The Arabidopsis BRAHMA Chromatin-Remodeling ATPase Is Involved in Repression of Seed Maturation Genes in Leaves[1][W][OA]

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Synthesis and accumulation of seed storage proteins (SSPs) is an important aspect of the seed maturation program. Genes encoding SSPs are specifically and highly expressed in the seed during maturation. However, the mechanisms that repress the expression of these genes in leaf tissue are not well understood. To gain insight into the repression mechanisms, we performed a genetic screen for mutants that express SSPs in leaves. Here, we show that mutations affecting BRAHMA (BRM), a SNF2 chromatin-remodeling ATPase, cause ectopic expression of a subset of SSPs and other embryogenesis-related genes in leaf tissue. Consistent with the notion that such SNF2-like ATPases form protein complexes in vivo, we observed similar phenotypes for mutations of AtSWI3C, a BRM-interacting partner, and BSH, a SNF5 homolog and essential SWI/SNF subunit. Chromatin immunoprecipitation experiments show that BRM is recruited to the promoters of a number of embryogenesis genes in wild-type leaves, including the *2S* genes, expressed in *brm* leaves. Consistent with its role in nucleosome remodeling, BRM appears to affect the chromatin structure of the *At2S2* promoter. Thus, the BRM-containing chromatin-remodeling ATPase complex involved in many aspects of plant development mediates the repression of SSPs in leaf tissue.

Seed storage proteins (SSPs) accumulate to high levels in seeds, in part, to provide a source of nutrients for the developing seedling after germination. Synthesis and accumulation of SSPs occurs specifically during the maturation phase of seed development and is not observed in vegetative organs of the plant (Harada, 1997; Laux and Jurgens, 1997; Vicente-Carbajosa and Carbonero, 2005). Although the regulatory network controlling SSP genes in developing seeds is being elucidated, little is known of the molecular mechanisms that prevent SSP gene expression in vegetative organs.

In Arabidopsis (*Arabidopsis thaliana*), SSPs are encoded by small gene families (Fujiwara et al., 2002). There are five genes encoding 2S albumin isoforms, designated as *At2S1* to *At2S5* (Krebbers et al., 1988; van der Klei et al., 1993). The *At2S1* to *At2S4* genes are tightly linked in a tandem array, while *At2S5* is not linked. The 12S globulins are encoded by three genes in the Columbia (Col) accession, designated as *CRA1*, *CRB*, and *CRC* (Pang et al., 1988). These genes are specifically and highly expressed in the seeds.

In Arabidopsis, ABI3, FUS3, LEC1, and LEC2 are master regulators of seed maturation (Giraudat et al., 1992; Lotan et al., 1998; Luerssen et al., 1998; Stone et al., 2001), and they regulate each other (Kagaya et al., 2005b; To et al., 2006). ABI3, FUS3, and LEC2 are closely related members of a plant-specific B3-domain transcription factor family (Giraudat et al., 1992; Luerssen et al., 1998; Stone et al., 2001). *LEC1* encodes a novel homolog of the CCAAT-binding factor HAP3 subunit (Lotan et al., 1998). Loss-of-function mutations in *ABI3*, *FUS3*, and *LEC1* give rise to pleiotropic seed phenotypes, including significant reduction of SSPs. These regulatory genes are mainly expressed in the seeds. When misexpressed in vegetative tissues, they are able to induce ectopic expression of the SSP genes, although in the case of ABI3, the plant hormone abscisic acid (ABA) is also required (Parcy et al., 1994; Lotan et al., 1998; Gazzarrini et al., 2004; Kagaya et al., 2005a). Monogenic *lec2* mutations affect maturation in specific spatial domains of the seed. When LEC2 is overexpressed in leaves, it is sufficient to induce embryo development, including the accumulation of SSPs (Stone et al., 2001; Santos Mendoza et al., 2005; Braybrook et al.,

[1] This work was supported by Agriculture and Agri-Food Canada's Canadian Crop Genomics Initiative and Genome Prairie/Genome Canada.

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The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Yuhai Cui (cuiy@agr.gc.ca).

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www.plantphysiol.org/cgi/doi/10.1104/pp.108.121996

2006). It is believed that these master regulators activate embryogenesis genes by directly binding to RY DNA elements in the promoters of the target genes (Suzuki et al., 1997; Reidt et al., 2000; Braybrook et al., 2006).

The in vivo mechanisms controlling the repression of embryonic traits in vegetative tissues are not well understood. One potential mode of regulation involves repression of genes encoding the master regulators through the action of Arabidopsis PICKLE (PKL; Ogas et al., 1997, 1999; Rider et al., 2003, 2004; Li et al., 2005). The primary roots of *pkl* mutants express embryonic traits and become enlarged due to accumulation of high levels of seed storage reserves including SSPs (Ogas et al., 1997; Rider et al., 2004). PKL is necessary for the repression of *LEC1*, *LEC2*, and *FUS3* genes. These genes are expressed at 100-fold higher levels in *pkl* mutant roots as compared with the wild type (Rider et al., 2003). PKL is a CHD3 chromatin-remodeling factor (Eshed et al., 1999; Ogas et al., 1999), suggesting the involvement of chromatin remodeling in the repression process.

A second potential mechanism to account for the absence of SSPs in vegetative organs is direct repression of the SSP genes. Studies with the β-phaseolin promoter from the bean *Phaseolus vulgaris* suggest the involvement of chromatin remodeling in the repression and activation of SSP genes in leaves (Li et al., 1998, 1999; Li and Hall, 1999; Ng et al., 2006). The β-phaseolin gene is not detectably expressed during vegetative phases of plant development (van der Geest et al., 1995), and the gene is in a repressive chromatin architecture in leaves (Li et al., 1998). Repressive β-phaseolin chromatin becomes remodeled concomitant with gene activation in the developing seed or in the leaf tissue misexpressing an ABI3 homolog (Li and Hall, 1999; Ng et al., 2006). However, the factor(s) responsible for these changes in chromatin architecture are not known.

To identify genes that repress the expression of SSP genes in leaf tissue, we took a genetic approach to screen for Arabidopsis mutants ectopically expressing a reporter gene driven by a SSP gene promoter. Mutants isolated from this screen express endogenous SSPs and other embryonic traits in leaves. Here, we report one of the mutations, *essp3* (*ectopic expression of seed storage proteins3*). *ESSP3* encodes BRAMHA (BRM), a member of the SNF2 chromatin-remodeling ATPase family. This result provides new evidence that chromatin remodeling plays an important role in plant developmental transitions.

RESULTS

Identification of the *essp3* Mutant

We first generated transgenic Arabidopsis plants expressing *GUS* under the control of a soybean (*Glycine max*) β-conglycinin β-subunit gene promoter (βCG_{pro}; Fig. 1A). β-Conglycinin (also referred to as 7S globulin) is a major SSP in soybean. The promoter has been previously shown to direct seed-specific reporter gene expression in tobacco (Lessard et al., 1993) and Arabidopsis (Hirai et al., 1994). Experiments with our transgenic line (βCG_{pro}:*GUS*) confirmed previous observations that transgene expression is embryo specific and not detectable in vegetative tissues (Fig. 1, B and C). In addition, using in vitro culture of immature siliques, we demonstrated that the promoter responded positively to ABA (Fig. 1D), consistent with earlier results obtained with cultured developing soybean cotyledons (Bray and Beachy, 1985). These results indicate that the seed-specific expression of the soybean SSP gene promoter is well conserved in transgenic Arabidopsis.

Seeds from the homozygous βCG_{pro}:*GUS* line were mutagenized with ethyl methanesulfonate (EMS), and the M_2 plants were screened for mutations that induce ectopic GUS activity in leaves. Out of approximately 300,000 M_2 plants tested, 150 showed GUS activity in leaves. In the M_3 generation, 14 lines maintained the mutant phenotype. These mutant lines were then back crossed with the progenitor βCG_{pro}:*GUS* line, and segregation in the F_2 generation was analyzed. Six lines showed an approximate 1:3 ratio of plants displaying the mutant phenotype versus wild type, suggesting single locus control of the phenotype. These mutants were further analyzed and mapped. The mutated loci underlying the mutant phenotypes were mapped to six different chromosomal locations by bulked-segregant analysis. In this article, we report the genetic and molecular characterization of mutant *essp3*.

essp3 mutant plants exhibited strong ectopic GUS activity in leaves, including both rosette and cauline leaves but little expression in other organs (Fig. 1, E and F). GUS staining in leaves occurred in the epidermis, mesophyll, bundle sheath cells, and veins (Supplemental Fig. S1). Staining in veins was observed in the xylem and phloem, with heavy staining occurring in xylem parenchyma cells. Morphologically, mutant plants had curly leaves, and they flowered earlier than wild-type plants (approximately 4 d earlier; Fig. 1G). At maturity, the mutant plants were smaller in size, with less branches than wild type (Fig. 1H), and the stems were not rigid or substantial, giving the plants a frail appearance. When the *essp3* plants were crossed with the progenitor βCG_{pro}:*GUS* line, the F_1 plants showed no GUS activity in leaves and morphologically resembled wild-type plants. In the F_2 generation, among the 312 plants tested, 74 were GUS positive, indicating that *essp3* is a single recessive mutation (expected ratio, 1:3; $\chi^2 = 0.27$; $P > 0.60$).

ESSP3 Encodes the SNF2-Like Chromatin-Remodeling ATPase BRM

To map the *essp3* mutation, *essp3* plants were crossed with Landsberg *erecta* (without the βCG_{pro}:*GUS* transgene) to generate a segregating population. First, a bulked segregant analysis (Lukowitz et al., 2000) was

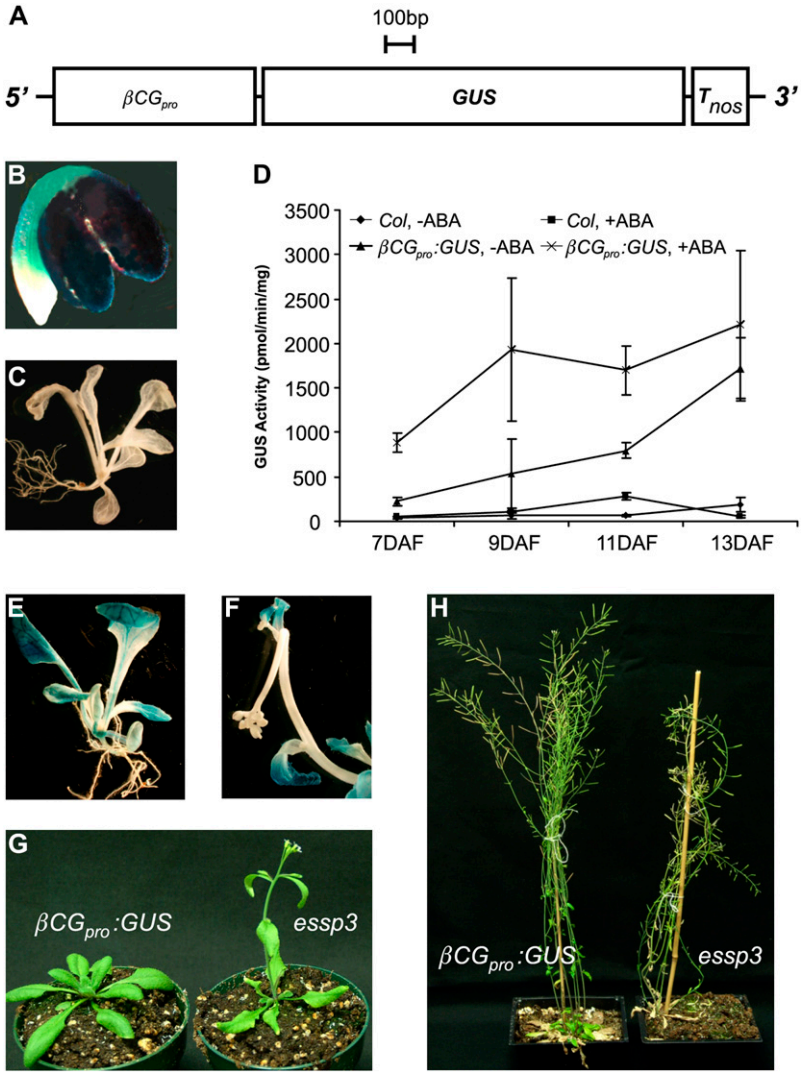

Figure 1. Phenotypes of the *essp3* mutant. A, Schematic representation of the βCG_{pro}:*GUS* reporter gene construct. B and C, Histochemical analysis of GUS expression in a representative wild-type βCG_{pro}:*GUS* transgenic embryo and a 14-d-old seedling grown on MS agar, respectively. D, Regulation of the βCG_{pro}:*GUS* reporter gene by ABA. Staged immature siliques were harvested from βCG_{pro}:*GUS* transgenic Arabidopsis plants, cultured on MS agar plates containing ABA (50 μM), and GUS activity was measured (picomoles/minutes/milligrams protein). DAF, Days after flowering. The mean and SE were determined from three measurements. Bars represent SEs. Siliques from wild-type (Col) Arabidopsis were used as control. E and F, GUS phenotype of the *essp3* mutant. Shown is representative histochemical staining of GUS activity of a 14-d-old seedling and cauline leaves from a mature plant grown on MS agar media with 1.5% Suc, respectively. G and H, Morphological comparison of wild-type βCG_{pro}:*GUS* and *essp3* mutant plants at 3 weeks and 6 weeks, respectively.

conducted that located the *essp3* mutation around the marker nga168 at the bottom of chromosome 2. For further mapping, 1,325 mutant plants were identified from the F_2 population based on the leaf morphology and GUS phenotype. Mapping with SSLP and SNP markers narrowed the mutation down to a genomic interval of about 100 kb (18,912–19,012 kb; Fig. 2A). There are 44 annotated protein-coding genes within this genomic region (www.arabidopsis.org). To identify the molecular lesion in *essp3*, all the coding regions were amplified by PCR and sequenced. A single G to A point mutation was identified in At2g46020, potentially leading to a sense mutation at the amino acid level, from Gly-1,137 to Arg-1,137. At2g46020 is identical to *BRM* (also called *AtBRM*), the Arabidopsis homolog of the *Drosophila* ATPase gene *BRM* (Farrona et al., 2004). BRM and its yeast counterpart SWI2/SNF2 belong to a conserved family of DNA-dependent ATPases termed the SNF2 family (Tamkun et al., 1992). The amino acid affected by the *essp3* mutation is a highly conserved residue across kingdoms in the ATPase domain (Fig. 2B).

BRM was recently demonstrated to be a regulator of shoot development, flowering time (Farrona et al., 2004; Hurtado et al., 2006), and cotyledon boundary formation (Kwon et al., 2006). Plants with *BRM* silenced by RNA interference exhibit a reduction in overall plant size with small and curled leaves, as well as a reduction in the size of the inflorescence meristem. *BRM*-silenced plants flower earlier than wild-type plants (Farrona et al., 2004). Thus, *BRM*-silenced plants share similarities with *essp3* mutants. To confirm that *ESSP3* is At2g46020, an 11.5-kb *Sac*I fragment (18,928,084–18,939,545 bp), which harbors At2g46020 and At2g46000 (no mutation was found within this gene), was subcloned from bacterial artificial chromosome (BAC) clone T3F17 and introduced into the *essp3* plants. In total, more than 200 transgenic lines were obtained, all of which showed wild-type morphology and no GUS activity in leaves (data not shown). In addition, two T-DNA insertion mutant alleles of At2g46020 were obtained from the Arabidopsis Biological Resource Center (ABRC; The Ohio

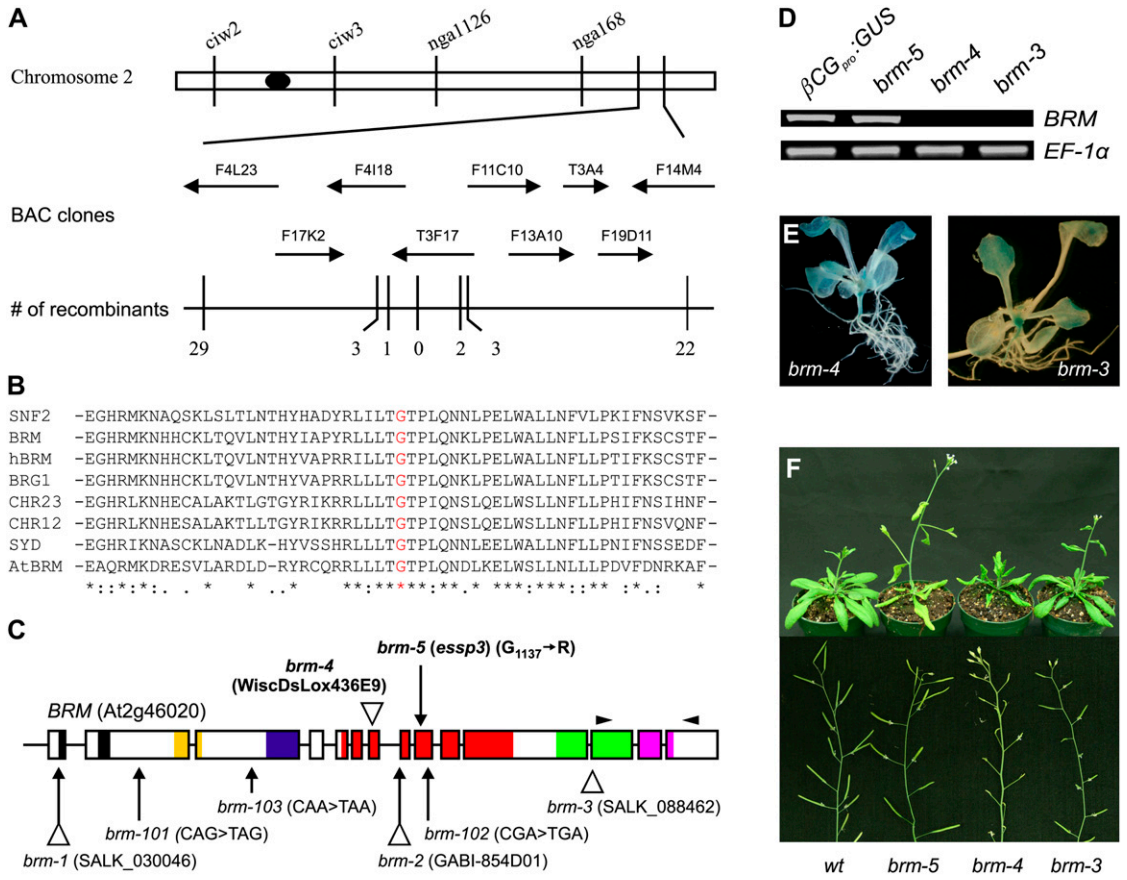

Figure 2. Map-based cloning of *essp3*. A, Fine genetic mapping with PCR-based markers locate the *essp3* locus to the bottom of chromosome 2, on BAC T3F17. The numbers of recombination events out of the total number of chromosomes examined (2,650) are indicated. B, Alignment of the deduced amino acid sequence of ESSP3/BRM with selected SNF2 homologs from yeast (SNF2), *Drosophila* (BRM), human (hBRM and BRG1), and three other members of the Arabidopsis SWI2/SNF2 subfamily (CHR12, CHR23, and SYD). Shown here is only the region flanking Gly-1,137 (highlighted in red) that is mutated to Arg in *essp3*. The asterisks indicate absolutely conserved residues; colons for high similarity; and dots for low similarity. C, Structure of the *BRM* gene and the location of mutation/T-DNA insertion sites of *brm* alleles. The two alleles (*brm-4*, *brm-5*) described in this work are depicted at the top and the six alleles documented previously are depicted at the bottom, including the three T-DNA alleles (*brm-1*–*brm-3*) characterized by Hurtado et al. (2006) and Farrona et al. (2007) and the three EMS alleles (*brm-101*–*brm-103*) identified by Kwon et al. (2006). Boxes indicate exons and lines represent introns and untranscribed regions. Protein domains of BRM are represented by colors: black, Gln-rich region; yellow, domain I; blue, domain II; red, ATPase domain; green, DNA-binding domains; pink, bromodomain. D, RT-PCR analysis of *BRM* expression in the three *brm* mutants and βCG_{pro}:*GUS*. Primer locations are indicated by black arrowheads in C. Elongation factor 1α was used as internal control. E, GUS phenotype of a representative F_2 progeny from the crosses of *brm-4* × βCG_{pro}:*GUS* and *brm-3* × βCG_{pro}:*GUS*, respectively. Plants were 2 weeks old and grown on MS agar plates. F, Morphological comparison of the *brm* mutants with wild-type plant at 4 weeks (top) and at maturity (bottom).

State University, Columbus): WiscDsLox436E9 and SALK_088462 (*brm-3*; Farrona et al., 2007), which contain T-DNA insertions in the seventh and 12th exons of At2g46020, respectively (Fig. 2C). Homozygous mutant plants were selected by PCR analysis, and expression of At2g46020 transcripts was examined by reverse transcription (RT)-PCR. No full-length transcripts of the At2g46020 gene could be detected (Fig. 2D). Plants homozygous for the T-DNA insertions were crossed with βCG_{pro}:*GUS*, and F_2 mutant plants selected based on morphological defects were stained for GUS activity in leaves. As shown in Figure 2E, mutant leaves were GUS positive. Furthermore, we performed genetic complementation experiments by reciprocally crossing *essp3* and WiscDsLox436E9 plants and analyzing F_1 plants. Because homozygous WiscDsLox436E9 plants are sterile, heterozygous plants were used for the crosses. In the F_1 generation, plants with wild-type and mutant (*essp3*-like) phenotypes segregated in a 1:1 ratio (108:101; $\chi^2 = 0.23$; $P > 0.6$), indicating that *essp3* is allelic to *brm*. Taken together, these data show that *BRM* (At2g46020) is *ESSP3*. To be consistent with the recently described *brm* mutant alleles (Hurtado et al., 2006; Kwon et al., 2006; Farrona et al., 2007), we designated the WiscDsLox436E9 insertion line as *brm-4*, and *essp3* as *brm-5* (Fig. 2C).

Phenotypically, *brm-4* is a strong allele and homozygous *brm-4* plants exhibit a more severe mutant phenotype than either *brm-5* or *brm-3*, including dwarfism and sterility (Fig. 2F). *brm-4* is very similar to the *brm* mutant described by Hurtado et al. (2006). *brm-3* is a weak allele, weaker than *brm-5/essp3.* *brm-3* plants display only subtle phenotypic differences compared to wild type (Fig. 2F; Farrona et al., 2007). However, the mutation clearly causes ectopic GUS expression in leaves when crossed into the βCG_{pro}:*GUS* background (Fig. 2E).

Expression of SSP Genes and Other Embryogenesis-Related Genes in *brm-5* Mutant Leaves

To obtain an overview of the effects of the *brm-5/essp3* mutation on endogenous SSP genes and other embryogenesis-related genes, a transcript profiling analysis was performed to compare gene expression at the whole genome level in mutant and wild-type (βCG_{pro}:*GUS*) leaves (http://www.ncbi.nlm.nih.gov/projects/geo/query/acc.cgi?acc=GSE11505). Total RNA was isolated from leaves of mutant and wild-type plants grown on Murashige and Skoog (MS) agar plates with 1.5% Suc for 2 weeks, and labeled RNAs were hybridized to the Affymetrix Arabidopsis ATH1 gene chip whole genome array (Affymetrix). Two-week-old plants were chosen, because we used plants at this age to examine the GUS phenotype in leaves. As listed in Supplemental Tables S1 and S2, 542 and 501 genes were significantly up- and down-regulated in the mutant ($\geq$2.0-fold; false discovery rate $\leq$ 0.05), respectively, accounting for approximately 4.6% of the genes encoded in the Arabidopsis genome (those present on the ATH1 chip). This number is similar to the percentage (5%) of genes affected by the loss of SWI and SNF proteins in yeast (Holstege et al., 1998; Sudarsanam et al., 2000) and consistent with the pleiotropic phenotypes caused by mutations in these genes. Interestingly, significantly fewer genes were reported to be affected in a *brm* null mutant in a very recent study (Bezhani et al., 2007). Differences between the two data sets could result from differences in the tissues analyzed (10-d-old whole seedlings versus leaves from 14-d-old seedlings) and the growth conditions (soil versus MS agar). Because the *brm* mutation is pleiotropic, changes in gene expression between the two samples may be large. Alternatively, differences could also arise from different methods of data analysis.

The differentially regulated genes were grouped based on the protein sequence similarity using the MIPS (http://mips.gsf.de/proj/thal/db/) Arabidopsis functional classification scheme. Importantly, among the up-regulated genes are a subset of SSP genes, a number of other nutrient reserve-related genes, and a few embryogenesis-related genes (Table I). The three 2S albumin genes, *At2S2*, *At2S3*, and *At2S5* identified, are well-established SSP genes (Krebbers et al., 1988; van der Klei et al., 1993). The cupin family member (At4g36700), encoding a 7S-like gene, has not been described in detail previously. Nevertheless, the gene, together with two others (At1g07750 and At2g18540), has been identified bioinformatically as potentially encoding additional globulin-like proteins besides the well-characterized 12S globulins in Arabidopsis (Fujiwara et al., 2002). According to the microarray transcript profiling data deposited in the public database (www.genesvestigator.ethz.ch/at), among the three putative *7S* globulin-like genes, only At4g36700 is expressed seed specifically, similar to other SSP genes, and we confirmed this expression pattern by RT-PCR (data not shown). For the convenience of description, we tentatively refer to this gene as *At7S1*, albeit the nature of the protein it encodes remains to be confirmed. A group of RNAs encoding lipid transfer proteins (LTPs)/protease inhibitors are also significantly elevated in *brm-5/essp3* leaves. These RNAs do not accumulate seed specifically but rather are present constitutively, particularly in leaves, where they play an important role in plant defense against pathogens (Arondel et al., 2000). They do, however, accumulate to high levels in mature seeds and are traditionally considered important for seed maturation as minor storage proteins.

Very interestingly, three genes that have been previously shown to be required for normal embryo development, *EMB2454*, *AtOPT3*, and *AtSIG5* (Tzafrir et al., 2003, 2004; www.seedgenes.org), are also among the genes whose mRNAs were significantly elevated in *brm-5/essp3* leaves. *EMB2454* encodes a RING-type ubiquitin ligase (Stone et al., 2005). *AtOPT3* encodes an oligopeptide transporter OPT family protein (Koh et al., 2002; Stacey et al., 2002) and is the only member of the family that is expressed in embryos (Stacey et al., 2006), consistent with its role as an *EMB* gene. AtSIG5 is a nuclear-encoded transcription initiation factor that functions in chloroplasts (Tsunoyama et al., 2004).

We validated the DNA microarray results for those genes listed in Table I and shown in Figure 3. Because the *2S* genes do not contain introns, we used RNA-blot analysis to examine their expression. Although the *2S1* and *2S4* RNAs were not detected in our microarray experiments, they were present in *essp3/brm* mutant leaves at a much lower level and were detectable by northern analysis (Fig. 3A). The ectopic expression of *At7S1* was confirmed by RT-PCR as shown in Figure 3B. In addition, two other T-DNA insertion mutants, *brm-4* and *-3*, also exhibited elevated levels of *2S* and *7S1* RNA, as shown in Figure 3, A and B, providing further evidence that *ESSP3* is *BRM*. It is worth noting that, for soil-grown *brm* plants, we could detect the ectopic GUS activity and the expression of *7S1* but not the transcripts of *2S* genes in leaves (data not shown), suggesting an overall lower level of ectopic expression of SSP genes under this growth condition. For the other genes listed in Table I that are normally expressed in leaves, data from real-time quantitative RT-PCR (qRT-PCR) experiments validated the microarray results (Fig. 3C). We also examined RNAs of the master regulators of seed maturation, *ABI3*, *FUS3*,

Table I. *Selected seed-related genes up-regulated in brm-5 leaves as revealed by microarray analysis*

Gene Identification	Locus	Fold Elevated[a]
SSP genes		
2S seed storage protein 2 (*At2S2*)	At4g27150[b]	59.8
At2S3	At4g27160[b]	10.1
At2S5	At5g54740[b]	8.2
Cupin family protein (*At7S1*)	At4g36700[b]	120.9
Other storage protein genes		
LTP	At5g38160[b]	4.5
LTP2	At2g38530[b]	3.0
LTP	At1g62500[b]	2.7
LTP	At5g48490[b]	2.6
LTP6	At3g08770[b]	2.0
Lipoxygenase (*LOX2*)	At3g45140	3.6
Em-like protein GEA1 (*Em1*)	At3g51810[b]	9.4
EMB genes[c]		
Oligopeptide transporter OPT family protein (*AtOPT3*)	At4g16370[b]	7.7
Zinc finger protein-related/E3 ligase (*EMB2454*)	At3g18290[b]	5.5
RNA polymerase σ-subunit SigE (*SIG5*)	At5g24120[b]	2.3
DNA-directed DNA polymerase ε catalytic subunit (*POL2B/TIL2*)	At2g27120	19.0
Metallo-β-lactamase family protein (*EMB2746*)	At5g63420	2.4

[a]Fold change is relative to wild type (βCG_{pro}:*GUS*). [b]Genes for which the elevated expression was validated by RNA-blot or PCR analyses (see Fig. 3). [c]Essential genes required for embryogenesis (Tzafrir et al., 2004; http://www.seedgenes.org).

LEC1, and *LEC2*, which were not detected in our microarray experiment. As shown in Figure 3D, with the exception of *FUS3*, none of these RNAs are detected in leaves of the three *brm* mutants. qRT-PCR experiments summarized in Figure 3E show that *FUS3* RNA is barely detectable in *brm-5/essp3* leaves but is present at significant levels in the two T-DNA mutant lines.

Functional Analysis of the BRM-Interacting Protein, AtSWI3C

BRM/SNF2-like proteins exist in multisubunit complexes (Mohrmann and Verrijzer, 2005). Consistent with its counterparts in other eukaryotic systems, Arabidopsis BRM was also shown to be associated with a high molecular mass complex in the nucleus (Farrona et al., 2004). Furthermore, the N terminus of BRM was demonstrated to interact, in the yeast two-hybrid system, with AtSWI3C (At1g21700, also called CHB4), an Arabidopsis homolog of the yeast SWI/SNF complex subunit SWI3 (Farrona et al., 2004; Hurtado et al., 2006). Based on this evidence, we reasoned that BRM likely works in conjunction with AtSWI3C in the same protein complex and that both *brm* and the *AtSWI3C* loss-of-function mutations may cause similar phenotypic defects. To test these hypotheses, we obtained a T-DNA insertion mutant line from ABRC, SAIL_224_B10, which contains a T-DNA insertion in the second exon of *AtSWI3C* (Fig. 4A). Because two T-DNA insertion mutant alleles have been documented (Sarnowski et al., 2005), we designated this line as *atswi3c-3*. Transcripts of *AtSWI3C* were not detected by RT-PCR (Fig. 4B), indicating that *atswi3c-3* is a knockout line. Homozygous *atswi3c-3* mutant plants were phenotypically similar to the two recently described alleles (Sarnowski et al., 2005), e.g. smaller in stature, slightly early flowering, with curled leaves (Fig. 4C), and greatly reduced fertility (Fig. 4D). The phenotype of *atswi3c-3* mutant was also similar to that of *brm* mutants, intermediate between *brm-5* and *brm-4*. To test whether the *atswi3c-3* mutation can cause the same GUS phenotype as *brm*, we introduced the *atswi3c-3* mutation into the βCG_{pro}:*GUS* background by genetic crossing. As shown in Figure 4E, homozygous *swi3c-3* mutation exhibited ectopic expression of *GUS* reporter in leaves. We also consistently detected ectopic expression of the endogenous *7S1* gene (Fig. 4F) and the *2S* genes (Fig. 4G) by RT-PCR and RNA-blot analysis, respectively. *atswi3c-3* is the only mutant allele available from public sources. To confirm the identity of the *swi3c-3* mutant allele, we obtained the two previously described alleles, *swi3c-1* and *swi3c-2* (Sarnowski et al., 2005) and examined the ectopic expression of *2S* genes in mutant leaves. As shown in Figure 4G, mRNAs for two representative *2S* genes, *2S2* and *2S3*, are clearly detected by RNA-blot analysis. Together, these results suggest that the mutant phenotype is caused by the T-DNA insertions in *AtSWI3C*.

Functional Analysis of the Arabidopsis SNF5 Homolog, BSH

We speculated that the SNF5 homolog, BSH, might be a subunit of the putative Arabidopsis SWI/SNF-like ATPase complex containing BRM. First, in all SWI/SNF-class complexes characterized, one of the key components is the SNF5-type protein (Brzeski et al.,

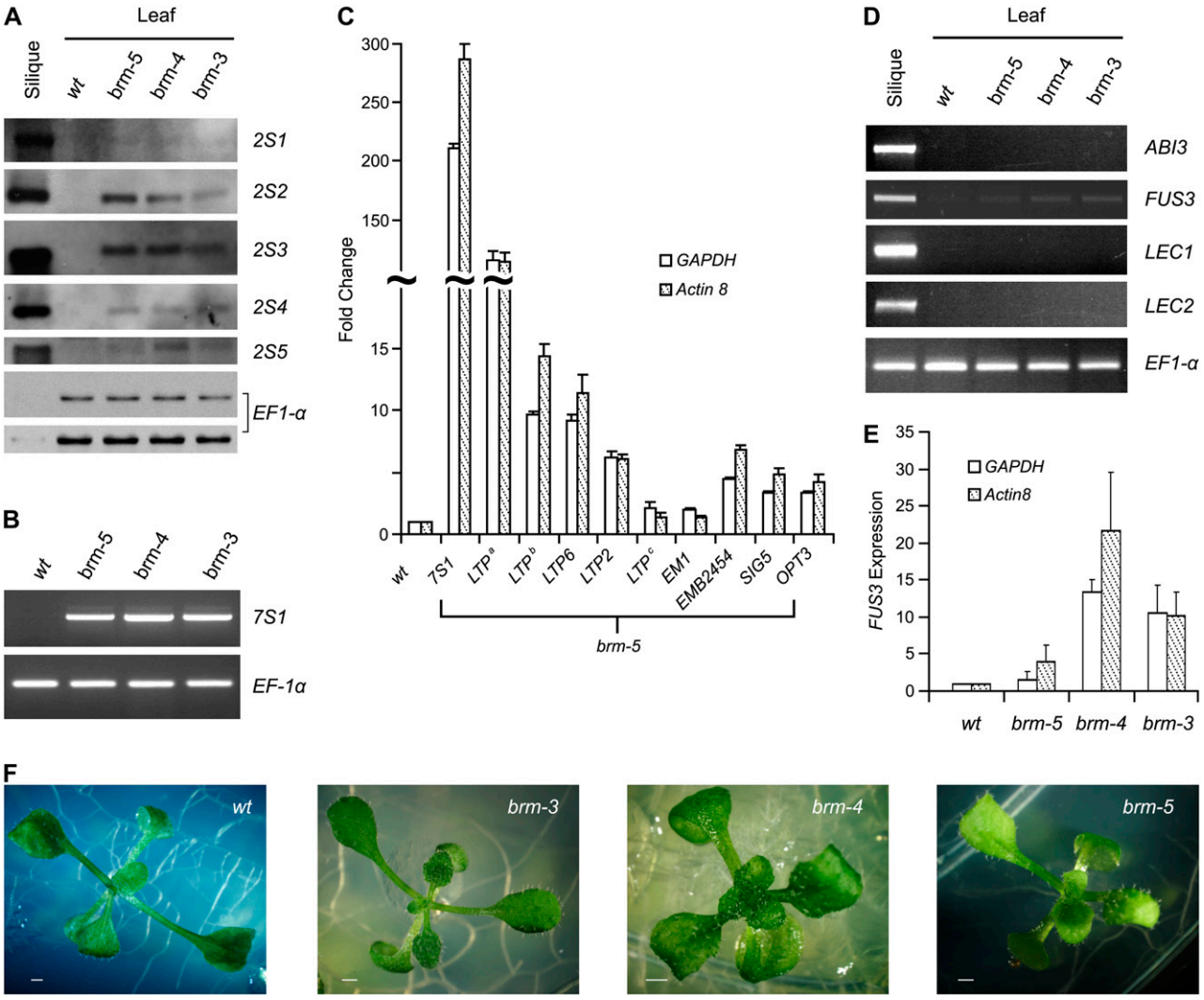

Figure 3. Validation of the DNA microarray results. A, RNA-blot analysis of the expression of the five *2S* genes in leaves of the three *brm* mutants grown for 14 d on MS agar. Wild-type leaves and siliques were used as negative and positive controls, respectively. Same amount of RNA (5 μg for leaf samples and 200 ng for siliques) was used for each blot. Elongation factor 1α was used as loading control. B, RT-PCR analysis of *At7S1* gene expression in the leaves of three *brm* mutants grown for 14 d on MS agar. C, Real-time qRT-PCR validation of the expression in *brm-5* leaves of seed-related genes revealed in our DNA microarray analysis. RNAs from leaves of 14-d-old plants grown on MS agar were used for PCR. Only those validated by qRT-PCR are shown here. Wild-type (βCG_{pro}:*GUS*) RNA levels are designated as 1-fold. The expression of *GAPDH* and *Actin-8* was used as internal controls. The mean and SE were determined from three biological replicates. Bars represent SES. Footnotes: a, At1g62500; b, At5g48490; c, At5g38160. D, RT-PCR analysis of the expression levels of the four positive regulators of maturation (*ABI3*, *FUS3*, *LEC1*, and *LEC2*) in *brm* mutant leaves of 14-d-old plants grown on MS agar. RNAs isolated from wild-type leaves and siliques were used as negative and positive controls, respectively. PCR cycle numbers: 32 for *FUS3*, 30 for *EF1-α*, and 38 for *ABI3*, *LEC1*, and *LEC2*. E, *FUS3* expression in *brm* mutant leaves was monitored and quantified by qRT-PCR. RNAs from leaves of 14-d-old plants grown on MS agar were used for PCR. Wild-type (βCG_{pro}:*GUS*) RNA levels are designated as 1-fold. The expression of *GAPDH* and *Actin-8* was used as internal controls. The mean and SE were determined from nine samples (three biological replicates and three real-time PCR reactions). Bars represent SES. F, Morphological comparison of 14-d-old wild type (βCG_{pro}:*GUS*) and *brm* mutant plants grown on MS agar. Bar = 1 mm.

1999). Second, in contrast to yeast and other systems, in Arabidopsis, most of the SWI/SNF-like proteins are encoded by gene families (Plant Chromatin Database, http://chromdb.org). For example, there are more than 40 ATPases of the SNF2 family, four SWI3 homologs, and two genes encoding SWP73 homologs, but there is only one gene encoding the SNF5 homolog BSH. Third, it has been demonstrated that *BSH*, when expressed in yeast, could partially complement the *snf5* mutation (Brzeski et al., 1999), indicating that BSH is a functional homolog of SNF5. Fourth, it has been demonstrated that BSH interacts with AtSWI3A and AtSWI3B,

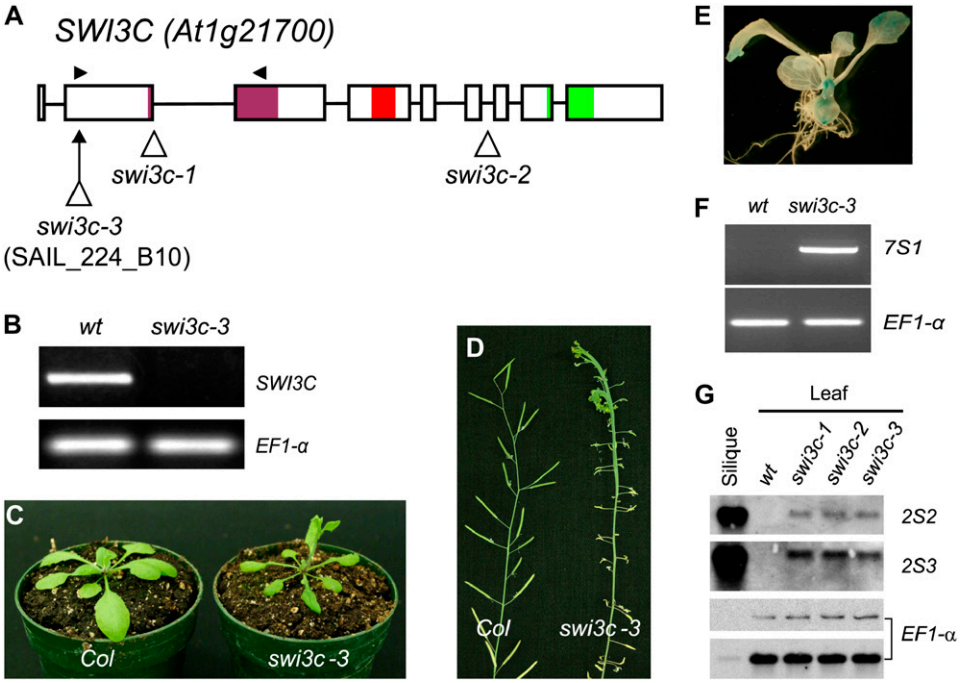

Figure 4. Analysis of an *AtSWI3C* null mutant. A, Structure of the *AtSWI3C* gene and T-DNA insertion sites. Protein domains are represented by colors: purple, SWIRM domain; red, SANT domain; green, Leu zipper domain. B, RT-PCR analysis of the *SWI3C* transcripts in *swi3c-3* mutant leaves of 14-d-old plants grown on MS agar. Primers used are indicated in A by arrowheads. C, Morphology of the 3-week-old *swi3c-3* mutant and wild-type plants. D, Siliques of *swi3c-3* mutant and wild-type plants. E, GUS phenotype of a representative F_2 progeny (grown for 14 d on MS agar) from the cross of βCG_{pro}:*GUS* × *swi3c-3*. F, RT-PCR analysis of the *At7S1* gene expression in leaves of 14-d-old *swi3c-3* plants grown on MS agar. G, RNA-blot analysis of the expression of the two representative *2S* genes, *2S2* and *2S3*, in leaves of 14-d-old *swi3c* mutant plants grown on MS agar. Wild-type leaves and siliques were used as negative and positive controls, respectively. The same amount of RNA (5 μg for leaf samples and 200 ng for siliques) was used for each blot. Elongation factor 1α was used as loading control.

which in turn bind AtSWI3C (Sarnowski et al., 2005). Therefore, we determined whether loss-of-function mutations of *BSH* could activate SSP genes in leaves. We obtained two T-DNA insertion lines, *bsh-1* (FLAG_298E02) and *bsh-2* (SALK_073635), that contained T-DNA insertions in the last intron and last exon of the *BSH* gene, respectively (Fig. 5A). No *BSH* transcripts were detected by RT-PCR with primers spanning the T-DNA insertion sites (Fig. 5B). *bsh-1* and *-2* homozygous plants displayed no significant morphological abnormalities as compared to wild type (data not shown). Because it was previously shown that *BSH* antisense transgenic plants were bushy and produced no seeds (Brzeski et al., 1999), these two T-DNA lines might be ''leaky'' weaker alleles, with truncated protein still being synthesized and partially functional. This might be the case, given the fact that *BSH* transcripts were detected with upstream primers (Fig. 5B) and that both T-DNA insertions only disrupt the C-terminal Box B, leaving the SNF5 domain intact (Fig. 5A). Nevertheless, when introduced into the βCG_{pro}:*GUS* background by crossing, the mutant alleles caused weak but clearly detectable GUS activity in leaves (Fig. 5C). Consistent with this observation, *At7S1* RNA was also detected in leaves of both mutants by RT-PCR, albeit at a lower level than in *brm-5* (Fig. 5D). We did not detect expression of the five *2S* genes by RNA-blot analysis in *bsh* mutant leaves (data not shown). Taken together, these data suggest that BSH functions like BRM and AtSWI3C in repressing SSPs. In summary, our genetic and molecular data are consistent with the interpretation that Arabidopsis BRM acts in a complex with AtSWI3C and BSH to repress embryonic traits in vegetative tissues.

Localization of BRM at the Promoters of *2S* and Other Embryogenesis Genes

Our genetic and molecular evidence strongly suggests that a SWI/SNF-like complex containing BRM acts to repress the expression of SSP genes in leaves. We then set out to examine whether the repression by BRM in planta is direct or indirect. Previous genetic and biochemical studies from both yeast and humans have provided strong evidence that the SWI/SNF complexes are recruited to the promoters of specific genes and once at a promoter, these complexes can remodel nucleosomes to affect accessibility to the basic transcription machinery (Peterson and Workman, 2000; Vignali et al., 2000). Although the SWI/SNF-like com-

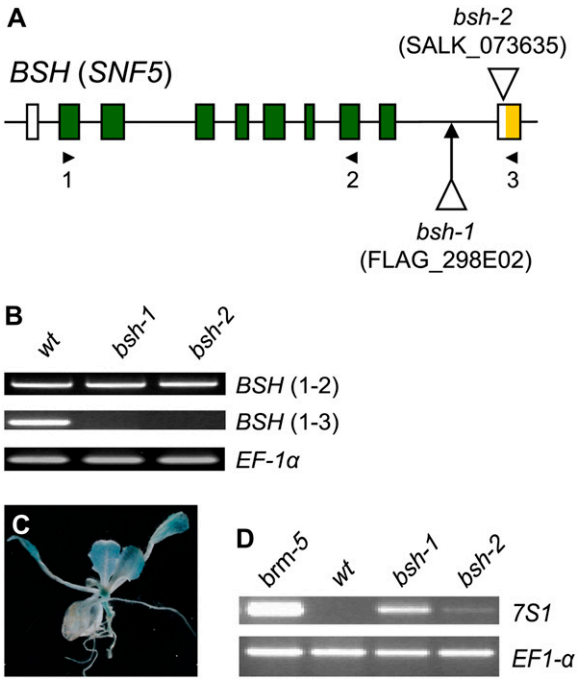

Figure 5. Analysis of *BSH/SNF5* mutants. A, Structure of the *BSH/SNF5* gene and T-DNA insertion sites of the mutants. Protein domains are represented by colors: green, SNF5 domain; yellow, Box B. B, RT-PCR analysis of *BSH* transcripts in leaves of 14-d-old *bsh* mutant plants grown on MS agar. Primers used are numbered and indicated in A. C, GUS phenotype of a representative F_2 progeny (grown for 14 d on MS agar) from the cross of βCG_{pro}:*GUS* × *bsh-2*. D, RT-PCR analysis of the *At7S1* gene expression in leaves of 14-d-old *bsh* mutant plants grown on MS agar. The *brm-5* sample was included for comparison.

plexes have been mainly characterized as transcriptional activators (Sudarsanam and Winston, 2000; Vignali et al., 2000; Narlikar et al., 2002), there has been increasing evidence that these complexes can also function as transcriptional repressors (Holstege et al., 1998; Sudarsanam et al., 2000; Underhill et al., 2000; Sif et al., 2001; Battaglioli et al., 2002; Kuzmichev et al., 2002; Pal et al., 2003; Watanabe et al., 2006; for review, see de la Serna et al., 2006; Simone 2006).

To address this question, we used the method of chromatin immunoprecipitation (ChIP) to examine the presence of BRM at the promoters of the *2S* genes and other selected embryogenesis-related genes (Table I) that are up-regulated in leaves of *brm* mutants (Fig. 3). For the ChIP assay, we used an antibody raised against amino acids 307 to 424 of BRM (α-BRM-N) that detects BRM in nuclear extracts of wild-type inflorescences but not in *brm* knockout mutants (Hurtado et al., 2006). We did protein-blot analysis and found that *brm-4* did not contain a detectable full-length BRM, but a truncated version was present (data not shown). Therefore, we decided to use *brm-1* for the ChIP assay, which is a null mutant producing no detectable BRM protein (Hurtado et al., 2006). Chromatin from wild-type (Col) and *brm-1* leaves was precipitated with the α-BRM-N antibody. We used qPCR to quantify the enrichment of DNA from the promoter regions of genes selected from Table I in the wild-type versus mutant ChIP samples. Consistently, the results show that the promoter regions of *2S2*, *2S5*, a *LTP* (At5g48490), *Em1*, and *AtOPT3* were significantly enriched in wild type relative to *brm-1* mutant leaves (Fig. 6). As a control, we showed that the promoter DNAs were not enriched in the ChIP experiment using preimmune serum (Supplemental Fig. S2). We also queried DNA regions further upstream and downstream, either at the 3′ end of the gene (*2S2*) or the intergenic regions (*2S5* and *AtOPT3*). As expected, enrichment was only detected for the promoter regions (Supplemental Fig. S2). Our results show that BRM was physically present at the promoters of the *2S* genes and other embryogenesis genes. The physical association of BRM with promoters of the seed genes, taken together with the requirement for BRM in SSP repression, suggests that BRM is likely involved in the direct repression of transcription of the seed genes in leaf tissue.

Altered Chromatin Structure at the *At2S2* Promoter in *brm* Mutant Leaves

Based on the ChIP results, we reasoned that the putative BRM-containing SWI/SNF complex contributes to the repression of SSP genes by regulating chromatin structure at the promoters, thereby preventing access of the transcription machinery. If this is the case, changes in chromatin structure, possibly to a relaxed state, are likely to occur in *brm* loss-of-function mutants to allow the binding of transcription machinery. To test this possibility, we chose *At2S2* as a working model, because it is the highest expressed *2S* gene in *brm* mutant leaves (Fig. 3; Table I). We compared sensitivity of the *At2S2* promoter to cleavage by DNase I in leaves of wild type (repressed) and two *brm* mutants (derepressed). As shown in Figure 7, sensitivity of *At2S2* chromatin to DNase I was significantly enhanced in *brm* mutants. Three major hypersensitive sites were revealed; one was in the coding region shortly downstream of the start codon, centered around +200 bp, and the other two were located in the proximal promoter region, centered around −270 and −620 bp, respectively, upstream of the transcription start site. Thus, BRM is required to maintain chromatin in a DNase I-insensitive conformation in leaves.

DISCUSSION

Comparison of the Roles of BRM and PKL in Repressing Embryonic Traits in Vegetative Tissues

The identification of BRM as a repressor of embryonic genes in leaves has provided an opportunity to make a comparison between BRM and PKL and to gain an understanding about the complex roles of ATP-dependent chromatin remodelers in the regulation of

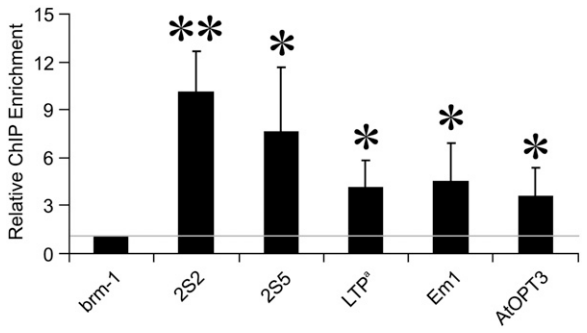

Figure 6. Recruitment of BRM to promoters of selected embryogenesis genes. ChIP analysis with antibodies against BRM (α-BRM-N). Leaves from 14-d-old wild-type and *brm-1* mutant plants grown on MS agar containing 1.5% Suc were analyzed with α-BRM-N. Promoter regions (within 700 bp upstream of the start codon) of the selected embryogenesis genes were analyzed by qPCR. Primer sequences are listed in Supplemental Table S3. *Ta3* was also amplified as a control for repressed loci (Johnson et al., 2002). Relative ChIP enrichment values for the embryonic gene promoter regions in wild type (Col) were calculated relative to the enrichment value for corresponding promoter regions in *brm-1* (see "Materials and Methods" for details). Value of ChIP enrichment in *brm-1* was set to 1. The gray line indicates level of background precipitation in *brm-1*. Each value is the average of three independent biological replicates, each of which was conducted in triplicate. Bars indicate SEs. ** and * denote ChIP enrichment values are significantly different at $P < 0.01$ and $P < 0.05$, respectively. Footnote a, At5g48490.

developmental transitions. Both BRM and PKL are involved in the regulation of embryogenesis genes, apparently through changes in chromatin structure, although they act through distinct mechanisms. PKL controls the transition from the embryonic to the vegetative phase by repressing *LEC1*, *LEC2*, and *FUS3* during germination (Rider et al., 2003). The *pkl* mutation confers embryonic traits mainly to primary roots (Ogas et al., 1997; Li et al., 2005) and, to a lesser extent, throughout seedlings in cotyledons, radicles, and hypocotyls, organs defined in embryos (Henderson et al., 2004). Our data show that BRM also represses embryogenesis genes in vegetative tissues, but it does so at a later developmental phase by repressing a subset of embryogenesis genes by targeting their promoters. However, *LEC1*, *LEC2*, and, possibly *FUS3* appear to be regulated through a different pathway. Thus, BRM, together with PKL and other chromatin-remodeling factors, promotes vegetative development by repressing embryonic and reproductive development.

The low level expression of *FUS3* in leaves of *brm* mutants is interesting, although its significance is unclear at the present time. Two lines of evidence suggest that this expression may not be very significant in terms of its contribution to the activation of *2S* genes. First, although we consistently detect *FUS3* expression in the *brm* T-DNA mutants, its expression in *brm-5/essp3* is barely detectable (Fig. 3, D and E). Second, in *pkl* leaves, a similar low level of *FUS3* expression (approximately 6-fold) was detected (Rider et al., 2003), but no embryonic traits were revealed by metabolite profiling (Rider et al., 2004). We also examined the expression of *2S* genes in *pkl* leaves by RNA-blot analysis and no signal was observed (data not shown). These observations, however, cannot rule out the possibility that a low level of FUS3 could cause derepression of SSP genes in the leaves.

The reason why there is no dramatic phenotype observed for *brm* mutants as compared to that of *pkl* is possibly 2-fold. First, BRM seems to control only a subset of seed maturation genes. Second, BRM is likely only one of the players involved in the repression process and, as we will discuss below, there are potentially other players that have redundant roles with BRM. That might be why the level of ectopic expression of the seed genes is low in *brm* leaves. Future work

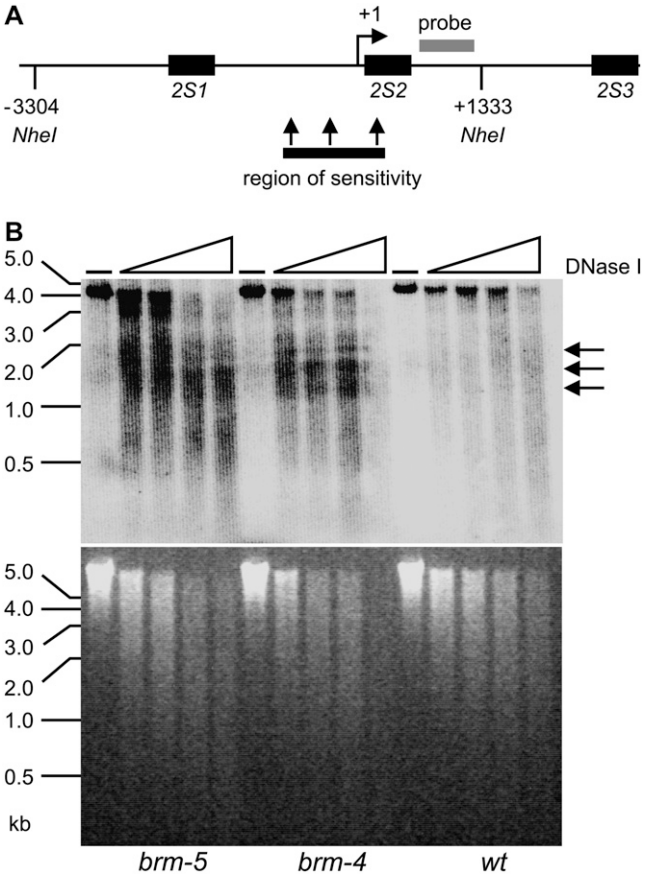

Figure 7. DNase I digestion profile of the *At2S2* gene. A, A schematic representation showing restriction sites, probe, coding regions (black boxes), and the region of DNase I sensitivity. B, Top, Nuclei isolated from leaves of wild type (Col) and two *brm* mutant plants were digested with increasing concentrations of DNase I (at 0, 5, 10, 20, and 40 units/mL, for 3 min at 22°C). DNA was purified and then restricted with *Nhe*I before Southern blotting. The resulting membrane was probed with a fragment as indicated in A. Arrows indicate the sites of DNase I sensitivity. DNA size markers are indicated at left in kilobases. B, Bottom, Ethidium bromide staining of the gel.

combining genetic, molecular, and biochemical approaches is needed to gain further understanding of the repression mechanisms.

Role of BRM in the Repression of SSPs in Leaves

Prevailing models suggest that the SWI/SNF complexes are recruited to specific in vivo targets through interactions with DNA-binding transcription factors, where the ATP-dependent activities of the complex assist in gene regulation through changes in DNA-histone contacts (Peterson and Workman, 2000; Peterson, 2002). Subsequently, the affected nucleosomes can be covalently modified to maintain active or repressed transcription through cooperation with histone acetyltransferases and histone deacetylase complexes (Narlikar et al., 2002). Our results presented here, combined with others (Brzeski et al., 1999; Farrona et al., 2004; Sarnowski et al., 2005; Hurtado et al., 2006; Bezhani et al., 2007), strongly suggest that a putative SWI/SNF-like complex, containing BRM, AtSWI3C, and BSH/SNF5, acts at the SSP promoters to repress their expression in leaf tissues by contributing to the maintenance of a repressive chromatin structure.

Previously documented data have shown that ATP-dependent chromatin-remodeling activities are typically associated with transcriptional activation by causing promoter-specific chromatin changes around the transcription start site (Peterson and Workman, 2000; Vignali et al., 2000). However, recent work by Ooi et al. (2006) suggests a similar role for SNF2-like ATPase when acting as a repressor. The authors studied the role of BRG1 (a human homolog of BRM) in REST-mediated transcriptional repression. Their results suggest that, when bound to chromatin, the BRG1 complex repositions nucleosomes with respect to DNA, allowing increased interaction of the REST repressor with its DNA binding site. Our results are in agreement with that of Ooi et al. (2006), and together, the data suggest that BRM plays similar roles in causing chromatin change in activator and repressor complexes. In addition, we note that the two *brm* mutants, *brm-5* (*essp3*) and *brm-4* (T-DNA knockout allele), showed similar DNase I sensitivity pattern. The mutation in *brm-5/essp3* is predicted to cause only one amino acid change (Fig. 2, B and C), possibly affecting only the ATPase domain. Taken together, these results strongly suggest the direct requirement of BRM, and more specifically its ATPase activity, to maintain a repressive chromatin structure over the *At2S2* promoter.

Future challenges will be to elucidate the components of the putative SWI/SNF-like complex, particularly those tissue- and loci-specific corepressors. It is not known how the BRM complex is recruited to specific promoters and what other players are involved, particularly histone deacetylase, which has been shown to be associated with SWI/SNF-like complexes in transcription repression (Harikrishnan et al., 2005; Ooi and Wood, 2007). Potential candidates for such corepressors have been implicated in a recent study. Suzuki et al. (2007) demonstrated that the B3 proteins VAL1 and VAL2 are repressors of seed maturation program. The *val1/val2* double mutants do not form leaves and develop embryo-like structures at the position of the apical meristems and on cotyledon margins. Moreover, the *SSP* genes and *LEC1*, *ABI3*, and *FUS3* are expressed at high levels in the double mutant. The *val1* single mutant plants lack a discernable morphological mutant phenotype, but weak expression of *SSPs* was detected in the seedlings and no expression of the *LEC1-B3* master regulators was observed. For these reasons, the authors proposed that the VAL and ABI3/FUS3/LEC2 families of B3 proteins may recognize the same or overlapping set of downstream targets, with the former functioning as repressors and the latter acting primarily as activators. In addition to the B3 domain, the VAL proteins also contain PHD and CW domains, which are frequently found in chromatin factors (Aasland et al., 1995; Perry and Zhao, 2003). The authors further suggested that VAL proteins could target genes for repression by recruiting a general chromatin-remodeling complex to repress the expression of specific sets of genes. VAL1 is identical to HSI2, which was recently identified as a transcriptional repressor of a sugar-inducible storage protein gene from sweet potato (*Ipomoea batatas*; Tsukagoshi et al., 2005). Coincidentally, we have also identified the same gene as an *essp* locus (*essp2*; X. Tang and Y. Cui, unpublished data). Thus, it is possible that VAL1/HSI2/ESSP2 might serve as an adaptor that recruits and/or stabilizes the SWI/SNF complex. In addition, very recently, Tanaka et al. (2008) demonstrated that the Arabidopsis histone deacetylases HDA6 and HDA19 act redundantly in contributing to the repression of embryonic properties after germination. All of this evidence points to a model for repressor complexes in plants that are similar in composition to what have been described in animal systems. Future molecular and biochemical work is needed to test the model.

Lastly, our observation that the BRM complex is required for SSP gene repression in vegetative organs suggests a coordinated process between ATP-dependent chromatin remodeling and epigenetic silencing mediated by the Polycomb group (PcG) protein complexes. In recent years, several Arabidopsis PcG complexes were shown to play a major role in vegetative development by maintaining the silencing of their target genes (for review, see Calonje and Sung, 2006; Pien and Grossniklaus, 2007). For example, it was shown that PcG proteins CURLY LEAF (CLF) and SWINGER (SWN), EMBRYONIC FLOWER2 (EMF2), and VERNALIZATION2 (VRN2) all play a (redundant) role in repressing embryonic traits in vegetative organs, based on the observation that *vrn2 emf2* and *clf swn* double mutants formed somatic embryos (Chanvivattana et al., 2004; Schubert et al., 2005). The molecular mechanisms are not completely understood, but one possible mechanism is through direct repression of seed maturation

genes such as *FUS3* (Makarevich et al., 2006). Thus, BRM and PcG proteins are both involved in the repression of seed genes. The functional and physical links between these proteins will be the subject of future investigations.

Relationship and Relevance to Seed Maturation Programs

We observed only a subset of SSP genes expressed in *brm* mutant leaves, i.e. *2S* genes are expressed at varied expression levels, but expression of the three *12S* genes was not detected. These results suggest that SSP genes are repressed by different mechanisms. Alternatively, it is also possible that the absence of *12S* gene expression is due to the lack of necessary activators in leaves. This interpretation is in agreement with several previous observations (Guerche et al., 1990; Kagaya et al., 2005a; Braybrook et al., 2006). Guerche et al. (1990) examined the temporal and spatial expression pattern of the *2S* albumin genes in Arabidopsis and found that although all the genes followed similar temporal profiles throughout development, they exhibited different spatial expression patterns and levels. Kagaya et al. (2005a) investigated the regulation of *At2S3* and *CRC* by FUS3, using transgenic plants in which *FUS3* could be ectopically induced in leaves by steroid hormones. They found that FUS3-mediated induction of *CRC* and *At2S3* followed different pathways, as evidenced by different requirements for ABA and differences in induction times. Braybrook et al. (2006) identified target genes of LEC2 employing transgenic Arabidopsis containing an inducible LEC2. It was found that 4 h after induction, all five *2S* albumin genes were significantly expressed, while among the *12S* cruciferin genes, only *CRA1* was induced, suggesting that the other two cruciferin genes were differentially regulated.

Yet another challenging question is how the putative BRM ATPase complex functions during embryogenesis. *BRM* is expressed in seeds (Farrona et al., 2004). Transcript profiling of developing Arabidopsis embryos shows that the gene is expressed throughout seed development, from ovule to mature seed, at a higher level than all other vegetative and floral tissues (http://estdb.biology.ucla.edu/genechip). A similar pattern is found for *SWI3C*, *BSH*, and the *VAL1/HSI2/ESSP2* genes, consistent with their possible roles in seeds. The seed maturation program is tightly controlled during embryogenesis. It is repressed in the earlier phase and active during the mid and later phase. The involvement of chromatin remodeling in controlling phase transition during embryogenesis from earlier stage to maturation was postulated by Braybrook et al. (2006) as one of the possible mechanisms to explain why *LEC2* is expressed at the earliest stage of seed development, well before the maturation (Stone et al., 2001), but no maturation genes are induced. Further studies are needed to test these possibilities.

In addition to SSP genes, a few *EMB* genes are also significantly elevated in *essp3/brm* leaves. The *EMB* gene class is a heterogeneous class encoding a diverse set of proteins whose function is required during embryogenesis by the virtue of their mutant phenotype (Tzafrir et al., 2004) and not a particular biochemical function. The molecular mechanisms linking these genes to embryogenesis are lacking. The new findings reported here suggest that a few of these genes are regulated by BRM, directly or indirectly. Further studies may reveal new mechanisms and how they could contribute to embryogenesis.

In conclusion, BRM is a chromatin-remodeling ATPase with pleiotropic effects on plant development. For example, BRM affects flowering, leaf architecture (Farrona et al., 2004), cotyledon separation (Kwon et al., 2006), and repression of embryonic genes in leaves (this work), and microarray analyses show that BRM affects the expression of a large number of genes. Thus, BRM plays vital roles in many different aspects of plant development.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Seeds of T-DNA insertion lines *brm-4* (WiscDsLox436E9), *brm-3* (SALK_088462), *swi3c-3* (SAIL_224_B10), and *bsh-2* (SALK-073635) were obtained from the ABRC. *bsh-1* (FLAG_298E02) was obtained from the Institute of Agronomic Research, Versailles, France. Homozygous T-DNA insertion mutants were identified by PCR. All plants were grown under 16-h-light (22°C)/8-h-dark (20°C) cycles, either on soil or on MS media containing 1.5% Suc and 0.8% agar with appropriate selection markers. All seeds were vernalized at 4°C for 3 d before placement in a growth environment.

Mutant Isolation

An 847-bp *Sac*I-*Bam*HI fragment containing the soybean (*Glycine max*) β-conglycinin β-subunit gene promoter (βCG_{pro}; Lessard et al., 1993) was inserted upstream of the GUS reporter gene in the binary vector pBI101 (CLONTECH). The *Bam*HI site is 18 bp upstream of the start codon (ATG). The resulting plasmid was then used to transform Arabidopsis (*Arabidopsis thaliana*) ecotype Col. Transformants were identified by growth in MS medium containing kanamycin. Seeds from a stable homozygous line were subjected to EMS mutagenesis according to standard protocols (Weigel and Glazebrook, 2002). Mutagenized seeds (M_1) were sown on soil and allowed to set seeds. The resulting M_2 seeds were collected in pools and germinated on MS agar. Two weeks after plating, seedlings were numbered and a single leaf was removed from each and stained for GUS activity. Those M_2 seedlings that showed GUS staining were transplanted to soil for seed setting. The leaf GUS phenotype of these putative mutants was then examined in the M_3 generation to ensure that the mutant phenotype was heritable and to identify homozygous mutants. Heritable homozygous mutants were backcrossed with βCG_{pro}:*GUS*, and the leaf GUS phenotype of the F_1 plants was analyzed to determine whether the mutation was dominant or recessive. Segregation in the F_2 generation was also examined to determine whether the phenotype is single-locus controlled. The homozygous *essp3* mutant was backcrossed twice with βCG_{pro}:*GUS* before further analysis.

Histochemical GUS Assay

Two-week-old seedlings or leaves were immersed in GUS staining solution (0.5 mg/mL 5-bromo-4-chloro-3-indolyl-glucuronide, 20% methanol, 0.01 M Tris-HCl, pH 7.0), which was modified for weak GUS expression (Jefferson et al., 1987) and then placed under vacuum for 15 min. After incubation at 37°C overnight, the staining solution was removed, and samples were cleared by sequential changes of 75% and 95% ethanol.

Immature Silique Cultures and ABA Response

The immature silique culture experiments were performed as described by Hirai et al. (1994). To determine the response of the βCG_{pro}:*GUS* reporter gene to ABA, 50 μM of ABA (mixed isomers, Sigma) was added to the basal medium plates. Fluorometric analysis of GUS activity was performed with 4-methylumbelliferyl-β-D-glucuronide as substrate (Jefferson et al., 1987), and GUS activity was calculated as picomoles of 4-methylumbelliferyl per hour per microgram of protein. At least 20 siliques per treatment were used for measuring GUS activity.

Map-Based Cloning of *essp3*

For genetic mapping of the *essp3* mutation, mutant plants from Col background were crossed with wild-type plants of the Landsberg *erecta* ecotype. A total of 1,325 homozygous *essp3* mutants were selected from the F_2 segregating population. Genomic DNA extracted from these seedlings was used for PCR-based mapping with simple sequence polymorphism markers, and the *essp3* locus was mapped to an approximately 100-kb area on BAC T3F17. Sequencing of the coding regions within this area revealed a mutation in the gene At2g46020, which encodes BRM ATPase. For the complementation test, an 11.5-kb *Sac*I fragment (18,928,084–18,939,545 bp) from the BAC T3F17, which harbors At2g46020 and At2g46000 (no mutation was found within this gene), was inserted into the pCAMBIA 3300 transformation vector. The resulting construct was introduced into *essp3* plants. More than 200 T_1 transgenic plants were obtained that all showed complete rescue of the *essp3* phenotype, i.e. wild-type morphology and no GUS activity in leaves.

Microarray Hybridization and Data Analysis

Total RNA was isolated in three biological replicates from leaves of 2-week-old wild-type (βCG_{pro}:*GUS*) and mutant (*essp3/brm-5*) seedlings grown on MS agar plates (1.5% Suc), using RNeasy Plant Mini kit (QIAGEN). Labeling, hybridization, and detection were performed at the McGill University and Genome Quebec Innovation Centre (http://genomequebec.mcgill.ca). The Affymetrix Arabidopsis ATH1-whole genome array, containing 22,810 probe sets representing approximately 24,000 genes, was used. The raw MAS 5.0 data files obtained from scanned array images are then imported into GeneSpring 7.3.1 (Silicon Genetics). Only genes with Present (P) calls were included in the analysis. Raw signals of each gene were normalized with the median of all measurements on the chip. The average normalized value of the signal intensity for each gene in three replicate hybridization experiments was adopted as the expression value of the gene. Expression data was analyzed by one-way ANOVA model to identify differentially regulated transcripts. False discovery rate multiple testing corrections (Benjamini and Hochberg, 1995) were calculated based on the *P* value generated from the one-way ANOVA. Using false discovery rate at 5% that corresponds to *P* value = 0.05, we selected only statistically significant genes that were regarded as differentially regulated only if their fold-change was $\geq$2.0 for up-regulated and $\leq$0.5-fold for down-regulated.

RT-PCR, Real-Time PCR, and RNA-Blot Analyses

Total RNAs were extracted from leaves or siliques using RNeasy Plant Mini kit (QIAGEN) or TRIzol (Invitrogen) and treated with DNase I. Typically, RT of 5 μg RNA in a 20-μL reaction was performed using the Superscript First Strand Synthesis kit (Invitrogen) and 1.5 μL cDNA was used for PCR. Real-time qPCR was performed and analyzed with the LightCycler real-time PCR system (Roche) following the manufacturer's instructions. The expression of *Actin-8* and *GAPDH* was used as internal controls. All PCR primers used in this work are listed in Supplemental Table S3. RNA-blot analysis was performed with Northern Starter kit (Roche) following the manufacturer's instructions. Probes for *2S1* to *2S4* were synthesized by PCR amplification of gene-specific regions as described by Guerche et al. (1990), while the one for *2S5* covered most of its coding region. Hybridization and washing were performed at 68°C with 0.1× SSC.

ChIP

Treatment of plant tissue and isolation of nuclei were performed essentially as previously described (Johnson et al., 2002). EZ-ChIP kit (Upstate) was used for the rest of the procedure following the manufacturer's protocols. Briefly, after 14-d growth on MS agar plates, leaves of wild-type and mutant plants were harvested and cross-linked in 1% formaldehyde solution for 15 min. Chromatin from 0.3 g of leaves was used for one immunoprecipitation assay. PCR cycles varied from 35 to 38, and primers used are listed in Supplemental Table S3. Input DNA and immunoprecipitated DNA were subjected to PCR and visualized by ethidium bromide staining on agarose gels. The DNAs were also subjected to qPCR for quantifying ChIP enrichment. *Actin2/7* and *Ta3* were also amplified as controls for actively expressed and repressed loci, respectively (Johnson et al., 2002). We confirmed by RT-PCR analysis that *Ta3* is not detectable in both wild-type and *brm* mutant leaves, while *Actin2/7* is uniformly expressed (data not shown). A standard curve was established for each pair of primers. The amount of ChIP DNA was calculated based on the standard curve and then normalized to the *Ta3* control locus for each sample. The relative fold-enrichment was calculated as the ChIP DNA in wide type (Col) versus *brm-1* for each gene. Similar results were obtained when using *Actin2/7* as a control locus. One-way ANOVA model was used to determine the significant level of each ChIP enrichment value.

DNase I Sensitivity Mapping

Nuclei from leaves of 14-d-old plants grown on MS agar plates (1.5% Suc) were isolated according to Li et al. (1998). DNase I hypersensitivity mapping was performed essentially as described (Zhang et al., 2000). Briefly, DNA from the DNase I (Worthington) treated nuclei was digested to completion with *Nhe*I, resolved on agarose gels, and then transferred to nylon membranes. The blot was hybridized with a 562-bp ^{32}P-labeled probe derived from the genomic sequence located 493 to 1,054 bp downstream of the transcriptional start site of *2S2*.

Microarray data from this article have been deposited with the NCBI Gene Expression Omnibus data repository (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE11505.

Supplemental Data

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

Supplemental Figure S1. Pattern of GUS expression in *essp3* plants.

Supplemental Figure S2. Recruitment of BRM to promoters of selected embryogenesis genes.

Supplemental Table S1. Genes up-regulated in *brm-5/essp3* leaves.

Supplemental Table S2. Genes down-regulated in *brm-5/essp3* leaves.

Supplemental Table S3. PCR primers used in this work.

Supplemental Table S4. Ct values (cycle threshold) for Figure 3C.

ACKNOWLEDGMENTS

We thank Roger Beachy and Toru Fujiwara for the conglycinin promoter; Andrzej Jerzmanowski for the *swi3c-1* and *swi3c-2* mutant seeds; Dario Bonetta for help with positional cloning; Guofu Li for advice on chromatin-remodeling assays; Fengshan Ma for Supplemental Figure S1; Dwayne Hegedus for suggesting a microarray analysis; ABRC for seeds of T-DNA insertion lines and BAC clones; Ida van Grinsven for sequencing service; Alex Molnar for help with preparing the figures; and Rima Menassa, Steven Rothstein, and anonymous reviewers for comments on the manuscript.

Received April 25, 2008; accepted May 22, 2008; published May 28, 2008.

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