which is especially true in many monocarpic plants. Hensel *et al.* (1994) found that meristems of all inflorescence branches in the wild-type *Arabidopsis* ecotype Landsberg *erecta* (L*er*) ceased to produce flowers coordinately, but such a coordinated proliferative arrest did not occur in the wild-type L*er* plants with their fruits surgically removed. Similarly, meristem arrest was not observed in a male-sterile line that never sets seeds. This result suggests that the arrest of inflorescence meristems is regulated by developing fruits/seeds (Hensel *et al.*, 1994). Hensel *et al.* further proposed two models to explain the effect of developing fruits on the mitotic activity of meristems. One model is that a factor necessary for sustaining mitotic activity at the SAM is gradually taken and eventually depleted by developing fruits, resulting in arrest. The other model is that developing fruits produce a negative regulator of mitotic activities, and that the negative regulator is transferred to and accumulated in the SAM to a threshold level so that the SAM is arrested. The factor, either positive or negative, is unknown.

Like postmitotic senescence that is hormonally regulated (Chapter 7), SAM arrest is controlled by plant hormones. It is known that both cytokinins and auxin can promote cell division (Trehin *et al.*, 1998). A mitotic cycle consists of G1 $\rightarrow$ S $\rightarrow$ G2 $\rightarrow$ M (and then back to G1). DNA is synthesized during S while mitosis occurs during M. Tissue culture studies have shown that auxin appears to promote advancement from G1 to S by up-regulating the expression of a cyclin-dependent kinase (CDK) gene. Cytokinins can advance the cycle through M, likely by maintaining cyclin homeostasis (Lee *et al.*, 2006). These data were largely obtained by tissue culture experiments. Whether these mechanisms are involved in the regulation of the cell division in SAM is unknown. In contrast to cytokinins and auxin, mitotic drugs also cause meristem arrest. For example, oryzalin, a chemical that can depolymerize microtubules, can very rapidly lead to meristem cell division arrest in *Arabidopsis* (Grandjean *et al.*, 2004).

In addition, many environmental factors, especially extreme conditions, regulate meristem arrest. For example, broccoli normally develops a ramified inflorescence

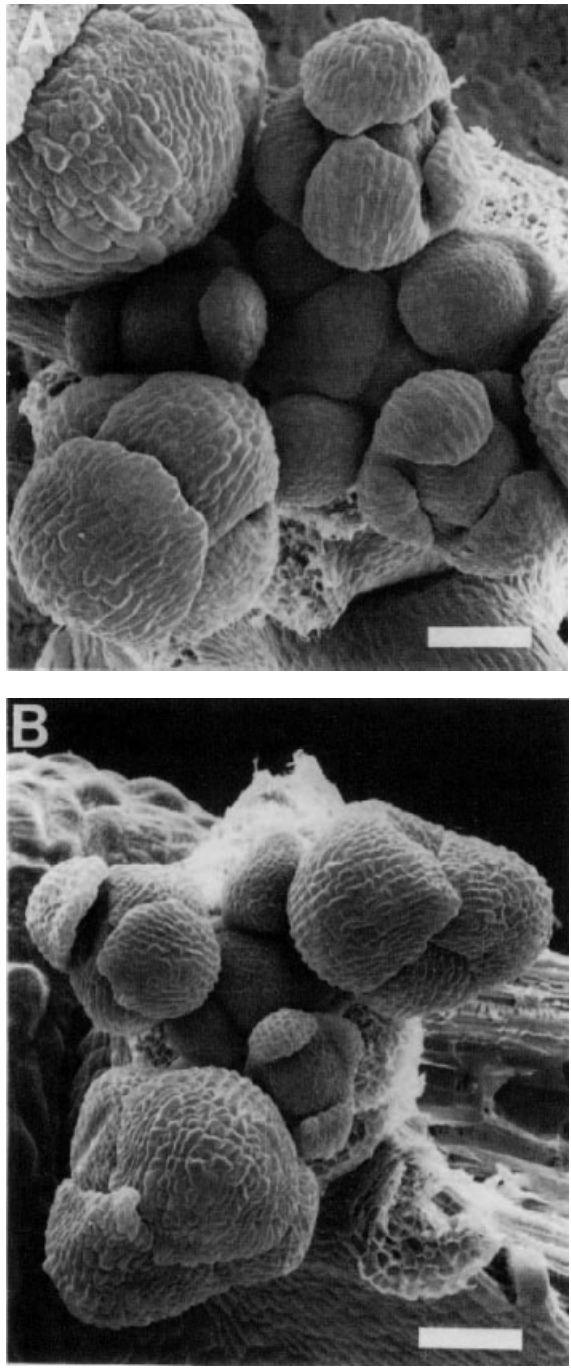

Figure 1.3 An arrested inflorescence apex (B) compared with a proliferating one (A) in *Arabidopsis thaliana* (strain: Landsberg *erecta*) as revealed by scanning electron microscope. (A) An apex 25 days after planting. Note the meristem is actively proliferating and there are nine floral buds at various developmental stages. (B) An apex 48 days after planting. The apex has been arrested for 1 week (Hensel *et al.*, 1994).

without flower bud development. After a certain period, meristems begin to make flower buds instead of more inflorescences. The meristem will be arrested at this transition if the temperature is too high (Bjorkman and Pearson, 1998). The temperature-sensitive arrest of meristem has also been observed in *Arabidopsis* (Pickett *et al.*, 1996).

1.4.3.2 Genetic regulation

In contrast to the initiation and growth, the arrest of apical meristems may be regulated in part by *FIREWORKS* (*FIW*). During a course of screening for mutants that exhibit premature cessation of inflorescence growth in *Arabidopsis*, Nakamura *et al.* (2000) isolated a novel mutant line named *fireworks* (*fiw*) that displayed earlier cessation of flower formation and inflorescence stem elongation. The recessive mutant *fiw/fiw* displayed an inflorescence meristem arrest 7 days earlier than wild-type *Arabidopsis* plants. Otherwise the vegetative growth and development in the mutant line were normal, and the mutant plants produced normal flowers and set fully matured siliques, although the flowers and siliques were clustered at the top of the inflorescence, looking like fireworks (thus so named). The early arrest in the *fiw/fiw* plants occurred globally, not only in the primary inflorescence but also in the lateral inflorescences (Nakamura *et al.*, 2000). In addition to the early mitotic senescence phenotype, the mutant plants also exhibited accelerated rosette leaf senescence (Nakamura *et al.*, 2000), suggesting that FIW may also have a role in regulating postmitotic senescence. The *fiw* mutation was mapped on the lower arm of chromosome 4 but the corresponding gene has not been cloned yet. The cloning and characterization of *FIW* will help us understand how a single gene may control both mitotic and postmitotic senescence.

1.5 Role of telomere and telomerase in mitotic senescence

1.5.1 Telomere

Telomeres are specialized structures consisting of proteins and highly repeated DNA at the ends of the linear eukaryotic chromosomes. The repeated sequences are relatively conserved, for example, the repeated sequence in vertebrates is TTAGGG, but the length of the telomere varies among different species, different individuals, different tissues and even among different chromosomes (Bekaert *et al.*, 2004). In humans, the telomere may be 3–20 kb in length. In yeasts, the repeated sequence is $T_{1-4}G_{1-4}$, not as highly conserved as that of humans. In many higher plants, the repeated sequence is TTTAGGG. Telomeres can serve as caps to prevent chromosomes from fusion with each other.

1.5.2 Telomerase

Chromosomal DNAs are replicated during S phase by DNA polymerases. DNA polymerases move from the 3′ to 5′ direction (polymerizing in the 5′ to 3′ direction),

so at a replication fork there are two new DNA strands: one is the leading strand that will have no problem to replicate the DNA to the end of the template, and the other is the lagging strand. The lagging strand will have problem to replicate the very end of the linear template DNA sequence. Therefore, the DNA sequence at the very end of a chromosome will be lost each time the chromosome is replicated. This is called telomere shortening.

Telomerases are special reverse transcriptases that add telomere DNA to chromosome ends. A telomerase contains both RNA and protein components. The RNA component is approximately 150 nucleotides long and contains about 1.5 copies of a specific telomeric repeat. The RNA component serves as a template to synthesize the corresponding telomeric repeat DNA sequence. In general, germ cells contain high telomerase activity and telomere length in the germ cells is maintained relatively stable because of the telomerase activity. In contrast, somatic cells in animals lack telomerase activity, which prevents somatic cells, such as skin cells, from developing into cancer cells, because the telomeres will be shortened after each division (Bekaert *et al.*, 2004).

1.5.3 Telomere shortening and replicative senescence in animals

In mammalian cells in culture, there is a molecular clock of senescence or aging that counts cell division numbers (Sedivy, 1998; Sherr and DePinho, 2000; Bekaert *et al.*, 2004). The nature of the molecular clock appears to be the telomere shortening. The length and amount of telomeric DNA in human fibroblasts decrease as a function of serial passage (division) during aging *in vitro* and possibly *in vivo* (Harley *et al.*, 1990). When the telomeres become very short, the DNA ends will be open, and the cell will perceive it as damaged DNA, and consequently the senescence process will be triggered. One strong line of evidence that supports this replicative senescence model involves the overexpression of a telomerase (Bodnar *et al.*, 1998). Normal human cells in culture undergo a certain number of mitotic divisions and then start replicative senescence. When the cells overexpressed *hTRT* that encodes the human telomerase catalytic subunit via transfection, the telomeres in these cells were elongated, and the cells kept dividing vigorously even after the control cells had entered nondividing status. The *hTRT*-overexpressing cells had a significantly prolonged replicative life span (Bodnar *et al.*, 1998).

1.5.4 Telomere biology in plants

The telomere length remains constant throughout the life cycle of, for example, *Arabidopsis* and *Silene latifolia* (Riha *et al.*, 1998; Fitzgerald *et al.*, 1999), although the exception has been reported in barley: there is a significant reduction (50 kb) in telomere length during embryogenesis (Kilian *et al.*, 1995). Telomere shortening in the SAM is likely trivial because the meristem cells, like stem cells in animals, possess telomerase activity. On the basis of homology to the human telomerase reverse transcriptase (hTERT), an *Arabidopsis thaliana* cDNA named *AtTERT* was cloned (Fitzgerald *et al.*, 1999; Oguchi *et al.*, 1999). The cDNA contains an open

reading frame of 3372 bp, encoding a protein with a predicted size of 131 kDa and isoelectric point of 9.9. The AtTERT protein contains the conserved reverse transcriptase motifs 1, 2 and A-E as well as the TERT-specific T motif. Reverse transcription polymerase chain reaction analysis and an assay of telomerase activity revealed that both *AtTERT* mRNA and telomerase activity are abundant in the SAM but are not detectable in rosette leaves. However, it should be noted that no detailed analysis of changes in telomere length in young versus senescent SAM has been reported, perhaps due to technical difficulties in collecting enough meristem tissues for analysis.

The cell culture system, like in animals, has been employed for the studies of telomere in plants, although the mitotic senescence process in the cultured plant cells has not been well characterized. Opposite to the situation in cultured animal cells, the telomere length in cultured plant cells does not shorten but increases upon a prolonged culture (Kilian *et al.*, 1995; Riha *et al.*, 1998).

The effect of telomere shortening on plant growth and development has been analyzed in *Arabidopsis* mutant plants in which the telomerase gene was knocked out due to T-DNA insertion. The telomerase-null plants displayed a slow loss of telomeric DNA, ∼500 bp per generation (the *Arabidopsis* telomeres are about 2–5 kb), which is 10 times slower than that observed in telomerase-deficient mice (Fitzgerald *et al.*, 1999). The first several generations of the telomerase-null plants developed normally. The later generations, beginning in the sixth generation, exhibited an extended life span compared with wild-type plants. However, the later generations also displayed some developmental abnormalities including altered phyllotaxy, abnormal leaf shape and reduced fertility (Riha *et al.*, 2001). Therefore, the extended life span might have resulted from reduced fertility; as discussed above, the SAM of male-sterile plants had much longer proliferative longevity (Hensel *et al.*, 1994). The meristems of telomerase-null plants of very late generations were enlarged (however disorganized) and, in some cases, dedifferentiated into a callusoid mass, and failed to produce leaves and/or flowers (Riha *et al.*, 2001). Only a few mutants were able to survive into the ninth generation and none survived later than the tenth generation (Riha *et al.*, 2001) because of genome instability (Siroky *et al.*, 2003; McKnight and Shippen, 2004).

It is therefore unlikely that telomere shortening plays an important role in controlling proliferative senescence in plants (Gan, 2003).

1.6 Closing remarks

The term *senescence* has been used by both plant and animal scientists, but the exact meanings of the term could be different. This chapter tried to clarify the difference. At the cellular level, there are two types of senescence: mitotic and postmitotic senescence. Although plants exhibit both these types of senescence, mitotic or replicative or proliferative senescence in plants has been much less studied than the comparable processes in yeasts, animals, and humans, and postmitotic senescence in plants. Nonetheless, reasonable progresses have been made toward the understanding of

physiological, molecular and genetic mechanisms of mitotic senescence in plants. It is known that many environmental stresses and fruit development can promote mitotic senescence in SAM, and that, unlike in animals, telomere and telomerase play little role in modulating plant mitotic senescence.

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2 Chlorophyll catabolism and leaf coloration

Stefan Hörtensteiner and David W. Lee

2.1 Introduction

Chlorophyll (chl) breakdown is an integral part of the senescence syndrome, characterized by physiological and biochemical changes that aim at the recycling of nutrients from senescing tissues, like leaves and fruits. Thus, worldwide, an estimated 10^9 tons of chl is degraded every year, but the fate of chl was enigmatic for a long time (Hendry *et al.*, 1987). Only 15 years ago, the first final degradation product could be identified as a linear tetrapyrrolic, nonfluorescent chlorophyll catabolite (NCC) (Kräutler *et al.*, 1991), and a pathway involved in the formation of NCCs has been elucidated gradually since then. Most helpful for the elucidation of breakdown intermediates and reactions (Table 2.1) was the availability of stay-green mutants that are affected in chl catabolic steps. Most of the reactions of chl breakdown are now known, and genes for some of the catabolic enzymes have been cloned recently. The current knowledge will be outlined in this review.

Autumnal leaf coloration in deciduous trees is a most spectacular phenomenon that attracts millions of people every year (Hendry *et al.*, 1987). The loss of chl and unmasking of retaining carotenoids together with the new synthesis of anthocyanins represent the biochemical basis of the polychromatic beauty of autumnal leaves. Whereas the chemical structures and the biosynthetic pathways of the involved pigments are rather well established, the biological function of leaf coloration is poorly understood. Several hypotheses have been presented in the literature and will be discussed here.

2.2 Chlorophyll catabolites

2.2.1 Green catabolites

2.2.1.1 Chlorins

Green-colored pigments that are derived from chl have been identified in a number of different species and include chlides, pheides, 13^2-hydroxy chl, pyropheide and pyropheophytin (Schoch *et al.*, 1981; Ziegler *et al.*, 1988). Their importance for a chl degradation pathway that ultimately leads to the disappearance of green color has not been unequivocally established for all of them. Whereas the occurrence of pigments like pheide, chlide and pheophytins well fits the concept of chl breakdown ending in the formation of NCCs (Figure 2.1), colorless derivatives of pyro forms or of 13^2-hydroxylated forms of chl have so far escaped detection. Arguably, the latter

Table 2.1 Overview over chl catabolic enzymes

	Enzyme	Abbreviation	Identification/gene locus in *Arabidopsis*	Localization	Reference
I	Chlorophyll *b* reductase	CBR	Enzyme activity	Plastids, thylakoid	Scheumann *et al.* (1998, 1999)
II	Hydroxychlorophyll *a* reductase	CAR	Enzyme activity	Plastids	Scheumann *et al.* (1998)
III	Chlorophyllase	CLH	*AtCLH1*: At1g19670 *AtCLH2*: At5g43860	Plastids, vacuole?	Tsuchiya *et al.* (1999)
IV	Mg-dechelatase (metal-chelating substance)	MCS	Enzyme activity	Plastids	Suzuki and Shioi (2002)
V	Pheophorbide *a* oxygenase	PAO	At3g44880	Plastids, inner envelope	Pružinská *et al.* (2003)
VI	Red chlorophyll catabolite reductase	RCCR	At4g37000	Plastids, stroma	Wüthrich *et al.* (2000)
VII	Catabolite exporter (ATP-hydrolyzing)		Activity	Plastids, envelope	Matile *et al.* (1992)
VIII	13^2-Demethylase (pheophorbidase)	—	Enzyme activity	Cytosol?	Suzuki *et al.* (2002)
IX	$C8^2$ Hydroxylase	—	—	—	
X	C3 Hydroxylase	—	—	—	
XI	Glucosyltransferase	—	—	—	
XII	Malonyltransferase	—	Enzyme activity	Cytosol	Hörtensteiner (1998)
XIII	ABC transporter	—	*AtMRP2*: At2g34660 *AtMRP3*: At3g13080	Tonoplast	Lu *et al.* (1998); Tommasini *et al.* (1998)