Dual role of tree florigen activation complex component *FD* in photoperiodic growth control and adaptive response pathways

Szymon Tylewicz[a,1], Hiroyuki Tsuji[b,1], Pál Miskolczi[a,1], Anna Petterle[a], Abdul Azeez[a], Kristoffer Jonsson[a], Ko Shimamoto[b,2], and Rishikesh P. Bhalerao[a,c,3]

[a]Umea Plant Science Center, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, S-901 87 Umea, Sweden; [b]Laboratory of Plant Molecular Genetics, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan; and [c]College of Science, King Saud University, Riyadh 11362, Saudi Arabia

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A complex consisting of evolutionarily conserved FD, FLOWERING LOCUS T (FT) proteins is a regulator of floral transition. Intriguingly, *FT* orthologs are also implicated in developmental transitions distinct from flowering, such as photoperiodic control of bulbing in onions, potato tuberization, and growth cessation in trees. However, whether an FT–FD complex participates in these transitions and, if so, its mode of action, are unknown. We identified two closely related *FD* homologs, *FD-like 1* (*FDL1*) and *FD-like 2* (*FDL2*), in the model tree hybrid aspen. Using gain of function and RNAi-suppressed *FDL1* and *FDL2* transgenic plants, we show that *FDL1* and *FDL2* have distinct functions and a complex consisting of FT and FDL1 mediates in photoperiodic control of seasonal growth. The downstream target of the FT–FD complex in photoperiodic control of growth is *Like AP1* (*LAP1*), a tree ortholog of the floral meristem identity gene *APETALA1*. Intriguingly, *FDL1* also participates in the transcriptional control of adaptive response and bud maturation pathways, independent of its interaction with FT, presumably via interaction with ABSCISIC ACID INSENSITIVE 3 (ABI3) transcription factor, a component of abscisic acid (ABA) signaling. Our data reveal that in contrast to its primary role in flowering, *FD* has dual roles in the photoperiodic control of seasonal growth and stress tolerance in trees. Thus, the functions of FT and FD have diversified during evolution, and *FD* homologs have acquired roles that are independent of their interaction with FT.

growth cessation | bud set | seasonal growth | adaptive response | hybrid aspen

The evolutionarily conserved protein FLOWERING LOCUS T (FT) plays a key role in the control of flowering in plants (1). Because FT lacks DNA binding activity, its interaction with transcription factors, such as FD (2–4) and more recently identified BRANCHED1 (BRC1) (5), is critical for the formation of protein complexes to control flowering via transcriptional control of downstream targets [e.g., floral meristem identity genes, transcription factors *APETALA1* (*AP1*) and *OsMADS1*]. Whereas the FT–FD complex promotes flowering at the shoot apical meristem (4), BRC1 appears to delay floral transition at the axillary meristem (5). These findings indicate that depending upon its interaction partner, FT-containing complexes can have distinct roles. The structure of FT–FD complex elucidated in rice has shown that a 14-3-3 protein mediates the interaction between rice FT homolog HEADING DATE 3a (Hd3a) and FD homolog OsFD1 via the C-terminally located SAP (serine alanine proline) motif in OsFD1 to generate the active nuclear localized florigen activation complex (3).

Interestingly, *FT* homologs are also involved in the control of diverse developmental transitions distinct from flowering, such as tuberization in potatoes (6), bulb formation in onions (7), stomatal opening (8), and photoperiodic control of seasonal growth in trees (9–11). These observations suggest that complexes of FT are not only important to the control of flowering but have a broader functionality. However, the mechanisms underlying the functional diversity of the FT complexes and how they can participate in the control of developmental pathways distinct from flowering are not well understood because, in contrast to FT, FD or BRC1 (or other interactors of FT), which provide DNA binding ability to the complexes of FT and thus are crucial for the function of these complexes, have not been well characterized in pathways distinct from flowering. Thus, the role of complexes of FT and their mode of action in these pathways remain poorly understood.

Cessation of growth before the onset of winter is essential for the survival of trees growing in temperate and boreal forests during subsequent periods of low temperatures (12, 13). Growth cessation is a photoperiodically controlled process (14, 15). In "short days" (SDs), when day length falls below the critical threshold allowing growth, elongation growth ceases (14–16). Moreover, the lamina of the last leaf primordia formed before the perception of SDs is aborted, and its stipules develop into bud scales that enclose the embryonic leaves inside a bud at the apex (17). Thus, bud set results in termination of the emergence of new leaves. SDs also concomitantly induce transcriptional changes resulting in the so-called "adaptive response," a metabolic shift toward the accumulation of vegetative proteins and acquisition of cold hardiness that protect the shoot apical meristem and embryonic leaves (18).

Significance

Perennial plants display seasonal cycles of growth. For example, in the trees of boreal temperate forests, growth must cease prior to the advent of winter and cold hardiness must be acquired to survive extreme low temperature. Growth cessation and activation of transcriptional programs underlying adaptive responses associated with cold hardiness are photoperiodically controlled. We show that the evolutionarily conserved protein FD implicated in the control of flowering mediates photoperiodic control of seasonal growth in trees by forming a complex with FLOWERING LOCUS T (FT) protein. *FD* genes of hybrid aspen display neofunctionalization and, in contrast to *Arabidopsis*, have evolved functions that are independent of their interaction with FT, such as transcriptional control of the adaptive response and bud maturation pathways in trees.

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[1]S.T., H.T., and P.M. contributed equally to this work.

[2]Deceased September 28, 2013.

[3]To whom correspondence should be addressed. Email: rishi.bhalerao@slu.se.

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Several studies have shown that the tree ortholog of *Arabidopsis FT* plays an important role in photoperiodic control of growth cessation (9–11). For example, down-regulation of *FT2* expression by SDs is a key early event in the induction of growth cessation (9). Importantly, functional studies have shown that overexpression of *FT2* or its paralog *FT1* in hybrid aspen (*P. tremula* × *tremuloides*) attenuates SD responses and abolishes growth cessation, whereas suppression of *FT* expression leads to earlier growth cessation than in WT plants (9–11). The MADS box transcription factor *LAP1* (*Like-APETALA1*), a tree homolog of *AP1*, has been recently identified as a target of SDs downstream of *FT* (19). Like *FT*, *LAP1* overexpression delays SD-mediated growth cessation, whereas its down-regulation causes early growth cessation in SDs (19). These findings clearly demonstrate that as in flowering, *FT* plays a central role in photoperiodic control of seasonal growth in trees.

In addition to *FT*, *FD* is a key component of the FT–FD complex, given its role in selection of downstream targets by the FT–FD complex. However, in contrast to *FT*, there are few studies addressing the role of *FD* other than in flowering (8, 20). Therefore, to improve understanding of the photoperiodic control of seasonal growth, we initiated analysis of *FD* homologs in the model tree hybrid aspen. Functional analyses in hybrid aspen of the two closely related *FD* homologs *FD-like 1* (*FDL1*) and *FD-like 1* (*FDL2*) show that they have distinct roles. We show that FDL1 interacts with FT2 to form a complex that mediates in photoperiodic control of seasonal growth. Most previously published data suggest that FD functions primarily in a complex with FT (2–4, 20). However, we show that *FDL1* has additional roles in trees, independent of its interaction with FT. Thus, the function of the FT–FD complex has diversified during evolution, and FD homologs have acquired novel roles that are independent of its interaction with FT.

Results

***FDL1* and *FDL2* Have Distinct Functions.** We cloned full-length cDNAs for two highly similar *FD*-like genes, *FDL1* and *FDL2*, from hybrid aspen, which encode 168-aa and 302-aa proteins, respectively. The larger size of FDL2 is due to an insertion at the C terminus (Fig. S1). Both FDL1 and FDL2 are highly similar to FD proteins from other plants and contain the functionally important conserved threonine (T)/SAP motif at the C terminus (20) (Fig. S1). To investigate the functions of *FDL1* and *FDL2*, we generated hybrid aspen plants overexpressing *FDL1* (FDL1oe) or *FDL2* (FDL2oe), as well as plants in which *FDL1* (FDL1RNAi) or *FDL2* (FDL2RNAi) expression was reduced (Fig. S2). In contrast to FDL1oe, FDL2oe plants were severe dwarfs when grown in long days (Fig. S3). We then investigated the function of FDL1 and FDL2 in photoperiodic control of growth cessation. Although WT plants clearly set buds, we saw no evidence of bud set in FDL1oe plants after 6 wk of SDs (Fig. 1). The FDL1oe plants eventually set buds after 10 wk of SDs, indicating that they can respond to SDs, but more slowly than WT plants (Fig. 1). The perception of SDs results in cessation of new leaf formation; thus, the number of leaves formed between initiation of SD treatment and bud set provides a sensitive measure of SD response (16, 19). Using this assay, we observed that FDL1oe plants formed more, whereas FDL1RNAi plants formed fewer, leaves than WT controls between initiation of the SD treatment and growth cessation (Fig. S4*A*). Thus, FDL1 overexpression leads to a delay, whereas FDL1 down-regulation leads to a faster SD response compared with the WT. In contrast to FDL1 transgenic plants, neither FDL2oe nor FDL2RNAi plants displayed any significant difference from WT in SD-mediated growth cessation (Fig. S4 *B* and *C*). Thus, despite high similarity, *FDL1* and *FDL2* have distinct functions, and *FDL1*, but not *FDL2*, mediates in photoperiodic control of growth.

FDL1 and FDL2 Can Interact with FT. Despite being highly similar, *FDL1*, but not *FDL2*, is involved in photoperiodic control of growth. This observation promoted us to investigate the molecular basis of the functional difference between FDL1 and FDL2. We investigated whether the difference between FDL1 and FDL2 stems from differences in their ability to interact with FT or, alternatively, whether both FDLs can interact with FT but the FDL1–FT complex differs in function from the FDL2–FT complex. To differentiate between these two possibilities, we used two approaches. First, we used bimolecular fluorescence complementation (BiFC) (21) to investigate interaction between hybrid aspen FT and FDL proteins (Fig. S5). BiFC assays indicate that YFP fluorescence is observed only when FT1 or FT2 fused to C-terminal YFP is coexpressed with FDL1 or FDL2 fused to N-terminal YFP (Fig. S5, top four rows), but not when FT1 or FT2 fused to C-terminal YFP is coexpressed with N-terminal YFP or when FDL1 or FDL2 fused to N-terminal YFP is coexpressed with C-terminal YFP controls. Thus, both hybrid aspen FT1 and FT2 could interact with FDL1 and FDL2. Second, we used a rice protoplast system developed previously, in which the activation of *OsMADs15* (a rice *AP1* homolog) expression, a downstream target of the FT–FD complex, is used as a transcriptional read-out for the ability of FT and FD to act together (3). We expressed FT1 and FT2 and FDL1 and FDL2 alone or together. As controls, we expressed Hd3a or OsFD1 alone (as a negative control) or coexpressed Hd3a and OsFD1 cDNAs (as a positive control) in rice protoplasts. Hd3a and OsFD1 coexpression activated *OsMADS15* transcription, unlike expression of Hd3a or OsFD1 alone, indicating that activation of *OsMADs15* depends on the expression of both proteins [Fig. 2, compare lane 8 with lanes 2 and 3 in agreement with previous data (3)]. When coexpressed with hybrid aspen *FT1* and *FDL1*, but not *FDL2*, activated *OsMADS15* transcription (Fig. 2, compare lane 9 with lane 10). Similarly, when cotransformed with hybrid aspen *FT2*, only *FDL1* activated *OsMADS15* (Fig. 2, compare lane 11 with lane 12). In contrast, the expression of *FT1*, *FT2*, *FDL1*, or *FDL2* alone did not lead to activation of *OsMADs15* expression. Moreover, FDL1 (but not FDL2) was also able to activate *OsMADS15* transcription when coexpressed with rice Hd3a (Fig. S6*A*). Expression of hybrid aspen *FT1*, *FT2*, *FDL1*, and *FDL2* cDNAs in the protoplasts was confirmed by RT-PCR (Fig. S6*B*). Taken together, these results show that although FDL1 and FDL2 can interact with FT1 and FT2, as demonstrated by BiFC assays, the FDL1–FT and FDL2–FT complexes differ from each other, because only FT–FDL1 can activate *OsMADS15* expression in the transcriptional read-out assays. Importantly, the differences between the FDL1–FT and

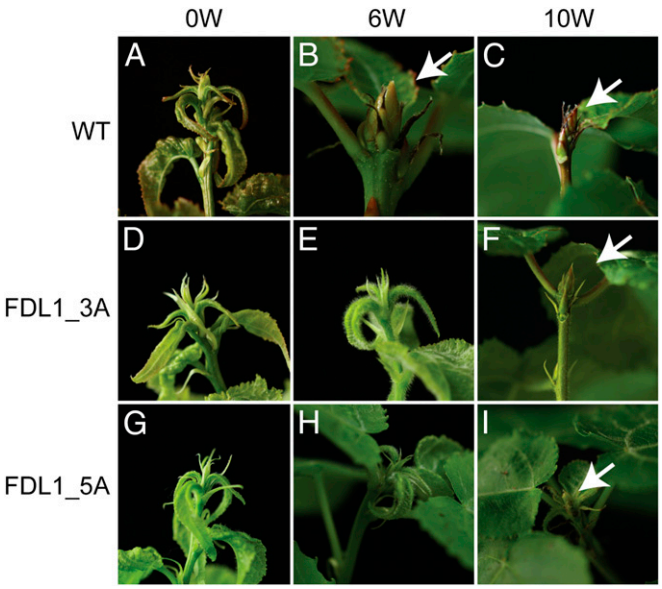

Fig. 1. Bud formation in WT and FDL1oe (lines 3A and 5A) plants. *A*, *D*, and *G* represent plants growing in long days (LD). WT plants had ceased growth and developed buds (*B* and *C*), but the FDL1oe plants had not (*E* and *H*). (*F* and *I*) FDL1oe plants set buds after 10 wk (W) of SDs. Arrows indicate apical buds.

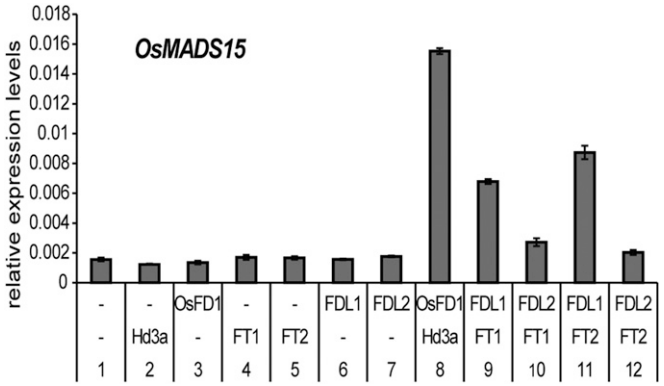

Fig. 2. Analysis of interaction between hybrid aspen FD and FT homologs. FDL1 interacts with hybrid aspen FT1 and FT2 to activate OsMADS15 expression when coexpressed in rice protoplasts. Plasmid DNA for expressing FT and FD homologs from rice or poplar was transformed into rice protoplasts, and OsMADS15 induction was assayed by quantitative RT-PCR analysis. cDNAs expressed and the OsMADS15/ubiquitin expression ratio 24 h after the transformation are shown on the *x* and *y* axes, respectively. Error bars indicate SDs of triplicate measurements.

FDL2–FT complexes suggested by the transcriptional read-out assays support the observed phenotypic differences between FDL1 and FDL2 transgenic plants, showing that FDL1, but not FDL2, mediates photoperiodic control of growth. To confirm the interaction of FDL1 with FT, we expressed hybrid aspen *FDL1* cDNA in the *Arabidopsis thaliana fd-2* mutant (Fig. S7). FDL1 expression (Fig. S7*A*) could partially suppress the late flowering phenotype of the *fd-2* mutant (Fig. S7*B*), indicating that, indeed, FDL1 can function like *Arabidopsis* FD and also supporting the hypothesis that FDL1 interaction with FT proteins is important for its function in photoperiodic control of growth.

***FDL1* Mediates in the Photoperiodic Regulation of *LAP1*.** *LAP1*, a tree *AP1* homolog, is targeted by SDs and is proposed to act downstream of FT in the photoperiodic control of growth in hybrid aspen (19). Moreover, inability of SDs to down-regulate *LAP1* expression underlies the attenuation of growth cessation responses in FT overexpressors (19). These findings prompted us to investigate the regulation of *LAP1* by SDs in FDL1oe and FDL1RNAi plants (Fig. 3). *LAP1* expression was reduced after 3 wk of SD treatment in WT apices, whereas SD-induced down-regulation of *LAP1* was severely attenuated in the FDL1oe plants (Fig. 3*A*). Conversely, there was a greater reduction in *LAP1* expression after SD treatment in the FDL1RNAi plants than in WT controls (Fig. 3*B*). Thus, like *FT*, *FDL1* clearly mediates in the photoperiodic control of *LAP1* expression.

SD Treatment Up-Regulates *FDL1* Expression. Down-regulation of *FT2* expression by SDs leads to growth cessation (9, 11), but the possibility that SDs may also down-regulate *FDL1* expression has not been tested. The observed attenuation of the growth cessation response in FDL1oe plants prompted us to investigate whether SD-induced growth cessation also involves down-regulation of *FDL1* expression in the apex. Although *FDL1* is clearly expressed in long days in the apex (Fig. 4 and Fig. S8), unexpectedly, we discovered that *FDL1* expression is not down-regulated but up-regulated after SD treatment (Fig. 4). Importantly, this up-regulation of *FDL1* expression after SD treatment was attenuated in FT1oe plants in which SD perception is defective (Fig. 4). These results indicate that SDs modulate *FDL1* expression.

***FDL1* Mediates in Transcriptional Regulation of Adaptive Response and Bud Maturation Pathways.** The up-regulation of *FDL1* expression after SD treatment suggested that it has functions in addition to its role in photoperiodic control of growth. Induction of *FDL1* after SDs coincides with bud maturation and activation of the adaptive response (18). Therefore, we tested the hypothesis that *FDL1* mediates SD-induced changes in the adaptive response and bud maturation pathways by monitoring expression of marker genes for the two pathways (18, 22, 23) in the WT and FDL1 transgenic plants. In WT plants, the expression of adaptive response markers, such as *OSMOTIN* (*OSM*) and *LATE EMBRYOGENESIS ABUNDANT* (*LEA*), was up-regulated after SD treatment, in accordance with earlier results (18) (Fig. 5*A*). Overexpression or down-regulation of *FDL1* clearly affected regulation of these markers after SDs. *OSM* and *LEA* were more strongly expressed in FDL1oe plants than in WT plants after SDs (Fig. 5*A*). In contrast, the FDL1RNAi plants displayed the opposite pattern (i.e., *OSM* and *LEA* were expressed at a lower level than in WT plants after SDs) (Fig. 5*B*).

As mentioned, SDs induce bud maturation concomitantly with the adaptive response, and accumulation of phenylpropanoids in the bud scales is a good marker for this process (18). Accordingly, expression of bud maturation markers, such as *CHALCONE SYNTHASE* (*CHS*) and *CINNAMATE ACID 4-HYDROXLASE* (*C4H*), enzymes of the phenylpropanoid pathway, was up-regulated after SDs in WT plants (Fig. 5*A*), and the buds were darker in color (Fig. 1). Further, up-regulation of these markers was reduced in the FDL1oe plants at one or more time points after SDs (Fig. 5*A*), and their buds were greener (Fig. 1), whereas the FDL1RNAi plants again exhibited opposite changes in their expression (Fig. 5*B*). Thus, FDL1 mediates in transcriptional control of the adaptive response and bud maturation pathways.

ABI3 and FDL1 Interact and Share Overlapping Targets in Adaptive Response and Bud Maturation Pathways. *FDL1* belongs to the A-group of basic leucine zipper domain (bZIP) transcription factors, which are known to interact with other transcription factors,

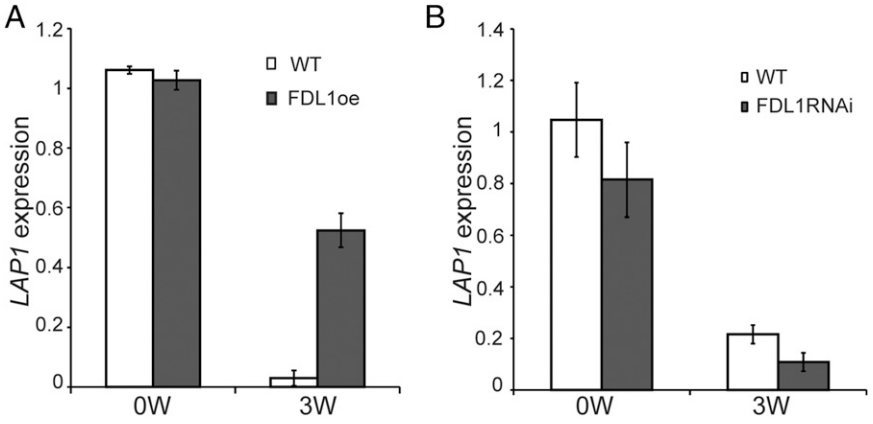

Fig. 3. FDL1 mediates in the photoperiodic control of LAP1 expression. Expression of LAP1 in the WT and FDL1oe plants (*A*) and expression of LAP1 in the WT and FDL1RNAi plants after SDs (*B*). The duration of SDs is shown (in weeks) on the *x* axis, and LAP1 expression (relative to the reference gene TIP41-like, average for three biological replicates ± SE) is shown on the *y* axis.

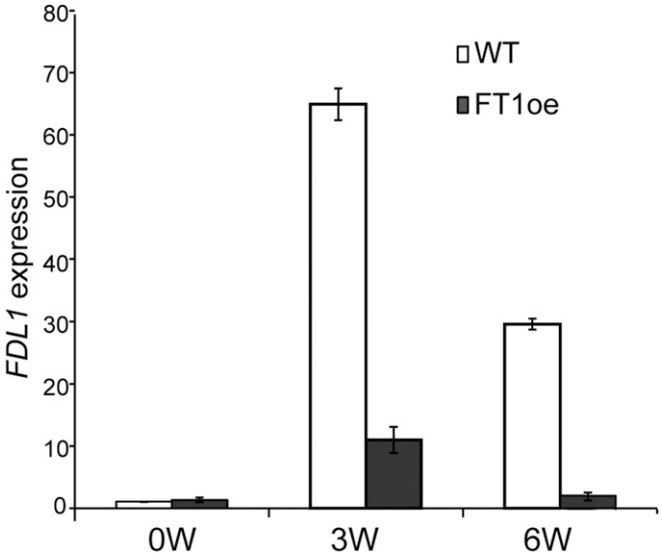

Fig. 4. Photoperiodic control of FDL1 expression. SD induction of FDL1 expression is attenuated in FT1oe plants. The duration of SDs is shown (in weeks) on the *x* axis. Induction of FDL1 expression in SDs (relative to the reference gene TIP41-like, average for three biological replicates ± SE) is plotted relative to expression in long days (0 wk SDs) on the *y* axis.

including *ABI3* (24). *ABI3* has been implicated in the transcriptional control of the adaptive response and bud maturation pathways (18). Interestingly, the phenotype of FDL1oe plants (e.g., green buds) (Fig. 1) is reminiscent of *ABI3* overexpressors (18). Therefore, we tested the hypothesis that FDL1 and ABI3 could interact and function in the same pathway. To test the interaction between FDL1 and ABI3, we coexpressed HA-tagged ABI3 and c-Myc epitope-tagged FDL1 (c-Myc–FDL1) in *Arabidopsis* protoplasts. Our data indicate that c-Myc–FDL1 can coimmunoprecipitate HA-ABI3 (Fig. 6). We then confirmed the FDL1–ABI3 interaction using BiFC assays. BiFC assays demonstrate that YFP fluorescence is observed only when ABI3 fused to C-terminal YFP is coexpressed with FDL1 fused to N-terminal YFP (Fig. S9, top row). In contrast no YFP fluorescence is observed when ABI3 fused to C-terminal YFP or FDL1 fused to C-terminal YFP is coexpressed with N-terminal or C-terminal YFP controls. Thus, the BiFC assays also demonstrate that FDL1 and ABI3 can interact. The interaction between FDL1 and ABI3, and the phenotypic similarity of FDL1 and ABI3 plants, led us to investigate whether FDL1 and ABI3 could mediate in regulation of the same set of downstream targets. After SD treatment, changes in the expression of adaptive response and bud maturation marker genes in ABI3-overexpressing (ABI3oe) plants, relative to WT responses, were similar to those changes in expression observed in the FDL1oe plants (Fig. S10) (i.e., *OSM* and *LEA* were more strongly expressed, whereas *C4H* and *CHS* were more weakly expressed). These findings suggest that *ABI3* and *FDL1* impinge on the same set of downstream target genes of the adaptive response and bud maturation pathways.

Discussion

***FDL1* Mediates in Photoperiodic Control of Growth.** Previously demonstrated functions of *FD* homologs are largely confined to the control of flowering (2–4). Delayed growth cessation in FDL1oe plants and early growth cessation in FDL1RNAi plants in SDs demonstrate that the hybrid aspen *FD* homolog *FDL1* mediates photoperiodic control of growth, a process distinct from flowering. Intriguingly, overexpression of *FDL1* delays the SD response even when *FT2* expression is highly reduced. This observation raises the possibility that *FDL1* could act independent of *FT* in photoperiodic control of growth in contrast to flowering, in which FD interaction with FT is essential (2, 4). However, several lines of evidences suggest the contrary. First, FDL1 interacts with FTs and mediates in the regulation of the same downstream target, LAP1, as FT (19). Second, FDL1RNAi plants display early growth cessation in SDs like FTRNAi plants (9). Third, FDL1 overexpression delays growth cessation in SDs (when FT expression is highly reduced) but cannot abolish the response to SDs, suggesting the dependence of FDL1

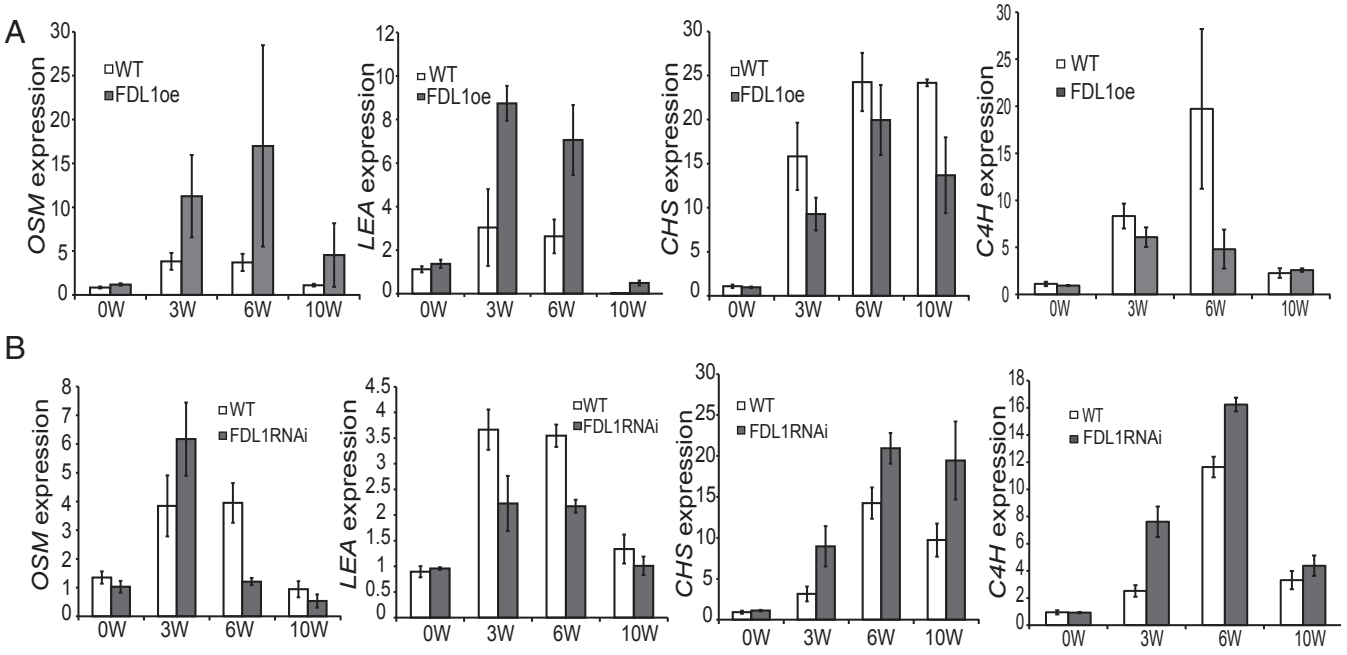

Fig. 5. FDL1 mediates in SD-controlled adaptive response and bud maturation pathways. Expression pattern of markers for adaptive response (LEA and OSM) and bud maturation (CHS and C4H) in FDL1oe (*A*; line) and FDL1RNAi (*B*; line) apices are compared with WT plants. Expression of the cited genes is shown relative to the reference gene TIP41-like on the *y* axis (average for three biological replicates ± SE), and duration of SDs (in weeks) is shown on the *x* axis.

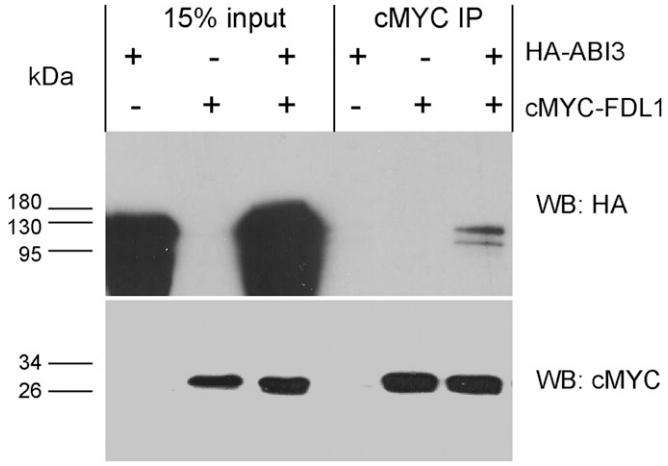

Fig. 6. Interaction of FDL1 and ABI3 proteins. c-Myc–FDL1 and HA-tagged ABI3 (HA-ABI3) were coexpressed in *Arabidopsis* protoplasts derived from cell suspension cultures. c-Myc–FDL1 was immunoprecipitated (IP) from the protein extracts using anti–c-Myc antibody, and HA-ABI3 was then assayed with anti-HA antibody by Western blot (WB). (*Lower*) c-Myc–FDL1 bound to the beads was revealed using anti–c-Myc antibody.

on FT2 in photoperiodic control of growth. Also, SDs induce *FDL1* expression in the WT, and if *FDL1* acted independent of *FT*, SDs would be unable to induce growth cessation. Additionally, FDL1 suppresses the *Arabidopsis fd-2* mutant phenotype, which would indicate that FDL1 must interact with FT like *Arabidopsis* FD. Finally, FT lacks DNA binding ability and must interact with a transcription factor to control gene expression, and all of the evidence would suggest that this transcription factor is FDL1 in photoperiodic control of growth. Therefore, we conclude that FDL1 interaction with FT mediates in photoperiodic control of growth and the delayed growth cessation in FDL1oe plants results from supraoptimal levels of FDL1 overcoming the lack of FT in SDs. Thus, the function of FD homologs is not limited to flowering control.

Functional Divergence of FDL1 and FDL2. Most plants contain multiple *FD* homologs (20), but their functions, apart from flowering, are not well characterized. FDL1 and FDL2 are highly similar to FD homologs from other plants and contain the conserved S/TAP motif necessary for 14-3-3 protein-mediated interaction with FT (3, 4). In agreement with the role of this motif in mediating interaction between FT and FD, both FDL1 and FDL2 can interact with FT1 and FT2. However, intriguingly, transcriptional read-out assays suggest that FT–FDL1 and FT–FDL2 differ from each other. These results lead us to hypothesize that the difference in FDL1 and FDL2 functions may not be due to differences between them to interact with FT. Rather, it is more likely that FDL1–FT and FDL2–FT complexes have distinct roles. Indeed, FT complexes can have distinct roles depending upon their interaction partner, as shown for FT–FD and FT–BRC1 complexes (5). Importantly, this hypothesis is supported by the differential photoperiodic responses of FDL1 and FDL2 transgenics, as well as the growth habits of these transgenics under long days. Currently, what underlies the differences between FDL1–FT and FDL2–FT complexes is not entirely clear. However, it is worth noting that the 3D structure of FDL2 could be distinct from FDL1 due to the presence of a sequence at the C terminus in FDL2 that FDL1 lacks (Fig. S1). As a result of this terminal extension, the bZIP domain of FDL2 is much longer than FDL1, which can contribute to the transcriptional differences between these two FDLs. It is not unusual for similar proteins to have distinct functions, as is already evident in the FT/TFL family (25). Nevertheless, these observations raise the possibility that despite high overall similarity and ability to interact with FT, not all members of the *FD* family or FT–FD complexes have the same functions.

Neofunctionalization of *FDL* Genes in Hybrid Aspen. Like hybrid aspen, *Arabidopsis* also has two closely related FD paralogs, both of which are involved in *FT*-mediated control of flowering (26). In contrast, neofunctionalization has occurred in the *FD* family in hybrid aspen. For example, FDL1 interacts with FT and mediates in photoperiodically controlled processes retaining the ancestral features of FD, whereas FDL2 can interact with FT but has acquired a role distinct from FDL1. Interestingly, *FD* partner *FT* has also undergone gene duplication in *Populus* and the two *FT* paralogs, like *FD*, are proposed to act in distinct processes (11). Whereas *FT1* is proposed to be involved in flowering, *FT2* mediates photoperiodic control of seasonal growth (11). However, differential function of *FT* paralogs is related to differential tissue and temporal expression patterns, with *FT1* being expressed during flowering in the apex and *FT2* during active growth in summer (11). In contrast, *FDL1* and *FDL2* have largely overlapping patterns of expression (Fig. S8); thus, neofunctionalization of *FDL2* is presumably due to structural differences resulting from insertion at the C terminus in FDL2.

Control of *LAP1* by the FT–FDL1 Module Is Involved in Photoperiodic Regulation of Growth. The targets of the FT–FD module in pathways distinct from flowering are not known as yet. Therefore, the mechanisms whereby this regulatory module mediates in bulbing or seasonal growth in trees are not well understood. *AP1* homolog *LAP1* is a target of SDs, and its down-regulation is essential for SD-mediated growth cessation (19). Our data indicate that *FDL1*, like *FT*, mediates in the photoperiodic regulation of *LAP1* (Fig. 3), suggesting that *LAP1* is a downstream target of the FT–FDL1 module in photoperiodic control of growth in hybrid aspen trees. Interestingly, the FT–FD module also acts on *AP1* in control of stomatal opening (8). Thus, the FT–FD module and its downstream target, *AP1*/*LAP1*, are conserved in the regulation of developmental pathways distinct from flowering, such as photoperiodic control of growth.

FT-Independent Role of *FDL1*. The function of FD has been explored primarily in flowering (2–4) and in photoperiodic control of seasonal growth (this study), in which FD functions together with FT. Moreover, FD homologs can also function in pathways other than flowering (e.g., stomatal opening in *Arabidopsis* and overexpression of the rice *FD* homolog *OsFD2* modulate leaf development) (8, 20). However, in contrast to these roles of *FD*, *FDL1* functions in adaptive response and bud maturation is independent of its interaction with FT, because *FT* expression is negligible after SDs. Therefore, the independence of FDL1 from interaction with FT in these SD responses contrasts with OsFD2's modulation of leaf development in rice, because control of the latter appears to involve OsFD2 interaction with the FT homolog Hd3a (20). Thus, not only can *FD* homologs function in processes other than flowering but, more importantly, some of these functions may be independent of their interaction with FT even in *FD* homologs, such as *FDL1*, that retain certain ancestral features of FD.

***FDL1* Is Involved in SD-Mediated Transcriptional Control of the Adaptive Response and Bud Maturation.** As yet, little is known about the functions of *FD* homologs that are independent of their interaction with FT. Our data demonstrate that *FDL1* participates in transcriptional control of the adaptive response pathway independent of FT. Interestingly, FDL1 transgenics share several phenotypic similarities with transgenic plants in which the expression of *ABI3*, a signaling intermediate in abscisic acid (ABA) responses, is perturbed (ref. 18 and this study). The phenotypic similarity between FDL1oe and ABI3oe plants, together with their overlapping downstream targets and coimmunoprecipitation and BiFC assays, suggests that *FDL1* and *ABI3* are components of a regulatory network underlying the SD-mediated transcriptional control of the adaptive response and bud maturation pathway. Such an interaction could also allow *FDL1*-mediated integration of photoperiodic and hormonal (ABA) signaling in control of the pathway.

***FDL1* Mediates Coordination of Growth Cessation and Other SD-Controlled Responses.** Intriguingly, *FT* has antagonistic effects on *FDL1* expression [e.g., up-regulation of *FDL1* after SDs mirrors down-regulation of *FT2*, increased expression of *FT* (as in FT-overexpressing plants) suppresses SD-mediated increase in *FDL1* expression]. To our knowledge, such an antagonistic effect of *FT* on *FD* has not been previously recorded, but it provides a possible mechanism for temporal coordination of the induction of growth cessation with concomitant transcriptional activation of the bud maturation and adaptive response pathways by the same environmental cues (SDs). In the long days, FDL1 and FT act together to promote growth. Subsequently, when down-regulating FT upon the shift to SDs, SDs may up-regulate *FDL1* expression, and in the resulting absence (or low levels) of FT, FDL1 can potentially be freed to interact with other factors (e.g., ABI3) involved in these pathways.

Conclusion

Based on our data, we present a model for the coordination of seasonal growth and adaptive response pathways by the photoperiodic signal in trees. In long days, FT interaction with FDL1 prevents growth cessation by maintaining the expression of *LAP1*. Perception of SDs promotes growth cessation by down-regulating the expression of *FT* and *LAP1*, the downstream target of the FT–FDL1 complex. Simultaneously, SDs induce *FDL1* expression, which, together with *ABI3*, activates the adaptive response pathway, thereby temporally coordinating these two processes. Hence, hybrid aspen *FDL1* has dual roles in photoperiodic control of pathways distinct from flowering, and, moreover, at least of some these functions of FDL1 are independent of its interaction with FT. Thus, *FD* homologs in hybrid aspen contrast to *Arabidopsis*, in which *FD* (and its paralog *FDP*) are both involved in the control of the same process, namely, flowering via interaction with FT (26). Although we have elucidated the role of FD, it remains to be seen whether tree homologs of BRC1 also have a role in SD-induced growth cessation or other related processes in the future. In summary, our results extend the functional repertoire of FD homologs and open new avenues to explore the function of FD in the future.

Experimental Procedures

Plant Material, Growth Conditions, and Tissue Sampling. WT hybrid aspen (*Populus tremula* × *tremuloides*, clone T89) and transgenic plants grown in soil were subjected to SDs (8-h day/16-h night, 20 °C during day/15 °C at night), and SD responses were investigated (19), as described in *SI Experimental Procedures*. Tissue samples for gene expression analyses were collected from shoot apices after 0, 3, 6, and 10 wk of SDs, frozen in liquid nitrogen, and stored at −80 °C, and RNA was isolated as described in *SI Experimental Procedures*.

Construction of Transgenic Plants and Plasmid Constructs. The cloning of *FDL1* and *FDL2* cDNAs, construction of FDL1oe and FDL2oe and FDL1RNAi and FDL2RNAi, and expression of *FDL1* cDNA in the *Arabidopsis fd-2* mutant are described in *SI Experimental Procedures*.

Transient Expression Assays in Rice Protoplasts. Transient expression in rice protoplasts was performed as described previously (3) and is described in detail in *SI Experimental Procedures*.

Transient Expression and Coimmunoprecipitation Assay in *Arabidopsis* Protoplasts. An *Arabidopsis* cell suspension culture derived from Col-0 roots was used in all experiments. Protoplast isolation and transient transfection were carried out as described (27), followed by coimmunoprecipitation performed using protein extracts from transfected protoplasts, as described in detail in *SI Experimental Procedures*.

RNA Isolation and Quantitative RT-PCR Analysis. Total RNA was extracted using an Aurum Total RNA Kit (Bio-Rad). RNA (5 μg) was treated with RNase-free TURBO DNase (Life Technologies, Ambion), and 1 μg was then utilized for cDNA synthesis using an iScript cDNA Synthesis Kit (BioRad). In all experiments, *TIP41-like* was selected by GeNorm software (Biogazelle) (28) as a reference gene. Quantitative RT-PCR experiments were conducted using LightCycler 480 SYBR Green I Master mix and a Light Cycler 480 II instrument (both supplied by Roche). The Δ-cq method was used to calculate relative expression values of genes of interest.

BiFC Assay. For the BiFC assay, full-length cDNA of FDL1, ABI3, and hybrid aspen FTs was cloned in pUC-SPYNE or pUC-SPYNE vector (21). *Arabidopsis* protoplasts were transfected as described (27), and YFP fluorescence was visualized 24 h after transfection on a Carl Zeiss LSM780 confocal microscope.

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