Long-range mobile signals mediate seasonal control of shoot growth

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In perennial plants, seasonal shifts provide cues that control adaptive growth patterns of the shoot apex. However, where these seasonal cues are sensed and communicated to the shoot apex remains unknown. We demonstrate that systemic signals from leaves play key roles in seasonal control of shoot growth in model tree hybrid aspen. Grafting experiments reveal that the tree ortholog of *Arabidopsis* flowering time regulator *FLOWERING LOCUS T* (*FT*) and the plant hormone gibberellic acid (GA) systemically convey seasonal cues to the shoot apex. GA (unlike *FT*) also acts locally in shoot apex, downstream of *FT* in seasonal growth control. At the shoot apex, antagonistic factors—*LAP1*, a target of *FT* and the *FT* antagonist *TERMINAL FLOWER 1* (*TFL1*)—act locally to promote and suppress seasonal growth, respectively. These data reveal seasonal changes perceived in leaves that are communicated to the shoot apex by systemic signals that, in concert with locally acting components, control adaptive growth patterns.

systemic signal | photoperiodic | FLOWERING LOCUS T | gibberellic acid | hybrid aspen

In perennials growing in boreal or temperate regions, low temperatures during the winter can severely damage vegetative and floral meristems. Therefore, in plants such as trees (some of which may survive thousands of winters) the shoot apical meristem (SAM) and leaf primordia are protected by cessation of growth before the onset of winter (1–4). Before the onset of winter, SAM activity is terminated, and the shoot apex undergoes a morphogenetic transition to a bud structure that encloses the SAM and arrested leaf primordia (1, 2). Several physiological studies have shown that photoperiodic shifts provide seasonal cues that play a key role in regulating the timing of these developmental transitions associated with annual growth cycle. While photoperiods longer than a critical day length (long days, LDs) are growth-permissive, the shift to photoperiods shorter than the critical day length (short days, SDs) heralding the onset of winter is growth-restrictive and induces growth cessation and bud set (3, 4). Although the role of photoperiodic signals in mediating seasonal control of growth is well established (5), it is not known where these seasonal cues are sensed and how the seasonal shifts are communicated to shoot apices to evoke the morphogenetic transitions associated with the annual growth cycle.

Molecular studies of annual growth cycles in the model tree *Populus* have identified a tree ortholog of the *Arabidopsis* flowering time regulator *FLOWERING LOCUS T* (*FT*) as the primary target of photoperiodic signals in the mediating of the control of seasonal growth (6). In *Populus*, two *FT* orthologs, *FT1* and *FT2*, have distinct expression patterns, with *FT2* being primarily expressed in the leaves. However, *FT1* and *FT2* are highly similar and functionally interchangeable as overexpression of either of them suppresses the growth cessation response to SDs. Moreover, overexpression of either of them initiates early flowering (6, 7). In LDs, *FT2* and the bZIP transcription factor *FDL1* interactively promote expression of *LAP1* (*LIKE-AP1*) (8). *LAP1* then promotes expression of *AIL1*, a tree ortholog of *AINTEGUMENTA*, which is a positive regulator of key cell-cycling genes such as genes encoding D-type cyclins (9). Shifts from LDs to SDs induce rapid down-regulation of *FT2* expression, resulting in suppression of *LAP1* and *AIL1*, leading to growth cessation.

The plant hormone gibberellin (GA) is also a target of the photoperiodic pathway in regulation of growth cessation. Rapid reductions in GA levels in apices of several plant genera, such as *Salix* and *Populus*, have been observed following exposure to SDs (10, 11). Moreover, plants overexpressing GA20 oxidase, a key GA biosynthetic enzyme, do not cease growth in response to SDs, and SD-insensitive *PHYA* overexpressors do not reduce their GA levels or cease growth in response to SDs (12, 13). Thus, in addition to *FT*, GA could also play a role in growth cessation and photoperiodic control of seasonal growth.

Intriguingly, *FT2*, the key target of seasonal cues in control of seasonal growth, is expressed exclusively in leaves and not in the shoot apex where the key developmental transitions associated with seasonal changes occur (14). Thus, the control of seasonal growth in shoot apices apparently involves an unknown systemic signaling mechanism mediated by changes in expression of *FT* in leaves. In contrast, GA metabolism-related genes are expressed in leaves as well as in shoot apices (11), but it is not known whether the GA pathway acts in the leaves or in shoot apices. Thus, identifying systemic and local signals involved in morphogenic transitions of the shoot apex, seasonal growth, and integration of the downstream signaling pathways is essential for

Significance

In perennial plants such as long-lived trees growing in boreal and temperate forest, transition from summer to winter is associated with induction of growth cessation and bud set at the shoot apex. Where in the plant these seasonal shifts are perceived and how these are communicated to the shoot apex remain unresolved. We identify leaves as a site for perception of seasonal shifts and reveal that components of floral transition such as *FLOWERING LOCUS T* (*FT*) and plant hormone GA have been recruited to function as long-range signals to communicate seasonal changes perceived in leaves to the shoot apical meristem to control its activity to synchronize bud set with the change of seasons in perennials.

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elucidating the mechanistic basis of the control of seasonal growth in trees.

Here we present evidence for leaf-mediated seasonal control of shoot growth by long-range mobile signals in the model tree hybrid aspen. We demonstrate the requirement for graft-transmissible movement of FT and its role in communicating growth-permissive conditions for shoot growth by seasonal cues perceived in leaves. Our experiments further reveal that, unlike FT, GA plays a dual signaling role, acting as both a long-range and a local mediator in seasonal control of growth of shoot apices. In contrast, *TERMINAL FLOWER 1* (*TFL*) and the *FT* target *LAP1* act locally and antagonistically in seasonal growth responses of the shoot apex.

Results

Long-Range Graft-Transmissible Signals Can Mediate Seasonal Control of Shoot Growth. To address the possibility that photoperiodic shifts providing seasonal cues are perceived in the leaves, we performed grafting experiments using southern and northern genotypes of Swedish aspen that have critical day lengths of ∼17 and ∼22 h, respectively, for short photoperiod-mediated growth cessation (15). Scions of a southern genotype of Swedish aspen were either grafted onto root stocks of a northern genotype (with ∼10 leaves) or self-grafted. To assess the effect of graft transmissible signals from root stock in grafting experiments, apices were grafted onto root stock with a similar number of leaves. The grafts were then exposed to 18-h photoperiods, which are shorter than the critical day length of the northern genotype (15). Under these conditions, growth ceased in scions of the southern genotype grafted onto root stocks of the northern genotype after 9 wk whereas scions of self-grafts of the southern genotype continued to grow under identical conditions (Fig. 1) as

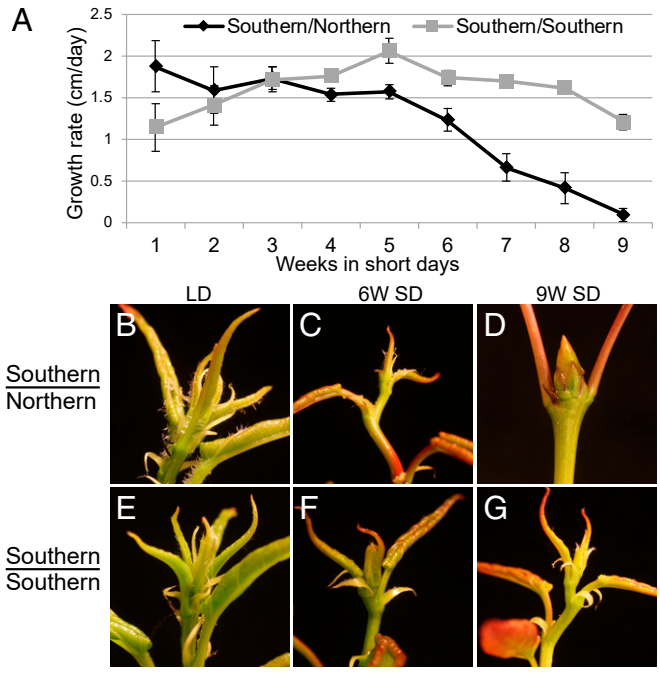

Fig. 1. Long-range graft-transmissible signals can mediate seasonal control of shoot growth. (*A*) Growth curves of the southern genotype scion on northern genotype stock (southern/northern) and the southern genotype scion grafted onto its own stock (southern/southern) after shift to short photoperiod ($n \geq 7$). Error bars indicate ±SE mean (SEM). (*B*–*G*) Pictures of representative graft apices were taken in LD and after the indicated 6 wk ("6W SD") and 9 wk ("9W SD") in short days. (*B*–*D*) Apices of grafts of the southern genotype scion on the northern genotype stock and (*E*–*G*) apices of southern/southern genotype grafts at the indicated time points. SwAsp line 5 and SwAsp line 115 were used as southern and northern genotypes, respectively.

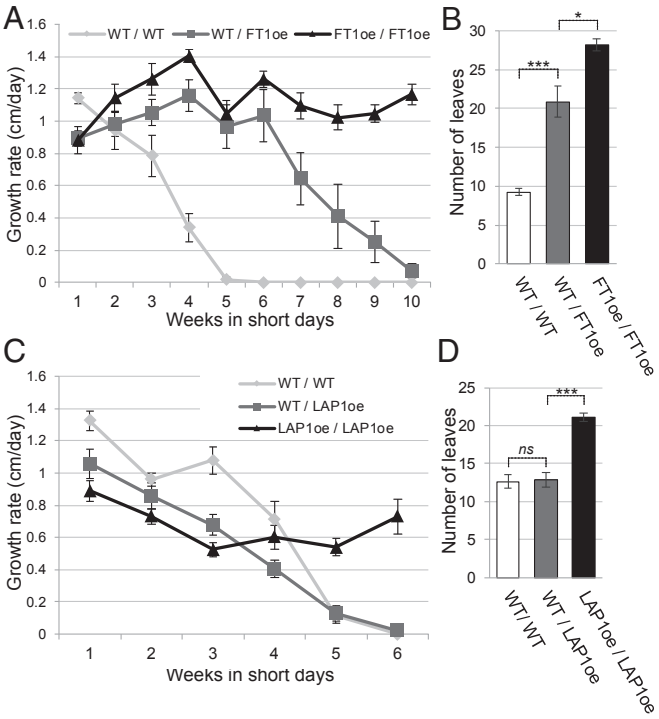

Fig. 2. Root stock-derived FT can modulate growth responses of shoot apices to photoperiodic shifts. (*A*) Growth curves of the WT and *FT1*oe graft combinations in SDs. The height of grafts was measured weekly. (*B*) Numbers of newly formed leaves after the initiation of the SD treatment in the indicated grafts of WT and *FT1*oe plants ($n \geq 7$). (*C*) Growth curves of the indicated combinations of grafts of WT and *LAP1*oe plants in 6 wk of SDs. The height of grafts was measured weekly. (*D*) Numbers of leaves formed in the generated graft combinations of WT and LAP1oe plants during 6 wk of SDs ($n \geq 9$). Error bars indicate SEM. $***P \leq 0.0001$) and $*P < 0.05$ indicate significant differences; "ns" indicates lack of significant difference using unpaired *t* test.

an 18-h photoperiod is longer than the southern genotype's critical day length. Thus, these results demonstrate that the genotype of root stocks can modulate the photoperiodic responses of scions and suggest that long-range graft-transmissible signals participate in seasonal control of shoot growth.

Root Stock-Derived FT, but Not LAP1, Can Modulate Growth Responses of Shoot Apices to Photoperiodic Shifts. SDs induce growth cessation by down-regulating *FT* expression in leaves, while overexpression of *FT* (*FT2* or *FT1*) prevents SD-induced growth cessation (6, 7, 16). *FT* expression is confined to leaves, but its downstream target *LAP1* is expressed primarily in the shoot apex and, to a lesser extent, in the leaves, as shown both here (*SI Appendix*, Fig. S1) and in previous studies (14, 16). These results prompted us to investigate whether leaf-expressed FT or LAP1 may participate in systemic control of photoperiodically controlled seasonal growth responses of shoot apices. For this, we grafted wild-type (WT) scions on WT (control), *FT*-overexpressing (*FT1*oe), and *FT* target *LAP1*-overexpressing (*LAP1*oe) root stocks and then assessed shoot growth of the grafts after exposure to SDs. Apices of scions grafted onto *FT*oe root stocks ceased growth in response to SDs significantly later than counterparts grafted on WT (control) (Fig. 2*A*) or *LAP1*oe root stocks (Fig. 2*C*). In accordance with the delayed growth cessation, WT shoots grafted onto *FT1*oe stocks also produced approximately two times more leaves than counterparts grafted on WT control stocks by the end of the experiment (Fig. 2*B*). In contrast, WT shoots grafted onto *LAP1*oe stocks produced similar numbers of leaves to WT control grafts in SDs (Fig. 2*D*). These results indicate

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that expression of *FT*, but not of *LAP1*, in the leaves can systemically mediate photoperiodic control of shoot growth.

FT Protein, but Not *FT* Transcripts, Is Graft-Transmissible. The apparent systemic effect of root stock-derived *FT* on the growth responses of shoot apices to photoperiodic shifts prompted us to investigate the mobility and graft-transmissibility of FT protein and *FT* transcripts. To be able to distinguish the graft transmissibility of FT protein derived from root stock, we generated transgenic hybrid aspen plants expressing FT protein fused with green fluorescent protein (GFP) and HA tags (FT-GFP-HA) (*SI Appendix*, Fig. S2). Whereas the GFP tag allows the microscopic localization of the fusion protein, the HA tag provides for a highly sensitive immunodetection of the resulting FT fusion protein. Subsequently, we grafted WT scions on rootstocks of these transgenic hybrid aspen plants expressing FT protein fused with the GFP-HA tag (*FT-GFP-HA*oe). The following analyses of the extracted protein and RNA samples showed that we could detect FT-GFP-HA protein (Fig. 3*A*), but not *FT-GFP* transcripts (*SI Appendix*, Fig. S3*A*), in the scions. In contrast, we detected no GFP protein in WT scions grafted onto unfused *GFP*-expressing root stocks (*SI Appendix*, Fig. S3*B*). Thus, movement of FT-GFP across the grafts is not a result of FT fusion with GFP. Taken together, these results demonstrate that FT protein, but not *FT* transcripts, is graft-transmissible.

Blockage of FT Mobility Prevents Its Mediation of Growth Responses in Shoot Apices. To investigate the requirement of graft transmissibility of FT protein for photoperiodic control of growth, we generated transgenic hybrid aspen plants expressing a FT fusion protein (NUC-FT-GFP-HA) that carries a nuclear localization signal (in addition to the GFP tag used for visualization). As a result, the NUC-FT-GFP-HA fusion protein was targeted to the nucleus, thereby trapping it within the cells and preventing its graft transmissibility. Then we confirmed whether this mobility-restricted FT (NUC-FT-GFP-HA) could function like WT FT, if expressed ectopically. Indeed, transgenic hybrid aspen plants, ectopically expressing *NUC-FT-GFP*, did not cease growth in SDs, as previously described for WT *FT1* and *FT2* overexpressors (6, 7) (*SI Appendix*, Fig. S4 *A* and *B*). Next, we confirmed that NUC-FT-GFP was targeted to the nucleus (*SI Appendix*, Fig. S5*A*) and not graft-transmissible (*SI Appendix*, Fig. S5*B*). We then grafted WT scions on WT or *NUC-FT-GFP*–expressing root stocks and (as controls) *NUC-FT-GFP* scions on *NUC-FT-GFP* root stocks and monitored growth responses of the shoot apices of the scions to SDs. In contrast to the effects of WT *FT*oe root stocks described before (Fig. 2 *A* and *B*), SD-induced growth cessation was not delayed in apices of the WT scions grafted onto root stocks expressing the nuclear-targeted, non–graft-transmissible NUC-FT-GFP (Fig. 3*B*). Moreover, growth cessation timing and numbers of leaves produced after SD exposure in the grafted scions did not significantly differ from those of WT self-graft controls (Fig. 3*C*). In contrast, self-grafted *NUC-FT-GFP*–expressing scions did not cease growth in response to identical SDs (Fig. 3*B*). Thus, graft-transmissible mobility is essential for FT to mediate in photoperiodically controlled growth of the shoot apex.

GA (Like *FT*) Can Systemically Modulate Photoperiodic Responses of the Shoot Apex. The plant hormone GA putatively acts in a parallel pathway to the *CO*/*FT* pathway in photoperiodic control of seasonal growth (13). However, unlike *FT*, GA is synthesized in leaves as well as in apices, and key enzymes like GA20 oxidase are expressed in both organs, as found both here (Fig. 4*A*) and previously (11). Thus, it remains unclear whether GA biosynthesis in the leaves can mediate photoperiodic responses in the shoot apex, as demonstrated for *FT*. To address this possibility, we grafted WT scions on root stocks of *GA20 oxidase*-overexpressing (*GA20ox1oe*) plants, which have high levels of GA and vice versa. The WT scions on *GA20ox1*oe root stocks ceased growth in response to SDs significantly later than those on WT root stocks (Fig. 4*B*).

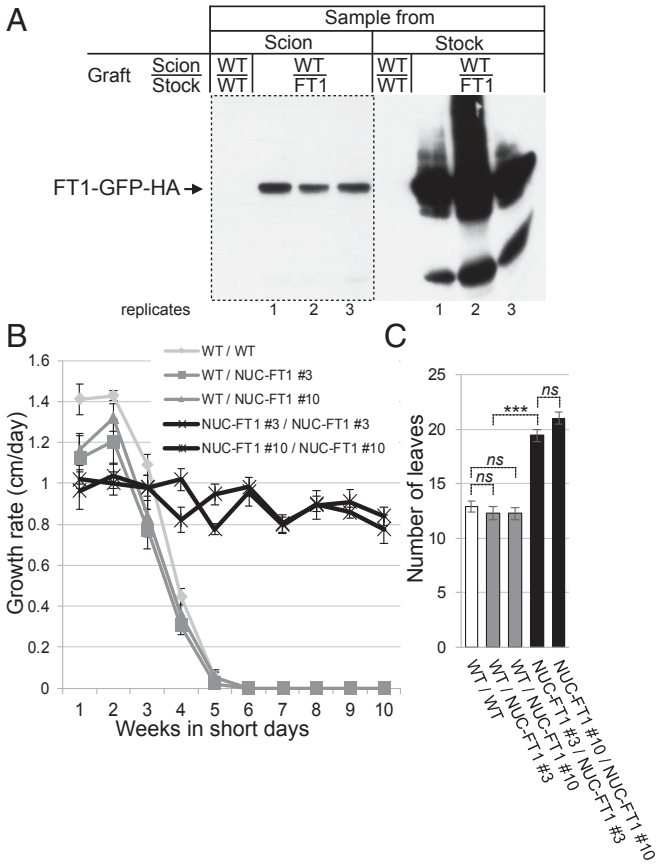

Fig. 3. FT protein is graft-transmissible while the blockage of FT protein mobility prevents its mediation of growth responses. (*A*) Western blot detected FT1-GFP-HA from stem samples of grafts of WT scion and the *FT1-GFP-HA*oe stock. Samples taken from the scion of WT grafted on itself were used as control. A longer exposition is shown in the dashed-line square for the membrane part of scion samples. Three biological replicates are indicated by numbers. (*B*) Growth curves of the grafts of WT scions on the stocks overexpressing the nuclear targeted *FT1* (*NUC-FT1*) (line #3 and line #10), the self-grafts of WT, and the self-grafts of the *NUC-FT1*–overexpressing lines. After shifting the grafts to short days, the height of the grafts was measured weekly for 10 wk. (*C*) The number of newly formed leaves after the initiation of the SD treatment is plotted for the generated grafts of WT and *NUC-FT1*oe plants (as indicated scion/stock) ($n \geq 7$). Error bars indicate SEM. $^{***}P \leq 0.0001$ indicates significant differences; "ns" indicates lack of significant difference between the indicated graft combinations using unpaired *t* test.

***FT* and *LAP1* Mediate in Transcriptional Regulation of the GA Metabolic Pathway.** GA levels are photoperiodically regulated in the apex, being down-regulated upon exposure to SDs. Since *FT* mediates in photoperiodic control of growth, we investigated if *FT* could also mediate in the photoperiodic regulation of GA levels. We also investigated if FT mediates in transcriptional regulation of GA metabolic pathway. As can be seen, photoperiodic control of several key GA metabolism-related genes is affected in *FT* overexpressors (Fig. 5). Since *LAP1* is a target of *FT*, we also checked if *LAP1* was involved in transcriptional regulation of the GA metabolic pathway like *FT*. As can be seen, like *FT*, photoperiodic control of key GA metabolism-related genes is affected in *LAP1* transgenics (*SI Appendix*, Fig. S6). Collectively, these results suggest that *FT* and its downstream target *LAP1* can mediate in transcriptional control of GA metabolism at the shoot apex by photoperiodic signal. Since systemic effect of *FT* in photoperiodic control of growth is suppressed by blocking its mobility, the effect of *FT* on the GA pathway is relevant at the apex, and thus GA could also act locally at the apex in seasonal control of growth.

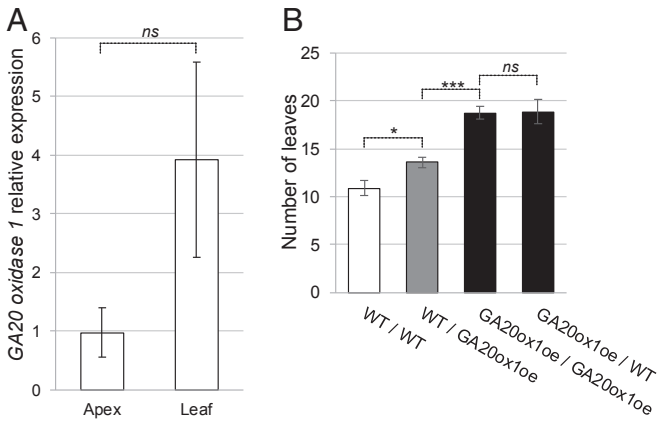

Fig. 4. GA can systemically modulate photoperiodic response of the shoot apex. (*A*) Relative expression of *GA20 oxidase 1* in the apex and leaf of hybrid aspen. The expression values are relative to the reference gene *UBQ* and the average of three biological replicates. (*B*) Numbers of newly formed leaves after the initiation of short days in the indicated graft combinations of WT and the GA20 oxidase 1 overexpressing plants ($n \geq 7$). Error bars indicate SEM. $*P < 0.05$ indicates significant difference from the corresponding controls; $***P \leq 0.0001$ indicates significant differences; and "ns" indicates lack of significant difference using unpaired *t* test.

***TFL* Acts Locally and Antagonistically to *FT* at Apices in Seasonal Control of Growth.** *TFL*, an antagonist of *FT*, has not been as intensively studied as *FT*. However, our experiments (Fig. 6*A*) and previous findings by Mohamed et al. (17) show that, in contrast to FT, it is predominantly expressed in the apex. Moreover, its ectopic overexpression and down-regulation, respectively, accelerate and delay growth cessation in response to SDs (*SI Appendix*, Fig. S7). Therefore, we investigated whether increasing *TFL* expression in the apex is sufficient to mediate its effect on the photoperiodic responses of the apices. For this, we grafted *TFL*-overexpressing (*TFL*oe) shoots onto WT stocks and exposed them to SDs. *TFL*oe scions ceased growth significantly earlier than WT self-graft controls (Fig. 6*B*). In contrast, growth cessation timing of WT scions grafted onto *TFL*oe root stocks (unlike that observed for *FT* grafts) or WT self-grafts did not significantly differ under identical conditions. Thus, unlike *FT*, *TFL* acts locally in the shoot apex in mediating seasonal growth.

Discussion

Proper temporal regulation of adaptive responses like autumnal growth cessation in the shoot apex is crucial for the survival of perennial plants in boreal and temperate ecosystems. Temporal regulation of these growth responses in the shoot apex relies on a highly sophisticated mechanism involving transduction of seasonal cues to cellular machinery controlling the activity of the shoot apical meristem. Sites of perception of seasonal cues, and the mechanism of their transduction to the shoot apex, were previously uncertain. Here we show that both systemic signals conveying seasonal shifts perceived in the leaves and agents acting locally in the shoot apex participate, in concert, in control of seasonal activity of the SAM in the model tree hybrid aspen.

Previous studies have identified the *CO*/*FT* module and plant hormone GA as two key mediators in seasonal control of the hybrid aspen's annual growth cycles (6, 13, 18). However, *FT2* is exclusively expressed in the leaves whereas GA biosynthesis-related genes are expressed in both leaves and shoot apices (11, 14). Thus, where seasonal shifts are sensed and how they are conveyed to the shoot apex has not been understood. Therefore, to clarify whether perception of the seasonal cues occurs only in leaves or in both leaves and shoot apices, we used Swasp genotypes from northern and southern Sweden that have distinct responses to seasonal cues, even when grown at the same geographical location (15). Scions of the southern genotype grafted onto root stocks of the northern genotype (which ceases growth earlier than the southern genotype) ceased growth significantly earlier than self-grafted scions when exposed to SDs (Fig. 1). These results clearly suggest that distant organs, e.g., leaves, participate in communication of seasonal cues in the control of shoot growth.

The grafting results suggested that systemically acting signals are conveyed to the shoot apex to mediate seasonal control of growth. This mechanism could involve either a reduction in production of a growth-promotive signal in distant organs following perception of growth-restrictive SDs resulting in cessation of growth in the shoot apex and bud set. Alternatively, an inhibitory signal may be produced in organs, such as leaves, that triggers growth cessation in the apex upon perception of SDs. There is little evidence for the production of any growth inhibitory signal in SDs so far, except indications presented by Eagles and Wareing (19) that a leaf-derived agent, probably ABA, may induce dormancy. However, growth cessation responses of abi1-1–overexpressing hybrid aspen plants with impaired responses to ABA are similar to those of WT plants (20). Thus, the first hypothesis seems likeliest. An obvious candidate for a putative growth-promoting signaling agent is the tree ortholog of *FT*, which, as shown here, is expressed exclusively in leaves and has a well-established role in seasonal control of growth in trees. Our demonstration that growth cessation is delayed in WT scions grafted onto *FT1*oe root stocks (Fig. 2 *A* and *B*) supports the participation of *FT* or *FT*-derived signals in systemic communication of seasonal cues to shoot apices in control of their growth cycles.

Although the results of grafting experiments indicate systemic signaling, presumably mediated by *FT*, there is surprisingly little evidence for mobility of *FT* in trees, unlike in other plants. In fact, previous evidence suggests that FT is not mobile, since grafting WT scions on *FT*-overexpressing root stocks does not lead to precocious flowering in contrast to ectopic expression of *FT* in poplar (21). Moreover, *LAP1*, a target of *FT*, and GA, which also participates in seasonal control of shoot growth, are produced in leaves and thus could systemically mediate *FT*'s effects. Therefore, we investigated whether *FT* itself is mobile or acts via *LAP1* (or other factors), and, if FT is mobile, whether its mobility is essential for *FT*-mediated seasonal regulation of shoot growth. Our results demonstrate that expression of *FT1* (used as a proxy for the nearly identical ortholog *FT2*), but not its downstream target *LAP1*, in root stocks can significantly delay SD-induced growth cessation in the shoot apex (Fig. 2).

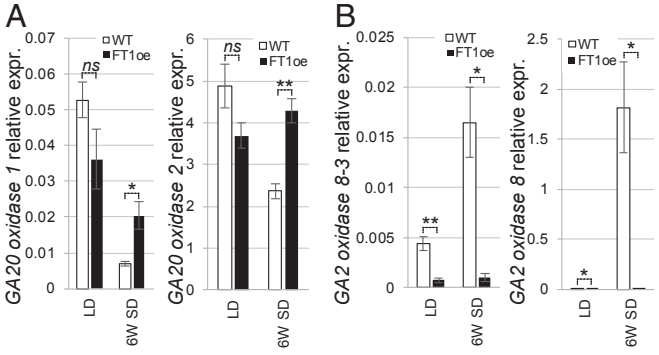

Fig. 5. *FT* mediates regulation of the GA metabolic pathway. (*A*) Relative expression of genes encoding GA20 oxidases (*GA20 oxidase 1* and *2*) that are GA biosynthesis enzymes and (*B*) the homolog genes of GA2 oxidases (*GA2 oxidase 8–3* and *8*) that encode GA deactivation enzymes in the apex of WT and *FT1*oe plants in LD and after 6 wk of SDs ("6W SD"). The expression values shown are relative to the *UBQ* reference gene and averages of three biological replicates. Error bars indicate SEM. $**P < 0.005$ and $*P < 0.05$ indicate significant differences and "ns" indicates lack of significant difference using unpaired *t* test.

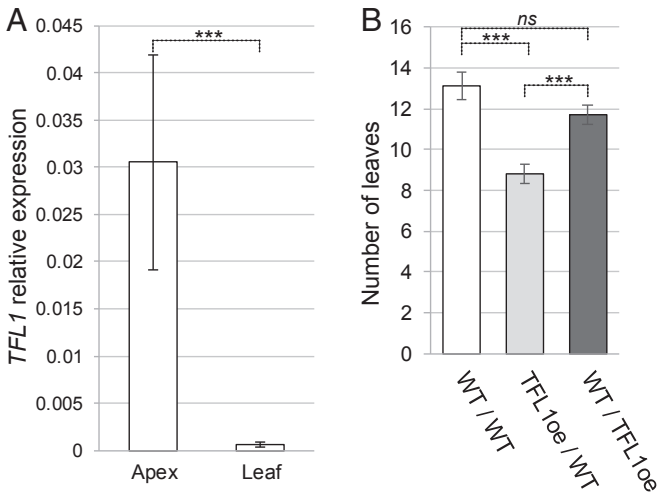

Fig. 6. *TFL1* acts locally at the apex in photoperiodic response. (*A*) Relative expression of *TFL1* in the apex and leaf of hybrid aspen. The expression values shown are relative to the reference gene *UBQ* and averages of three biological replicates ±SEM. (*B*) Numbers of newly formed leaves after the initiation of the SDs in the indicated graft combinations of WT and *TFL1* overexpressing plants ($n \geq 10$). Error bars indicate SEM. ***$P < 0.001$ indicates significant and "ns" indicates lack of significant difference using unpaired *t* test.

Furthermore, FT displayed graft-transmissible mobility (Fig. 3*A*), and its effect on shoot growth could be abolished by targeting FT to the nucleus (Fig. 3 *B* and *C*), thereby blocking its graft-transmissibility from root stocks. Thus, the effects of root stocks on shoot apices that we observed are directly mediated by FT, rather than *FT*-induced production of *LAP1* or other factors followed by their movement or induction of production of another mobile intermediate.

Like *FT*, the GA pathway has been implicated in control of seasonal growth in trees. In GA overexpressors, *FT* can be down-regulated in response to SDs, suggesting that GA either acts in a parallel pathway or downstream of the *FT* (13). GA has demonstrated mobility in *Arabidopsis* (22), so if *FT* can induce its production, GA could potentially move to shoot apices from root stocks. Our grafting data indicate that *GA20 oxidase*-overexpressing root stocks can indeed delay growth cessation in WT shoots following shifts to growth-restrictive SDs (Fig. 4*B*). Furthermore, it has been shown that GA levels and expression of *GA20 oxidase* is down-regulated after SDs in the leaves (11). Altogether, these results support the possibility that GAs may act as systemic signals in control of seasonal growth, either independently or downstream of *FT*. In agreement, however, since mobility of FT is essential for its systemic mediation of seasonal growth responses, GAs are unlikely to act as mediators of *FT* in this process. Thus, GA can act systemically but independently of *FT*. However, GA is considerably less effective than FT in modulating growth responses of shoots when provided from root stocks, in contrast with increasing GA levels at the apex as in *GA20 oxidase* overexpressors (13). Moreover, GA biosynthesis can occur in leaves and shoot apices (11) (unlike *FT*, which is exclusively expressed in leaves), so there is not apparently an absolute requirement for systemic control of shoot growth by GA. Moreover, both *FT* and its target *LAP1* can participate in transcriptional control of the GA pathway at the apex (Fig. 5 and *SI Appendix*, Fig. S6). Therefore, while we cannot exclude the possibility that GA may act as a systemic signal, we favor the hypothesis that FT is the predominant systemic signaling agent in seasonal control of shoot growth in hybrid aspen and that GA could act locally in the apex, presumably downstream of *FT* and *LAP1*.

At the shoot apex, *LAP1*, which promotes growth in LDs, appears to be a key local mediator downstream of the systemically transduced signals from leaves (16). However, our results show that *TFL1* also mediates growth responses, acting locally in the shoot apex (Fig. 6*B*). Moreover, as in flowering (23), the *Populus TFL1* homolog appears to play an antagonistic role to *FT* in seasonal control of growth in trees. *TFL1* overexpression induces early growth cessation, whereas its down-regulation delays growth cessation (*SI Appendix*, Fig. S7). However, there is a major difference between *TFL1*-mediated control of flowering in *Arabidopsis* and seasonal growth control in trees. While *TFL1* (like *FT*) also displays mobility in *Arabidopsis* (24), our data indicate that in hybrid aspen it acts locally (unlike *FT*) in the apex in seasonal control of growth. Thus, *LAP1* and *TFL1* are antagonistically acting local mediators of environmental cues regulating seasonal growth in shoot apices.

Our results suggest the following model for the control of seasonally synchronized growth transitions, involving both long-range and local signaling components (*SI Appendix*, Fig. S8). Signals of seasonal changes perceived in the leaves are conveyed systemically by FT to the shoot apex where TFL1 and LAP1 act locally in the coordination of anticipatory growth responses. We propose that, unlike *FT*, GA has a dual role, participating not only systemically but also locally in the shoot apex. During summer, under growth-permissive LDs, FT, presumably by interacting with FDL1 (8), directly binds the *LAP1* promoter in shoot apices (*SI Appendix*, Fig. S9) to positively regulate *LAP1* expression. Furthermore, *FT* participates in transcriptional control of the growth-promotive GA pathway either via *LAP1* or independently of *LAP1*. Positive regulation of *LAP1*, which positively regulates cell-cycle–related genes via *AIL* transcription factors and that of the GA pathway (9, 16), results in promotion of growth during summer. Transition to winter, as day length becomes shorter, results in suppression of *FT* expression (and the GA pathway), thereby switching off long-range growth-promotive signaling to the apex. Consequently, the FT/TFL ratio falls, and *LAP1* is down-regulated in the apex. Additionally, the suppression of *FT* and its target *LAP1* results in GA down-regulation in the apex, thereby reinforcing the switch to growth repression, inducing the growth cessation program culminating in morphogenetic transformation of the shoot apex to a bud structure. The selective pressures that resulted in shoot apices' seasonal growth dependence on leaf-derived signals are unclear, but may be linked to a general growth regulation mechanism that enables coupling of shoot growth, leaf production, and metabolic status with seasonal cues.

Anticipating the change of seasons and modulating development accordingly is central to adaptation and thus survival in plants. The induction of flowering by vernalization has provided a paradigm for the control of a key developmental transition by seasonal cues (25). Vernalization in *Arabidopsis* acts via repression of the floral repressor *FLC* (26) by chromatin remodeling whereas our results now demonstrate the role of long-range signals and systemic signaling in seasonal control of growth cycles that define perennial habit and provide evidence strongly implicating *FT* and GA as systemic mediators of seasonal shifts. Previously, there had been scant evidence of FT movement in trees. Thus, its potential role in long-range systemic signaling in seasonal control of the growth cycles of perennials has not been explored. However, *FT* homologs have been implicated in various developmental transitions, inter alia flowering, tuber induction, and bulbing (27–29). In each of these cases, an inductive environmental signal activates expression of *FT* homologs, which are then transported to the site of activation of the transitional process. In trees, *FT* is required for maintenance of vegetative growth, and, in contrast with examples outlined above, suppression of *FT* induces the transition to growth cessation (6). Thus, while *FT* may regulate developmental transitions that are highly distinct—e.g., flowering, bulbing, tuberization, or growth control—its movement appears to be a key evolutionarily conserved feature of systemic control of developmental transitions in plants. In summary, our studies have addressed two key questions: where the signals that herald seasonal shifts are perceived and how the

perception of these seasonal shifts is communicated to the shoot apex to control seasonal growth by demonstrating the role of long-range mobile signals and providing evidence strongly supporting *FT* and GA as mobile mediators in control of seasonal growth transitions.

Materials and Methods

Plant Material and Growth Conditions. Hybrid aspen (*Populus tremula x Populus tremuloides*) clone T89 (WT) and the transgenic plants grown in soil for 5 wk were subjected to SD (8 h, 20 °C light/16 h, 15 °C dark cycles) for growth cessation analysis as detailed in *SI Appendix, Supplementary Materials and Methods*. Aspen plants of SwAsp line 5 and line 115 from southern and northern regions of Sweden (15), respectively, were grown in the greenhouse for 5 wk (23 h light, 20 °C, and 1 h dark, 80% relative humidity) before grafting them.

Grafting Experiments. Soil-grown plants were grown in the greenhouse (18 h light, 22 °C, and 60% relative humidity), and then scions were grafted onto root stocks that had about 10 developed leaves, as previously reported (30). After 2 wk in LDs, the growing grafts were transferred to the SD chamber (8 h, 20 °C light/16 h, 15 °C dark cycles, 80% relative humidity) and monitored for growth cessation. The grafts of SwAsp lines were treated with 18-h light and 6-h dark cycles in SDs as described in detail in *SI Appendix, Supplementary Materials and Methods*.

RNA Isolation and Quantitative Real-Time PCR Analysis. Total RNA extracted using the Spectrum Plant Total RNA Kit (Sigma-Aldrich) was used for quantitative real-time PCR analyses as described in *SI Appendix, Supplementary Materials and Methods*. Relative expression values were calculated by using the d-ct-method (31). The complete list of primers used in real-time PCR analysis is presented in *SI Appendix*, Table S1.

Generation of Plasmid Constructs and Plant Transformation. The generation of the *FT1-GFP-HA* construct has been described earlier (16). For nuclear targeting of *FT1,* nuclear localization signal sequence was inserted in the front of the N-terminal of *FT1-GFP-HA* sequence, and hybrid aspen were transformed as described in detail in *SI Appendix, Supplementary Materials and Methods*. The generation of the other transformant lines used in the experiments has been previously described: *FT*RNAi (6), control *GFP*oe (32), *GA20oxidase1*oe (18), and *TFL1*oe (17).

Western Blot Analysis. Western blot analysis was performed on total extracts isolated from leaves of untransformed control and independent transformed lines to detect the FT-GFP-HA and NLS-FT-GPF-HA protein levels using anti–HA-peroxidase antibody (3F10; Roche). To detect the FT-GFP-HA, protein levels from the scion and stock stem segments of grafts were taken 5 cm below and above the joints, and the presence of HA-tagged protein was detected by Western blot using anti-HA-peroxidase–conjugated antibody after GFP-Trap precipitation as detailed in *SI Appendix, Supplementary Materials and Methods*.

Chromatin Immunoprecipitation. Chromatin immunoprecipitation assays were carried out generally as previously described by Gendrel et al. (33) with further details and modifications described in *SI Appendix, Supplementary Materials and Methods*.

Confocal Microscopy. Fluorescence was visualized by confocal laser-scanning microscopy using a Carl Zeiss LSM780 confocal microscope as detailed in *SI Appendix, Supplementary Materials and Methods*.

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