

# SAUR36, a SMALL AUXIN UP RNA Gene, Is Involved in the Promotion of Leaf Senescence in Arabidopsis<sup>1</sup>[C][W][OA]

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Small Auxin Up RNA genes (*SAURs*) are early auxin-responsive genes, but whether any of them are involved in leaf senescence is not known. Auxin, on the other hand, has been shown to have a role in leaf senescence. Some of the external application experiments indicated that auxin can inhibit leaf senescence, whereas other experiments indicated that auxin can promote leaf senescence. Here, we report the identification and characterization of an Arabidopsis (*Arabidopsis thaliana*) leaf senescence-associated gene named *SAG201*, which is highly up-regulated during leaf senescence and can be induced by 1-naphthaleneacetic acid, a synthetic auxin. It encodes a functionally uncharacterized *SAUR* that has been annotated as *SAUR36*. Leaf senescence in transfer DNA insertion *saur36* knockout lines was delayed as revealed by analyses of chlorophyll content,  $F_v/F_m$  ratio (a parameter for photosystem II activity), ion leakage, and the expression of leaf senescence marker genes. In contrast, transgenic Arabidopsis plants overexpressing *SAUR36* (without its 3' untranslated region [UTR]) displayed an early leaf senescence phenotype. However, plants overexpressing *SAUR36* with its 3' UTR were normal and did not exhibit the early-senescence phenotype. These data suggest that *SAUR36* is a positive regulator of leaf senescence and may mediate auxin-induced leaf senescence and that the 3' UTR containing a highly conserved downstream destabilizes the *SAUR36* transcripts in young leaves.

Leaf senescence is the final phase of leaf development. Like other plant developmental processes, leaf senescence is genetically programmed; a subset of *Senescence-Associated Genes* (*SAGs*) are up-regulated at the onset of and during leaf senescence, while the vast majority of genes (including photosynthesis-related genes) are down-regulated (Gan and Amasino, 1997). Thousands of *SAGs* have been isolated, including genes encoding transcription factors, signal transduction components, proteinases and other catabolic enzymes, and various transporters of sugars, amino acids, and other nutrients (Gepstein et al., 2003; Guo et al., 2004; Buchanan-Wollaston et al., 2005; Guo and Gan, 2012). Some of these *SAGs*, such as NAC and WRKY transcription factor genes (Guo and Gan, 2006; Uauy et al., 2006; Zentgraf et al., 2010; Besseau et al.,

2012; Zhang and Gan, 2012), protein kinase and phosphatase genes (Hajouj et al., 2000; Zhou et al., 2009; Xu et al., 2011; Zhang et al., 2012), lipase genes (He and Gan, 2002), and nuclease genes (Lers et al., 2006), have been shown biochemically, genetically, and/or physiologically to have a role in leaf senescence. The functions of the vast majority of *SAGs* (some of which encode Small Auxin Up RNAs [*SAURs*]) have yet to be characterized.

Leaf senescence can be regulated by various internal signals and environmental cues (Xu et al., 2011; Guo and Gan, 2012). Among the internal factors are plant hormones that promote or inhibit leaf senescence. Ethylene, abscisic acid, jasmonic acid, and salicylic acid can promote leaf senescence, and cytokinins and gibberellin inhibit senescence (Gan, 2010). However, the role of auxin in regulating leaf senescence remains elusive. In previous studies, exogenous application of auxin often delayed chlorophyll loss and protein degradation and suppressed the transcription of some *SAGs* in detached leaves of various plant species. Some correlative studies also showed that the total auxin levels decrease as leaf senescence progresses (Gan, 2010). These findings suggest that auxin may be a negative regulator of leaf senescence. However, the levels of free indole-3-acetic acid (IAA) in senescing leaves were shown to be higher than those in young leaves, although the levels of total IAA species decreased during Arabidopsis (*Arabidopsis thaliana*) leaf senescence (Quirino et al., 1999). Transcription analysis found that many genes involved in auxin biosynthesis such as Trp synthase (*AtTSA1*) and nitrilases (*NIT1–NIT4*) are

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up-regulated during age-dependent senescence (Quirino et al., 1999; van der Graaff et al., 2006). These results suggest that auxin may be a positive regulator of leaf senescence.

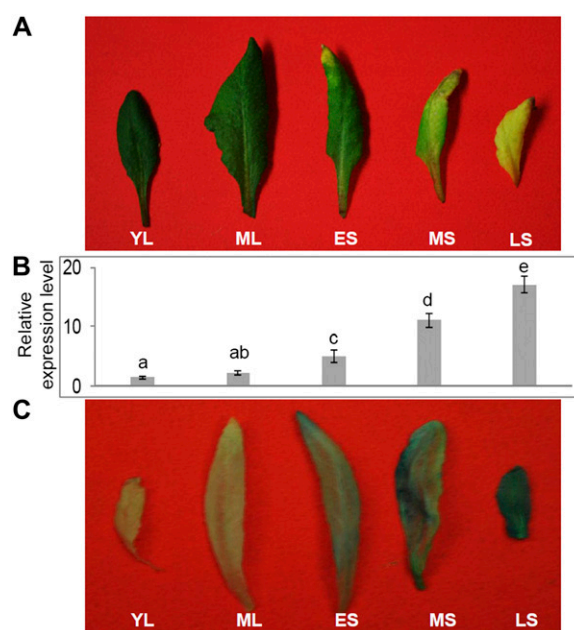
Auxin exerts its roles in cell division, elongation, and differentiation in part by altering gene expression (Kant et al., 2009; Scarpella et al., 2010). There are three major early auxin-responsive gene families, including the *SAUR* family (Hagen et al., 2010). *SAUR* genes can be readily induced by exogenous auxin (Jain et al., 2006) and have been widely used as auxin-inducible reporters. Studies have correlated some *SAUR* genes with auxin-mediated cell expansion based on their temporal and spatial expression and their regulation at the transcriptional, posttranscriptional, and protein levels (Esmon et al., 2006; Spartz et al., 2012). Molecular studies have also demonstrated that many *SAUR* genes contain a highly conserved downstream (DST) element in their 3' untranslated region (UTR) that contributes to mRNA instability in an auxin-independent manner (Sullivan and Green, 1996; Park et al., 2012). Analysis of Arabidopsis plants expressing a SAUR15-luciferase fusion protein suggested that SAUR proteins are unstable (Zenser et al., 2003). Studies of the maize (*Zea mays*) ZmSAUR2 protein revealed that *SAUR* expression may be regulated at the protein level (Knauss et al., 2003). Recent studies showed that *OsSAUR39* acts as a negative regulator of auxin synthesis and transport; overexpression of this gene in rice (*Oryza sativa*) confers phenotypes including reductions in lateral root development, yield, and shoot and root lengths (Kant et al., 2009). The addition of an N-terminal GFP or epitope tag dramatically increases the stability of SAUR19 to SAUR24 proteins and their ability to function as positive effectors of cell expansion through the modulation of auxin transport (Spartz et al., 2012). However, the function and underlying mechanisms of many *SAUR* genes remain unknown.

Here, we report our molecular genetic analyses of Arabidopsis *SAUR36*, which shows *SAUR36* to be a positive regulator of leaf senescence, which implies that auxin, via the action of *SAUR36*, has a role in promoting leaf senescence.

## RESULTS

### The Expression of *SAG201/SAUR36* Is Up-Regulated during Leaf Senescence in Arabidopsis

A gene, initially named *SAG201*, was identified from our previous transcriptomic analyses of leaf senescence in Arabidopsis (Guo et al., 2004). This gene is *SAUR36*. Quantitative PCR (qPCR) analyses revealed that the transcript levels of *SAG201/SAUR36* were very low in expanding young leaves and fully expanded mature leaves but increased with the progression of senescence (Fig. 1, A and B).

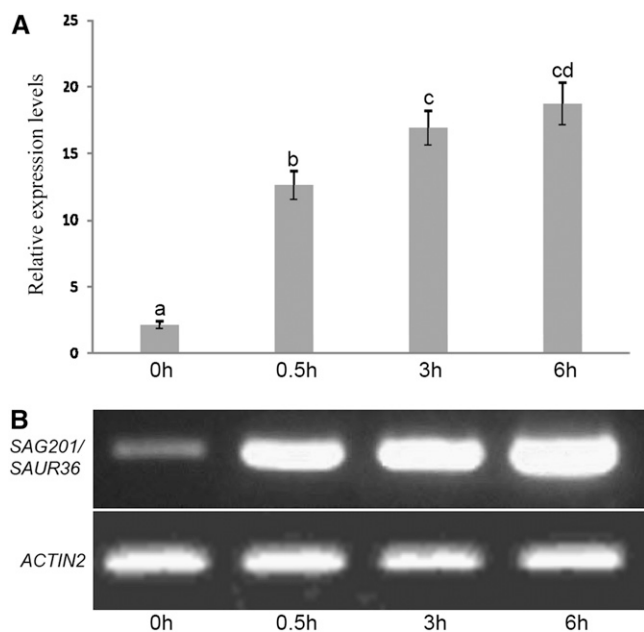


**Figure 1.** Expression of *SAG201/SAUR36* during leaf senescence in Arabidopsis. A, Phenotypes of leaf senescence in wild-type plants. YL, Expanding young leaf; ML, mature leaf that is fully expanded but with no visible yellowing; ES, early senescence leaf, with about 25% leaf area yellowing; MS, middle senescence leaf, with about 50% leaf area yellowing; LS, late senescence leaf, with more than 50% leaf area yellowing. B, Relative expression of *SAG201/SAUR36* during leaf senescence. The expression levels were determined using qPCR. The expression levels in young leaves were set to 1. Data represent mean values  $\pm$  SE of three replicates. Letters indicate significant differences by Student's *t* test ( $P = 0.05$ ). C, GUS staining in senescing leaves of  $P_{SAG201/SAUR36}$ -*GUS* transgenic Arabidopsis plants at 40 DAE. [See online article for color version of this figure.]

We also generated transgenic Arabidopsis plants containing the promoter of *SAUR36* fused with the GUS reporter gene. Histochemical staining revealed that the expression of *SAUR36* was only evident in senescing leaves and not in the nonsenescent ones (Fig. 1C), which is consistent with the above qPCR data (Fig. 1B).

### *SAUR36* Is Induced by Exogenous Auxin

Studies have shown that some of the *SAUR* family genes can be rapidly induced by auxin treatment. To investigate whether *SAUR36* is inducible by auxin, first we performed sequence analysis and found that there is the TGTCTC sequence in the 5' UTR of *SAUR36*; the TGTCTC-containing sequence is an auxin response element (Ulmasov et al., 1995; Hagen and Guilfoyle, 2002). To confirm the inducibility of *SAUR36* by auxin, 3-week-old wild-type plants were treated with  $\alpha$ -naphthalene acetic acid (NAA; a synthetic auxin). Semiquantitative and quantitative PCR analyses revealed that the transcript levels of the gene increased readily after 30 min of NAA application and reached a high level (nearly a 20-fold increase relative to the expression at 0 h) 6 h after treatment (Fig. 2).



**Figure 2.** Inducible expression of *SAUR36* by NAA treatments in Arabidopsis leaves. The sixth rosette leaves of wild-type plants (30 DAE) were incubated in 3 mM MES buffer (pH 5.7) containing 20  $\mu$ M NAA. The accumulation of *SAG201/SAUR36* transcripts was determined using qPCR (A) and semi-quantitative PCR (B). The expression levels in the 0-h leaves were set to 1. Letters indicate significant differences by Student's *t* test ( $P = 0.05$ ).

### *SAUR36* Is Knocked Out in Two T-DNA Insertion Lines

The *SAUR36* gene of Arabidopsis is located on chromosome II. Like most *SAUR* genes in Arabidopsis (Knauss et al., 2003), the *SAUR36* gene consists of only one exon (Fig. 3A) that encodes a protein with 162 amino acid residues. To investigate the biological function of the gene in leaf senescence, we obtained and identified two independent SALK transfer DNA (T-DNA) insertion lines (Columbia background) from the Arabidopsis Biological Resource Center at Ohio State University (Alonso et al., 2003). Line 1 (SALK\_011960) has a T-DNA insertion in the 5' UTR, and line 2 (SALK\_118552) has a T-DNA insertion in the open reading frame (ORF; Fig. 3A). Plants homozygous for the T-DNA insertions were identified using PCR genotyping (Alonso et al., 2003). Semi-quantitative PCR analyses showed that the *SAUR36* transcripts in senescing leaves of two homozygous mutant lines were not detectable (Fig. 3B), suggesting that these lines are *SAUR36* knockout or null mutants.

### Leaf Senescence Is Remarkably Delayed in the *SAUR36* Knockout Mutant Plants

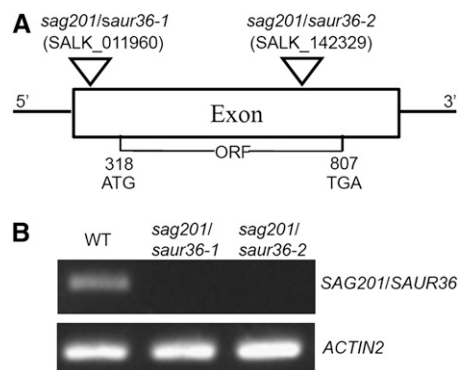
The above mutants were grown side by side in a growth chamber with corresponding wild-type plants to compare phenotypic changes in growth and development. The early stages of growth and development

of both knockout lines appeared normal (Fig. 4A), but leaf senescence was significantly delayed compared with that of the wild type (Fig. 4B). The delay in leaf senescence was also obvious when the rosette leaves were excised from age-matched mutants and the wild type: the fifth leaf (numbered from the bottom; the first leaf is the oldest and the 11th leaf is the youngest) of the wild type became senescent while the third leaf of the knockout remained green (Fig. 4C).

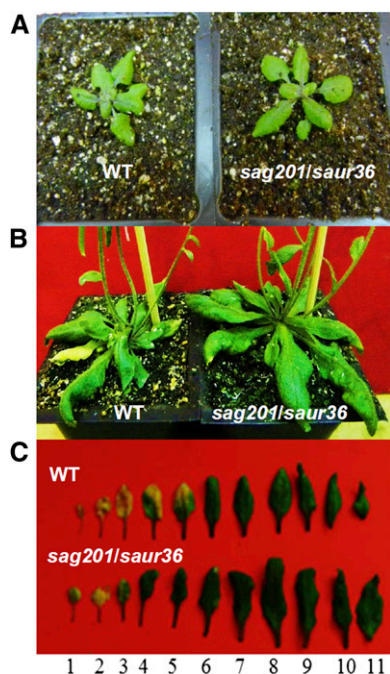
Further physiological analyses of the knockout mutant plants were performed. The leaf mortality curves (Fig. 5A) revealed that the leaves of *saur36* started to senesce 3 to 4 d later than those of the wild type. Fifty percent of wild-type and null mutant leaves became senescent at 34 and 41 d after emergence (DAE), respectively, which means a 7-d delay in leaf senescence as measured by mortality analysis (Fig. 5A). The delay in leaf senescence of the null plants is also supported by changes in the total chlorophyll contents (Fig. 5B), in the ion leakage assays (Fig. 5C), and in the maximum photochemical efficiency of PSII in the dark-adapted state ( $F_v/F_m$ ; Fig. 5D). The  $F_v/F_m$  ratio is an indicator of the photochemical quantum efficiency of PSII and the photoreduction efficiency of the primary electron-accepting plastoquinone of PSII; a senescing leaf often loses its photosynthetic activity, as reflected by the decline in  $F_v/F_m$  ratios (He and Gan, 2002).

### Leaves of *saur36* Null Mutant Plants Are Much Larger

In addition to a significant delay in leaf senescence, the individual leaves of the *saur36* null plants were larger compared with those of the wild type (Figs. 4 and 6A), which was confirmed by ImageJ (<http://rsbweb.nih.gov/ij>) leaf area measurements. The leaf area of the mutant plants was 83% greater than that of the wild type (Fig. 6A). Further microscopic analyses of the size of the epidermal cells (excluding stomatal



**Figure 3.** Gene structure and expression of *SAUR36* in two T-DNA insertion lines. A, Gene structure of *SAUR36* and locations of two T-DNA insertions. B, Semi-quantitative PCR analysis of *SAUR36* expression in senescence leaves of the wild type (WT) and *saur36-1* and *saur36-2* T-DNA insertion lines.



**Figure 4.** Delayed leaf senescence phenotype of the T-DNA insertion lines compared with that of the wild type. A, Phenotypes of null mutant and wild-type (WT) plants at approximately 10 DAE. Note that *saur36* mutant plants are larger than the wild type. B, Phenotypes of null mutant and wild-type plants at approximately 40 DAE. Note the senescing leaves in the wild type and the larger leaves in the mutant plants. C, Phenotypes of the rosette leaves detached from age-matched plants shown in B. Leaves were numbered from bottom to top, with the first leaf being the oldest and the 11th leaf the youngest. [See online article for color version of this figure.]

cells) revealed that the individual cells were approximately 67% larger in the null mutant leaves than in wild-type leaves (Fig. 6B). These data suggest that the larger leaf area in the mutant plants results mainly from the larger cell size. The data also suggest that *SAUR36* may inhibit cell expansion.

#### Inducible Overexpression of *SAUR36* Causes Precocious Leaf Senescence

To further analyze the role of *SAUR36* in leaf senescence, we performed gain-of-function analysis of *SAUR36*. Sequence analysis revealed that there is a DST element in the 3' UTR of *SAUR36* (Supplemental Fig. S1). Because highly conserved DST elements have been shown to be important for SAUR function (Newman et al., 1993), we generated inducible overexpression lines containing the *SAUR36* ORF with [OE(+DST)] or without [OE(–DST)] its DST sequence. The glucocorticoid-inducible gene expression system (Aoyama and Chua, 1997) was used to avoid possible complications caused by constitutive expression (Guo and Gan, 2006; Zhang and Gan, 2012; Zhang et al., 2012).

The effect of inducible overexpression of *SAUR36* with or without the DST element on leaf senescence

was analyzed after spraying whole plants (20 DAE) with dexamethasone (DEX; a synthetic glucocorticoid). Precocious leaf yellowing was readily observed 3 d after the DEX treatment in the OE(–DST) lines but not in the OE(+DST) plants or the wild-type plants (Fig. 7A). qPCR analysis showed that the *SAUR36* transcripts accumulated to high levels in the OE(–DST) leaves but not in the OE(+DST) leaves or the wild-type plants (Fig. 7B). The DEX-treated OE(–DST) leaves displayed significantly reduced chlorophyll content, markedly declined  $F_v/F_m$  ratios, and high levels of ion leakage (Fig. 8). The senescence-specific marker gene *SAG12* was also highly expressed in the OE(–DST) leaves (Fig. 7B). All these data suggest that the inducible expression of *SAUR36* is sufficient to promote leaf senescence and that the DST element may cause rapid turnover of the *SAUR36* transcripts.

## DISCUSSION

### *SAUR36* Positively Regulates Leaf Senescence in Arabidopsis

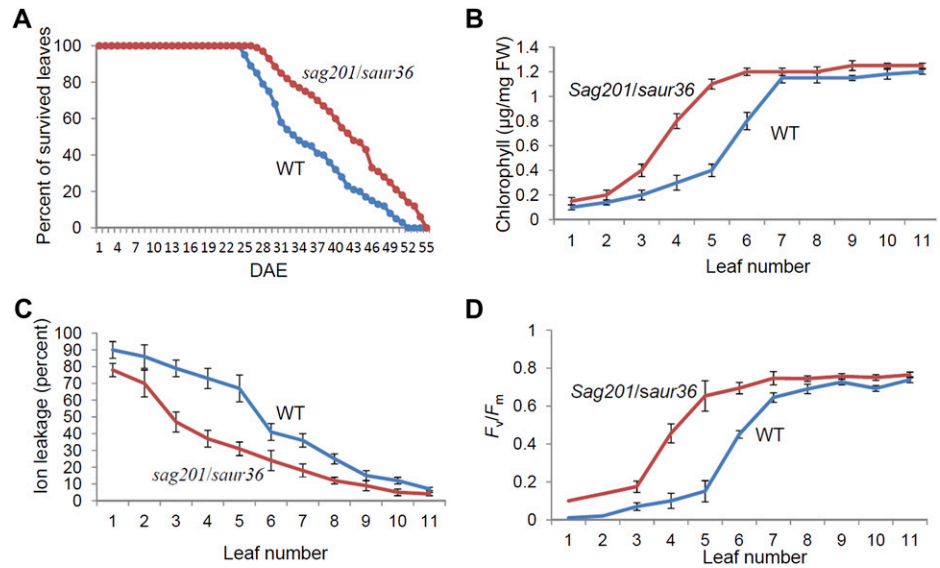
There are more than 70 SAUR family genes in the Arabidopsis genome that encode small 9- to 15-kD proteins (Hagen et al., 2010). These proteins have been shown to bind with  $Ca^{2+}$ /calmodulin proteins in maize (Yang and Poovaiah, 2000) and Arabidopsis (Reddy et al., 2002), to regulate auxin synthesis and transport in rice (Kant et al., 2009), or to control apical hook development in Arabidopsis (Park et al., 2007), but the roles of most of the SAUR proteins in plant growth and development have yet to be investigated. In this report, we demonstrate that *SAUR36* plays a role in promoting leaf senescence in Arabidopsis, because leaf senescence is substantially delayed in the *saur36* null mutant plants both phenotypically and physiologically (Fig. 4), whereas the inducible expression of this gene (excluding its 3' UTR such that the transcripts can accumulate, as seen in Fig. 7) promotes young leaves to undergo premature senescence (Figs. 7 and 8). As discussed below, it is likely that *SAUR36*, together with other genes, mediates auxin-promoted leaf senescence in Arabidopsis.

### Auxin May Promote Leaf Senescence in Arabidopsis

Auxin has pervasive roles in regulating many aspects of plant growth and development and in response to various environmental cues (Zhao, 2008), and its involvement in senescence has long been observed (Sacher, 1973). However, how auxin affects leaf senescence is not clear. The identification and characterization of *SAUR36* as a SAG reveals that *SAUR36* mediates auxin-promoted leaf senescence.

There are several lines of evidence for an inhibitory role of auxin in leaf senescence. First, external application of synthetic or natural auxin can generally delay chlorophyll degradation and protein turnover in

**Figure 5.** Physiological analyses of leaves from *saur36* mutant plants. A, Leaf survival curves of mutant and wild-type (WT) plants ( $n = 30$ ). B to D, Chlorophyll content (B;  $n = 6$ ), ion leakage (C;  $n = 6$ ), and  $F_v/F_m$  ratio (D;  $n = 30$ ) in individual rosette leaves of age-matched wild-type and mutant plants. Data represent mean values  $\pm$  SE. FW, Fresh weight. [See online article for color version of this figure.]

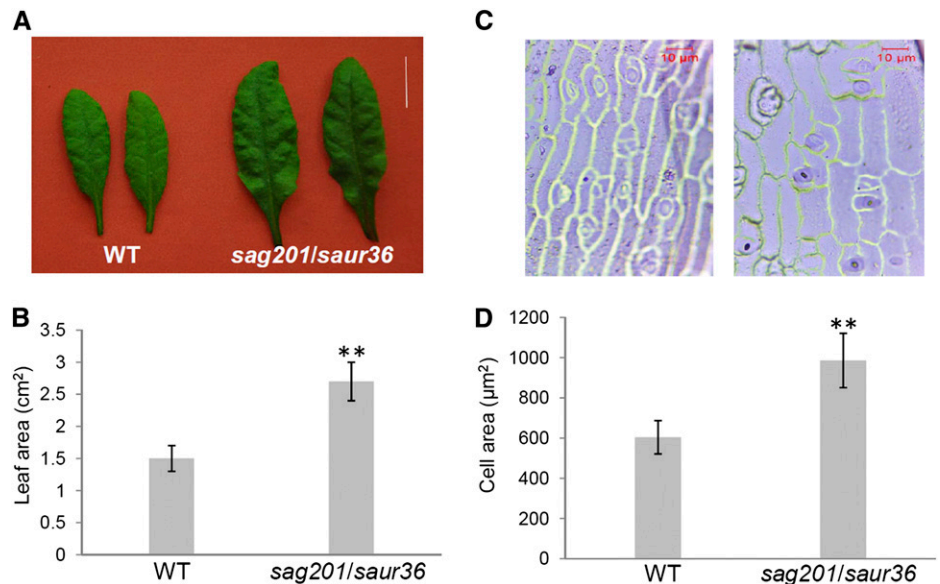


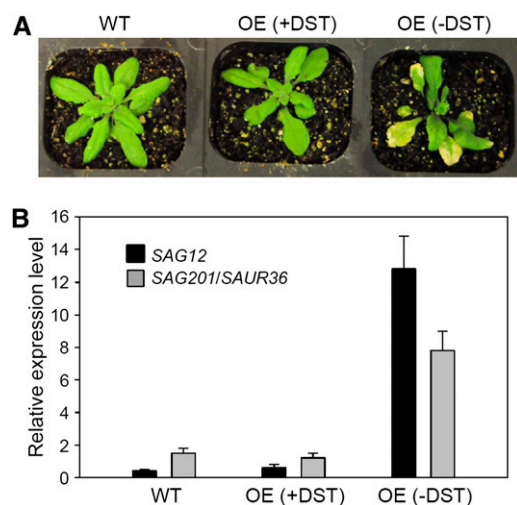
detached leaves (for review, see Gan, 2010). For example, NAA delays senescence in detached Arabidopsis leaves (Kim et al., 2011). Second, total auxin levels decrease in senescing leaves (Gan, 2010). Third, Arabidopsis plants overexpressing *YUCCA6* contain high levels of auxin and display a prolonged leaf longevity phenotype in dark (Kim et al., 2011). Last, auxin treatment can down-regulate the transcript levels of *SAG12* in detached senescing leaves (Noh and Amasino, 1999; Gan, 2010); *SAG12* is a highly senescence-specific marker gene (Gan and Amasino, 1995). The

suppression of leaf senescence by auxin appears to decline with aging (Woo et al., 2010).

However, there are also lines of evidence for a promoting role of auxin in leaf senescence. It is not unusual to observe that application of auxin promotes leaf senescence, and the endogenous auxin activity increases just prior to or at the onset of leaf senescence, as revealed in some early experiments (Atsumi and Hayashi, 1979; Gan, 2010). In fact, the levels of free IAA (the biologically active form) increase during leaf senescence in Arabidopsis and reach approximately

**Figure 6.** Increased leaf and cell size of the *saur36* mutants measured using ImageJ. A, Examples of fully expanded mature leaves of wild-type (WT) and mutant plants. Bar = 1 cm. B, Average areas of fully expanded mature wild-type and *saur36* leaves. The sixth leaves of 35-DAE plants were used. Data represent mean values  $\pm$  SE ( $n = 6$ ).  $**P \leq 0.01$  (significant difference). C, Examples of leaf epidermal cells from wild-type (left) and *saur36* mutant (right) plants. D, Average cell areas of wild-type and *saur36* leaves. Data represent mean values  $\pm$  SE ( $n = 30$ ).  $**P \leq 0.01$  (significant difference). [See online article for color version of this figure.]





**Figure 7.** Analyses of Arabidopsis plants with induced overexpression of *SAUR36* with [OE(+DST)] or without [OE(-DST)] DST. **A**, Phenotypes of wild-type (WT) and transgenic plants containing *SAUR36* ORF with or without its DST sequence. The plants were 20 DAE, and the photograph was taken 3 d after treatment with 30  $\mu$ m DEX. **B**, qPCR analysis of the transcript levels of *SAUR36* and *SAG12* in the fifth leaves of the plants shown in **A**. The expression level of *SAUR36* in the OE(+DST) plants treated with DEX was set to 1. Data represent mean values  $\pm$  SE ( $n = 3$ ). [See online article for color version of this figure.]

2-fold higher levels in leaves that are 50% yellowing compared with fully expanded green leaves, although the total auxin levels decrease (Quirino et al., 1999). Consistent with these findings, genes whose products are involved in auxin biosynthesis, such as Trp synthase (*AtTSA1*) and nitrilases (*NIT1-NIT4*), are up-regulated during leaf senescence (Quirino et al., 1999; van der Graaff et al., 2006); nitrilase converts indole-3-acetonitrile to IAA. Furthermore, several auxin response factor genes, such as *ARF1*, *ARF2*, *ARF7*, and *ARF19*, are expressed in senescing leaves in Arabidopsis. The T-DNA knockout *arf2* mutant exhibits a delay in leaf senescence, and the delay in senescence becomes more striking in the *arf1arf2* double mutant plants (Ellis et al., 2005). Most recently, a Senescence-Associated Receptor-Like Kinase (SARK) from soybean (*Glycine max*) and its ortholog in Arabidopsis have been shown to promote leaf senescence, and auxin (and ethylene) biosynthesis and signaling pathways are required for SARK to cause leaf senescence (Xu et al., 2011).

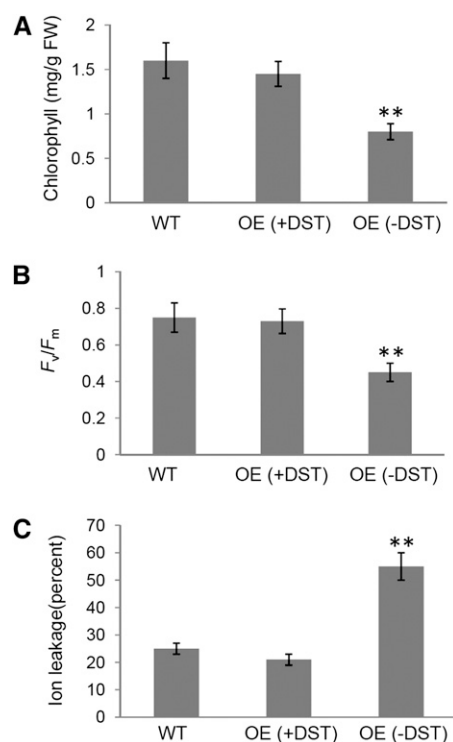
Our data reported here further support that auxin promotes leaf senescence. As discussed above, *SAUR36* plays a critical role in promoting leaf senescence. Like many other *SAURs* that can be rapidly induced by exogenously applied auxin (Li et al., 1994; Hagen and Guilfoyle, 2002), *SAUR36* is an early auxin-responsive gene that can be readily up-regulated by NAA treatment (Fig. 2). The expression of *SAUR36* is up-regulated during the progression of leaf senescence (Fig. 1), which likely results from the elevated levels of the endogenous

active IAA (Quirino et al., 1999). In short, auxin appears to promote leaf senescence in part by inducing the expression of *SAUR36* and possibly other positive senescence-regulating genes.

How *SAUR36* mediates auxin-promoted leaf senescence is not clear. The *SAUR36* protein contains a nuclear localization signal, 59-RRRS-62 (Supplemental Fig. S1), predicted by the NLStradamus program (Nguyen Ba et al., 2009). A GFP fusion protein confirms that the *SAUR36* protein is targeted to the nucleus (Narsai et al., 2011). Auxin can activate the 1-aminocyclopropane-1-carboxylate biosynthetic step to stimulate ethylene production, which in turn promotes senescence (Abel et al., 1995). Whether this nuclear protein functions through or is independent of ethylene has yet to be investigated.

### *SAUR36* May Also Be Involved in Leaf Cell Expansion

In addition to a positive role in leaf senescence, *SAUR36* appears to be involved in leaf cell expansion. qPCR analysis revealed that this gene is expressed in expanding leaves at low levels prior to the up-regulation at the onset of senescence (Fig. 1B). When *SAUR36* is knocked out due to T-DNA insertion, the leaves are 83% larger than wild-type leaves in terms of



**Figure 8.** Chlorophyll content (**A**),  $F_v/F_m$  ratio (**B**), and ion leakage (**C**) in the fifth leaves of various plants shown in Figure 7A. Data represent mean values  $\pm$  SE ( $n = 6$ ). \*\* $P \leq 0.01$ , indicating significant difference by Student's  $t$  test. FW, Fresh weight; WT, wild type.

area (Fig. 6, A and B), which is mostly due to the formation of larger cells (Fig. 6, C and D). These data suggest that *SAUR36* may inhibit cell enlargement. Early studies have also shown that some SAUR proteins play a role in auxin-modulated cell elongation (Hagen and Guilfoyle, 2002; Knauss et al., 2003; Hagen et al., 2010).

### Transcriptional and Posttranscriptional Regulation of *SAUR36*

*SAUR* transcripts are unstable (McClure and Guilfoyle, 1987; Feldbrügge et al., 2002), and their high turnover rate may be due to the DST element in the 3' UTR (Newman et al., 1993). DST is generally a 30- to 40-bp sequence consisting of three highly conserved boxes that are separated by two variable regions. The DNA sequence of the DST element and its location are highly conserved in different plant species (Newman et al., 1993). The sequences AUAGAU and GUA, which are considered invariable in DST, are present in the 3' UTR of *SAUR36* (Supplemental Fig. S1). Inducible overexpression of *SAUR36* with its 3' UTR did not cause precocious leaf senescence, because its transcripts were barely detectable (Fig. 7), presumably being rapidly degraded. However, the low levels of *SAUR36* in nonsenescent leaves and the high levels in senescing leaves appeared to be regulated at the transcriptional level, because the GUS reporter directed by the *SAUR36* promoter was highly detected in senescing leaves and very low in young leaves (Fig. 1C); the  $P_{SAUR36}$ -*GUS* chimeric gene did not contain the DST element.

## MATERIALS AND METHODS

### Plant Materials and Growth Conditions

*Arabidopsis* (*Arabidopsis thaliana*) ecotype Columbia was used in this study. Two T-DNA insertion lines (SALK\_011960 and SALK\_142329, Columbia background) were obtained from the Arabidopsis Biological Resource Center (Alonso et al., 2003). As suggested by the Salk Institute Genomic Analysis Laboratory (<http://signal.salk.edu/tdnaprimers.2.html>), a PCR-based method was used to identify homozygous T-DNA insertion mutants. The T-DNA left border primer G2325 and the gene-specific primers G3516 and G3517 for *saur36-1* (SALK\_011960) and G3518 and G3519 for *saur36-2* (SALK\_142329) were used. Plants homozygous for the T-DNA insertion were used in this study. The primers used in this research are listed in Supplemental Table S1.

Seeds were surface sterilized and then sown on one-half-strength Murashige and Skoog salts with 0.7% (w/v) phytoagar (Sigma) and appropriate antibiotics. The dishes were kept at 4°C for 2 d and then moved to a growth chamber at 22°C with 60% relative humidity under continuous light ( $110 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) from a mixture of fluorescent and incandescent bulbs. The 10-d-old seedlings were transferred into Cornell mix soil (3:2:1 peat moss:vermiculite:perlite, v/v/v) and grown under the same conditions. The mutants, transgenic plants, and the wild type were grown side by side.

### Plasmid Construction and Transformation of Arabidopsis

To create the  $P_{SAUR36}$ -*GUS* construct, PCR products of G3556 and G3557 were cloned into pGEM-T Easy vector (Promega). The coding sequence was then released with *Xba*I and *Bam*HI and subsequently cloned into pBI101 to form the *GUS* chimeric gene.

To make the inducible *SAUR36* overexpression constructs, the *SAUR36* coding regions with or without the DST element were cloned into the inducible binary vector pGL1152 (Guo and Gan, 2006).

The above binary vectors were transferred into *Agrobacterium tumefaciens* strain ABI. The *A. tumefaciens* cells containing the related binary vector constructs were used to transform Arabidopsis plants via floral dipping (Clough and Bent, 1998). Plant transformants were selected on one-half-strength Murashige and Skoog medium containing suitable antibiotics and then transferred to soil. Approximately 30 T1 transgenic lines for each transgene were selected; phenotypic analyses were performed in the T2 generation and further confirmed in the T3 generation. Homozygous plants were used in all experiments.

### Chemical Induction of Gene Expression

Glucocorticoid treatments were performed as described previously (Aoyama and Chua, 1997). Twenty-day-old plants were sprayed with 30  $\mu\text{M}$  DEX (synthetic glucocorticoid). The images were recorded after 3 d, and the seedlings were sampled for chlorophyll content, fluorescence, and ion leakage assays.

### Histochemical GUS Staining, Chlorophyll Content, Fluorescence, and Ion Leakage Assay

Chlorophyll, fluorescence, and ion leakage analyses as well as histochemical GUS staining were performed as described previously (Zhang and Gan, 2012; Zhang et al., 2012).

### Transcript Analysis

RNA extraction, complementary DNA synthesis, and qPCR analysis were carried out as described previously (He and Gan, 2002). First-strand complementary DNA was synthesized from 3  $\mu\text{g}$  of total RNA (treated with RNase-free DNase; New England Biolabs) at 42°C with Promega MV-Reverse Transcriptase. For qPCR, 1  $\mu\text{L}$  of each diluted sample was used as a template in a 25- $\mu\text{L}$  reaction following the standard methods. All PCRs were performed on a Bio-Rad IQ-5 thermocycler with 40 cycles and an annealing temperature of 55°C. Cycle threshold values were determined by IQ-5 Bio-Rad software assuming 100% primer efficiency. Three mRNA samples from three independently harvested leaf samples were qPCR analyzed.

### Auxin Treatment

The synthetic auxin NAA (Sigma) was used. The sixth leaves from approximately 30-d-old wild-type plants were incubated for various times at room temperature in 3 mM MES buffer (pH 5.7) containing 20  $\mu\text{M}$  NAA.

Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under the following accession numbers: *SAG201/SAUR36* (AT2G45210), *SAG12* (AT5G45890), *ACTIN2* (AT3G18780).

### Supplemental Data

The following materials are available in the online version of this article.

**Supplemental Figure S1.** Nucleotide and deduced amino acid sequence of *SAG201/SAUR36* and the alignment of highly conserved DST sequence.

**Supplemental Table S1.** Primers used in this study.

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