

**Rapid Paper**

## LEAFY COTYLEDON1 Controls Seed Storage Protein Genes through Its Regulation of *FUSCA3* and *ABSCISIC ACID INSENSITIVE3*

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*Arabidopsis ABSCISIC ACID INSENSITIVE3 (ABI3)*, *FUSCA3 (FUS3)* and *LEAFY COTYLEDON1 (LEC1)* encode key transcription factors that control seed maturation events, including seed storage protein (SSP) accumulation. Although *lec1* mutations are known to down-regulate *SSP* gene expression as the *fus3* or *abi3* mutation does, the mechanisms by which *LEC1* regulates *SSP* gene expression are largely unknown compared with the mechanisms utilized by *FUS3* or *ABI3*. We expressed *LEC1* ectopically in transgenic plants using an artificial dexamethasone (Dex) induction system. The ectopic expression of *LEC1* also resulted in the induction of *FUS3* and *ABI3* expression, which preceded the induction of *SSP* expression. The expression of *FUS3* and *ABI3* was found to be down-regulated in developing siliques of the *lec1* mutant. Furthermore, the levels of ectopic *SSP* induction by *LEC1* were greatly or moderately reduced in transgenic plants with an *abi3* or *fus3* mutant background, respectively. *LEC1*-induced ectopic expression of the *Atlg62290* aspartic protease gene, which was identified to be regulated preferentially by *FUS3*, was more severely affected in the *fus3* mutant than in the *abi3* mutant. From these data, we suggest that *LEC1* controls the expression of the *SSP* genes in a hierarchical manner, which involves *ABI3* and *FUS3*.

**Keywords:** *ABI3* — *Arabidopsis thaliana* — *FUS3* — *LEC1* — Seed development — Seed storage protein.

Abbreviations: CaMV, cauliflower mosaic virus; Dex, dexamethasone; SSP, seed storage protein.

### Introduction

Accumulation of specific proteins and other compounds for nutrient storage to high levels is one of the characteristic events during seed development. Seed storage material accumulation occurs during the latter half of embryogenesis or the maturation phase where morphogenesis has already been

accomplished (Harada 1997). The embryo also acquires desiccation tolerance and dormancy during the maturation phase (Harada 1997). These events, including seed storage protein (SSP) accumulation, are considered to be regulated by a common, at least in part, genetic program because mutations are known which affect a wide range of events that occur during the maturation phase (Koornneef et al. 1984, Finkelstein and Somerville 1990, Meinke 1992, Nambara et al. 1992, Bäumllein et al. 1994, Keith et al. 1994, Meinke et al. 1994, Parcy et al. 1994, West et al. 1994, Nambara et al. 1995, Parcy et al. 1997, Raz et al. 2001). In *Arabidopsis*, *ABI3*, *FUS3* and *LEC1* are the three major regulatory genetic loci of the maturation program (Koornneef et al. 1984, Meinke et al. 1994, Bäumllein et al. 1994, Keith et al. 1994, Meinke et al. 1994, West et al. 1994). Also, the *LEC2* gene is reported to have a similar function (Meinke et al. 1994). The plant hormone abscisic acid (ABA) participates in the regulation of seed maturation and is closely linked to the function of *ABI3* (Koornneef et al. 1984, Giraudat et al. 1992, Parcy et al. 1994, Parcy et al. 1997). All of these regulatory genes encode transcription factors. *ABI3*, *FUS3* and *LEC2* are members of a transcription factor family, which contains a plant-specific DNA-binding domain called B3 (Giraudat et al. 1992, Luerßen et al. 1998, Stone et al. 2001). *LEC1* encodes a homolog of the CCAAT-binding factor HAP3 subunit (Lotan et al. 1998). The B3 domains of *ABI3*, *FUS3* and *LEC2* are most closely related to each other among the B3-containing protein family (Giraudat et al. 1992, Luerßen et al. 1998, Stone et al. 2001). In addition to direct binding to DNA via the B3 domain (Suzuki et al. 1997, Monke et al. 2004), *ABI3* also functions as a coactivator by interacting with the ABA-response element (ABRE)-binding factors (Hattori et al. 1992, Hattori et al. 1995, Carson et al. 1997, Hobo et al. 1999, Nakamura et al. 2001).

Despite the molecular similarities between *FUS3* and *ABI3*, *fus3* mutants resemble *lec1* mutants morphologically in that they exhibit leafy traits in cotyledons, as represented by trichome formation and anthocyanin accumulation (Meinke 1992, Bäumllein et al. 1994, Keith et al. 1994, West et al. 1994). *abi3* mutant seeds also display unique phenotypes, such as failure in chlorophyll degradation and ABA insensitivity upon ger-

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mination, which distinguish *abi3* mutants from *fus3* and *lec1* mutants (Koornneef et al. 1984, Finkelstein and Somerville 1990, Meinke 1992, Nambara et al. 1992, Parcy et al. 1994, Nambara et al. 1995, Parcy et al. 1997). The similarity between the *fus3* and *lec1* mutant phenotypes suggests that wild-type *fus3* and *lec1* regulate a common set of downstream genes. However, genetic studies have revealed no epistatic relationships between them at least for the morphological phenotypes (West et al. 1994, Lotan et al. 1998, Raz et al. 2001). Despite the phenotypic differences between *fus3/lec1* and *abi3* mutants, these loci genetically interact as the phenotypes specific to the monogenic mutant of either type are enhanced in digenic mutants (Parcy et al. 1997, Nambara et al. 2000, Raz et al. 2001). Synergistic interactions are also observed for phenotypes common to *lec*-type and *abi3* mutants such as the suppressive effect on *SSP* gene expression (Parcy et al. 1997, Nambara et al. 2000).

12S globulins (cruciferins) and 2S albumins are the two abundant classes of SSP in *Arabidopsis* and are encoded by gene families with 4–5 members: *Cruciferins* consist of *CRA-CRC* and an unnamed member, and *2S albumins* consist of *At2S1–At2S4* and an unnamed member (Pang et al. 1988, Guerche et al. 1990). The expression of these storage protein genes is greatly reduced in the mutants of the above-mentioned seed maturation regulatory genes (Nambara et al. 1992, Bäumlein et al. 1994, Keith et al. 1994, Meinke et al. 1994, Parcy et al. 1994, West et al. 1994, Nambara et al. 1995, Parcy et al. 1997, Nambara et al. 2000). Interactions of *FUS3* or *ABI3* with the promoters of the *SSP* genes have been investigated by experimental methods such as the transient co-transfection assay and DNA–protein interaction assays in yeast cells or in vitro (Bobb et al. 1997, Luerßen et al. 1998, Ezcurra et al. 2000, Reidt et al. 2000, Kroj et al. 2003, Monke et al. 2004). Such analyses have revealed that both *FUS3* and *ABI3* bind to a *cis*-element, called the RY repeat (CATGCA), present in the promoters of 2S albumin genes and other seed-specific promoters. When *ABI3* or *FUS3* is ectopically expressed, the *SSP* genes are activated in vegetative tissues in a completely or partially ABA-dependent manner (Parcy et al. 1994, Parcy et al. 1997, Kagaya et al. 2005). In addition to the direct interaction with the promoters, we have demonstrated that the activation of the *SSP* genes by *FUS3* requires secondary regulatory factor(s), which are induced by *FUS3*.

In contrast to the increasing knowledge about the molecular action of *FUS3* and *ABI3*, the mechanisms through which *LEC1* regulates *SSP* and other seed-specific genes are largely unknown. When *LEC1* is ectopically expressed using the cauliflower mosaic virus (CaMV) 35S promoter, somatic embryos are formed in vegetative tissues, and seed-specific genes, including the *SSP* genes, are activated (Lotan et al. 1998). However, it is unknown whether such ectopic expression of the *SSP* genes is a result of ectopic embryogenesis or can be induced by *LEC1* without embryogenesis. In addition, no genes

under direct regulation by *LEC1* have been identified. Thus, no *cis*-elements through which *LEC1* acts have been identified, though *LEC1* is known to be a homolog of a subunit of the CCAAT-binding factor.

In the present study, *LEC1* was ectopically expressed using an artificial dexamethasone (Dex) induction system (Aoyama and Chua 1997). This induction system showed that the *SSP* genes are induced by *LEC1* with slower kinetics compared with the induction by *FUS3* or *ABI3*, but within a time range where no ectopic embryogenesis is initiated. Furthermore, we show that *LEC1* induction of the *SSP* genes requires functional *FUS3* and *ABI3* genes, which were also demonstrated to be rapidly induced by *LEC1*. From these data, we suggest that *LEC1* controls the expression of the *SSP* genes in a hierarchical manner, which involves *ABI3* and *FUS3*.

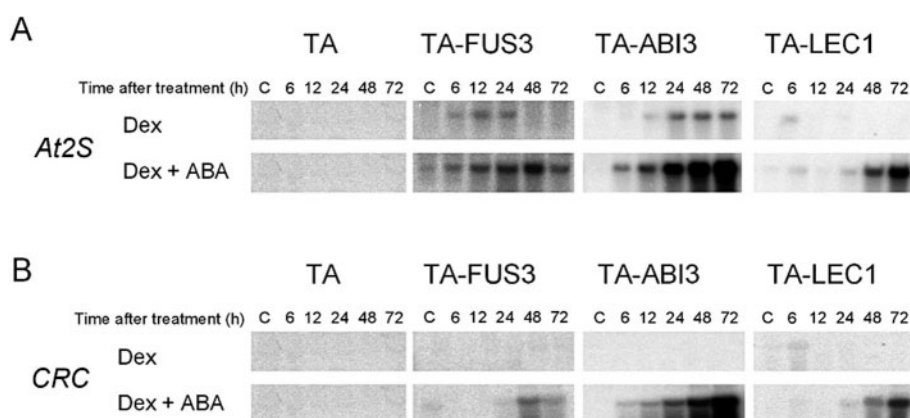
## Results

### *Ectopic expression of LEC1 results in the induction of the SSP genes*

Previously, Lotan et al. (1998) reported that constitutive expression of *LEC1*, driven by the CaMV 35S promoter, resulted in the formation of somatic embryos in vegetative tissues. As expected, the *SSP* genes were expressed in these somatic embryos. However, these results do not provide much information about the mechanisms of how *LEC1* is involved in the regulation of the *SSP* genes because the *SSP* gene expression could be regarded as a consequence of embryogenesis resulting from the long-term effect of ectopic *LEC1* expression. In order to study the effect of *LEC1* ectopic expression for a defined time period, transgenic *Arabidopsis* lines (TA-*LEC1*) were generated, in which *LEC1* expression could be induced by Dex treatment.

When young TA-*LEC1* plants (7 d old) were treated with Dex in the presence of ABA, the transcripts of two *SSP* genes, *At2S3* and *CRC*, were found to be induced (Fig. 1A, B). Very low levels of induction were observed at 6 h of treatment, but the expression of *CRC* and *At2S3* became significant after 24 h. The induction levels of these *SSP* transcripts in TA-*LEC1* lines were comparable with or somewhat lower than those in the TA-*ABI3* or TA-*FUS3* plants in which *ABI3* or *FUS3*, respectively, could be induced by Dex treatment. Although accurate comparisons were difficult because of the differences in the expression levels, the induction kinetics of *CRC* and *At2S3* expression in TA-*LEC1* plants appeared to be slower than those in TA-*FUS3* and *ABI3* plants (Fig. 1).

While the *SSP* transcripts accumulated to significant levels after 72 h of *LEC1* induction, no ectopic embryogenesis or other morphological abnormalities were observed in TA-*LEC1* plants within this time period (Fig. 2). Thus, the ectopic activation of *SSP* genes by *LEC1* occurred independently of embryogenesis.



**Fig. 1** Induction of *SSP* mRNA by Dex and ABA in TA-FUS3, TA-ABI3 or TA-LEC1 transgenic plants. Seven-day-old transgenic *Arabidopsis* plants carrying glucocorticoid-inducible FUS3 (TA-FUS3), ABI3 (TA-ABI3) or LEC1 (TA-LEC1) transgenes, or transformed with empty pTA7001 vector (TA) were treated with 30  $\mu$ M Dex alone or simultaneously with 50  $\mu$ M ABA for the indicated times. RNA was prepared from these plants and analyzed by Northern blot analysis using  $^{32}$ P-labeled probes specific to the indicated genes. Lanes labeled 'C' were loaded with RNA samples from plants before treatment. No variation in RNA loading and quality between samples, which might affect the interpretation of the results, was observed based on the examination of ethidium bromide-stained rRNA bands (data not shown).

#### *Ectopic expression of LEC1 induces the expression of FUS3, ABI3 and LEC2*

Cross-regulation among seed maturation-related transcription factors has been described (Parcy et al. 1997, Söderman et al. 2000, Brocard et al. 2002, Brocard-Gifford et al. 2003, Kroj et al. 2003). In addition to those observations, the seemingly slower induction kinetics of the *SSP* genes by LEC1 compared with those by FUS3 or ABI3 led us to hypothesize that the LEC1 activation of *SSP* genes occurred via the activation of FUS3 and/or ABI3. In agreement with the hypothesis, the expression of FUS3 and ABI3 was found to be induced by Dex in TA-LEC1 but not in the control TA plants (Fig. 3). The induction of FUS3 and ABI3 was already observed at plateau levels after 6 h of Dex treatment (Fig. 3B) in the absence of ABA. Therefore, the activation of FUS3 and ABI3 by LEC1 was significantly rapid and likely to be caused by direct action

of LEC1 on the FUS3 and ABI3 promoters. The LEC1-induced expression of FUS3, but not that of ABI3, was enhanced after 48 h in the presence of ABA. In addition to FUS3 and ABI3, induction of LEC2 by Dex treatment was also observed in TA-LEC1 plants with slower kinetics than that of FUS3 or ABI3.

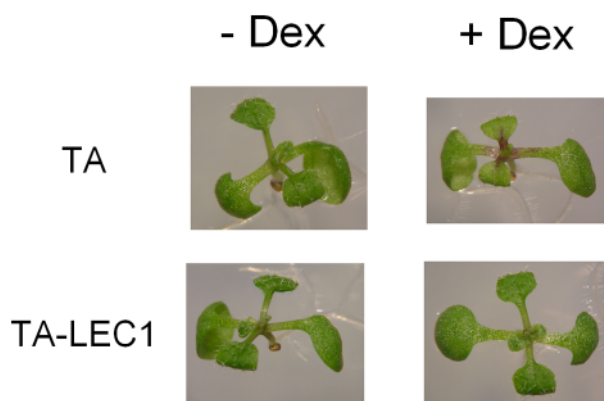
In contrast to the previous observation with TA-ABI3 and TA-FUS3 plants, in which the transgene expression levels declined at later times after Dex treatment (Kagaya et al. 2005), LEC1 transcript levels in TA-LEC1 plants tended to increase after 48 h of Dex treatment, particularly in the absence of ABA (Fig. 3D). Therefore, it could be possible that LEC1 may up-regulate its own gene.

#### *Expression of FUS3 and ABI3 is controlled by LEC1 in developing seeds*

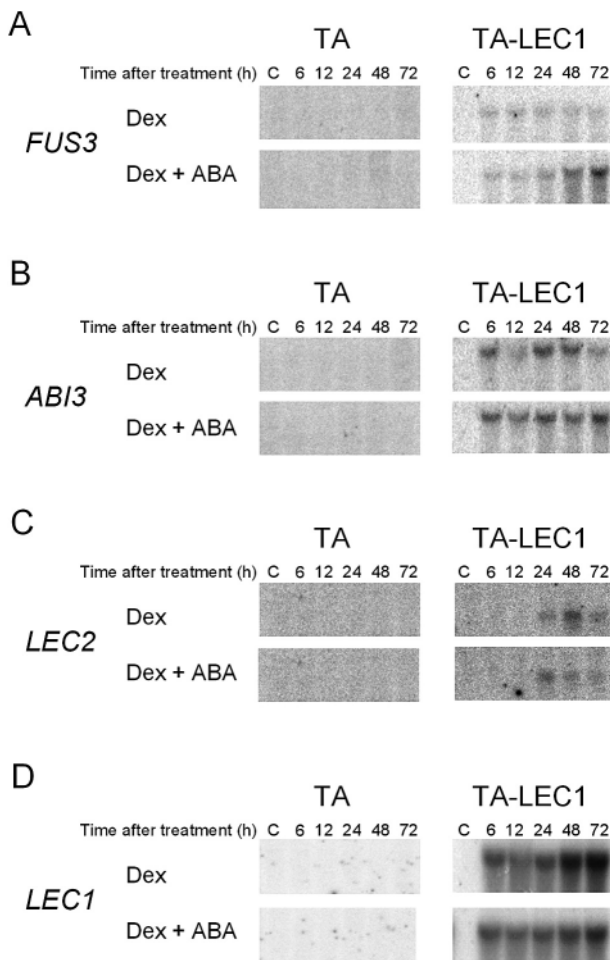
Since FUS3 and ABI3 were found to be ectopically induced by LEC1 in vegetative tissues, we tested if LEC1 regulates FUS3 and ABI3 during seed development. Northern analysis performed with RNA from developing siliques of the wild-type and *lec1-1* mutant plants (Meinke 1992, Meinke et al. 1994) showed that the levels of ABI3 and FUS3 transcripts were significantly reduced in the *lec1* mutant (Fig. 4). These results supported that LEC1 controls the expression of the *SSP* genes via the regulation of FUS3 and ABI3 in developing seeds.

#### *Ectopic induction of the SSP genes by LEC1 requires functional ABI3 and FUS3 alleles*

To confirm further that ectopic induction of the *SSP* genes by LEC1 is mediated by FUS3 and ABI3, the TA-LEC1 transgene was transferred into the *abi3* or *fus3* mutant background by crossing. The levels of *CRC* and *At2S3* induction by Dex treatment in vegetative tissues were reduced moderately in the *fus3-3* mutant and greatly in the *abi3-6* mutant as compared



**Fig. 2** Photographs of 10-day-old TA-LEC1 or the control TA plants that had been treated with 30  $\mu$ M Dex (+Dex) for 72 h and the untreated plants (-Dex).

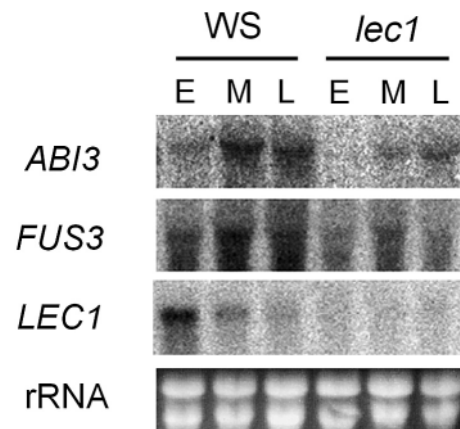


**Fig. 3** Induction of *FUS3* (A), *ABI3* (B), *LEC2* (C) or *LEC1* (D) mRNAs by Dex and ABA in TA-LEC1 transgenic plants. TA-LEC1 or the control TA transgenic plants were treated and analyzed as described for Fig. 1. No variation in RNA loading and quality between samples, which might affect the interpretation of the results, was observed based on the examination of ethidium bromide-stained rRNA bands (data not shown).

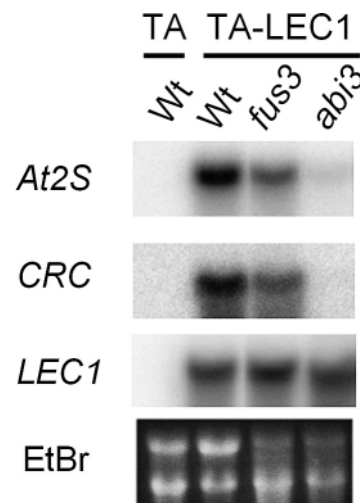
with TA-LEC1 plants with the wild-type background (Fig. 5). This indicated that the ectopic induction of *CRC* and *At2S3* in vegetative tissues by LEC1 required, at least in part, functional *FUS3* and *ABI3*, thus showing that the regulation of the *SSP* genes by ectopic expression of LEC1 was mediated by *FUS3* and *ABI3*, the induction of which by LEC1 preceded the *SSP* gene induction.

*LEC1 regulation of an aspartic protease gene is also mediated by FUS3*

From preliminary microarray analysis using TA-*FUS3* transgenic plants, we identified that the gene encoding an isoform of aspartic protease (*At1g62290*) was ectopically induced by *FUS3* in either the presence or absence of ABA. However, it was not, or only to very low levels, induced in TA-*ABI3* plants



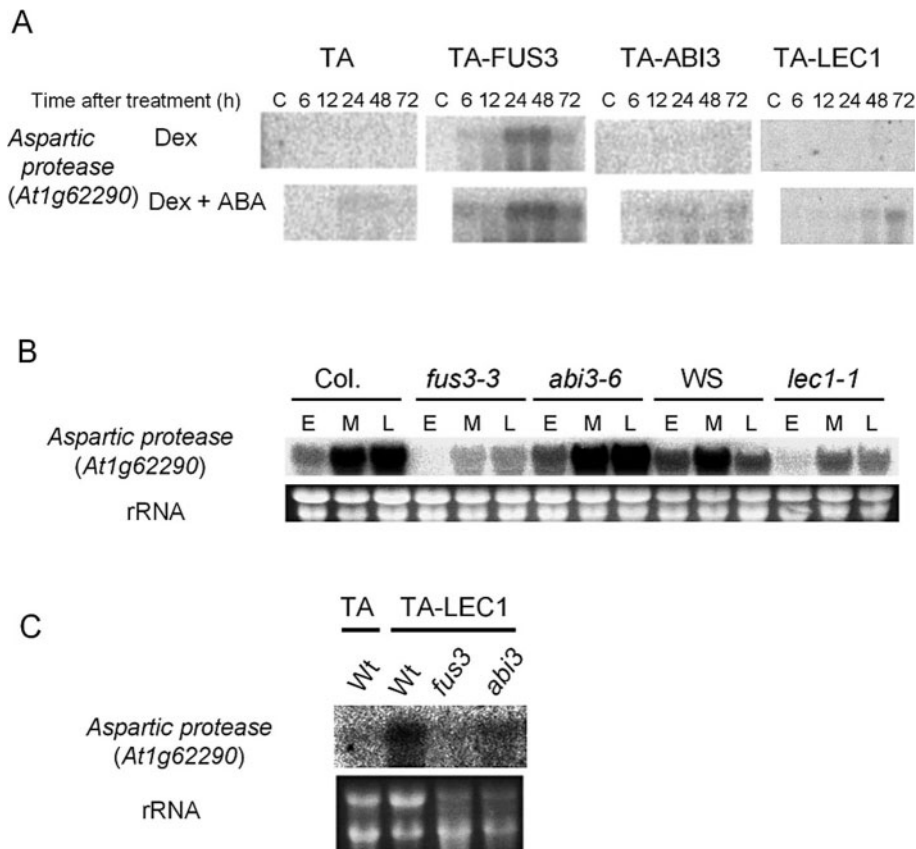
**Fig. 4** Regulation of *ABI3* and *FUS3* expression by *LEC1* in developing seed. RNA was prepared from developing siliques of wild-type (ecotype WS) *Arabidopsis* plants (WS) or the *lec1-1* mutant at 4–7 days after flowering (DAF, E), 8–11 DAF (M) or 12–15 DAF (L), and analyzed by Northern analysis using  $^{32}$ P-labeled probes specific for the indicated genes. Ethidium bromide (EtBr)-stained rRNA bands are shown to verify RNA loading and quality.



**Fig. 5** Dependence of ectopic *At2S3* and *CRC* induction on the functional allele of *ABI3* or *FUS3*. Seven-day-old TA-LEC1 transgenic plants with wild-type Columbia (Wt) or the *fus3-3* or *abi3-6* mutant background, or the control TA plants were treated with 30  $\mu$ M Dex alone or simultaneously with 50  $\mu$ M ABA for 72 h and analyzed by gel blot hybridization using  $^{32}$ P-labeled probes specific to the indicated genes. Ethidium bromide (EtBr)-stained rRNA bands are shown to verify RNA loading and quality.

upon Dex treatment (Fig. 6A). In accordance with this observation, the level of the *At1g62290* transcripts in developing siliques was found to be greatly lowered in the *fus3-3* plants but not in the *abi3-6* plants (Fig 6B). Therefore, the *At1g62290* aspartic protease gene was considered to be preferentially regulated by *FUS3*.

When the expression of the *At1g62290* aspartic protease gene was analyzed in TA-LEC1 plants, an induction was



**Fig. 6** FUS3-dependent LEC1 regulation of the *At1g62290* aspartic protease gene. (A) The expression of the *At1g62290* aspartic protease gene in TA, TA-FUS3, TA-ABI3 or TA-LEC1 transgenic plants was analyzed as in Fig. 1. No variation in RNA loading and quality between samples was observed based on the examination of ethidium bromide (EtBr)-stained rRNA bands (data not shown). (B) The expression of the *At1g62290* aspartic protease gene in developing siliques of wild type (Col. or WS) or the indicated mutants was analyzed as in Fig. 4. (C) Seven-day-old TA-LEC1 transgenic plants with wild type Columbia (Wt) or the *fus3-3* or *abi3-6* mutant background, or the control TA plants were treated and analyzed as in Fig. 5. EtBr-stained rRNA bands are shown to verify RNA loading and quality (B and C).

observed by Dex treatment in the presence of ABA with slower kinetics than that in TA-FUS3 plants (Fig. 6A). LEC1 regulation of the aspartic protease gene was confirmed by the decreased expression in the *lec1-1* mutant siliques (Fig. 6B). These results suggested that LEC1 regulation of the *At1g62290* gene was mediated preferentially by FUS3. ABA dependence of the *At1g62290* induction in TA-LEC1 plants, which was not observed in TA-FUS3 plants, was considered to be due to the enhancement of LEC1-induced FUS3 expression by ABA (Fig. 3A).

#### *Ectopic induction of the aspartic protease gene by LEC1 also preferentially depends on FUS3*

The ectopic *SSP* gene induction by LEC1 appeared to depend more on ABI3 than FUS3 judging from the more severe effect on the transcript levels in the *abi3-6* background than in the *fus3-3* background (Fig. 5), although the effects of the mutations in the *SSP* transcript levels in seeds were not as significantly different between the two mutants (Parcy et al. 1997). In contrast, the expression of the *At1g62290* aspartic protease gene depended more strongly on functional FUS3 than ABI3 in developing seeds (Fig. 6B). To correlate the LEC1-induced ectopic gene induction mediated by FUS3 and/or ABI3 with the gene regulation in developing seeds, LEC1-induced ectopic expression of the *At1g62290* gene in TA-LEC1 plants

was compared between the wild type and the *fus3* or *abi3* mutant background (Fig. 6C). The level of LEC1-induced expression of the *At1g62290* gene after Dex treatment was significantly lower in TA-LEC1 plants with the *fus3-3* background than in those with the wild-type or *abi3-6* background. Thus, the *At1g62290* gene also preferentially required FUS3 for LEC1-induced ectopic expression. Such correlation in the FUS3 preference between the ectopic expression and the expression in developing seeds supported the hypothesis that LEC1 controls the expression of certain sets of genes during seed development via its regulation of *FUS3* and/or *ABI3* as seen for the ectopic expression.

#### Discussion

In the present study, we showed that LEC1 was capable of activating *SSP* gene expression when it was ectopically induced in vegetative tissues of transgenic plants. The use of the artificial Dex induction system enabled us to perform kinetic analyses of LEC1-induced *SSP* gene expression. Such analyses revealed that *SSP* gene induction by LEC1 was slower than that by ABI3 or FUS3 but was not a result of ectopic embryogenesis caused by the long-term effect of LEC1 expression. Furthermore, we demonstrated that LEC1 was capable of activating the expression of *ABI3* and *FUS3* with rapid kinet-

ics. The reduced levels of LEC1-induced ectopic expression of the *SSP* genes in TA-LEC1 plants with an *abi3* or *fus3* mutant background indicated that the *SSP* genes were activated by ectopic expression of LEC1 via the induction of the *ABI3* and *FUS3* transcription factors. In addition, the following results supported that such a regulatory cascade is likely to represent a part of the regulatory networks for *SSP* expression operating in the developing embryo. First, the expression of *ABI3* and *FUS3* was regulated by LEC1 because the *ABI3* and *FUS3* transcript levels were lowered in the *lec1* mutant embryo. Secondly, the relative dependency on *FUS3* or *ABI3* of ectopic induction of the *At1g62290* aspartic protease gene by LEC1 correlated with those in developing seed.

LEC1 regulation of *FUS3* was consistent with the report by Brocard-Gifford et al. (2003). However, the down-regulation of the *ABI3* transcript in *lec1* mutants appeared contradictory to the results of Parcy et al. (1997), in which no difference was observed in the *ABI3* protein levels between the wild-type and the *lec1* mutant embryo. The reason for the apparent discrepancy is not clear, but it may be derived from the differences in the stages of embryo samples, which had not been clearly defined in their report.

The *LEC2* transcript was also found to be induced by LEC1. The regulation of *LEC2* by LEC1 had not been known previously. However, such regulation remains to be investigated in developing seeds. Ectopic *LEC2* induction by LEC1 was significantly slower than that of *FUS3* and *ABI3*. Therefore, LEC1 may regulate *LEC2* via intermediate regulatory factor(s), whose synthesis depends on LEC1.

The *FUS3* and *ABI3* transcripts were still present in *lec1-1* developing seeds, although they were significantly reduced. Therefore, LEC1 regulation of *FUS3* and *ABI3* is considered to be quantitative. Nevertheless, the effects of the *lec1* mutation on *SSP* expression at both the mRNA and protein level were as severe as the *fus3* or *abi3* single mutation (Parcy et al. 1997). One explanation for the apparently greater effect of the *lec1* mutation on *SSP* gene expression than that on *FUS3* or *ABI3* expression is that LEC1 regulates *SSP* expression through both *FUS3/ABI3*-dependent and -independent mechanisms. However, combination of the *abi3* and *fus3* mutation has been reported to affect the *SSP* expression synergistically as well as other maturation-related phenotypes (Keith et al. 1994, Parcy et al. 1997, Nambara et al. 2000). Therefore, an alternative possibility is that partial inhibition of both *FUS3* and *ABI3* expression in the *lec1* mutant may synergistically affect *SSP* expression in developing seed, thereby resulting in the greater reduction of *SSP* expression than that expected from the additive effects of the decrease in the *FUS3* and *ABI3* transcript levels.

It should be noted that the hierarchical LEC1 regulation of the *SSP* genes via *FUS3* and *ABI3* is likely to be a part of the regulatory networks of the *SSP* gene regulation because the LEC1 regulation of *ABI3* and *FUS3* is only quantitative, as mentioned above. It remains to be assessed as to what extent

*FUS3/ABI3*-dependent LEC1 regulation of the *SSP* genes contributes to the overall network, although it is not easy to do so. Yet, the inducible ectopic expression of LEC1 in the present study effectively uncovered the presence of such a transcription cascade by isolating it from the complex regulatory network operating in developing seed. Despite the fact that very similar phenotypes are displayed by *fus3* and *lec1* mutants, no epistatic relationships have been revealed by genetic studies (West et al. 1994, Lotan et al. 1998, Raz et al. 2001). However, the results of these genetic studies are not necessarily inconsistent with our results if we envisage the hierarchical regulation as an element of the complex regulatory network. Additionally, it should be noted that no epistasis analysis has actually been performed with respect to the *SSP* phenotypes.

The *abi3* mutation affected the LEC1-induced ectopic expression of the *SSP* genes more severely than the *fus3* mutation did. Thus, it appeared that the contribution of *ABI3* to the LEC1-regulated *SSP* gene expression is greater than that of *FUS3*. However, the difference may not necessarily reflect the relative contribution of *ABI3* and *FUS3* to the LEC1 regulation of the *SSP* genes in developing seeds because the regulation observed in the ectopic expression experiments was considered as an element of the regulatory network operating in the developing seeds.

LEC1 is a homolog of the HAP3 subunit of the trimeric CCAAT-binding factor that is widely distributed from fungi to mammals and may function in a similar complex in plants (Lotan et al. 1998). However, such a complex with the LEC1 subunit is expected to regulate a specific set of genes mainly related to embryo development, but not to function as a general transcription factor because of the seed-specific phenotypes of the mutants. Although the downstream genes of *LEC1* are known, neither direct targets nor the *cis*-elements of the putative LEC1 complex have been identified. The induction of *ABI3* and *FUS3* in TA-LEC1 plants by Dex was extremely rapid. In an independent experiment, we observed *ABI3* induction in TA-LEC1 plants within 3 h (Y. Kagaya and T. Hattori, unpublished result), comparable with the induction kinetics of *AtEm1* and *AtEm6* genes by *ABI3*, which probably represented a direct activation via the interaction with ABRE-binding factor (Kagaya et al. 2005). Therefore, *ABI3* and *FUS3* are likely candidates for the direct targets of the LEC1 complex. Analyses of the *ABI3* or *FUS3* promoters with respect to LEC1 regulation will identify the *cis*-element for the LEC1 complex and ultimately lead to a fundamental understanding of the molecular mechanism of LEC1 action.

## Materials and Methods

### Plasmid construction

The *LEC1* coding sequence was amplified by polymerase chain reaction (PCR) from the expressed sequence tag (EST) clone SQ243a11F (Kazusa DNA Research Institute) and synthetic primers T3 (5'-AAT TAA CCC TCA CTA AAG GG-3') and Rev LEC1 Salt

(5'-TGC TGT CGA CTA TAG AAA TGT TAT TGA TAAG-3'), digested with *Bam*HI and *Sal*I, and cloned into the corresponding sites p35S-DH-His-NosT (Kagaya et al. 2005). A dHA-His-tagged *LEC1* sequence was excised with *Bam*HI and *Not*I, blunt-ended with *Kle*now, and cloned into the *Eco*RV site of pBluescript. The insert in the pBluescript subclone was excised with *Xho*I and *Spe*I, and cloned into the corresponding sites of pTA7001, which subsequently was used to transform *Arabidopsis*.

#### Transgenic plants

TA-LEC1 transgenic plants were produced using the LEC1 construct in pTA7001 (see above) as described previously (Kagaya et al. 2005). Ectopic induction of *CRC*, *ABI3* and *FUS3* was confirmed in eight independent TA-LEC1 lines, from which a representative line was selected for detailed analyses.

#### Genetic cross

TA-LEC1 transgenic *Arabidopsis* was crossed with the *abi3-6* (Nambara et al. 1994) or *fus3-3* (Keith et al. 1994, Luerßen et al. 1998) mutant. The F<sub>2</sub> seedlings homozygous for *fus3-3* or *abi3-6* were selected by the visible phenotypes or by PCR that discriminates the presence or absence of the internal deletion specific to the *abi3-6* allele, respectively. F<sub>3</sub> generations that carried the TA-LEC1 transgene and showed levels of transgene expression comparable with those with the wild-type Columbia background were selected and utilized for experiments.

#### Other methods

Plant treatments, RNA isolation and Northern analysis were performed as described previously (Kagaya et al. 2005).

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