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Epigenetic and small RNA regulation of senescence

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Abstract Leaf senescence is regulated through a complex regulatory network triggered by internal and external signals for the reprogramming of gene expression. In plants, the major developmental phase transitions and stress responses are under epigenetic control. In this review, the underlying molecular mechanisms are briefly discussed and evidence is shown that epigenetic processes are also involved in the regulation of leaf senescence. Changes in the chromatin structure during senescence, differential histone modifications determining active and inactive sites at senescence-associated genes and DNA methylation are addressed. In addition, the role of small RNAs in senescence regulation is discussed.

Keywords Chromatin · DNA-methylation · Epigenetics · Histone modifications · Leaf senescence · Small RNAs

Senescence, triggered by internal and external signals, requires complex regulation

Senescence, the last step in leaf development, is characterised by degradation and recycling processes. Photosynthesis is reduced and chloroplasts are decomposed. Chloroplast proteins and other biomolecules are catabolised into transport forms, which are exported to the growing parts of the plant. The switch from a mature, photosynthetically active leaf to a senescing leaf is a major phase transition in plant development, consistent with substantial changes in the structure and functions of the leaf cells. This complex phase transition requires the massive reprogramming of gene expression. We now know that the senescence programme involves the regulation of thousands of genes, which are, when up-regulated, called SAGs (senescence-associated genes), and when down-regulated, referred to as SDGs (senescence down-regulated genes). Global transcriptome analyses (Buchanan-Wollaston et al. 2005; van der Graaff et al. 2006; Breeze et al. 2011) revealed classes of differentially transcribed genes during senescence. While the genes for photosynthesis and chloroplast development are down-regulated, the genes for the degradation and recycling of resources and the genes encoding protective factors to facilitate efficient recycling under the dangerous conditions during chloroplast dismantling are up-regulated. In addition, many regulatory genes, e.g., members of the WRKY and NAC transcription factor families and the components of upstream signalling networks, are induced at onset of senescence (Lim et al. 2007a, b; Balazadeh et al. 2008). The transcription factors act as central regulators, which are induced through upstream regulatory pathways, and subsequently induce senescence signals via the up- and down-regulation of many downstream target genes encoding proteins involved in the senescence programme. But, there is not only one senescence signal and more than one signalling pathway leading to senescence. In fact, there are several pathways associated with senescence that are initiated through internal and external signals. In addition to pure age-dependent, also called developmental senescence pathways, environmental stress factors (e.g., drought, darkness, biotic stress) can also trigger senescence (Guo and Gan 2005; Wingler and Roitsch 2008; Gregersen et al. 2008; Guiboileau et al. 2010), which partly function via separate, but interacting regulatory pathways. These pathways form a complex, environment-sensitive

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regulatory network for leaf senescence. Cross talk between the developmental and stress-related regulatory pathways in plants facilitates the optimisation and fine-tuning of leaf senescence in response to the changing environmental conditions. Recently, Guo and Gan (2012) analysed the changes in transcriptomes, comparing 27 different, senescence-inducing conditions. This meta-analysis clearly showed that early pathways for the induction of senescence differ, but later converge into a shared senescence programme.

The senescence-specific and environment-dependent reprogramming of gene expression requires complex regulation. Transcription factors are key players in this process (Balazadeh et al. 2008). However, as an increasing amount of evidence indicates, there is an additional higher-order mechanism that regulates the reprogramming of gene expression at major developmental phase transitions, e.g., germination and flowering, and in response to stress. The epigenetic and small RNA-mediated regulation of gene expression, as discussed below, is also involved in the regulation of leaf senescence.

Epigenetic regulation of gene expression in plants

Epigenetics

Epigenetic mechanisms are central regulators of gene expression, responsible for a major part of the phenotype of higher organisms. The work of many research groups has shown that epigenetic regulation occurs via a set of different molecular mechanisms at the DNA, RNA and histone levels, and changes in three-dimensional structure of chromatin are the basis for the epigenetic regulation of gene expression. Recently, it has been shown that this type of regulation, which is principally different from gene regulation via *cis*- and *trans*-acting factors, is also dynamic. Dynamic changes in the chromatin structure determining transcriptionally active or inactive DNA sites via epigenetic mechanisms also play a role in the regulation of leaf senescence. In the following chapters, the underlying mechanisms and their role in plant development and stress responses are briefly summarised, and findings concerning epigenetic control of leaf senescence are discussed.

Modifications in histones and DNA determine chromatin structure

Eukaryotic DNA is wrapped around an octamer of histone proteins forming a central structural chromatin element, the nucleosome (Luger et al. 1997). While approximately 146 bp of DNA directly contact the central histone octamer, approximately 50 bp form a linker between two nucleosomes, interacting with another histone type, histone H1. The packaging of DNA around histones, which determines the three-dimensional structure of the chromatin and thereby gene expression, is under the control of different epigenetic mechanisms, including DNA-methylation, post-translational histone modifications, nucleosome remodelling and the regulatory action of small RNA molecules.

DNA methyltransferases (e.g., MET1, CMT3, DRM1 or DRM2; Finnegan and Kovac 2000) methylate cytosine bases in plant DNA, forming 5-methylcytosine. Three types of DNA methylation can be distinguished: symmetric CG or CHG (H is A, T or C) and asymmetric CHH DNA-methylation (Vanyushin and Ashapkin 2011). DNA-methylation occurs at repeats and transposons, ensuring stable heterochromatic structure. Through this mechanism repeats and transposons are silenced, and the plant genome is protected. However, DNA-methylation also occurs in the gene body. Zhang et al. (2006) showed that over 30 % of the expressed genes contain methylation within transcribed regions, particularly symmetric CG methylation. Zubko et al. (2012) recently showed that MET1 catalyses the methylation of this gene body de novo. However, the significance of CG methylation in transcribed regions remains a matter of debate. The methyl groups in DNA are not inevitably stable and can be cleaved through demethylating enzymes, e.g., DME, DML2 or ROS1 (Choi et al. 2002; Morales-Ruiz et al. 2006; Penterman et al. 2007).

In addition to DNA-methylation, the many forms of post-translational histone modifications regulate the dynamic expression of genes during plant development and in response to the environment. The amino acids at the N-terminal tails of histones are covalently modified. The most prominent types of modifications are methylation, acetylation, H2B monoubiquitination and phosphorylation at different amino acids, but other types of modifications are also known. In addition, multiple methylations (di- and tri-methylation) are possible. The different modifications are listed as short forms, e.g., H3K4me3, which indicates trimethylation at lysine 4 of histone 3. The different types of post-translational modifications are collectively referred to as the histone code (Jenuwein and Allis 2001; Berger 2007). The dynamic establishment of histone modifications at promoter and gene body regions involves many histone modification enzymes and interacting proteins. Prominent families of histone modifying enzymes include histone acetyltransferases, histone deacetylases (HDAs), histone methyltransferases and histone demethylases (Pandey et al. 2002; Thorstensen et al. 2011; Lauria and Rossi 2011). Currently, a plethora of histone modifying enzymes have been identified, but the exact functions of the specific enzymes remain unclear.

Typical euchromatic modifications, such as H3K9Ac, have been associated with open chromatin, while typical heterochromatic modifications, such as H3K9me2, represent densely packed chromatin. The chromatin structure at specific DNA sites might determine the transcriptional activity of the genes at this site. Both, DNA-methylation and histone modifications, together with other regulatory proteins, which detect these marks and bind additional proteins involved in chromatin remodelling, determine the chromatin structure and either facilitate or inhibit the binding of transcription factors and RNA polymerase II machinery, thereby inducing or suppressing transcription. This dynamic action, involving the promoter and gene body, also changes the nucleosome positioning and composition (Kusch and Workman 2007; Clapier and Cairns 2009; Chodavarapu et al. 2010).

Small RNAs

In addition to proteins acting either as transcription factors or in the epigenetic modification of DNA and histones, small RNAs (sRNAs) of approximately 20–30 nucleotides play a role in regulating gene expression in animals and plants. In recent years, it has become clear that sRNAs are key regulators in plant development and plant stress responses (Pilido and Laufs 2010; Rubio-Somoza and Weigel 2011; Khraiwesh et al. 2012). There are different classes of sRNAs with specific sizes and functions, including microRNAs (miRNA) and small interfering RNAs (siRNAs). These small RNAs bind to specific target mRNAs and act either via the cleavage and/or translational inhibition of these mRNAs. In addition small RNAs interact with epigenetic DNA-methylation for RNA-directed DNA methylation (RdDM).

Many reports show that mutations in the genes involved in the biogenesis and action of small RNAs (sRNAs) often drastically affect developmental phase transitions in plants. In these mutants, developmental timing is disrupted at different phases, e.g., the juvenile to adult phase transition, flowering, fruit ripening, leaf development and trichome formation (Huijser and Schmid 2011). Similar to higher-order regulation via epigenetic mechanisms, sRNAs also play a major role in the stress response of plants (Sunkar et al. 2007). Many studies have focused on the characterisation of the expression of sRNAs and their target transcripts, using mutant analyses to demonstrate the involvement of sRNAs in different stress pathways, e.g., drought, cold, salt, UV, heavy metal and biotic attack (Khraiwesh et al. 2012). This regulatory network of stress-responsive sRNAs also interacts with regulatory pathways under the control of the stress hormone abscisic acid (ABA).

Plant development and stress responses are regulated through epigenetic mechanisms

In recent years, it has become more evident that major developmental phase transitions in plants, e.g., gametogenesis, seed development and transition to generative growth, are under epigenetic control (He et al. 2011). The best-characterised developmental step under epigenetic control is vernalisation-induced flowering (Oliver et al. 2009; Groszmann et al. 2011), which establishes the flowering time after the cold winter period. One central switch in this process is the regulation of *Flowering Locus C* (*FLC*), which is the major floral repressor. Before cold-exposure, *FLC* is actively transcribed. During transcription, two complexes are associated with *FLC*, containing histone methyltransferases that mediate methylation at H3K4. Consequently, *FLC* is marked with active H3K4me3 modifications. During cold treatment, inactive histone marks (H3K9me2 and H3K27me3) are established, which inhibit *FLC* expression and lead to the activation of the photoperiodic flowering pathway. Recent evidence has shown that flowering time is also under epigenetic control in ecotypes that do not require vernalisation, indicating the action of epigenetic regulatory mechanisms in different flowering pathways (Adrian et al. 2010, Yang et al. 2011). In recent years, the epigenetic control of plants responses to the environment has been shown. Two examples have been well characterised: the role of epigenetic control mechanisms in photomorphogenesis and ABA-mediated epigenetic processes in plant stress responses. Light is a central external factor for plants that affects the expression of many light-responsive genes via different signalling pathways. Recent studies have shown that epigenetic control mechanisms are involved in light-responsive gene expression. ChIP analyses have demonstrated the light-dependent acetylation of H3K9 and H3K27 at several light-induced genes, including *PetE* (plastocyanine) (Chua et al. 2003) and two central transcription factors of light response, *HY5* and *HYH* (Benhamed et al. 2006; Servet et al. 2010). Studies using mutations in epigenetic control factors have also illustrated that epigenetic factors are involved in photomorphogenesis (Li et al. 2012). However, it has also been shown that, in darkness active marks (e.g., H3K9ac, H3K27ac and also H3K4me3) are established at the *phyA* gene, while light-treatment results in the rapid disappearance of these marks and the establishment of inactive marks, e.g., H3K27me3 (Jang et al. 2011). Mutant analyses indicate that a histone deacetylase (HDA19/HD1) mediates the light-dependent deacetylation of H3K9. Interestingly, the plants response to environmental stress conditions is also partially controlled via epigenetic mechanisms. The results obtained from many mutant studies and analyses of DNA-methylation and histone modification patterns have shown that various stress

response pathways, e.g., cold, freezing, salt and drought, involve chromatin modifications and remodelling proteins. The phytohormone ABA has been implicated as a key factor in plant stress responses. Epigenetic control of stress responses via ABA occurs via different mechanisms. In the first mechanism, ABA influences the expression of histone deacetylases
and causes changes in acetylation patterns in stress genes (e.g., *RD29A*; Chinnusamy et al. 2008; Kim et al. 2008). In the second mechanism, ABA also affects DNA methylation patterns, and microRNAs play a role in ABA signalling pathways (Yaish et al. 2011; Luo et al. 2012).

In the last 5 years, exciting new findings have shown that in addition to the regulation through transcription factors, reversible epigenetic mechanisms play a major role in the regulation of gene expression during plant development and plant responses to the environment. Leaf senescence is a major developmental phase transition associated with other developmental processes, e.g., flowering. In addition, leaf senescence is highly responsive to environmental stress conditions. The regulatory pathways of developmental- and stress-induced senescence are linked, but the molecular basis for this cross talk remains elusive. As discussed below, several recent studies have demonstrated the involvement of epigenetic mechanisms, acting as higher-order regulatory switches in both developmental and stress-related induction of leaf senescence.

Epigenetic control of leaf senescence

Changes in global chromatin structure during leaf senescence

Chromatin structure, i.e., the complex three-dimensional packaging of DNA in association with histones and other proteins, determines nuclear functions. The remodelling of this structure regulates the accessibility of DNA sequences and controls gene expression. In this chapter, changes in the global chromatin structure of senescing nuclei, as detailed using electron or confocal laser scanning microscopy with specific antibodies, will be discussed. Several publications describe a change in chromatin organisation during senescence (Drumm and Nagl 1982; Kolodziejek et al. 2007; Damri et al. 2009). Electron microscopy shows the re-structuring of hetero- and euchromatic areas in the nucleus of senescing cells, associated with the spreading of dense areas. Ay et al. (2009) reported similar results. DAPI staining of the nuclei in the cells of mature *Arabidopsis thaliana* leaves shows clear bright spots at dense chromocenters. While these heterochromatic regions are primarily transcriptional inactive, the surrounding euchromatic areas are less densely stained and transcriptionally active. This clear separation disintegrates during leaf senescence (Ay et al. 2009). Heterochromatic and euchromatic areas are characterised by certain histone modification marks. While the constitutive heterochromatin at pericentric regions is loaded with typical heterochromatic marks, such as H3K9me2, the pure euchromatic regions bear marks, such as H3K4me2 and H3K4me3. Using labelled antibodies against different histone modifications, Ay et al. (2009) showed that the distribution of eu- and heterochromatic marks in the nuclei changes after the onset of senescence. The typical heterochromatic mark H3K9me2 in mature leaves is only visible at clear spots in chromocenters. When senescence begins, this pattern changes, and the mark can be detected in larger areas of the nuclei. The pattern of euchromatic marks also changes. These data were confirmed using FISH analyses against the typical heterochromatin 180-bp core repeat sequence, showing the drastic restructuring of the chromatin architecture during senescence. A similar effect on the global chromatin structure was shown in an *Arabidopsis* mutant overexpressing the AT-hook protein *ORE7/ESC* and showing a clear senescence phenotype (Lim et al. 2007a, b). The AT-hook is a DNA binding motif involved in epigenetic changes of chromatin structure. Using the YFP-labelled histone2B variant, these authors showed that this senescence-associated protein affects the chromatin architecture. The microscopic studies of global chromatin structure show that during senescence, the chromatin architecture changes. The restructuring of chromatin architecture during senescence, as visualised using microscopic and immunocytological techniques, occurs at early stages of senescence when gene expression is reprogrammed, indicating that a major change in chromatin architecture is involved in the senescence programme.

Epigenetic mutants show a senescence phenotype

In *Arabidopsis thaliana* a wide range of mutants in epigenetic control factors, showing effects in development and stress responses, are available. However, in most cases, senescence processes were not analysed. Nevertheless, there are some studies that have shown an effect on the course of leaf senescence. Two of these reports are focused on histone deacetylases. Tian and Chen (2001) constructed antisense AtHD1 plants with approximately 90 % reduced expression. AtHD1 (also referred to as HDA19) belongs to the RPD3 class of histone deacetylases (Pandey et al. 2002). The antisense plants showed an accumulation of tetra-acetylated histone H4. These plants exhibited a pleiotropic developmental phenotype, including early senescence, serration, aerial rosette formation, and delayed and abnormal flowering. Tian et al. (2005) analysed global transcriptome changes in the AtHD1 mutant using microarrays. These

experiments revealed that a loss of AtHD1 expression in the mutant affects several classes of genes involved in plant development and stress, and AtHD1 is responsible for both the positive and negative regulation of genes. Consistent with the observed senescence phenotype, a sub-set of these genes could be associated with cell death and aging. Another well-known key player in the epigenetic control of plant development and stress response is the RPD3-type histone deacetylase HDA6. In a detailed study, Wu et al. (2008) investigated the effects of a *loss-of-function* mutation in this enzyme. These authors analysed both, a splice site HDA6 mutant and HDA6 RNAi lines. The reduction in HDA6 expression consequently increased the acetylation of histone H3. These plants showed effects in the jasmonate response, senescence and flowering. In the mutants, the expression of jasmonate responsive genes *PDF1*.2, JIN1, ERF1 and *VSP2* was down-regulated (Wu et al. 2008). The finding that HDA6 expression is induced through jasmonate treatment supports the idea that HDA6 is involved in jasmonate signalling. Interestingly, leaf senescence and flowering were delayed in the HDA6 mutant and RNAi-lines. Future work is needed to clarify whether the delay in leaf senescence in these mutants is a direct effect on senescence-specific gene expression or a more indirect effect of the delayed developmental programme. The authors further showed that a *loss-of-function* mutation in HDA6 reduced the expression of typical senescence-associated genes (*SAG12* and *SEN4*), while the expression of *RPS17*, which is typically down-regulated during senescence, remained high in the mutant and RNAi plants. Wu et al. (2008) analysed the acetylation of the floral repressor gene *FLC* and demonstrated that a *loss-of-function* mutation in HDA6 induces the hyperacetylation of this gene. This result suggests that HDA6 is required for the deacetylation of genes, such as *FLC*. Following the deacetylation of *FLC*, active marks are removed and chromatin changes to an inactive stage, resulting in the down-regulation of the floral repressor. Indeed, in the mutants flowering is delayed. These experiments indicate that the epigenetic regulation via histone acetylation modulates the expression of senescence-related genes and affects the course of leaf senescence. Wu et al. (2008) showed that this epigenetic mechanism functions in different developmental and stress response processes and implies that epigenetic control might act as a central regulator. The divergent phenotypes (early vs. delayed senescence) furthermore show that different histone deacetylases might have different targets and functions, reflecting the large families of different histones and DNA modifying enzymes in plants. Further studies are needed to analyse the distinct functions of the various epigenetic factors and identify the primary targets of these enzymes.

However, not only histone deacetylase mutants show a senescence phenotype. Lim et al. (2007a, b) reported that the overexpression of a chromatin architecture-controlling AT-hook protein alters the course of leaf senescence. This gene, *ore7*, was initially identified in a screen with activation-tagged Arabidopsis lines showing delayed leaf senescence. The extended leaf longevity could be clearly demonstrated through the physiological senescence parameters of chlorophyll content and photosystem II efficiency and the delayed expression of senescence-associated genes. Another study showed that the overexpression of *ore7* (also called *ESCAROLA*) causes delayed flowering (Weigel et al. 2000). As previously discussed, Lim et al. (2007a, b) demonstrated that a *gain-of-function* mutation in *ore7* which contains the AT-hook DNA-binding motif, indeed modifies chromatin structure. Microarray analyses comparing the *ore7* mutant with wild type plants confirmed an effect on senescence-specific gene expression. Among 481 genes showing increased expression levels in the *ore7* mutant, 131 genes were associated with senescence, while among the 615 down-regulated genes, 237 genes were senescence-related genes. These findings indicate that senescence is controlled through the AT-hook protein via changes in chromatin architecture. However, as previously discussed, the histone deacetylase and *ore7* mutants also showed delayed flowering, potentially indicating a delay in the entire developmental programme.

Another major class of epigenetic control factors comprises histone methyltransferases. A central histone methyltransferase is SUVH2, which belongs to a plant sub-group of histone methyltransferases homologous to the animal SU(VAR)3-9 (KMTase1) type (Naumann et al. 2005). Naumann et al. (2005) showed that the overexpression of SUVH2 in *Arabidopsis thaliana* causes ectopic heterochromatin formation through an increase in the heterochromatic histone methylation marks at H3K9, H3K27 and H4K20. Ay et al. (2009) investigated the effects of the overexpression of SUVH2 on leaf senescence. These authors demonstrated that in these plants, leaf senescence is delayed approximately 2 weeks. Consequently, typical senescence-associated genes (e.g., *WRKY53*, *SIRK*, *SAG101*, *ANAC083*, *SAG12* and *SAG24*) were not induced or the expression of these genes was drastically repressed in SUVH2 overexpression plants. But, SUVH2 overexpression lines showed a pleiotropic phenotype (delayed senescence, smaller curled leaves, and elongated petioles), which might indicate that SUVH2 does not directly affect senescence-specific gene expression, but rather affects superior developmental regulators, e.g., hormones.

Senescence-specific alterations in histone modifications at senescence-associated genes

Local changes in epigenetic histone marks at specific genes can be analysed using chromatin immunoprecipitation

(ChIP) analyses and quantified through subsequent real-time PCR analyses. Ay et al. (2009) used this approach to analyse one central regulatory gene of leaf senescence in *Arabidopsis thaliana*, the transcription factor *WRKY53*, which is induced at early stages of senescence (Hinderhofer and Zentgraf 2001). These authors showed that during senescence, when *WRKY53* is induced, the active marks H3K4me2 and H3K4me3 are at the 5′ end of the gene and at coding regions. These data show a correlation between histone modifications and the expression of specific SAGs. When overriding the euchromatic marks through the overexpression of SUVH2, which establishes silencing marks (H3K27me2 and H3K27me3; Zhang et al. 2007) at *WRKY53*, this gene was not induced at onset of senescence. Similar changes in the histone marks of other senescence-related genes were observed in *Arabidopsis thaliana* and *Hordeum vulgare* (unpublished data), indicating that the epigenetic control of senescence-associated genes plays a role in the regulation of senescence. However, more studies are needed to determine the precise schedule of epigenetic regulatory events during the transition from a mature photosynthetically active leaf to a senescing leaf. It will be important to precisely define the time points of events involving specific genes. To analyse differential histone modifications on a more global scale, genome-wide ChIP-on-chip or ChIP-seq technology is used. Brusslan et al. (2012) used ChIP-seq to observe the genome-wide changes in the euchromatic mark H3K4me3 and the silencing mark H3K27me3 using green leaf material obtained from mature and senescing plants. These results show that for a portion of the known SAGs, the euchromatic mark H3K4me3 is higher in the older leaves, while this mark is higher in the younger leaves for down-regulated genes (SDGs) during senescence. Similar have been shown for the silencing mark H3K27me3, which at some SAGs is lost and at some SDGs is established in the older leaves. This initial genome-wide study clearly confirms that the reprogramming of gene expression during leaf senescence is under epigenetic control. The results show that at least at the time point analysed, alterations in the specific histone modifications H3K4me3 and H3K27me3 occur at some, but not all, SAGs and SDGs. Further studies are needed to identify other epigenetic marks and define various senescence time points.

Changes in DNA-methylation during senescence

DNA-methylation occurs predominantly in stable heterochromatic regions, silencing repeats and transposons. However, some reports have shown that DNA-methylation can also dynamically regulate gene expression (Penterman et al. 2007). Little is known about the role of DNA-methylation in the senescence programme. Previous studies have shown that the extent of DNA-methylation changes during aging (Diaz-Sala et al. 1995; Fraga et al. 2002). However, evidence of senescence-specific changes in DNA-methylation either on a global genome-wide, or local scale at distinct genes has not been reported.

microRNA networks controlling leaf senescence

Several studies have shown microRNAs also control senescence. Kim et al. (2009) demonstrated that expression of ORE1, which is a NAC transcription factor regulating downstream senescence-associated genes, such as *SAG12*, is repressed by the microRNA miR164 via cleavage of *ORE1* mRNA. Consequently, these authors showed that in the *miR164* mutant, senescence was accelerated. Interestingly, the expression of microR164 in wild-type plants is decreased with increasing leaf age in an ethylene-dependent manner (Kim et al. 2009). The overexpression of another microRNA, miR319, causes a stay-green phenotype (Schommer et al. 2008). This microRNA increases the levels of jasmonic acid (JA) via the regulation of the TEOSINTE BRANCHED1 CYCLOIDEA, PCF (TCP) transcription factor family. Another target of sRNA action is the auxin regulatory pathway. In this case, miR390 triggers the production of the trans-acting siRNA *TAS3*, which results in the mRNA degradation of the auxin response factors, ARF 2, ARF3 and ARF4 (Adenot et al. 2006; Fahlgren et al. 2006). ARF2 is a negative regulator of auxin responses, and auxin responses are involved in the timing of senescence and flowering (Ellis et al. 2005; Lim et al. 2010).

Concluding remarks and future perspectives

In recent years, it has become clear that major developmental steps in plants are epigenetically controlled. Recent studies have revealed senescence-specific changes in chromatin structure, histone modifications and abundant small RNA expression. These results, and the data obtained from mutant analyses, indicate that epigenetic mechanisms are also involved in the regulation of leaf senescence, acting in response to external and internal signals and controlling the downstream reprogramming of gene expression (Fig. 1). Thus, in addition to the senescence-specific regulation of gene expression via *trans*-acting transcription factors, another, higher-order epigenetic control has been proposed. The recent findings indicate that the regulatory network of leaf senescence is more complex. However, an understanding of the molecular mechanisms underlying this new epigenetic level of senescence regulation and its association to the regulatory network remains unclear. Future work will address the following questions: Which senescence-associated genes are under epigenetic control? Which control mechanisms are involved (histone modifications,

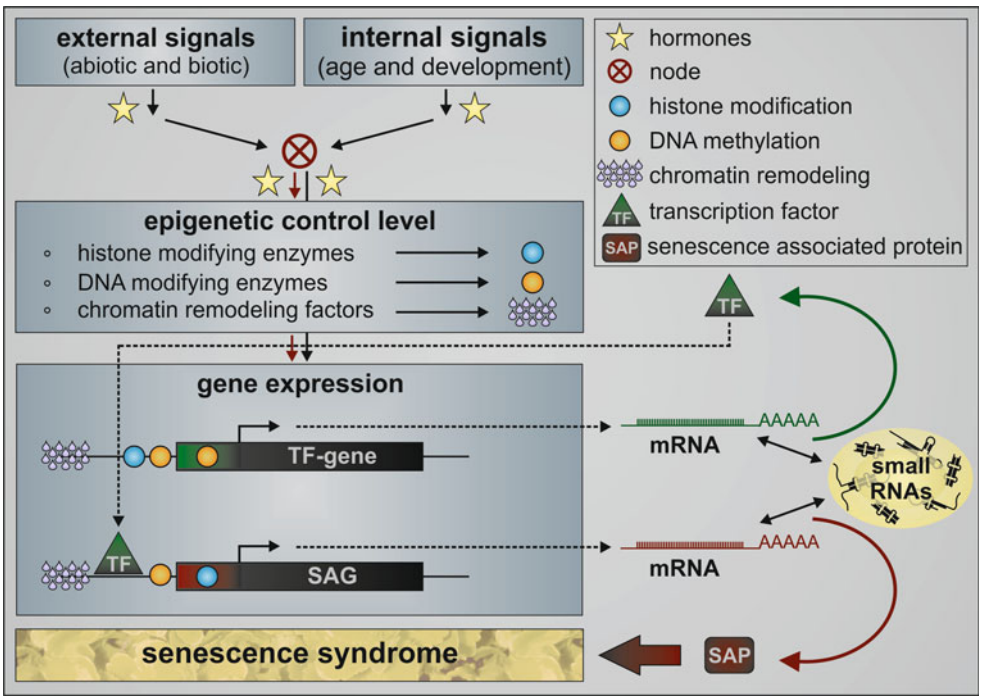

Fig. 1 Model of the regulatory network of leaf senescence. Leaf senescence is induced through external and internal signals. Recent findings indicate that downstream of signalling pathways, including hormone activity, and upstream of transcription factors, regulating the expression of SAGs, there is an epigenetic control level. This regulation occurs through histone modifications at SAGs (transcription factors and other senescence-related genes) via the methylation of specific DNA sequences and the interactions of small RNAs with senescence-associated mRNAs. Histone and DNA modifying enzymes and chromatin remodelling factors are involved in the epigenetic regulation of leaf senescence

DNA methylation, small RNAs)? Which epigenetic control factors are acting in a senescence-specific manner? How is epigenetic control associated with upstream signalling pathways? Is the epigenetic regulation of senescence conserved in all plant species? Future studies will reveal novel and exciting results in this field to provide further insight into the complex regulatory network of leaf senescence.

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