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MOTHER-OF-FT-AND-TFL1 represses seed germination under far-red light by modulating phytohormone responses in Arabidopsis thaliana

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Seed germination in many plant species is triggered by sunlight, which is rich in the red (R) wavelength and repressed by under-the-canopy light rich in far red (FR). R:FR ratios are sensed by phytochromes to regulate levels of gibberellins (GAs) and abscisic acid (ABA), which induce and inhibit germination respectively. In this study we have discovered that, under FR light conditions, germination is repressed by MOTHER-OF-FT-AND-TFL1 (MFT) through the regulation of the ABA and GA signaling pathways. We also show that MFT gene expression is tightly regulated by light quality. Previous work has shown that under FR light conditions the transcription factor PHYCHROME-INTERACTING-FACTOR1 (PIF1) accumulates and promotes expression of SOMNUS (SOM) that, in turn, leads to increased ABA and decreased GA levels. PIF1 also promotes expression of genes encoding ABA-INSENSITIVES (ABI5) and DELLA growth-repressor proteins, which act in the ABA and GA signaling pathways, respectively. Here we show that MFT gene expression is promoted by FR light through the PIF1/SOM/ABI5/DELLA pathway and is repressed by R light via the transcription factor SPATULA (SPT). Consistent with this, we also show that SPT gene expression is repressed under FR light in a PIF1-dependent manner. Furthermore, transcriptional analyses presented in this study indicate that MFT exerts its function by promoting expression of known ABA-induced genes and repressing cell wall expansion-related genes.

Significance

Seeds sense environmental conditions, such as light quality, to regulate their germination. Light enriched in the far-red wavelength, such as under-the-canopy light (shade), increases abscisic acid (ABA) and decreases gibberellin (GA) levels, the phytohormones repressing and promoting germination, respectively. We have discovered that MFT functions as a key repressor of germination under far-red light conditions by modulating ABA and GA responses.

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The authors declare no conflict of interest.

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Data deposition: The sequences reported in this paper have been deposited in The National Center for Biotechnology Information (NCBI) RBA database (accession nos. SRR7175460–SRR7175468).

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and is also involved in regulating vegetative growth and seed dormancy (22–24). SPT regulation of seed dormancy involves the direct repression of MOTHER-OF-FT-AND-TFL1 (MFT) (25). MFT is a member of the phosphatidylethanolamine-binding protein (PEBP) family, which is involved in diverse growth and differentiation signaling pathways in bacteria, animals, and plants. In Arabidopsis there are six PEBP proteins and extensive analyses of two of them, FLOWERING-LOCUS-T (FT) and TERMINAL-FLORER1 (TFL1), have demonstrated that they antagonistically regulate flowering time and seed dormancy (26–29). In wheat it has been established that up-regulation of MFT expression by cold during seed development or by a mutation in its promoter leads to strong seed dormancy (30, 31). In Arabidopsis MFT is also a strong promoter of seed dormancy (25), but its expression is only weakly promoted by low temperature during seed development (32). However, MFT expression in the soil seed bank is seasonally regulated and correlates with low temperatures and high levels of cycling secondary dormancy (33). Furthermore, expression of MFT has also been shown to be promoted by ABA and DELLA proteins (34). Intriguingly, we and others have found that while freshly matured MFT-deficient seeds are less dormant than wild-type seeds, afterripened (nondormant) seeds are, counterintuitively, more sensitive to ABA treatments (25, 34). This raised the possibility that MFT has opposite roles in regulating seed dormancy and nondormant seed germination.

In the present study, we investigate the role of the MFT gene in regulating germination of afterripened seeds under R and FR light. We demonstrate that MFT is a key component of the PIF1-dependent pathway of repression of germination and acts by integrating ABA and GA signals. Furthermore, we show that expression of the MFT gene is regulated by light quality: While it is promoted by the PIF1/SOM/DELLA pathway under FR light, it is repressed by SPT under R light. Consistent with this, we also show that FR light represses SPT gene expression also in a PIF1-dependent manner. Our genetic, physiological and transcriptomic analyses reveal that MFT plays a major role in repressing seed germination by integrating the ABA and GA signaling pathways.

Results and Discussion

MFT Represses Germination Under FR Light. We reported previously that freshly matured Arabidopsis loss-of-function mft-2 mutant seeds are less dormant than wild-type seeds, which led us to conclude that MFT promotes dormancy (25). However, ABA treatments of mft-2 nondormant seeds indicated that MFT promotes germination of afterripened seeds (25, 34). This prompted us to investigate the role of MFT in regulating germination of nondormant seeds under shade light conditions, which repress germination by inducing accumulation of endogenous ABA. To do this, germination assays were performed on control and mft-2 afterripened seeds (in the Col and Ler backgrounds) under different light treatments (SI Appendix, Fig. S1). Seeds were imbibed for 3 h under white light, then treated with: (i) two consecutive short (5 min) pulses of FR and R light (FR/R) to activate PHYB; (ii) a single FR light pulse (FR) to deactivate PHYB; and (iii) an initial short FR pulse followed [46 h after imbibition (hai)] by a long (120 min) FR light exposure (FR–FR) to activate PHYA while also deactivating PHYB. We found that under FR/R light conditions, wild-type and mft-2 seeds germinate at similarly high levels (80%) (Fig. 1 A and B). Upon FR treatments, germination of wild-type seeds was, as expected, severely repressed (less than 5%), whereas mft-2 seeds germinated at higher rates (30–70%) (Fig. 1 A and B).

We used an mft-2 complemented line (33) to confirm that this mutant phenotype is due to a mutation in the MFT gene (SI Appendix, Fig. S2). Under FR–FR conditions, germination rates of control seeds were, as expected, approximately intermediate (40%) to those of FR/R- and FR-treated seeds; and mft-2 seeds germinated at higher rates (70%) than the controls (Fig. 1 A and B). Taken together these observations demonstrate that MFT is required to repress germination under FR light conditions.

MFT Promotes Hypocotyl Elongation in the Dark. In addition to their role in seed germination, phytochromes and the PIF1-dependent pathway also control seedling hypocotyl elongation (35, 36). Thus, we investigated whether MFT also regulates seedling development and measured wild-type and mft-2 hypocotyls of seedlings grown under dark conditions, which are known to trigger PHYB inactivation (14). We found that mft-2 hypocotyls were significantly shorter than the wild-type controls (SI Appendix, Fig. S3) supporting the hypothesis that MFT promotes seedling hypocotyl elongation. These roles for MFT in repressing seed germination and promoting seedling hypocotyl elongation parallel the established roles of the phytochrome interacting factor PIF1 (16, 17, 37, 38) and further indicates that MFT operates in the PIF1 pathway.

MFT Is a Positive Regulator of ABA Signaling. To investigate how MFT inhibits germination, we measured phytohormone levels in afterripened seeds under FR light conditions (24 hai). ABA levels were actually elevated in FR-treated mft-2 seeds compared with the wild type (Fig. 2A), even though germination is higher in the mutant (Fig. 1 A and B). This suggests that the inhibitory effect of MFT on germination of FR-treated seeds acts downstream of ABA. To test this hypothesis, we analyzed triple cyp707a1-1 cyp707a2-1 mft-2 mutant seeds germination. Both CYP707A1 and CYP707A2 are involved in ABA degradation, and double cyp707a1-1 cyp707a2-1 (ala2) mutant seeds germinate poorly due to their elevated levels of endogenous ABA (39). Under FR–FR light conditions, we found that the almost zero percentage germination phenotype of the ala2 double mutant is rescued by mft-2 to levels that are intermediate between the wild type and the mft-2 single mutant (Fig. 2B). We also performed germination assays of the afterripened seeds under white light, and observed that, whereas ala2 seed germination was impaired, mft-2 ala2 seed germination was almost as high as mft-2 and wild-type control seeds (Fig. 3C). Considering that ABA induces MFT expression (34), these observations indicate that MFT is involved in the transduction of the ABA signal that results in germination inhibition. However, since the repression of germination in ala2 seeds is not completely removed in the mft-2 background, we conclude that not all of the ABA repression of germination is due to its effect on MFT. We and others have previously shown that mft-2 seeds are hypersensitive to exogenous ABA treatment (25, 34). This contrasts with our present observation, which indicates mft-2 to be hypo- rather than hypersensitive to elevated endogenous ABA in ala2 seeds (Fig. 2 B and C). The hypersensitivity of mft-2 to exogenous ABA also contrasts with studies analyzing sensitivity of mft-2 seeds to exogenous ABA during dormancy cycling (40).
MFT Expression Is Promoted by FR Light in a PIF1 Pathway-Dependent Manner. Publicly available transcriptomic data indicate that MFT gene expression is high during seed development when primary dormancy is set and, conversely, low in imbibed seeds when germination is triggered (seed.nottingham.ac.uk). This prompted us to investigate whether light quality plays a role in regulating MFT expression in imbibed seeds. To test this, we quantified MFT transcript accumulation in FR/R and FR light treated afterripened seeds 12 and 24 hai. We observed that, compared with FR/R, MFT transcript accumulation is strongly induced under FR light conditions (Fig. 3A). Moreover, we established that this FR-triggered increase of MFT expression is strongly compromised (over 20-fold) in pif1-1 mutant seeds (Fig. 3B), which are impaired in the FR-dependent repression of germination (18). Since active PHYA represses PIF1 accumulation, we also analyzed phyA-211 mutant seeds under FR–FR light conditions and found increased MFT transcript accumulation (SI Appendix, Fig. S4). This is consistent with our finding that MFT expression is positively regulated by PIF1 (Fig. 3B).

As a member of the bHLH family of transcription factors, PIF1 preferentially binds to G-box motifs (41). Since the MFT promoter harbors three G-box motifs (25), we investigated by chromatin immunoprecipitation (ChIP) followed by quantitative PCR (qPCR) whether PIF1 binds the MFT promoter in FR-treated seeds but we found no evidence of such an interaction (SI Appendix, Fig. S5). This indicates that the PIF1 involvement in promoting MFT expression under FR conditions is likely to be indirect. It is known that PIF1 does interact directly with the promoters of SOM and ABI5 as well as the DELLA-encoding RGA and GAI genes (18, 19). It was previously shown in imbibed seeds under white light that MFT expression is promoted by ABA and repressed by GA in an ABI5- and DELLA-dependent manner, respectively (34). This prompted us to test whether this regulation also occurs under FR light conditions. We analyzed som-3, abi5-7, and quadruple rgl1-1 rgl2-2 gai-6 rga-2 (hereafter referred to as del1a4) mutant seeds and found that MFT expression is decreased in all three mutant backgrounds (Fig. 3 C–E), which indicates that MFT expression is promoted by these factors acting downstream of PIF1.

It is worth noting that the decrease of MFT transcript accumulation in pif1-1, som-3, and del1a4 seeds is much more pronounced (15- to 20-fold) than its decrease in abi5–7 seeds (3-fold) (Fig. 3 B–E). This shows that, relative to PIF1/SOM/DELLA, ABI5 is a weak promoter of MFT expression. Interestingly, while pif1-1, som-3, and multiple combinations of del1a4 mutant seeds germinate under FR light (17, 19, 42), abi5 mutant seeds do not (8). This suggests that the relatively high levels of residual MFT transcript in abi5 mutant seeds may be sufficient to repress germination under FR light conditions.

SPT Represses both MFT and ABI5 Gene Expression in Imbibed Seeds. We showed previously that freshly matured spt-2 mutant seeds (in the Ler background) are extremely dormant, but their dormancy is lost over dry storage, and afterripened spt-2 seeds germinate as much as Ler control seeds (22). In freshly matured dry seeds, SPT binds to the MFT and ABI5 promoters to repress and promote their expression, respectively. However, at least in the case of SPT-MFT, this direct interaction is lost in imbibed seeds (25). We therefore reassessed the role of SPT in regulating these genes in developing and imbibed seeds. As previously reported in dry seeds, MFT transcript accumulation is increased in both developing and imbibed spt-2 seeds (Fig. 4), which indicates that SPT represses MFT expression in seeds irrespective of their developmental stage. Regarding ABI5, we found that its expression is decreased in developing spt-2 seeds, which is consistent with our previous findings (8).
with our previous finding for dry seeds. However, ABI5 expression is increased in imbibed spt-2 seeds (Fig. 4B). Thus, SPT promotes ABI5 expression in developing seeds and represses it in imbibed seeds. Using ChIP-qPCR assays, we found no evidence for direct interaction of SPT with MFT or ABI5 in imbibed seeds (SI Appendix, Fig. S5).

**SPT Expression Is Repressed by FR Light in a PIF1 Pathway-Dependent Manner.** We next investigated whether SPT expression is regulated by light quality. We established that, compared with FR/R conditions, SPT transcript accumulation is inhibited by FR light in a PIF1 pathway-dependent manner (Fig. 4C and D). Consistent with this, we also established that PHYA promotes SPT expression (SI Appendix, Fig. S4). ChIP-qPCR assays established that repression of SPT by PIF1 is likely to be indirect (SI Appendix, Fig. S5). Furthermore, genetic analysis revealed that SPT gene expression is repressed by SOM and DELLA proteins, although the repressing effect of the latter appears to be weaker (Fig. 4E and F). Finally, we also assessed germination under FR–FR light conditions of spt-2 mft-2 double mutant and Ler and parental control seeds. We found that spt-2 seeds germinate at a lower rate than Ler control seeds and, importantly, spt-2 mft-2 seeds germinate as much as the single mft-2 mutant seeds (Fig. 4G), which is consistent with our previous observation in freshly harvested seeds and shows that MFT acts downstream of SPT in the light quality regulation of germination.

**MFT Regulates Expression of Growth-Related Genes.** It has been established that MFT localizes in the nucleus and interacts indirectly with DNA to regulate gene expression (34, 43). To gain insight into the MFT mode of action, we performed RNA sequencing-based transcriptomic analyses to identify genes regulated by MFT. We analyzed wild-type (Col) and mft-2 afterripened seeds under FR/R and FR light conditions (24 hai). We made two sets of gene expression profile comparisons from three transcriptomic datasets: (i) FR- vs. FR/R-treated Col seeds (ColFR vs. ColFR/R) and (ii) FR-treated Col seeds vs. FR-treated mft-2 seeds (ColFR vs. mft-2FR). We reasoned that genes that are up-regulated by the MFT-mediated FR light response will show increased expression in ColFR compared with ColFR/R and mft-2FR. Similarly, genes that are down-regulated by MFT will have decreased expression in ColFR. Using at least a twofold-difference threshold in each comparison, we found 175 MFT up-regulated genes and 74 MFT down-regulated genes (Datasets S1 and S2).

Among the MFT up-regulated group are MFT itself and genes known to be induced by ABA and involved in ABA signaling, such as ABI2, PYL13/RCAR7, and GCR2/GPCR, although the role of the latter in this pathway has been controversial (44–46). The gene most strongly up-regulated by MFT is PLP1/PLAV1A, which encodes a phospholipase known to promote hypocotyl elongation (47). Also among this group of MFT up-regulated genes are CATA1, which is known to act downstream of ABI5 to regulate reactive oxygen species (ROS) homeostasis in germinating seeds (48), and DOGL1, DOGL2, and DOGL3, which are closely related to the strong dormancy promoter DELAY-OF-GERMINATION1 (DOG1) (49). Notably, another of the genes up-regulated by MFT, NCED4/CCD4, encodes a negative regulator of β-carotene, a biosynthetic precursor of ABA (50, 51). Thus, it is possible that the higher-than-normal ABA levels found in mft-2 seeds is a consequence of the decreased NCED4/CCD4...
transcript abundance and a concomitant increase of β-carotene. MFT also promotes expression of genes involved in seed development and seed storage, as well as in lipid metabolism, including oxylipin biosynthesis. AOS/CYP74A, which is involved in an early step in the biosynthesis of the oxylipin 12-oxo-phytodienoic acid (OPDA) (52), is up-regulated by MFT. We reported previously that OPDA is a potent repressor of germination that interacts with MFT and ABA in a complex network of feedback interactions (53, 54).

SPT gene expression is 11-fold decreased by FR light and MFT partially contributes to this repression, highlighting the complex feedback interactions between these two key players. Many of the other MFT down-regulated genes are predicted to be involved in cell wall metabolism, response to GA, and root growth. The DVL19/RTFL10 gene, belonging to the DEVIL/ROTUNDIFOLIA family, is the most strongly down-regulated by FR light and this appears to be completely dependent on MFT. Little is known about the function of this gene; however, overexpression of other members of this gene family results in elongated cells at the base of trichomes (55). Many genes involved in auxin signaling (for example AUX1, HLS3/COP3, GHS3/1/WES1, WOX11, SAUR77, and PAP1/IAA26) are also repressed by MFT, as well as CYP90A1, involved in brassinosteroid biosynthesis. Both auxin and brassinosteroid phytohormones regulate a wide range of plant development and physiological processes and are recognized as positive growth regulators of root and shoot development (56). GASA6, GASA14, and EXPA1 are also repressed by MFT and are known to be promoted by GA and repressed by ABA and DELLA proteins. It has been reported that GASA6 and GASA14 promote elongation and leaf expansion, as well as seed germination (57, 58). GASA6 requires the cell wall loosening function of EXP1A (59) and it is expressed in the hypocotyl–radicular transition zone of the embryonic axis (58). This is consistent with the reported tissue specificity of MFT gene promoter activity in ABA-treated seeds (34).

Conclusion

The integration of the data presented in this study with current knowledge of FR light signaling allows us to propose a model (Fig. 5) in which MFT plays a key role in the integration of signals downstream of core signaling components and the antagonistically acting phytohormones ABA and GA. Previous reports have established that light-activated phytochromes lead to PIF1 degradation (17) and, when phytochromes are deactivated, PIF1 accumulates and induces SOM expression. SOM, in turn, promotes and represses ABA and GA accumulation, respectively (19). This then leads to repression of seed germination through a mechanism involving AIB5 and DELLA proteins (2, 4). In the present work, we have shown that SOM also represses germination through the promotion of the germination inhibitor MFT. We show that this effect of SOM on MFT is most likely due to SOM repressing SPT, which is a negative regulator of MFT expression. Our data also demonstrate that the growth-repressing DELLA proteins inhibit and promote SPT and MFT expression, respectively, which is entirely consistent with the fact that DELLAAs accumulate under light conditions that repress germination. Furthermore, MFT is also promoted, albeit weakly, by AIB5, which itself is repressed by SPT. Our data also highlight that, as well as acting downstream of ABA, MFT represses its accumulation. Thus, MFT counteracts the effect of SOM on ABA accumulation. Finally, while we show that MFT plays a key role in the integration of signaling downstream of ABA and GA, both phytohormones can still affect germination independently of MFT.

In summary, our findings establish that shade (FR light) promotes expression of MFT, which in turn represses germination by up- and down-regulating expression of genes involved in ABA signaling and cell wall expansion, respectively. Moreover, we show that this MFT-dependent mechanism of germination inhibition is actively repressed by SPT under R light conditions that induce germination. Publicly available data indicate that MFT is strongly expressed in the endosperm during the late stages of seed development (see seed.nottingham.ac.uk/). It has been established that the endosperm plays a crucial role in promoting dormancy and repressing germination of nondormant seeds under FR light conditions (7, 8). Whether MFT plays a role in this endosperm-based pathway leading to repression of germination remains to be established.

Experimental Procedures

Growth Conditions and Biological Materials. Plants were grown in a greenhouse supplemented with artificial light to give a photoperiod of 16 h light at a temperature of 20–22 °C. Seeds were harvested and afterripened for at least 8 wk before analyses. Original mutant and transgenic lines were derived previously: MFT-2 (Col-gMFT-1), SPT-2 (Col-gSPT-1 (a1a2) (39); pif1-1/pil5-1 (16); som-3 (19) and cyp707a1-1 cyp707a2-1 (abi2) (2); ab1-5 (60); sp1-2 (61); 35S:PIF1myc (18); and 35S:SPTmyc (25).

Germination Assays. Sterilized seeds were plated on water agar (0.9% wt/vol) and allowed to imbibe under dim light for 3 h and then LED irradiated with FR (4.5 μmol m−2 s−1) and R (20 μmol m−2 s−1) as indicated in Fig. 1A. After FR/R, FR, and FR–FR treatments, plates were wrapped in foil and kept at 20 °C. Germination was scored on the basis of radicle emergence of 50–100 seeds per replica. Paclorotrazol (Sigma-Aldrich) was included in the water agar medium where indicated.

Phytohormone Analyses. Phytohormones were extracted and quantified from four biological replicates of 100 mg of treated seeds according to the protocol described previously (53).

Molecular Biology Techniques. RNA extractions were performed as described previously (25). Standard protocols were used for RQ1 RNase-Free DNase (Promega), cDNA synthesis (SuperScriptII, Invitrogen), and qPCRs (Taq Universal Syber Green, Bio-Rad). Expression of UBQ11 was used for normalization, and gene expression was expressed as a fold change relative to the control sample. CHIP assays were performed as described previously (16) followed by qPCR and DNA amplification normalized to the amplification of UBQ11 and expressed relative to the negative control (ΔΔCt). Relative qPCR amplifications were calculated following the delta–delta Ct method (2−ΔΔCt). Primer sequences for the qPCRs are described in SI Appendix, Table S1.

Transcriptomic Analyses. RNA yield from three independent biological samples was quantified and quality checked using a Nanodrop Spectrophotometer and Agilent Bioanalyser RNA nanochip. A total of 500 ng of total RNA was used for library preparation using the NEBNext Ultra Library preparation kit for Illumina in conjunction with the NEBNext Poly(A) mRNA Magnetic Isolation Module (New England BioLabs, Inc.) and NEBNext single 6-bp indexing primers, according to the manufacturer’s instructions. Libraries were pooled at equimolar ratios, and the pool was sent for 2 × 150 base-paired-end sequencing on a HiSeq 3000 at the University of Leeds Next Generation Sequencing Facility. Ribosomal RNA was filtered with mapping to rRNA_115_tax_silva_v1.0 downloaded from SILVA database (https://www.arb-silva.de/). The remaining RNAseq reads were mapped to the cDNA reference sequence from TAIR10. BWA (62) was used with default parameters for mapping. SAMTools (63) was used for read quantification (SI Appendix, Table S2). The methods in the Bioconductor package EdgeR (64) were used to identify the differentially expressed genes from comparisons described in the main text. Only genes with at least a twofold change in each of the two comparisons, and a minimum of 5 reads per kilobase million (RPKM) in C0wp3 (for MFR promotion) or C0wp5 and mft-2a (for MFT repression), were considered in the analyses. Raw RNAseq data have been deposited at the National Center for Biotechnology Information (accession nos. SRRT175460–SRRT175468).

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