Differential control of seed primary dormancy in *Arabidopsis* ecotypes by the transcription factor SPATULA

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Edited by Mark Estelle, University of California at San Diego, La Jolla, CA, and approved May 9, 2013 (received for review January 31, 2013)

Freshly matured seeds exhibit primary dormancy, which prevents germination until environmental conditions are favorable. The establishment of dormancy occurs during seed development and involves both genetic and environmental factors that impact on the ratio of two antagonistic phytohormones: abscisic acid (ABA), which promotes dormancy, and gibberellic acid, which promotes germination. Although our understanding of dormancy breakage in mature seeds is well advanced, relatively little is known about the mechanisms involved in establishing dormancy during seed maturation. We previously showed that the SPATULA (SPT) transcription factor plays a key role in regulating seed germination. Here we investigate its role during seed development and find that, surprisingly, it has opposite roles in setting dormancy in Landsberg *erecta* and Columbia *Arabidopsis* ecotypes. We also find that SPT regulates expression of five transcription factor encoding genes: *ABA-INSENSITIVE4* (*ABI4*) and *ABI5*, which mediate ABA signaling; *REPRESSOR-OF-GA* (*RGA*) and *RGA-LIKE3* involved in gibberellic acid signaling; and *MOTHER-OF-FT-AND-TFL1* (*MFT*) that we show here promotes *Arabidopsis* seed dormancy. Although *ABI4*, *RGA*, and *MFT* are repressed by SPT, *ABI5* and *RGL3* are induced. Furthermore, we show that *RGA*, *MFT*, and *ABI5* are direct targets of SPT in vivo. We present a model in which SPT drives two antagonistic "dormancy-repressing" and "dormancy-promoting" routes that operate simultaneously in freshly matured seeds. Each of these routes has different impacts and this in turn explains the opposite effect of SPT on seed dormancy of the two ecotypes analyzed here.

phytohormone analyses | chromatin immunoprecipitation | transcriptomic analyses

Plants have evolved to alter how they grow and develop in response to signals from their environment. A good example of this is the process of seed germination, which marks the start of growth following a period of quiescence or primary dormancy. This dormant state enables seeds to survive in the soil until conditions are favorable for growth. In *Arabidopsis* and many other species, dormancy can be released by either low temperature of imbibed seeds (stratification) or by an extended period of dry seed storage (after-ripening). Regulation of germination results from a balance between levels of and sensitivity to the antagonistic phytohormones abscisic acid (ABA) and gibberellic acid (GA) (1). ABA is synthesized during seed development leading to induction of primary dormancy, which inhibits precocious germination. ABA acts through ABA-response transcription factors, such as ABA-INSENSITIVE3 (ABI3), ABI4, and ABI5, which are all known to affect seed dormancy and germination (2–9). Opposite to the action of ABA, GA promotes germination by triggering 26S-proteosome degradation of the growth-repressing DELLA transcription factors. In *Arabidopsis* (*Arabidopsis thaliana*) there are five different *DELLA* genes and four of them [*GA-INSENSITIVE* (*GAI*), *REPRESSOR-OF-GA,* (*RGA*), *RGA-LIKE2* (*RGL2*), and *RGL3*] have a role in repressing germination (10–15). The mechanism for establishing primary dormancy during seed maturation remains poorly understood with only a few genes, such as *DELAY-OF-GERMINATION* (*DOG1*) being identified that are not directly involved in seed maturation or phytohormone metabolism (16–22). Environmental conditions during seed set influence dormancy status. For example, low temperature conditions result in increased primary dormancy of mature *Arabidopsis* seeds (23–25) by inducing expression of genes associated with dormancy and influencing ABA and GA levels (24).

The phosphatidyl ethanolamine-binding protein (PEBP) family is an evolutionary conserved group of proteins present from bacteria to animals and plants. In *Arabidopsis*, the better characterized PEBP-family members so far are FLOWERING-LOCUS (FT), TERMINAL-FLOWER1 (TFL1), and MOTHER-OF-FT-AND-TFL1 (MFT). Although FT and TFL1 are antagonistic regulators of flowering time control (26–28), MFT is involved in regulating seed germination (29, 30), localizing in the nucleus and acting as a transcription factor that directly binds to the *ABI5* promoter to repress its expression in nondormant seeds (30). SPATULA (SPT) is a transcription factor belonging to the basic helix–loop–helix (bHLH) subfamily, which also includes PHYTOCHROME-INTERACTING-(LIKE)-FACTORS (PIFs and PILs) (31). SPT was originally described as a regulator of septum, style, and stigma growth during gynoecium development (32–34) and promotes seed dispersal (35). SPT also plays a role integrating day time and temperature signaling to repress growth of vegetative tissue, such as hypocotyls, cotyledons, and leaves (36–39). We showed previously that SPT is also involved in germination of freshly matured (but not after-ripened) seeds by regulating expression of genes involved in GA biosynthesis in imbibed seeds (38). Interestingly, expression of *SPT* peaks during seed development (40) as well as seed germination (38), which suggested to us that it may be involved in the poorly understood process of primary dormancy establishment.

In the present study, we investigated further the role of SPT in seed dormancy/germination using mutant and overexpressing lines in two different *Arabidopsis* ecotype backgrounds (Landsberg

Author contributions: F.E.V., Y.G., S.P., A.D., K.J.H., and I.A.G. designed research; F.E.V., Y.G., S.P., A.D.G., and A.D. performed research; G.C. contributed new reagents/analytic tools; F.E.V., Y.G., S.P., A.D.G., A.D., Z.H., E.-M.J., G.C., K.J.H., and I.A.G. analyzed data; and F.E.V. and I.A.G. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1301647110/-/DCSupplemental.

erecta, L*er*, and Columbia, Col). Unexpectedly, we found that SPT has opposite effects in germination in the two ecotypes analyzed: although in L*er* SPT represses dormancy, in Col SPT promotes it. We also investigated the role of SPT in gene expression and found that it promotes *ABI5* and *RGL3* and represses *ABI4*, *RGA*, and *MFT* in freshly matured seeds before germination in both L*er* and Col ecotypes. We also found that MFT promotes primary dormancy and germination in freshly matured and after-ripened seeds, respectively. We propose a model in which the role of SPT on gene expression explains the opposite *spt* mutant phenotypes in the L*er* and Col ecotypes.

Results and Discussion

SPT Has Opposite Effects on Seed Primary Dormancy in L*er* and Col. We previously described the role of SPT in germination of freshly matured L*er* seeds (38). Here we extend our phenotypic analysis to the Col ecotype. As reported earlier (38), L*er* seeds carrying the *spt*-2 mutation, resulting in an $R^{209}K$ amino acid change in the DNA-binding domain, are extremely dormant (Fig. 1*A*). However, seeds from SPT overexpressing (SPToe) lines exhibit increased germination (Fig. 1*A*). In contrast, Col seeds of the *spt*-11 and *spt*-12 T-DNA insertion loss-of-function mutants are less dormant and SPToe seeds are more dormant than WT under unstratified conditions (Fig. 1*C*). After stratification, all of the genetic backgrounds analyzed in Col germinated at 100% (Fig. 1*C*). Comparison of L*er* and Col nucleotide sequences determined that the germination differences between these ecotypes are not because of allelic variation at the *SPT* locus. The *spt*-12 T-DNA insertion null mutant was introgressed from Col into L*er* and in this ecotype *spt*-12 seeds are more dormant than WT (Fig. 1*B*). To assess the effect of *spt*-2 in Col, we took advantage of the fact that it is a semidominant mutation (38) by overexpressing the *spt*-2 cDNA (SPT2oe) in Col plants: SPT2oe seeds are less dormant than WT-Col controls (Fig. 1*D*). Taken together, these results underline the importance of background ecotype in determining the role played by SPT, which results in more germination in L*er* and less in Col. These results led us to retest the experimental materials we used to report on the role of SPT in germination (38). Consistent with the results shown in Fig. 1*C*, we found that the SPToe data reported previously was derived from a Col rather than a L*er* background. Furthermore, *spt*-10, which was reported as a loss-of-function allele in L*er* because of a transposon insertion near the 5′ end of the first exon, actually results in increased transcript levels (Fig. S1). The similarity of the *spt*-10 and L*er* overexpressor germination phenotypes lead us to conclude that this particular allele acts as a SPT2oe at this stage of development.

To gain more information about the mode of action of SPT, exogenously applied GA and the ABA biosynthesis inhibitor

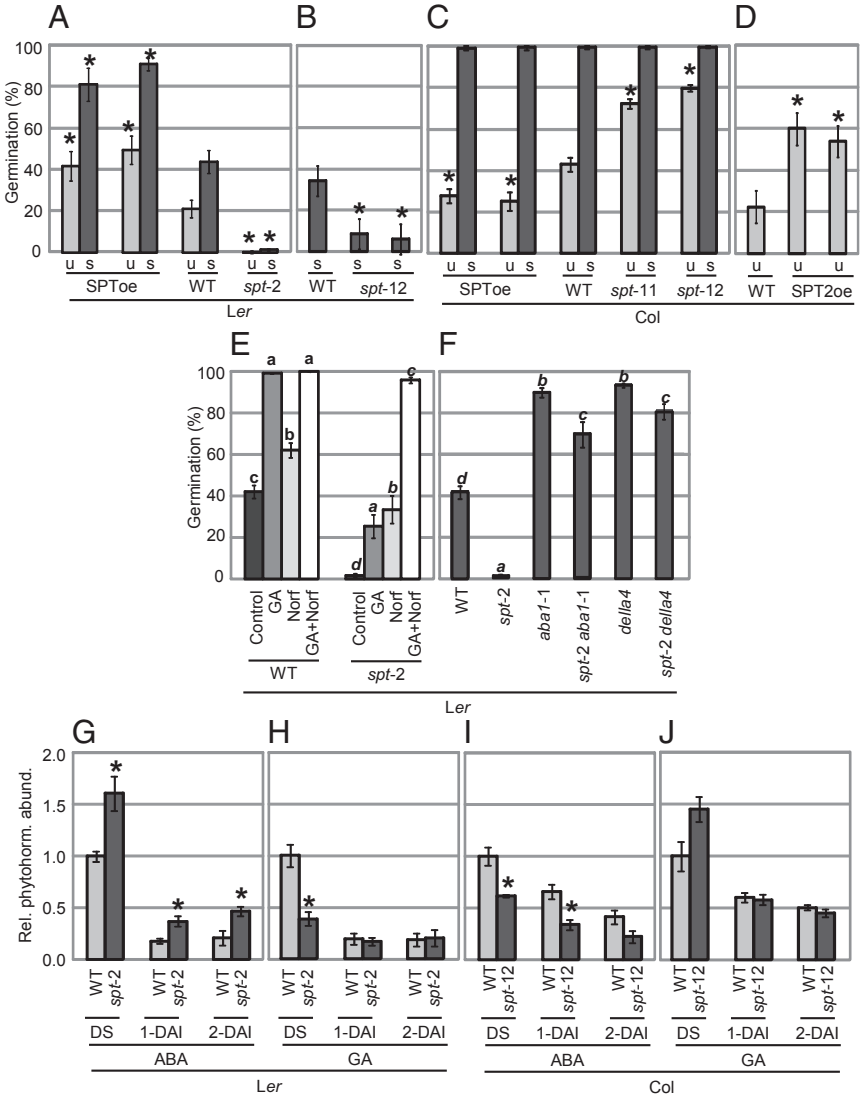

Fig. 1. Different dormancy effects of SPT depending on ecotype background. Germination assays of: (*A*) SPToe, WT, and *spt*-2 in L*er* (unstratified, u; or stratified, s); (*B*) *spt*-12 retrogressed into L*er* (stratified, s; two independent retrogressions are shown); (*C*) SPToe, WT, *spt*-11 and *spt*-12 in Columbia (Col) (unstratified, u; or stratified, s); (*D*) two independent SPT2oe lines in Col (unstratified, u); (*E*) WT and *spt*-2 in L*er* (stratified) supplemented with GA (100 μM), Norflurazon (Norf; 50 μM), or both; and (*F*) WT, *spt*-2 and *aba1*-1 single-mutants, *spt*-2 *aba1*-1 double-mutant, *gai*-6 *rga*-2 *rgl1*-1 *rgl2*-1 quadruple-mutant (*della4*), and *spt*-2 *della4* quintuple-mutant in L*er* (stratified). Assays were performed on freshly matured seeds; germination was counted 7 d after imbibition in continuous light. (*G–J*) ABA and GA in freshly matured dry seed (DS) and 1- and 2-d after imbibition (DAI) of WT and *spt* mutants in L*er* and Col. Relative phytohormone abundance (Rel. phytohorm. abund.) values were obtained by dividing absolute amounts of all samples by the WT DS amount, which was defined as the reference point. In all panels error bars represent standard deviation (SD) of at least three determinations. Asterisks and letters above the bars indicate statistically significant differences ($P < 0.05$). See *Experimental Procedures* for details.

Norflurazon (100 and 50 μM, respectively) were used in germination assays. Both these compounds promote germination of WT seeds and rescue the extreme *spt*-2 dormancy phenotype (Fig. 1*E*). Furthermore, the germination promoting effect of these compounds on *spt*-2 is synergistic resulting in close to 100% germination (Fig. 1*E*). A series of genetic analyses were also carried out by introducing *spt*-2 into *aba1*-1 (disrupted in ABA biosynthesis) and a quadruple *rgl1*-1/*rgl2*-1/*gai*-6/*rga*-2 *DELLA* mutant (*della4*; having a constitutive GA response). Germination assays determined that the extreme *spt*-2 dormancy phenotype is alleviated by decreasing endogenous ABA levels or constitutively activating the GA response pathway (Fig. 1*F*).

To better characterize the *spt* mutants, endogenous levels of ABA and GA were measured in freshly matured dry and imbibed seeds. Because stratification abolishes the *spt* mutant phenotype in Col (Fig. 1*C*), the analyses were performed on unstratified samples. The ABA and GA levels are presented relative to the respective WT dry-seed samples (for absolute values, see Table S1): In *spt*-2, ABA levels are significantly higher in dry and imbibed seeds (Fig. 1*G*); GA levels are significantly lower in dry seeds but unchanged in imbibed seeds (Fig. 1*H*). In *spt*-12, ABA levels are lower throughout the time course (Fig. 1*I*) and GA abundance is higher in dry seeds but unchanged in imbibed seeds (Fig. 1*J*). Although the differences in ABA and GA accumulation in *spt* mutant backgrounds compared with their respective WTs is generally less than twofold, the same trends were observed in independent experiments. Thus, ABA and GA levels in dry seeds are consistent with the dormancy status of the *spt* mutants in L*er* and Col (Fig. 1 *A* and *C*). These observations, together with the fact that *SPT* is expressed during seed development (Fig. S2), led us to further investigate its possible role at this developmental stage.

***ABI5* and *RGA* Are Direct Targets of SPT.** To find possible direct targets of SPT, we assessed expression of selected genes involved in ABA and GA biosynthesis [*ABA1*, *GA-3-OXIDASE1* (*GA3OX1*), and *GA3OX2*], ABA signaling (*ABI3*, *ABI4*, and *ABI5*), GA signaling (*GAI*, *RGA*, *RGL2*, and *RGL3*), and seed dormancy (*DOG1*) that are expressed during seed development and germination (Fig. S2). We measured transcript abundance of these genes in freshly matured dry seeds as an indicator of expression during late stages of seed development (Fig. 2 *A* and *B*). *ABA1* expression is increased in *spt*-2 and decreased in *spt*-12, consistent with ABA levels in these two mutants, but there is no difference in *GA3OX1* and *GA3OX2* expression despite GA levels being altered. Regarding the ABA-signaling genes, *ABI3* expression is unchanged, *ABI4* is increased, and *ABI5* decreased in both L*er* and Col *spt* mutants. Of the GA-signaling genes, the expression of *GAI* and *RGL2* are unchanged in the mutant backgrounds, *RGL3* expression is repressed in both L*er* and Col *spt* mutants, and *RGA* transcript levels are increased twofold in L*er* *spt*-2 but unchanged in Col *spt*-12. *DOG1* transcript accumulation is unchanged in *spt* mutant irrespective of ecotype.

The bHLH-type transcription factors bind to the E-box (CANNTG) motif present in target gene promoters (41). The members of the PIF/PIL subfamily, to which SPT belongs, preferentially bind to the G-box (CACGTG) motif (42–44). We screened the promoter regions of the SPT-regulated genes for these motifs. Although *ABI5* and *RGA* promoters have five and three G-boxes, respectively, *ABA1*, *ABI4*, and *RGL3* promoters only contain E-boxes (Fig. 2*C* and Table S2). To test whether SPT binds directly to these promoters in vivo, ChIP followed by quantitative PCR (qPCR) analyses were performed on fully expanded green siliques of a MYC-epitope tagged SPT line (SPTmyc) in L*er*. Primers spanning different regions of each of the promoters (Fig. 2*C*) were

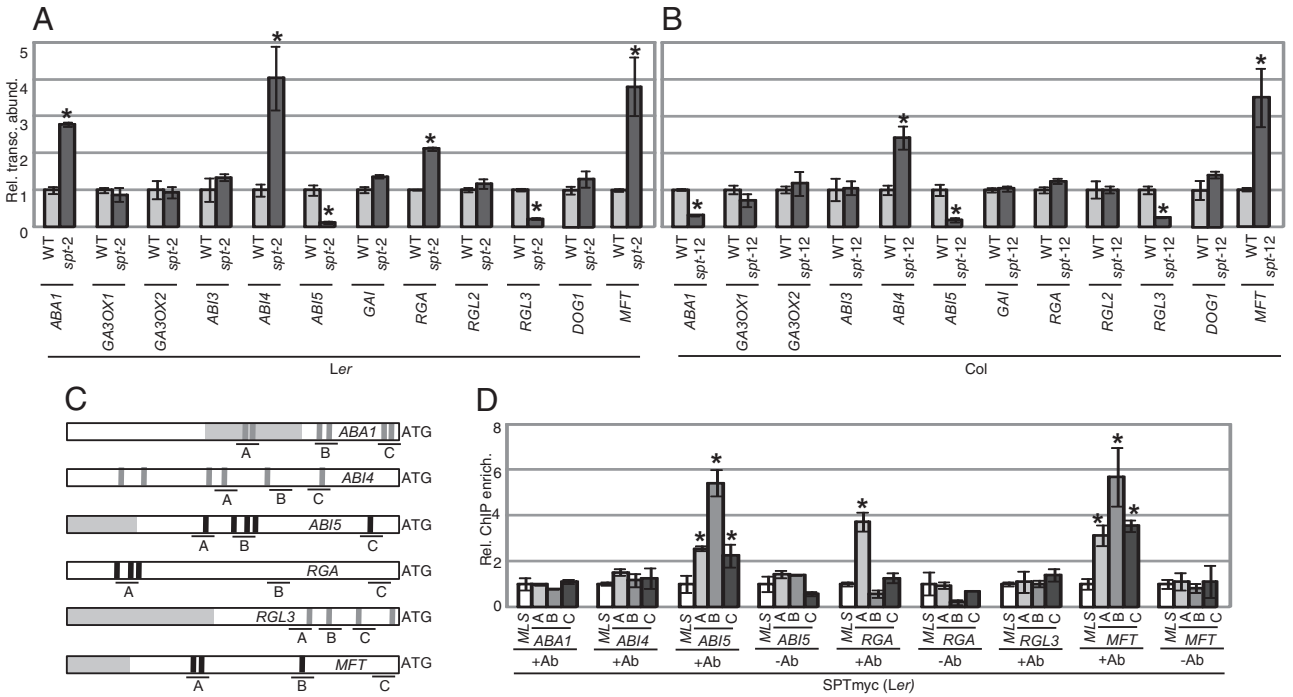

Fig. 2. *ABI5*, *RGA*, and *MFT* are direct targets of SPT. (*A* and *B*) Relative transcript abundance (Rel. transc. abund.) in L*er* and Col freshly matured dry seeds of selected genes involved in ABA and GA biosynthetic and signaling pathways, *DOG1* and *MFT*. Error bars represent SD of three determinations. (*C*) Schematic representation of the 2,500-bp region upstream of the ATG start codon of the indicated genes: Vertical thick black bars represent G-box motifs; vertical thick gray bars represent E-box motifs in G-box free promoters; light gray rectangles indicate the presence of an upstream gene; horizontal thin black lines represent amplicons assayed for SPT binding. (*D*) Relative ChIP-qPCR enrichment (Rel. ChIP enrich.) of the indicated promoter regions compared with the negative MLS control. Assays were performed on silique samples from a SPTmyc epitope-tagged transgenic line (L*er*) with (+Ab) or without (−Ab) anti-MYC antibodies. Error bars represent standard error (SE) of three replicates (similar results were obtained in independent experiments). Asterisks above the bars indicate statistically significant differences ($P < 0.05$). See *Experimental Procedures* for details.

used, as well as an internal control region of the unrelated G-box free *MALATE SYNTHASE* (*MLS*) gene promoter. These experiments show enrichment of only the *ABI5* and *RGA* G-Box specific amplicons (Fig. 2*D*). We found that the promoters of both *ABI5* and *RGA* are also direct targets of SPT in Col (Fig. S3). Interestingly, despite the in vivo SPT–*RGA* interaction in Col, we did not find *RGA* expression to be altered in *spt*-12 (Fig. 2*B* and Fig. S3).

During seed imbibition, *SPT* expression coincides with that of *GA3OX1* and *GA3OX2* (Fig. S2). Expression of both these genes is altered by SPT in stratified imbibed germinating L*er* seeds (38) (Fig. S1) and their promoters each contain a G-box motif (Fig. S3 and Table S2). To test whether SPT binds directly to these promoters in imbibed seeds, ChIP-qPCR analyses were performed but failed to detect any SPT-*GA3OX1* or SPT-*GA3OX2* interactions, suggesting that these genes are not primary targets of SPT (Fig. S3).

Transcriptomic and ChIP Analyses Reveal *MFT* as a Direct Target of SPT. To find other SPT targets, we analyzed transcriptomic data available in our laboratory from freshly matured stratified L*er* seeds sampled 1 d after imbibition, which is the time point when *SPT* expression is highest in germinating L*er* seeds (38). Two sets of transcriptomic comparisons were carried out: (*i*) SPToe vs. WT vs. *spt*-2, and (*ii*) *aba1*-1 vs. *spt*-2 *aba1*-1. The first comparison identifies many genes that are up- or down-regulated depending on levels of SPT, but because this material shows large differences in germination capacity (Fig. 1*A*), many of these genes will not be direct targets of SPT. In the second comparison germination capacity is similar, because *aba1*-1 rescues the strong dormancy phenotype of *spt*-2 (Fig. 1*F*). We found that only 10 genes had their profiles altered in the same pattern between the two comparison sets (Table S3). Six of these genes contain G-boxes within their promoters (Tables S2 and S3) with one of these, *MFT*, which is repressed by SPT, having three (Fig. 2*C* and Table S2). Interestingly, the *MFT* expression profile parallels that of *SPT* during seed development (Fig. S2). Transcript abundance in freshly matured dry seeds and ChIP-qPCR analyses on silique material showed that *MFT* expression increases in the absence of SPT (Fig. 2 *A* and *B*) and SPTmyc binds to the three *MFT* promoter regions assessed in L*er* and Col backgrounds (Fig. 2*D* and Fig. S3). However, these SPT-*MFT* interactions were not observed in material from germinating seeds (Fig. S3). Of the other G-box containing putative primary SPT targets described in Table S3, ChIP-qPCR analyses indicate that only the *At4g33980* gene is a direct target of SPT (Fig. S3) and although the gene function is not known, it is interesting to note that it is mainly expressed during seed development (Fig. S2).

MFT Promotes Primary Dormancy. MFT was recently shown to be involved in seed germination (29, 30). However, although MFT has been found to negatively regulate ABA signaling and promote germination in *Arabidopsis* (30), in wheat (*Triticum aestivum*) an MFT homolog (Ta-MFT) has been shown to be a key factor in promoting dormancy (29). The *Arabidopsis* study used after-ripened nondormant seeds but the wheat study used freshly

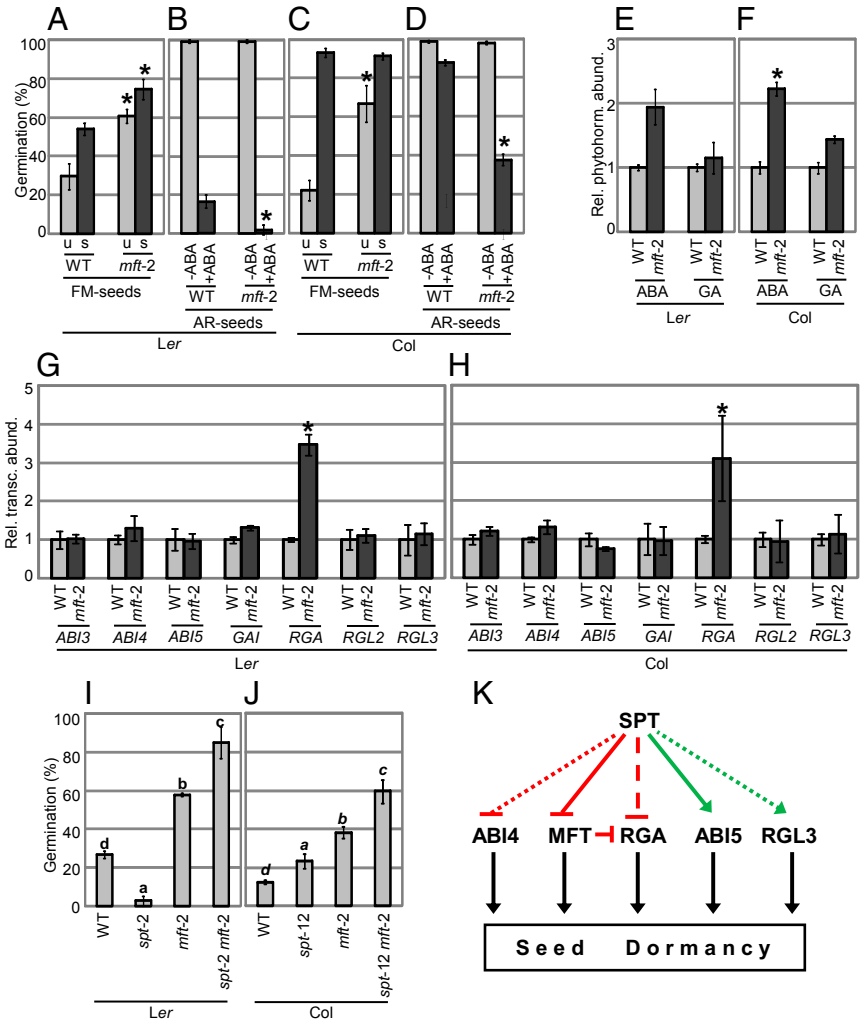

Fig. 3. MFT regulates seed primary dormancy. (*A* and *C*) Germination assays of freshly mature (FM) unstratified (u) and stratified (s) WT and *mft*-2 L*er* and Col seeds. (*B* and *D*) Germination assays of after-ripened (AR) stratified WT and *mft*-2 L*er* and Col seeds supplemented with 10 μM ABA (+ABA) or without (–ABA). (*E* and *F*) ABA and GA in freshly matured dry seed of WT and *mft* mutants in L*er* and Col. Absolute amounts were divided by the reference (WT DS) to obtain relative phytohormone abundance (Rel. phytohorm. abund.). (*G* and *H*) Relative transcript abundance (Rel. transc. abund.) of selected genes involved in ABA and GA signaling pathways in freshly matured WT and *mft*-2 L*er* and Col dry seeds. (*I* and *J*) Germination assays of freshly matured unstratified WT, *spt*, and *mft* single mutants and the *spt mft* double-mutants L*er* and Col seeds. Error bars represent SD of at least three determinations. Asterisks and letters above the bars indicate statistically significant differences ($P < 0.05$). See *Experimental Procedures* for details. (*K*) Model of SPT-mediated regulation of seed primary dormancy. Red and green lines from SPT denote repression and activation, respectively, by this transcription factor of *ABI4*, *MFT*, *RGA*, *ABI5*, and *RGL3* gene expression. These red and green lines also represent the dormancy-repressing and dormancy-promoting routes, respectively. *MFT* and *ABI5* are direct SPT targets (solid lines); *RGA* is a direct SPT target in L*er* and Col but gene expression is only altered in L*er* (dashed line); *ABI4* and *RGL3* are indirect SPT targets (dotted lines). Red horizontal line from MFT denotes the repressive action on *RGA* expression (whether *RGA* is a direct or indirect target of MFT is still unknown). Solid black lines from ABI4, MFT, RGA, ABI5, and RGL3 denote the promotion of primary seed dormancy by these transcription factors.

matured dormant seeds. These differences prompted us to assess whether MFT plays a role in regulating primary dormancy in *Arabidopsis*. We found that *mft*-2 L*er* and Col seeds are less dormant than the corresponding WTs (Fig. 3 *A* and *C*). We also analyzed sensitivity to exogenously applied ABA (10 μM) on 2-mo-old after-ripened seeds and found that, as previously reported for Col (30), *mft*-2 nondormant L*er* seeds are hypersensitive to the phytohormone (Fig. 3 *B* and *D*). We therefore conclude that MFT operates to promote (*i*) dormancy during seed development and (*ii*) germination in after-ripened imbibed seeds with exogenous ABA. Surprisingly, given the less-dormant phenotype of *mft-2*, relative endogenous levels of ABA in freshly matured dry seeds are approximately twofold higher in the *mft*-2 mutants compared with the L*er* and Col WTs, and GA levels are similar (Fig. 3 *E* and *F*; for absolute values see Table S1). The role of MFT in promoting germination in after-ripened seeds is mediated at least in part by directly repressing *ABI5* expression (30). Hence, we assessed *ABI5* expression (as well as other ABA and GA signaling related-genes) in freshly matured dry seeds. We found that of all these genes only *RGA* expression is altered, showing an increase in *mft*-2 in both ecotype backgrounds (Fig. 3 *G* and *H*). As with the elevated ABA levels, the increase in *RGA* expression is unexpected given that this gene promotes dormancy but in *mft*-2 dormancy levels are decreased. These results suggest that to promote dormancy, MFT acts downstream with or parallel to the ABA and GA response pathways. We reported recently that the *pxa1* mutant (that is disrupted in a peroxisomal ABC transporter activity) has low levels of seed germination despite accumulating higher GA levels than WT (45). Hence, the high ABA and GA levels in *mft* and *pxa1* mutants, respectively, may be a consequence of compensatory mechanisms attempting to rescue germination back to WT levels.

To gain insight into the combined action of SPT and MFT, we obtained the *spt*-2 *mft*-2 (in L*er*) and *spt*-12 *mft*-2 (in Col) double-mutants and assessed germination of freshly matured seeds. We found the effect of the two mutations to be additive in Col as expected from the single-mutant phenotypes (Fig. 3*J*). However, in L*er*, the two mutations are not additive and the double-mutant is less dormant than the *mft*-2 single-mutant despite the strong dormancy phenotype of *spt*-2 in this ecotype (Fig. 3*I*).

Arabidopsis seed primary dormancy is promoted by low-temperature conditions during seed development (23–25). Low temperature also increases seed dormancy in wheat and this correlates with an increase in Ta-*MFT* gene expression (29). Once primary dormancy is released, secondary dormancy can be induced depending on environmental conditions (46). A recent study has demonstrated that secondary dormancy of *Arabidopsis* Cvi ecotype seeds correlated with low seasonal soil temperatures and high *MFT* expression (47). Thus, MFT appears to be associated with the induction of both primary and secondary dormancy in *Arabidopsis*. Another recent study has described a temperature-mediated promotion of *FT* expression by PIF4 (48). Interestingly, PIF4 and FT belong to the same protein families as SPT and MFT, respectively. Our demonstration that SPT and MFT control the transition from seed to seedling parallels the control of the transition from vegetative to floral development by PIF4 and FT. Allelic variation at the *PIF4* locus is known to be associated with several ecological traits, including flowering time (49). Here we find ecotype differences in SPT function between L*er* and Col in the absence of allelic variation at the *SPT* locus.

Antagonistic Routes Mediated by SPT Control Primary Dormancy. Integrating the data reported here, we propose the following model for the role of SPT in setting seed primary dormancy (Fig. 3*K*). During seed development, SPT controls expression of transcription factors encoding genes that promote seed dormancy in both L*er* and Col: SPT promotes *ABI5* and represses *MFT* and *RGA* expression by binding to their respective promoters. This process creates two antagonistic "dormancy-repressing" and "dormancy-promoting" routes that operate in both ecotypes simultaneously. Both routes are reinforced by the indirect action of SPT in repressing *ABI4* and promoting *RGL3*. These routes have different outcomes in L*er* and Col seeds and this, in turn, influences *ABA1* expression and ABA/GA ratios in dry seeds (Figs. 1 *G* and *I*, and 2 *A* and *B*). RGA enhances RGL2 function and they are both known to stimulate ABA biosynthesis (50), but it remains to be established whether this is through a direct effect on *ABA1* expression. We propose that the low germination rates of *spt* mutant L*er* seeds (Fig. 1 *A* and *B*) result from higher dormancy levels because of the elevated expression of *MFT*, *RGA*, and *ABI4*, which override the low expression of *ABI5* and *RGL3* (Fig. 2*A*). In our model the L*er*-specific increase of *RGA* expression in the *spt* mutant background (Fig. 2 *A* and *B*) may be a factor reinforcing the dormancy-repressing route in this ecotype. This role for RGA is supported by the rescue of the strong dormant phenotype of *spt*-2 when crossed into *della4* (Fig. 1*F*). In contrast to what happens in L*er*, the high germination rates of *spt* mutant Col seeds (Fig. 1 *C* and *D*) result from lower dormancy levels because of the low *ABI5* and *RGL3* expression, which are dominant over the increased *MFT* and *ABI4* expression (Fig. 2*B*). Our model is backed-up by the outcome of the *spt mft* double-mutant analyses. In Col the additive effect of the double-mutant is a consequence of the removal of these two dormancy-promoting factors. Furthermore, the fact that in L*er* the *spt mft* double-mutant is not additive (the double-mutant germinates even more that the single *mft*-mutant and overrides the strong dormant phenotype of *spt*-2), suggests that MFT is a major component of the dormancy-repressing route in this ecotype.

Apart from its role in dormancy/germination, SPT is also involved in fruit development (32–34) and repression of hypocotyl elongation and cotyledon and leaf size (36–38). Hence, the role of SPT as a growth repressor of vegetative developmental stages parallels its effect in promoting seed dormancy (i.e., repressing growth) in Col seeds. The nonseed-related roles of SPT do not appear to vary between ecotypes. The fact that perturbation of SPT has different outcomes in terms of seed dormancy in L*er* and Col perhaps reflects adaptation to different environmental conditions. Our double-mutant and gene-expression analysis implicate MFT and RGA as candidates for the ecotype differences. Genetic variation in seed-germination sensitivity of L*er* and Col to the GA-biosynthesis inhibitor paclobutrazol has been mapped to three quantitative trait loci, but none of these colocalize with *MFT* or *RGA* (51). This study highlights the central role played by the transcription factor SPT in controlling seed dormancy and demonstrates how fine-tuning of five other transcription factors can lead to very different outcomes depending on ecotypes.

Experimental Procedures

All mutant lines used in this study were described previously: *spt*-2 (32); *spt*-11 and *spt*-12 (36); *abi3*-4 (52); *aba1*-1 (53); *rgl1*-1 *rgl2*-1 *gai*-6 *rga*-2 (*della4*) (10); *spt*-2 *della4* (37); *mft*-2 (30). The SPToe and SPT2oe transgenic lines were obtained by transforming plants with the pK2GW7 and pH2GW7 vectors carrying the *SPT* and *spt*-2 coding sequences, respectively, downstream of the 35S promoter. SPTmyc containing L*er* plants were produced by transformation with a pGREENII based binary vector carrying *SPT*-12xMYC and Col plants with a derivative of the pBI121 binary vector in which the GUS sequence was replaced with the *SPT*-9xMYC. In both ecotypes the SPTmyc genes are driven by the 35S promoter.

Plants were grown in the departmental greenhouse facilities supplemented with artificial light to give a photoperiod of 16-h light at a temperature of ∼20–22 °C. Germination assays were performed as described previously (38). Stratification was performed during imbibition of seeds for 2–3 d in the dark at 4 °C. RNA extractions from 1-d after-imbibition seeds and ABA and GA (GA4) measurements were performed as described previously (38, 45). For RNA extractions from developing seeds the method was scaled down to use ∼200 seeds and 15% of the buffer volumes. cDNA synthesis was performed using standard methods. qPCR was performed using iQ SYBR GreenSupermix and the MyiQ Real-Time PCR detection system (Bio-Rad) according to the manufacturer's instructions. Expression of *ACTIN2* was used for normalization. Transcriptomic analyses and ChIP assays were

performed as described previously (42, 45). Public domain Affymetrix ATH1 data sets were obtained from NascArrays (http://affymetrix.arabidopsis.info/narrays/experimentbrowse.pl) and TAIR (www.arabidopsis.org) and used to produce Fig. S2. A *MLS* specific amplicon was used as negative control. Sequence of primers used in RT-qPCRs and ChIP-qPCRs are listed in Table S4. Relative positions of the G-box and E-box motifs are indicated in Table S2.

In relevant figures, asterisks indicate results of two tailed Student *t* test analyses performed in Microsoft Excel, comparing overexpressors, mutants, or treatments to corresponding controls. Grouped matching letters indicate nonsignificant differences from ANOVA followed by Tukey's honestly significant difference tests performed in R (v2.15.2; www.R-project.org).

ACKNOWLEDGMENTS. We thank Hao Yu for supplying *mft*-2 mutant seeds; Wim Soppe for constructive comments on the manuscript; Jeongmoo Park for advice on ChIP; and departmental horticultural and technology facility services for plant care and transcriptomic assays, respectively. This work was funded by the Biotechnology and Biological Sciences Research Council (BB/E000541/1, BB/J000949/1, BB/F005296/1, BB/F005237/1) and the Garfield Weston Foundation.

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