Arabidopsis CPR5 is a senescence-regulatory gene with pleiotropic functions as predicted by the evolutionary theory of senescence

Hai-Chun Jing1,2, Lisa Anderson3, Marcel J.G. Sturre1, Jacques Hille1 and Paul P. Dijkwel1,*

1 Molecular Biology of Plants, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Kerklaan 30, 9751 NN, Haren, The Netherlands
2 Wheat Pathogenesis Programme, Rothamsted Research, Plant–Pathogen Interaction Division, Harpenden, Herts AL5 2JQ, UK
3 Developmental, Cell, and Molecular Biology Group, Department of Biology, Duke University, Durham, NC 27708-1000, USA

Received 5 July 2007; Revised 30 August 2007; Accepted 31 August 2007

Abstract

Evolutionary theories of senescence predict that genes with pleiotropic functions are important for senescence regulation. In plants there is no direct molecular genetic test for the existence of such senescence-regulatory genes. Arabidopsis cpr5 mutants exhibit multiple phenotypes including hypersensitivity to various signalling molecules, constitutive expression of pathogen-related genes, abnormal trichome development, spontaneous lesion formation, and accelerated leaf senescence. These indicate that CPR5 is a beneficial gene which controls multiple facets of the Arabidopsis life cycle. Ectopic expression of CPR5 restored all the mutant phenotypes. However, in transgenic plants with increased CPR5 transcripts, accelerated leaf senescence was observed in detached leaves and at late development around 50 d after germination, as illustrated by the earlier onset of senescence-associated physiological and molecular markers. Thus, CPR5 has early-life beneficial effects by repressing cell death and insuring normal plant development, but late-life deleterious effects by promoting developmental senescence. As such, CPR5 appears to function as a typical senescence-regulatory gene as predicted by the evolutionary theories of senescence.

Key words: Arabidopsis, cell death, CPR5/OLD1, evolutionary senescence, hormones, leaf senescence.

Introduction

In animal and evolutionary biology, senescence is defined as a decline in age-specific fitness components due to internal physiological deterioration (Rose, 1991). Studies on evolutionary senescence aim to address why the mortality rates of individuals increase with advancing age and vary within populations and among species. Based on research, mainly in the animal field, two major theories of evolutionary senescence have been developed and are widely acknowledged (Kirkwood and Austad, 2000). The Antagonistic Pleiotropy Theory points out that evolution acts to maximize reproductive fitness and will allow the existence of mutations that have beneficial effects for early-life survival and reproduction despite the fact that these mutations may have deleterious late-life effects to promote senescence. The Mutation Accumulation Theory is based on the observation that the force of natural selection diminishes with age and predicts that a mutation with deleterious late-life effects which lead to senescence is acceptable if it will allow the carrier to reproduce before death. These two theories suggest that two classes of mutations are responsible for senescence: those with beneficial early-life effects but deleterious late-life effects; and late-acting mutations with purely deleterious effects (Kirkwood and Austad, 2000).

In yeast and animal ageing paradigms, both types of gene action have been validated and genes involved in the insulin/IGF (insulin growth factor)-1 signalling, metabolic...
flux, and resistance to oxidative stress have been shown to be the important players for lifespan regulation (Sgro and Partridge, 1999; Guarente and Kenyon, 2000; Gems and Partridge, 2001; Kenyon, 2001; Arantes-Oliveira et al., 2002; Biesalski, 2002; Hughes et al., 2002; Tatar et al., 2003). In plants, the term senescence is prevalently used in a physiological context to describe a genetically controlled developmental programme that leads to the death of plant cells, tissues, organs, and whole plants. There is a debate as to whether studies on leaf senescence can validate the evolutionary theories of senescence in plants (Thomas, 2002). Senescence in the evolutionary sense is based on studies on individuals at the population and species levels. One doubt is whether this definition can be ‘scaled down’ to individual leaves. However, it has been argued that leaves have a clear lifespan and demographic features, and hence can be viewed as cohorts in a population (Bleecker, 1998). Furthermore, leaf senescence is marked by the massive mobilization and recycling of the assimilated nutrients in the senescing leaf, and hence considered to be essential for ensuring survivability of a species (Buchanan-Wollaston et al., 2003; Hopkins et al., 2007). Due to such a strong adaptive advantage, leaf senescence appears to define the evolutionary theories of senescence that occurs in the absence of natural selection and is non-adaptive. This conflict might be reconciled by considering leaf senescence as a deleterious consequence of the selection for the traits that enable nutrient mobilization for the success of reproduction (Bleecker, 1998; Schippers et al., 2007).

A direct test of the evolutionary theories of senescence in plants is to characterize the functionality of senescence-regulatory genes. In an effort to isolate Arabidopsis mutants that exhibit altered ethylene (ET)-induced leaf senescence, old1 mutants were obtained, which have two distinct alterations: accelerated age-regulated senescence and an enhanced ET response (Jing et al., 2002, 2005). Subsequent map-based cloning showed that old1 is allelic to cpr5, which has been recovered in screens for mutants with enhanced pathogen defence responses (Bowling et al., 1997), abnormal trichome development (Kirik et al., 2001), and dark-induced leaf senescence (Yoshida et al., 2002). Thus, CPR5 appears to be involved in multiple processes of plant growth and development and responses to biotic and abiotic stresses. The pleiotropic effects of CPR5 make it an ideal candidate to test whether this gene is a senescence-regulatory gene as predicted by the evolutionary theories of senescence. Taking advantage of the availability of multiple cpr5 mutants from different genetic backgrounds, cpr5-induced phenotypes were explored in detail. Transgenic plants with increased CPR5 mRNA levels were constructed to examine the effects of CPR5 during late life, especially during plant senescence. It was shown that CPR5 ensures normal growth and development by controlling hormonal signalling and by repressing cell death in early plant life. However, CPR5 can promote leaf senescence in late development. These results are consistent with the notion that CPR5 may be a senescence-regulatory gene as predicted by the Antagonistic Pleiotropy Theory of Evolutionary Senescence.

Materials and methods

Plant materials and growth conditions

Arabidopsis thaliana accessions Ler-0 and Col-0 were the wild types. The mutant alleles and transgenic plants used were old1-1 (renamed cpr5-11 in this paper) (Jing et al., 2002), cpr5-12, cpr5-13, cpr5-1 (Bowling et al., 1997), cpr5-2 (Boch et al., 1998), hysl-1 (Yoshida et al., 2002), ein2-1 (Guzman and Ecker, 1990), ctrl-1 (Kieber et al., 1993), jar1-1 (Staswick et al., 1992), and abi4-1 (Finkelstein et al., 1998). In order to compare the effect of different cpr5 mutations on various phenotypes, the cpr5-1 allele was crossed with Col-0, and cpr5-1/C was selected from an F2 population and thus contains a mixed Ler-0 and Col-0 background.

Plants were grown in an organic-rich soil (TULIP PROFI No. 4, BOGRO B.V., Hardenberg, The Netherlands) or in Murashige and Skoog (MS) medium containing 0.8% agar under the conditions described by Jing et al. (2002).

Map-based cloning, complementation test, and construction of transgenic lines

cpr5-11 was originally placed ~3 cM south of the single nucleotide polymorphism (SNP) marker SGCSNP84 at the bottom of chromosome 5. To perform fine mapping, 2000 F2 cpr5-11 seedlings were selected from a mapping population generated by crossing cpr5-11 with Col-0. DNA was isolated using the SHORTY quick preparation method (http://www.biotech.wisc.edu/Arabidopsis). By comparing the genomes of Col-0 (TAIR database) and Cereon Ler-0 (Monsanto SNPs and Ler) (http://arabidopsis.org; Jander et al., 2002), potential SNPs were selected. Primers were designed, using the WebSNAPER program, that specifically amplified Col-0 DNA fragments, and used for PCR (Drenkard et al., 2000; http://ausubellab.mgh.harvard.edu/resources). The mutation was mapped onto a 15 kb region spanning three open reading frames including CPR5. Sequence analyses revealed a single nucleotide change inside CPR5. The other two Ler-0 cpr5 alleles were subsequently sequenced (Table 1). Agrobacterium-mediated transformation was performed to confirm further the identity of old1 as a cpr5 allele.

For constructing CPR5 transgenic plants, full-length CPR5 cDNA was amplified using primers designed either with or without an in-frame fusion of the HA epitope tag (YPYDVPDYA) and cloned behind a modified 35S CaMV promoter in the plant transformation vector pHBl-4T. All constructs were verified by sequencing and subsequently electroporated into Agrobacterium tumefaciens strain GV3101. The resulting bacteria were used to transform wild-type Col-0 (Clough and Bent, 1998). Transformants were selected on MS media containing 50 µg ml−1 kanamycin.

Table 1. cpr5 alleles identified or studied in this paper

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Accession</th>
<th>Nucleotide change</th>
<th>AA change</th>
</tr>
</thead>
<tbody>
<tr>
<td>cpr5-11</td>
<td>Ler-0</td>
<td>GGT to AGT</td>
<td>459G to S</td>
</tr>
<tr>
<td>cpr5-12</td>
<td>Ler-0</td>
<td>TGG to TGA</td>
<td>391W to Stop</td>
</tr>
<tr>
<td>cpr5-13</td>
<td>Ler-0</td>
<td>GGG to GAG</td>
<td>120F to D</td>
</tr>
<tr>
<td>cpr5-2</td>
<td>Col-0</td>
<td>TGG to TAG</td>
<td>473W to Stop</td>
</tr>
<tr>
<td>hysl-1</td>
<td>Col-0</td>
<td>TGG to TGA</td>
<td>477W to Stop</td>
</tr>
</tbody>
</table>
Hormonal sensitivity assay
For ET sensitivity, seedlings were grown on MS media containing 1 \textmu M ACC (1-amino cyclopropane-1-carboxylic acid) in the dark for 5 d, and the triple response was observed (Guzman and Ecker, 1990). Sugar sensitivity was determined by growth for 5 d in darkness on MS medium containing 1% sucrose. The hypocotyl lengths of the seedlings were subsequently compared (Dijkstra et al., 1997). The effect of JA on the inhibition of root elongation of light-grown seedlings was examined as described (Staswick et al., 1992). Briefly, seeds were germinated in light in vertical plates containing MS medium, 0.5% sucrose, and 20 \textmu M MeJA, and the root elongation of 7-d-old light-grown seedlings was examined. A low concentration of sucrose (0.5%) was used to minimize the inhibiting effect of sugar. For ABA sensitivity, seeds were germinated in light on vertical plates containing MS medium, 0.5% sucrose, and 0.3 \textmu M ABA. The growth of 7-d-old light-grown seedlings was examined. Seedlings and detached leaves were incubated in a growth chamber of 22 °C and, when grown in light, the intensity was ~60 \textmu mol m^{-2} s^{-1}.

Chlorophyll content measurement and gene expression analysis
For chlorophyll content measurement and northern blotting, rosette leaf samples were collected from 30-d-old soil-grown Arabidopsis plants. For northern blotting, whole rosettes were used. Leaf samples were prepared and analysed as described by Jing et al. (2002).

For real-time PCR measurements of CPR5 mRNA levels, approximately 150 mg of tissue was harvested from 3-week-old soil-grown wild-type Col-0, mutant cpr5-1, and wild-type plants overexpressing CPR5 cDNA (C5-7, C4-3, and C8-6) or HA-tagged CPR5 cDNA (N5-6). Subsequently, total RNA was extracted as described by Cao et al. (1997). Ten micrograms of RNA was treated with DNase I according to the manufacturer’s instructions (Ambion Inc., Austin, TX, USA). One microgram of RNA was incubated with Superscript II reverse transcriptase and ligo(dT) in a 20 \textmu l reaction to synthesize cDNA (Invitrogen, Carlsbad, CA, USA). For the quantitative PCR, 2 \textmu l of the cDNA product was used as template with the CPR5-specific primers, whereas 2 \textmu l of a 20-fold dilution was used for reactions with Ubiqutin5 (UBQ5, At3g62250.1)-specific primers. The final primer concentration in all reactions was 0.5 \textmu M. Quantitative PCR was carried out using the SYBR green PCR kit (QIAGEN, Valencia, CA, USA) and a Roche Lightcycler real-time PCR machine according to the manufacturer’s instructions (Roche, Mannheim, Germany). The relative number of CPR5-specific transcripts was determined in three replicate experiments by normalization to UBQ transcript levels.

HPLC-MS (high pressure liquid chromatograph–mass spectrometry) analyses of salicylic acid (SA) and jasmonic acid (JA)
Rosette leaves numbers 3 and 4 without any signs of visible yellowing were taken from 21-d-old soil-grown plants and used to measure the SA and JA concentrations according to a procedure derived from Wilbert et al. (1998). Briefly, ~200 mg of leaf tissues were ground in liquid nitrogen into fine powder and extracted with 500 \textmu l of acidified MeOH (methanol with 0.1% concentrated HCl) overnight at 4 °C. After centrifugation, the supernatant was collected, diluted to 35% with water, and centrifuged before injecting 100 \textmu l of it into HPLC coupled on-line with a mass spectrometer for quantification. The injection was done with a PerkinElmer series 200 autosampler. Before and after injection the injector and the needle were flushed twice with 0.1% NH4OH in 50% MeOH to remove the residual SA or JA. MeOH (gradient grade), formic acid (p.a., 98–100%) and ammonia solution (p.a., 25%) were purchased from Merck, Darmstadt.

For HPLC, both JA and SA were negatively charged by post-column addition of 1% NH4OH solution in MeOH with a flow of 100 \textmu l min^{-1}, delivered by a Kratos spectroflow 400-pump. JA and SA were separated under acidic conditions by running a gradient of aqueous 0.1% formic acid and 0.1% formic acid in MeOH over a 2.1 mm column (Alltech Alltima C18 5\textmu m). The gradient was delivered by two Perkin-Elmer series 200 LC pumps at a flow rate of 200 \textmu l min^{-1}, started with 30% MeOH for 1 min, raised to 95% in 5 min, and retained for 5 min, then dropped to 30% in 2 min. A 6 min interval was used for equilibration.

For mass spectrometry, the free acids JA and SA were analysed in the negative ion-mode by measuring a small range in a Q1-profile-scan-mode combined with ‘up-front’ collision to see the M-44-ion of SA (loss of CO2) and to avoid association of the formate-ion with JA (M+45). The M-44-ion of JA was not observed. The MS system consisted of an API3000 mass spectrometer (Applied Biosystems/MDS-SCIEX, Toronto, Canada) and a triple quadrupole mass spectrometer equipped with a Turbo Ion-spray interface. The 200 \textmu l min^{-1} HPLC flow, combined with a 100 \textmu l min^{-1} post-column flow, were introduced through the ion-spray interface with the temperature of the heater set to 450 °C. The state file was as follows: NEB (zero air)¼14, CUR¼14, IS¼-4500, TEM¼450, OR¼-50, RNG¼-200, QO¼11, IQ1¼11, IQ2¼20, RO2¼100, St3¼120, RO3¼102, DF¼300, CEM¼2500. For SA, the range of 136–139 amu with the same step-size and IQ2¼25, RO2¼102, Q0¼11, St3¼120, RO3¼102, DF¼300, CEM¼2500. For JA, the range of 208–211 amu with a step-size of 0.100 amu and a dwell-time of 1 ms was analysed. The molecular weight of SA is 138. The M-1-ion is m/z 137 and the M-1-44 is m/z 93. For JA, the range of 208–211 amu with the same step-size and dwell-time was analysed. The molecular weight of JA is 210. The M-1-ion is m/z 209. SIM was avoided to double-check the isotope patterns of the free acids. Due to the interference of many unknown products, slight shifting of the retention time for the same ions was observed. The area under the ion-signals was calculated with MacQuan 1.7 (PE SCIEX). To confirm the authenticity, SA and JA standards were added to the plant extracts as controls.

Results
Identification of Ler-0 alleles of cpr5
Three old1 alleles were isolated in a screen for leaf senescence mutants that showed accelerated visible yellowing upon exposure to 10 \textmu l l^{-1} ET for 3 d; the details of this screen were reported previously (Jing et al., 2002). The OLD1 gene was cloned by map-based cloning as described in Materials and methods, and complementation tests showed that old1 mutants are allelic to cpr5 (data not shown). Therefore, the old1-1, old1-2, and old1-3 alleles were renamed as cpr5-11, cpr5-12, and cpr5-13, respectively (Table 1).

Mutations in CPR5 induce defects in multiple developmental processes
The responses of cpr5 mutants to multiple stimuli involved in plant growth and development and stress responses such as hormones and sugar were examined.
To examine the potential effects of different genetic backgrounds on cpr5 mutations, the cpr5-l1/C line was generated as described in Materials and methods. Thus, the cpr5-l1/C line contains the cpr5-l1 mutation with a mixed Col-0/Ler background. Regardless of genetic background and the nature of mutations, all the examined cpr5 mutants are hypersensitive to ET and sugar (Fig. 1A–C), which is consistent with previous reports (Jing et al., 2002; Yoshida et al., 2002). The root growth of cpr5 seedlings was strongly inhibited in media containing low levels of exogenously applied ABA and MeJA (Fig. 1D, E). Thus, cpr5 mutants exhibit enhanced responses to ET, sugar, JA, and ABA, indicating that CPR5 is involved in seedling growth and development by controlling responses to multiple signalling pathways.

It was reported previously that SA accumulates to a substantially high level in cpr5 mutants (Bowling et al., 1997; Clarke et al., 2000, 2001; Jirage et al., 2001). The endogenous levels of several hormones in cpr5 mutants were then quantified to test the hypothesis that the altered responses may be associated with the alteration of hormonal production. Figure 2 shows that besides SA, the cpr5 mutants had increased JA levels as well. The ET production was also measured in etiolated cpr5 mutants and the results showed that cpr5 mutants had a wild-type ET production level (data not shown). Thus, cpr5 mutations impose different effects on SA, JA, and ET production in Arabidopsis.

Mutations in CPR5 also cause precocious cell death in the form of spontaneous lesions and senescence (Fig. 3A). This was reflected by decreases in leaf chlorophyll contents (Fig. 3B). Analyses of the expression profiles of senescence-associated genes showed that cpr5 mutants similarly exhibited earlier and higher transcription levels of SAG12, SAG13, SAG14, and SAG21 in comparison with wild-type plants. PR-1 mRNA levels were also higher in cpr5 mutants (Fig. 3C). Thus, mutations in CPR5 result in precocious developmental cell death.
Overexpression of CPR5 restores all the mutant phenotypes

Utilizing the transcriptome data on the Genevestigator website (Zimmermann et al., 2004), the transcription profiles of CPR5 during development were examined. As shown in Fig. 4, CPR5 is constitutively expressed during the Arabidopsis life cycle. The CPR5 transcripts increase towards the end of development at age 45–50, which is characteristic of senescence-associated genes. Interestingly, the transcripts of two classical SAGs, SAG12 and SAG13, increase two to three developmental stages earlier than CPR5, indicating that the induction of SAG12 and SAG13 expression during senescence may not be associated with the induction of CPR5 expression.

The expression pattern of CPR5 indicates that it may have a role in promoting senescence during late development only, while its function in the regulation of the onset of senescence during early development may be limited. To test this hypothesis, transgenic plants expressing various amounts of CPR5 transcripts were constructed as described in Materials and methods. Four representative transgenic lines were used in this study. Quantitative PCR measurements indicated that lines C5-7, C4-3, and N5-6 had higher than wild-type CPR5 mRNA levels, whereas line C8-6 and cpr5-1 mutants had lower transcript levels (Fig. 6B). As shown in Fig. 5, the C8-6 plants displayed all the cpr5-mutant phenotypes such as enhanced hormonal sensitivity, reduced leaf and plant size, and abnormal trichome development, consistent with knocked-down CPR5 transcription. By contrast, in the C5-7, C4-3, and N5-6 plants, cpr5 mutant phenotypes were not observed. Thus, overexpression of CPR5 does not affect early seedling and plant development.

CPR5 overexpression accelerates senescence at late development

Senescence phenotypes were examined in the transgenic lines and in the cpr5-1 mutant. Leaf senescence was induced by placing detached leaves in continuous light for 7 d. Figure 6A shows the results of the detachment-induced senescence experiment. As would be expected, the cpr5-1 mutant and line C8-6 contained less chlorophyll in comparison with the wild type. The chlorophyll contents in the overexpression lines C5-7, C4-3, and N5-6 were lower than that of the wild type, but were higher than those of the cpr5-1 mutant and C8-6 plants. Similar results were obtained when the detached leaves were incubated with JA or ABA (data not shown). Thus, leaves of the transgenic lines exhibited accelerated drops in chlorophyll content upon detachment, demonstrating that...
increasing CPR5 transcripts promote senescence in detached leaves.

Developmental senescence was further characterized. At ~40 d after germination visible yellowing was observed both in the wild type and the transgenic lines (Fig. 6C), but chlorosis proceeded faster and occurred in more leaves in the overexpression lines (Fig. 6D). The correlation between CPR5 expression and the advanced senescence syndrome was studied further using molecular markers (Fig. 6E). RNA gel blot analysis showed that at this developmental stage, the CPR5 mRNA level was highest in C5-7, slightly lower in N5-6, and comparable with the wild type in C4-3. There was a good correlation between the CPR5 and SAG12 mRNA levels; the low CPR5 levels in cpr5-1 and C8-6 correlated with appreciable SAG12 expression, whereas high CPR5 transcript levels in C5-7 and N5-6 correlated with increased SAG12 mRNA levels. SAG12 mRNA was not detected in the wild-type Col-0 tissue. The results suggest that increasing CPR5 levels cause accelerated senescence at late developmental stages. The general defense response marker PR-1 mRNA levels were also enhanced in the transgenic lines and the cpr5-1 mutant.

Taken together, plants with reduced CPR5 mRNA levels had phenotypes similar to cpr5 mutants, whereas CPR5 overexpression only caused early leaf senescence during later stages of plant development.

Discussion

It has been argued that a plant or animal genome is optimized for early survival and reproduction and that senescence is a consequence of such genome optimization (de Magalhaes and Church, 2005; Schippers et al., 2007). At biochemical and molecular levels leaf senescence resembles animal ageing in various aspects. In most cases the strategies used by plants to regulate senescence are similar to those in animals (Gan, 2003; Jing et al., 2003; Lim et al., 2003). Leaf senescence is marked by changes in gene expression profiles. Many senescence-associated genes (SAGs) have been isolated and shown to include genes involved in protein and lipid degradation, transport, cellular stress- and defence-related responses, transcriptional regulation, and signalling pathways (Buchanan-Wollaston, 1997; Nam, 1997; Quirino et al., 2000; Chen et al., 2002; Buchanan-Wollaston et al., 2003). In ageing yeast and animals, similar groups of genes displayed such senescence-associated changes in their expression profiles (Zou et al., 2000; Weindruch et al., 2001; Pletcher et al., 2002; Kyng et al., 2003). Thus, similar molecular and cellular processes may take place during leaf senescence and animal ageing, which argues that genes regulating leaf senescence and animal ageing may be similar in nature.

Here this issue is addressed by studying the functions of CPR5 during development. The results show that CPR5 is a senescence-regulatory gene with pleiotropic functions as predicted by the evolutionary theories of senescence, which were developed from studying animal ageing.

**CPR5 has early-life beneficial effects**

The cpr5 mutants have many phenotypes and were recovered in several mutant screens, indicative of the involvement of CPR5 in various aspects of the plant life cycle. The beneficial effects of CPR5 are illustrated by the following observations. (i) Seedlings carrying cpr5 alleles were found to be hypersensitive to ET, sugar, JA, and ABA responses (Jing et al., 2002; Yoshida et al., 2002;
Fig. 5. Characterization of hormonal sensitivity and growth of CPR5 transgenic lines, cpr5 mutants, and wild type. (A–F) The first eight seedlings are Ler-0, cpr5-l1, Col-0, cpr5-1, C5-7, C8-6, C4-3, and N5-6, and the rest are ctr1-1, ein2, and abi4-1 (A), ctr1-1 and ein2 (B), abi4-1 (C), jar1-1 and abi4-1 (D), jar1-1 (E), and abi4-1 (F). Seedlings were grown on plates containing the components indicated in darkness for 5 d (A–C) or in light for 7 d (D–F). (G) Representative mature leaves from the lines indicated. White bars represent 0.5 cm.

Fig. 6. Characterization of the senescence syndrome in cpr5 mutants, CPR5 transgenic lines, and the wild type. (A) Comparison of detachment-induced senescence between wild type, cpr5-1 mutants, and Arabidopsis transgenic lines with varied CPR5 mRNA levels. Shown are the chlorophyll contents of the first and second pairs of rosette leaves detached from 21-d-old soil-grown plants. Leaves were incubated in light on two layers of Whatman filter papers saturated with MES solution (pH 5.7) for 7 d and collected for chlorophyll content measurement. Four replicates of two pairs of leaves were analysed for each line. Results are shown as mean ± SD. (B) Quantification of CPR5 mRNA levels of Arabidopsis transgenic lines, the cpr5-1 mutant, and the wild type. The abundance of CPR5 relative to ubiquitin (UBQ) mRNA, expressed as mean ± SD, is shown. (C, D) Comparison of in planta senescence of CPR5 transgenic lines, the cpr5-1 mutant, and the wild type. Plants were grown on soil for 50 d and the number of yellow leaves was recorded as described in Materials and methods. (C) Representative plants whose inflorescence stems were removed are shown. (D) The results of visible yellowing quantification that are presented as mean ± SD from observations on at least 10 plants per line are shown. (E) RNA gel blot analysis showing the abundance of the mRNA of various genes in CPR5 transgenic lines, the cpr5-1 mutant, and the wild type. Rosette leaves 1–6 were harvested from 50-d-old soil-grown plants for total RNA isolation, and 10 μg of total RNA was used for RNA gel blot analysis. 32P-labelled probes were used. The rRNA picture is shown as a loading control.
this study). These signalling molecules all contribute to seedling growth and development in various ways (Creelman and Mullet, 1997; Johnson and Ecker, 1999; Rolland et al., 2002). Thus, it appears that CPR5 controls normal seedling growth and development by mediating responses to multiple hormonal and sugar signalling components. (ii) Adult cpr5 mutants have a stunted plant status and reduced trichome development. The cpr5 mutants also produce fewer seeds and a smaller rosette under various conditions (Heidel et al., 2004). Thus, cpr5 mutations reduce the fitness of adult plants. (iii) Overexpression of CPR5 restored all the phenotypes induced by cpr5 mutations, and transgenic lines with increased CPR5 transcripts displayed normal growth and development. The reduced growth and development of adult cpr5 plants could be caused by a number of factors. They could be due to the enhanced responses to the aforementioned multiple signalling molecules and/or elevated SA and JA levels. They could also result from the occurrence of precocious cell death as envisaged by lesion formation and accelerated senescence. Whatever the reasons are, CPR5 appears to be essential for Arabidopsis to develop into a normal adult plant. These observations clearly demonstrate that CPR5 has predominant early-life beneficial effects.

CPR5 has late-life deleterious effects

CPR5 transcripts increase during late developmental stages, which is consistent with a role in promoting senescence. This is in agreement with observations that CPR5 levels increased in mature leaves of transgenic plants harbouring CPR5 promoter-GUS reporter constructs (H-C Jing et al., unpublished results). Construction and study of CPR5 overexpression lines provides important clues to the function of CPR5 during late Arabidopsis life. The examined transgenic lines with constitutively enhanced CPR5 transcripts exhibited accelerated leaf senescence, both upon detachment and in planta. This demonstrates that CPR5 has late-life deleterious effects. It is important to note that the timing of senescence in CPR5 overexpression lines is different from that of cpr5 mutants. In CPR5 transgenic lines, senescence only occurs after reproduction (bolting), whereas in cpr5 mutants senescence starts in young plants before reproduction. In this sense, CPR5 represses cell death before reproduction and promotes cell death after reproduction.

Evolution of senescence-regulatory genes in plants

The results obtained in this study showed that CPR5 differentially exerts its functions during Arabidopsis growth and development. CPR5 exhibits early-life beneficial effects by ensuring normal seedling growth and repressing cell death in adult plants. However, after reproduction a functional CPR5 may promote ‘normal’ senescence and hence is deleterious. Such a separation of the functions of CPR5 throughout development mimics the action of the insulin/IGF-1 signalling pathway and p53 in animal and human cells (Zafon, 2007). The insulin/IGF-1 signalling pathway has pleiotropic functions and is shown to employ independent mechanisms to regulate lifespan and reproduction (Dillin et al., 2002). p53 is a genome guardian; the deficiency of p53 proteins leads to cancer and tumour development due to increased cellular damage, suggesting that p53 has clear early-life beneficial effects (Levine, 1997; Sharpless and DePinho, 2002). Nonetheless, a p53 mutant mouse line (p53+/−), in which the stability and activities of the wild-type p53 protein were augmented in the presence of a mutant allele, developed fewer tumours than wild-type (p53+/+) homozygotes, but exhibited faster ageing (Tyner et al., 2002). Clearly, maintaining a higher p53 level in late life is deleterious. Thus, CPR5 seems to function as a typical senescence-regulatory gene as predicted by the evolutionary theory of senescence. However, at the DNA and protein levels, CPR5 shares no similarities with any genes in the insulin/IGF-1 signalling pathway, or p53, in agreement with the notion that, although plants may use similar strategies to control senescence, the particular molecular mechanisms can be different (Jing et al., 2003). Further molecular genetic and biochemical studies that unravel how CPR5 works in a plant cell will allow a better comparison of senescence-regulatory mechanisms across kingdoms.

So far, the cloned senescence-regulatory genes in plants share a common feature, which is that they are involved in plant growth and development as well. For instance, ORE9 encodes an F-box protein and is part of the ubiquitination protein degradation machinery (Woo et al., 2001). ore9/max2 alleles were also recovered in a screen for mutants with altered shoot lateral branching (Stirnberg et al., 2002). SAG101 was shown to be involved in lipid metabolism (He and Gan, 2002), and ORE4 encodes the plastid ribosomal small subunit protein 17 (PRPS17) that is important for protein synthesis (Woo et al., 2002). Furthermore, a recent characterization of the SGR gene in rice indicates the possible existence of genes with deleterious effects late in life (Park et al., 2007). However, it is not clear whether these genes are the sensu stricto senescence-regulatory genes as defined by the evolutionary theories of senescence. It is hoped the study presented here will stimulate more research to address this issue.

Acknowledgements

We thank Bert Venema and Annemiek Loos for their technical support. We thank Margot C Jeronimus-Strating and Andries Bruins from of MS for HPLC-MS quantification of SA and JA, and the laboratory of Dr Paul E Staswick at the University of Nebraska for the information on the jar1-1 mutation. Several mutants were obtained from NASC and ABRC.