FLOWERING LOCUS T duplication coordinates reproductive and vegetative growth in perennial poplar

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Annual plants grow vegetatively at early developmental stages and then transition to the reproductive stage, followed by senescence in the same year. In contrast, after successive years of vegetative growth at early ages, woody perennial shoot meristems begin repeated transitions between vegetative and reproductive growth at sexual maturity. However, it is unknown how these repeated transitions occur without a developmental conflict between vegetative and reproductive growth. We report that functionally diverged paralogs *FLOWERING LOCUS T1* (*FT1*) and *FLOWERING LOCUS T2* (*FT2*), products of whole-genome duplication and homologs of *Arabidopsis thaliana* gene *FLOWERING LOCUS T* (*FT*), coordinate the repeated cycles of vegetative and reproductive growth in woody perennial poplar (*Populus* spp.). Our manipulative physiological and genetic experiments coupled with field studies, expression profiling, and network analysis reveal that reproductive onset is determined by *FT1* in response to winter temperatures, whereas vegetative growth and inhibition of bud set are promoted by *FT2* in response to warm temperatures and long days in the growing season. The basis for functional differentiation between *FT1* and *FT2* appears to be expression pattern shifts, changes in proteins, and divergence in gene regulatory networks. Thus, temporal separation of reproductive onset and vegetative growth into different seasons via *FT1* and *FT2* provides seasonality and demonstrates the evolution of a complex perennial adaptive trait after genome duplication.

perennialism | tree | dormancy | gene duplication | signaling

Life cycles of higher plants display a great diversity in morphological and seasonal adaptation. Annual plants grow, reproduce, and senesce within a growing season, whereas woody perennials display successive years of vegetative growth before reaching sexual maturity (1–3). After this time, shoot meristems begin cyclical transitions between vegetative and reproductive growth. Consequently, shoots may repeatedly form early vegetative buds (Vegetative Zone I), reproductive buds (Floral Zone), and late vegetative buds (Vegetative Zone II) in a sequential manner (3). However, our understanding of the mechanisms underlying such complex phenotypes, and thus variation in growth habits and adaptation, remain rudimentary. In the herbaceous perennial *Arabis alpina*, repeated transcriptional repression and activation of *PERPETUAL FLOWERING 1* (*PEP1*), an ortholog of the floral repressor *FLOWERING LOCUS C* (*FLC*) in annual *Arabidopsis thaliana* (4), controls recurring seasonal transitions between reproductive and vegetative phases (5). However, a true functional ortholog of *FLC* has not been reported in trees, nor does phylogenetic analysis point to a clear structural ortholog of *FLC* in poplar (*Populus* spp.) (6).

Previous results showed that *FLOWERING LOCUS T1* (*FT1*) (7) and *FLOWERING LOCUS T2* (*FT2*) (8) under the cauliflower mosaic virus 35S (CaMV 35S) constitutive overexpression promoter induce early flowering in poplar. Transcript abundance of both genes gradually increases in the growing season as poplar trees mature. These findings imply that *FT1* and *FT2* redundantly control the transition from juvenile to reproductive stage during the growing season. Moreover, short-day–induced growth cessation and bud set are attributed to the *FT1*/*CONSTANS 2* regulon in poplar (7). *FT1* and *FT2*, products of a whole-genome salicoid duplication event (9), are located on paralogous chromosomes VIII and X, respectively (Fig. S1*A*). *FT1* and *FT2* are homologs of paralogous *FLOWERING LOCUS T* (*FT*) and *TWIN SISTER OF FT* (*TSF*) (Fig. S1*B*). The onset of reproduction in *Arabidopsis* is induced redundantly by *FT* (10, 11) and *TSF* (12) under warm-temperature and long-day conditions. No other functions of *FT* or *TSF* have been reported. Through elucidating the detailed roles of *FT1* and *FT2* in reproductive and vegetative growth, we report a mechanism indicating that cycles of reproductive and vegetative growth in perennial poplar are coordinated by the transient expression of the functionally diverged paralogs *FT1* and *FT2* in contrasting seasons.

Results

***FT1* and *FT2* Diverged in Regulation.** To identify normal temporal and spatial expression of *FT1* and *FT2*, we first designed and tested gene-specific primers (Fig. S2 *A* and *B*). We then conducted year-round transcript analyses of *FT1* and *FT2* in the same tissues using normally growing mature *Populus deltoides*. In all five tissues analyzed, *FT1* transcripts were abundant only in winter (dormant season) when day length was the shortest (<12 h) and mean monthly low and high temperatures were <6 °C and <15 °C, respectively (Fig. 1 *A* and *B* and Fig. S2*C*). Conversely, *FT2* transcripts were abundant only in leaves and reproductive buds in the growing season when day length was >12 h and mean monthly low and high temperatures were >10 °C and >25 °C, respectively (Fig. 1 *A* and *C*). After abundant expression in spring, *FT2* continued

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to be expressed at lower levels in the same tissues until mid-fall, when day length became shorter (<12 h), and air temperature began dropping. These findings show that *FT1* transcripts were abundant in all tissues analyzed when the days were short and temperatures were cold, whereas *FT2* transcripts were abundant in leaves and developing reproductive buds when days were long and temperatures were warm. Similarly, in leaves of two other poplars (*Populus trichocarpa* and *Populus tremula* × *Populus tremuloides*), *FT1* transcripts were abundant in February, whereas *FT2* was abundant in May, suggesting similar regulation of *FT1* and *FT2* in different poplar taxa (Fig. S2*D*). These results suggest that transcription of *FT1* and *FT2* is temporally and spatially separated.

We then tested whether temperature, day length, and internal factors regulate *FT1* and *FT2* transcription in mature *P. deltoides*. Trees in the field were allowed to set terminal buds normally in late summer/early fall under short-day conditions. Then, in November, one group of dormant trees was moved to either warm (25 °C) or cold (4 °C) temperature under short-day conditions (8 h light) for 161 d. *FT1* transcription began to increase in preformed leaves enclosed in vegetative buds within 45 d at 4 °C but was undetectable at 25 °C throughout the experimental period (Fig. 2*A*). When some trees were transferred to 25 °C after 90 d at 4 °C, *FT1* transcription diminished rapidly, resembling the decline in normal *FT1* transcription from winter to spring (Fig. 1*B*). *FT2* transcripts were undetectable in the identical tissues in these experiments. The treatment of a second group of normally dormant trees in winter (November–March) showed that *FT1* transcripts were abundant in cold temperature under continuous darkness or ambient conditions (Fig. 2*B*). However, *FT1* transcription was significantly ($P \leq 0.0001$) less at 25 °C under short-day conditions. Day length did not affect *FT1* expression, because trees treated in short-day (8 h light) and long-day (16 h light) conditions in cold temperature showed no significant ($P = 0.45$) differences in transcript levels (Fig. 2*B*). Similarly, the presence or absence of light did not affect *FT1* transcription, because trees grown in dark and in light did not differ significantly ($P = 0.107$) in transcript abundance (Fig. 2*B*). *FT2* transcripts were not detected in the identical tissues in these experiments. A third group of actively growing trees was placed under long-day or short-day conditions at 25 °C for 42 d in spring, when *FT2* is normally induced. *FT2* transcripts were significantly ($P \leq 0.0001$) abundant in leaves in long-day conditions but were undetectable in short-day conditions (Fig. 2*C*). *FT1* transcripts were undetectable in the identical tissues. The fourth group of actively growing trees also was placed in long-day conditions at 25 °C or at 4 °C for 14 d in May. *FT2* transcripts in expanding leaves were abundant at 25 °C but were decreased significantly ($P \leq 0.001$) at 4 °C (Fig. 2*D*). *FT1* transcripts were slightly detectable in trees grown for 14 d at 4 °C. These results show that, although cold temperature activates and warm temperature suppresses *FT1* transcription, day length or presence or absence of light does not affect expression. Conversely, long-day conditions or warm temperatures promote *FT2* transcription, whereas short-day conditions or cold temperatures suppress expression. These findings are consistent with normal winter expression of *FT1* and growing-season expression of *FT2* (Fig. 1). Moreover, *FT1* expression does not show a rhythm in daily transcript abundance (Fig. S3*A*), whereas *FT2* expression shows a semidian rhythm with a periodicity of about 12 h (Fig. S3*B*). Taken together, these experiments reveal that *FT1* and *FT2* have diverged in regulation, implying changes in regulatory DNA regions of the paralogs after the duplication event.

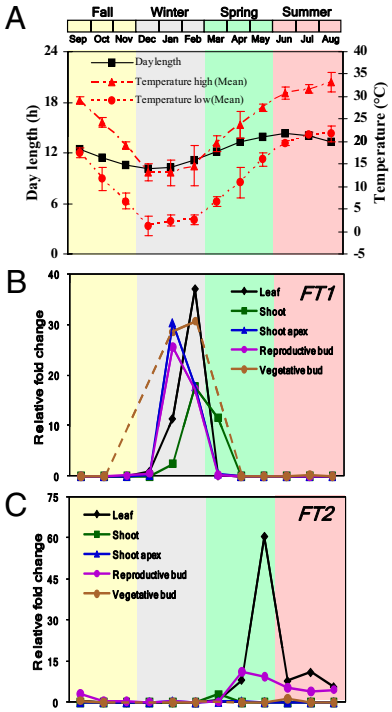

Fig. 1. Year-round normal expression of *FT1* and *FT2* in the same five above-ground tissues of mature *P. deltoides*. (*A*) Monthly high/low temperatures and day length in Mississippi, where experimental trees were grown. Error bars show SD about the mean. (*B* and *C*) Relative fold change in transcript levels of *FT1* (*B*) or *FT2* (*C*) relative to the lowest amount of expression within a tissue. (*B*) *FT1* transcripts are abundant in all the analyzed tissues in winter. Dashed lines indicate missing samples. (*C*) *FT2* transcripts are abundant in leaves and reproductive buds in spring and summer.

***FT1* Signals Reproductive Onset.** To define *FT1* and *FT2* functions further, we genetically perturbed their expression in poplar. To avoid potential complications caused by constitutive overexpression using the CaMV 35S promoter, we used the heat-inducible promoter of *HEAT SHOCK PROTEIN* (*HSP*) gene to make Pro_{HSP}:*FT1* and Pro_{HSP}:*FT2* constructs for transformation. Unlike Pro_{HSP}:*FT2*, Pro_{HSP}:*FT1* induced flowers within 30 d of cyclical heat treatment at 37 °C (Fig. 3*A* and Dataset S1). Transcripts of both genes were significantly ($P \leq 0.0001$) abundant in transgenic trees. We note that, compared with extremely abundant overexpression of *FT1* and *FT2* under the CaMV 35S promoter (Pro_{35S}:*FT1* and Pro_{35S}:*FT2*, respectively), Pro_{HSP}:*FT1* and Pro_{HSP}:*FT2* constructs induced only a very moderate overexpression, much closer to normal peak expression of *FT1* and *FT2* (Fig. 3*A*). Pro_{HSP}:*FT1* trees continuously formed axillary inflorescences (catkins) and eventually formed a terminal inflorescence on the new shoot growth as long as *FT1* signaling was available (Fig. S4*A*). Axillary vegetative buds that had formed before heat treatment did not produce inflorescences or overcome dormancy. When the temperature was increased to 40 °C to test whether higher abundance of *FT2* transcripts triggers flowering, *FT2* transcript levels increased significantly ($P \leq 0.0001$), and trees showed a weak flowering phenotype, mainly forming incomplete inflorescences (Fig. 3*A*, Fig. S4*A*, and Dataset S1). Thus, in poplar relatively low *FT1* signaling induces reproductive onset in undifferentiated meristems, whereas abnormally abundant *FT2* transcripts are required for this process to occur. Our results suggest that a pulse of *FT1* expression in winter initiates the transition of vegetative meristems to the reproductive phase, resulting in a limited number of reproductive buds in the Floral Zone (Fig. S4*B*). Buds that are produced under warm temperatures before and after *FT1* expression are vegetative (Vegetative Zones I and II).

If *FT2* signal is required for reproductive onset in poplar, suppression of *FT2* transcription following *FT1* signaling should produce no reproductive buds. Because short-day conditions repress *FT2* transcription (Fig. 2*C*), we maintained branches of field-grown mature *P. deltoides* under short-day conditions in spring (March–May) when *FT2* expression normally is abundant (Fig. S5*A*). Control branches were kept under ambient long-day conditions (12–14 h). The short-day treatment was effective, because *FT2* transcription was significantly ($P \leq 0.005$) lower in short-day–treated shoots than in controls (Fig. S5*B*). The controls

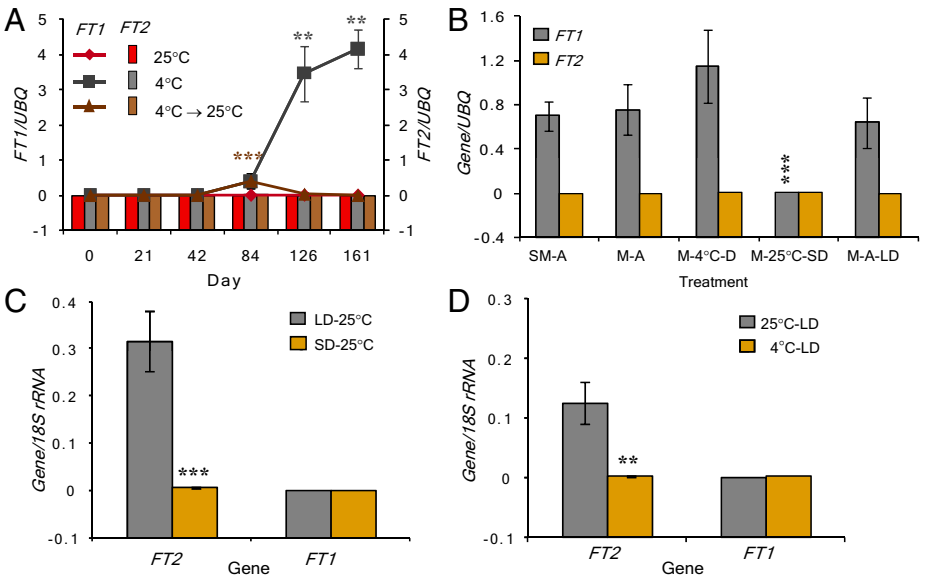

Fig. 2. Regulation of *FT1* and *FT2* in *P. deltoides*. (*A*) *FT1* transcript abundance increased in dormant trees ($n = 12$) at 4 °C under short-day conditions. When six trees were transferred to 25 °C after 90 d of 4 °C treatment, *FT1* was undetectable. *FT2* transcripts were undetectable in the identical tissues. (*B*) In winter, *FT1* transcripts were more abundant in mature dormant trees in the field at ambient conditions (SM-A; $n = 3$), in mature dormant trees in pots at ambient conditions (M-A; $n = 3$), in mature dormant trees in pots at 4 °C in continuous darkness (M-4 °C-D; $n = 3$), or in mature dormant trees in pots at ambient conditions in long-day conditions (M-A-LD; $n = 3$) than in mature dormant trees in pots at 25 °C under short-day conditions (M-25 °C-SD; $n = 3$). *FT2* transcripts were not detected in the identical tissues. (*C*) *FT2* transcripts were more abundant in long-day than in short-day conditions at 25 °C. *FT1* transcripts were undetectable in the identical tissues. (*D*) Treatment at 4 °C repressed *FT2* transcription ($n = 3$) in trees grown for 14 d at 4 °C and 25 °C. In contrast, *FT1* transcripts increased slightly in abundance at 4 °C. Error bars indicate SD. $^{**}P \leq 0.005$ and $^{***}P \leq 0.0005$ within a treatment.

ceased shoot growth within 56 d, but the short-day–treated shoots did so within 35 d and produced significantly ($P \leq 0.0001$) shorter shoots and fewer vegetative buds (Fig. S5 *C–E*). Reproduction was not eliminated; however, there were significantly ($P \leq 0.005$) fewer reproductive buds in the short-day treatment (Fig. S5 *C–E*). In the second experiment, Pro_{HSP}:*FT1* and *FT2*-RNAi constructs were coexpressed in the same trees to increase *FT1* and reduce *FT2* transcript abundance, respectively. *FT2* knockdown ranged from 15–45% compared with controls, and *FT1* transcripts were abundant during the heat treatment at 37 °C (Fig. S6 *A* and *B*). Unlike controls, 10 of 11 Pro_{HSP}:*FT1*/*FT2*-RNAi lines formed inflorescences (Fig. S6*C*), suggesting that *FT1* signaling is sufficient for reproductive onset for which *FT2* signaling is not necessary. In the third experiment, when Pro_{HSP}:*FT1* trees were heat-treated to 40 °C under short-day conditions in which *FT2* is not normally expressed (Fig. 2*C*), flowering still was induced (Fig. S6*D* and Dataset S1). Finally, poplar trees (*P. tremula* × *Populus alba*) with relatively less *FT2* overexpression (Pro_{35S}:*FT2*) produced inflorescences at the same age (5 y) as the controls in the field. We would have expected Pro_{35S}:*FT2* trees to transition to the sexually mature stage at an earlier age because of the greater *FT2* transcript output by both transgene and endogenous alleles. These results show that *FT2* signal is not essential for reproductive onset but may play a role in normal development of reproductive buds and/or flowers, because *FT2* transcripts are abundant in reproductive buds during the growing season (Fig. 1*C*).

***FT1* and *FT2* Molecular Networks Diverged.** To determine whether the molecular networks of *FT1* and *FT2* have diverged and reflect their function, we conducted microarray experiments to compare constitutive and inducible constructs with controls and subsequently to identify common genes downstream of Pro_{35S}:*FT1* and Pro_{HSP}:*FT1* or Pro_{35S}:*FT2* and Pro_{HSP}:*FT2* in poplar (Fig. S7*A* and Dataset S2). Leaf tissues from heat-treated (inducible constructs) plants were sampled on the day immediately following heat treatment (day 21). We then mapped year-round normal expression of such downstream genes in leaves of mature *P. deltoides* by conducting another set of microarray experiments, followed by cluster analysis and functional classification (Fig. 3*B*). Genes downstream of *FT1* mostly were down-regulated, whereas genes downstream of *FT2* and genes downstream of both *FT1* and *FT2* were mainly up-regulated. Unlike *FT2*, 18 genes downstream of *FT1* are related to reproduction (Fig. 3*B*), supporting *FT1*'s main function in reproductive onset. *FT1* up-regulated genes include *MADS49*, a homolog of *Arabidopsis SEPALLATA* involved in floral organ formation (Fig. S7*B*) (13). *MADS49* transcripts were abundant in reproductive buds throughout inflorescence development after the formation of floral meristems on flanks of inflorescence shoots (Fig. S7*C*) (3)]. In contrast, *MADS7*, similar to the *Arabidopsis* floral repressor *SHORT VEGETATIVE PHASE* (Fig. S7*B*) (14, 15), was down-regulated. *MADS7* was expressed mainly in juvenile trees (Fig. S7*D*) and showed an inverse relationship with *FT1* (Fig. S7*E*), suggesting that *MADS7* may be a negative regulator of reproductive onset. Moreover, 15 auxin-related genes involved in signaling and transport established a unique network with *FT1* and were down-regulated when *FT1* was up-regulated via Pro_{35S}:*FT1* or Pro_{HSP}:*FT1* (Fig. 3*B*). These genes were suppressed when *FT1* was normally activated in winter but were up-regulated in the following growing season (turquoise and red modules in Fig. 3*B*). Although the mechanism is not clear, auxin has been known since the 1940s to be a repressor of reproductive onset in leaves but a promoter of reproductive development (16–20). These auxin-related genes might act as negative regulators of poplar reproductive onset in winter, and thus need to be transiently repressed by *FT1*, but are subsequently needed during reproductive development in the growing season. Upon up-regulation of *FT1*, down-regulation of methyltransferase and histone genes (Dataset S2) indicates an epigenetic change in chromatin, probably enabling reproductive development. Of the 27% of the genes downstream of *FT1* that are involved in metabolism, 63% were down-regulated when *FT1* was activated, and 52% were up-regulated in the following growing season (turquoise and red modules in Fig. 3*B*), suggesting that *FT1* influences metabolic networks into the growing season that support rapidly developing reproductive buds. These results show that *FT1* and *FT2* molecular networks have diverged, are highly modulated, and show a dynamic year-round expression pattern.

***FT2* Regulates Vegetative Growth.** What is the primary function of *FT2*? The abundance of *FT2* transcripts during rapid shoot growth in the growing season and the observation during aforementioned experiments that increased *FT2* transcription accelerated vegetative growth prompted us to conduct the following experiments to test whether *FT2* regulates vegetative growth. First, actively growing trees harboring Pro_{HSP}:*FT1* or Pro_{HSP}:*FT2* were transferred for 105 d into short-day conditions at 30 °C, which is compatible with growing-season temperatures (Fig. 1*A*) and is high enough to promote *FT1* and *FT2* transcription via Pro_{HSP} without inducing flowering. To repress endogenous expression of *FT1* and *FT2* and to ensure that the treatment effect is

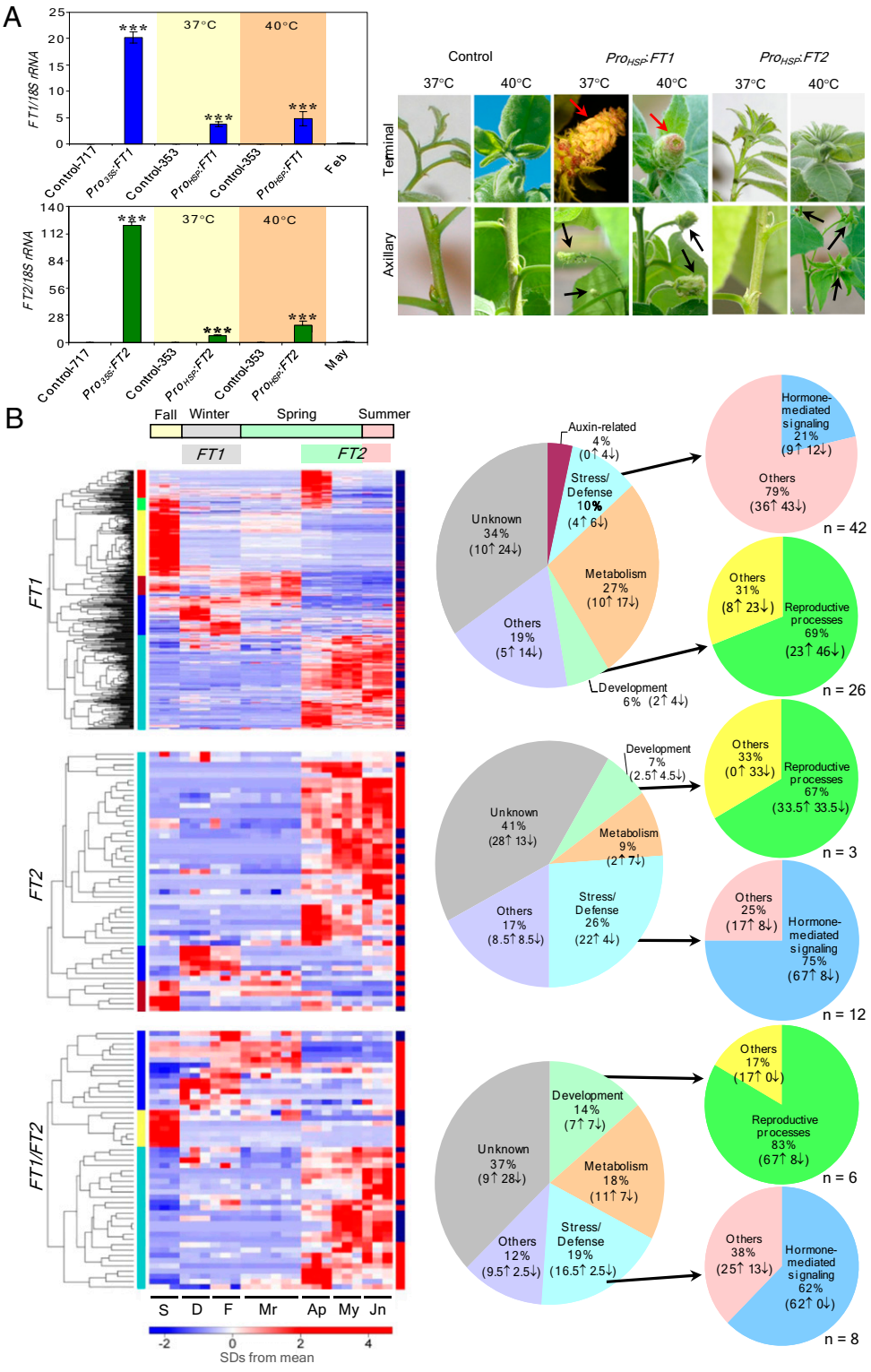

Fig. 3. Functional and network analyses of *FT1* and *FT2* in poplar. (*A*) Trees (*P. tremula* × *P. tremuloides* 353) harboring Pro_{HSP}:*FT1* and Pro_{HSP}:*FT2* (n = 30) were treated at 37 °C and 40 °C under long-day conditions to determine reproductive onset. (*Right*) (*Upper*) Red arrows show terminal inflorescences. (*Lower*) Black arrows show axillary inflorescences. (*Left*) *FT1* (*Upper*) and *FT2* (*Lower*) transcript abundance was determined in leaves of trees (*P. tremula* x *P. tremuloides* 353) harboring Pro_{HSP}:*FT1* and Pro_{HSP}:*FT2*, in leaves of trees (*P. tremula* × *P. alba* 717) harboring Pro_{35S}:*FT1* and Pro_{35S}:*FT2*, and in leaves of normally growing mature *P. deltoides* (controls) in February and May. ***$P \leq 0.0001$ within a treatment. (*B*) (*Left*) Heat maps showing year-round normal expression of genes downstream of *FT1* and *FT2* (Dataset S2) in mature *P. deltoides*. (*Left*) Clusters on the left represent modules. The column on the right shows up-regulated (red) and down-regulated (blue) genes downstream of *FT1*, downstream of *FT2*, or downstream of both *FT1* and *FT2* commonly expressed in Pro_{35S}:*FT1* and Pro_{HSP}:*FT1*, and Pro_{35S}:*FT2* and Pro_{HSP}:*FT2*. Months from September (S) to June (Jn) are identified below the heat maps. SDs are shown below the heat maps. (*Right*) Pie charts show functional categorization of similar Gene Ontology Biological Process terms. Numbers in parenthesis represent partitioning of overall percentages into up (↑) and down (↓) percentages. *n*, number of genes.

caused only by Pro_{HSP}, we used warm-temperature, short-day conditions, because *FT1* normally is not expressed in warm temperature (Fig. 2 *A* and *B*), nor is *FT2* normally expressed in short-day conditions (Fig. 2*C*). The treatment was effective, because *FT1* and *FT2* transcripts were significantly ($P \leq 0.001$) more abundant in transgenic trees than in controls (Fig. S8*A*). Control trees normally ceased shoot growth within 35 d because of short-day conditions. Pro_{HSP}:*FT2* trees grew continuously, whereas Pro_{HSP}:*FT1* trees ceased shoot growth by day 105. Consequently, Pro_{HSP}:*FT2* trees produced significantly ($P \leq 0.0001$) more shoot, internode, and stem diameter growth (Fig. S8*A*). When returned to 23 °C and short-day conditions, Pro_{HSP}:*FT2* trees ceased shoot growth within 35 d. Second, Pro_{35S}:*FT2* or $Pro_{35S2\times}$:*FT2*-C_{tag} trees with no early flowering did not cease shoot growth or form terminal buds in response to short photoperiods and cold temperatures in the field, resulting in no induction of winter dormancy (Fig. S8 *B* and *C*). Consequently, they grew year-round as long as air temperatures stayed above freezing. Winter frost killed

growing leaves and shoot tips on mature trees and often killed shoots and above-ground stems of juvenile trees. However, when the air temperature became warmer in the winter, undamaged axillary buds began to grow rapidly. Thus, constitutive expression of *FT2* is sufficient to prevent tree growth cessation induced by adverse environmental conditions (e.g., short days and cold temperature). In contrast, $Pro_{35S2\times}$:*FT1*-C_{tag} trees did not show year-round growth (Fig. S8*D*). Control trees normally induced dormancy in late summer or early fall and did not resume growth until the following spring. Third, Pro_{35S}:*FT2* trees showed strong apical dominance and produced significantly ($P \leq 0.0001$) shorter axillary shoots than controls (Fig. S9*A*). Finally, Pro_{HSP}:*FT1*/*FT2*-RNAi trees with fewer *FT2* transcripts (Fig. S6*A*) produced significantly ($P \leq 0.007$) less shoot growth than controls when grown at 30 °C and long-day conditions (Fig. S9*B*). A temperature of 30 ° C was used to drive *FT1* expression via Pro_{HSP}, and long-day conditions were used to enable normal expression of *FT2* so that the RNAi construct would reduce endogenous *FT2* expression. *FT2* knockdown resulted in less vegetative growth in trees. Considered together, these results reveal that vegetative growth, including growth cessation, bud set, and dormancy induction, is controlled by *FT2*, consistent with seasonal timing of its normal regulation in poplar (Fig. 1*C*).

What are the genetic mechanisms by which *FT2* controls vegetative growth? A majority (26%) of the known genes downstream of *FT2*, mainly expressed in the growing season (turquoise module in Fig. 3*B*), are related to stress defense (Fig. 3*B*). Growth cessation and bud set are induced when environmental factors are limiting (i.e., ecodormancy); thus, they may share regulatory elements (21). To determine whether genes downstream of *FT2* respond to stress that reduces or arrests shoot growth (22, 23), we conducted the following experiments in poplar. First, when day-length–treated tissues from mature trees grown in the field (Fig. S5) were reanalyzed, *FT2* and *JASMONATE-ZIM-DOMAIN PROTEIN 1* transcripts were significantly ($P \leq 0.05$) less abundant under short-day conditions that induced growth cessation (Fig. S9*C*). Second, poplar is a fast-growing pioneer species and normally is intolerant of shading by neighboring plants, but during the growing season, leaves in the interior tree crown often are shaded, or cloud covers shade trees. When the ambient light intensity was decreased from 1,700 to 500 μmol s^{-1} m^{-2} via shading of whole trees in the field, the transcript abundance of *FT2* and the antimicrobial extrusion efflux protein *ZF14* was reduced significantly ($P \leq 0.05$) (Fig. S9*D*). Shaded plants produced significantly ($P \leq 0.05$) shorter shoots. Third, trees often experience heat stress (temperatures >30 °C) coupled with water stress during summer days (Fig. 1*A*). *FT2* and *MAPK3* transcripts were significantly ($P \leq 0.05$) less at 38 °C (heat stress) than at 25 °C (Fig. S9*E*). Fourth, the abundance of *FT2* transcripts was significantly ($P \leq 0.05$) reduced, whereas that of *ETHYLENE RESPONSE FACTOR-APETALA2* was significantly ($P \leq 0.005$) increased under low, medium, and severe water stress that induced cessation of shoot growth (Fig. S9*F*). Finally, cold temperature significantly ($P \leq 0.001$) repressed *FT2* transcription (Fig. 2*D*). *FT1* transcripts were undetectable in these experiments (e.g., Fig. S9 *C*–*F*). These results demonstrate that *FT2* acts as a multistress sensor and selectively forms molecular networks with different genes in response to various stress factors to control vegetative growth during the growing season.

Discussion

Our results suggest that repeated cycles of reproductive and vegetative growth in sexually mature poplar are coordinated by the transient functioning of the duplication products *FT1* and *FT2*. Reproductive onset is determined by *FT1* signaling in response to winter temperature, resulting in the formation of a limited number of reproductive buds in the Floral Zone (Fig. 4). Cold-temperature signaling also is used by other trees for reproduction (24). The gradual onset of warm spring temperatures rapidly suppresses *FT1* transcription, ending reproductive onset and marking the beginning of reproductive bud development during the growing season when internal and external resources are abundant for rapid development. If *FT1* were expressed during the growing season, poplar could not form true vegetative shoots and buds, and all the buds would be reproductive, as our data show. In contrast to *FT1*, with the gradual onset of warm temperatures and long days in early spring, *FT2* signaling promotes rapid vegetative growth.

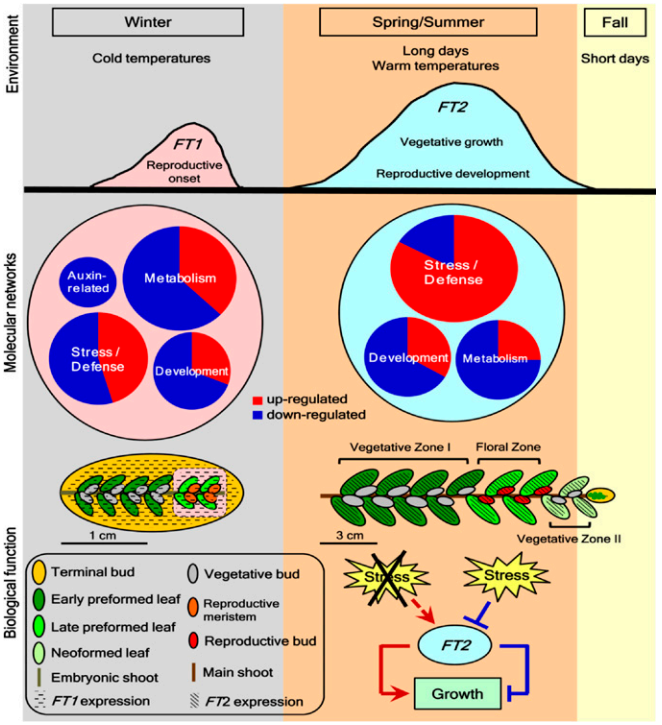

Fig. 4. A schematic integrated model showing that *FT1* and *FT2* regulate cycles of reproductive and vegetative growth. When *FT1* transcription is triggered by winter temperature, it induces reproductive onset through a network of downstream genes in a small number of axillary meristems in dormant buds, resulting in reproductive buds in the Floral Zone. Conversely, in response to warm temperatures, long days, and multiple stress factors in the following growing season, *FT2*, through its molecular networks, regulates vegetative growth.

However, *FT2* expression is either reduced or completely suppressed under stress, such as high temperature and drought that are prevalent in late spring and summer or the gradual shortening of days accompanied by cooling temperature that occurs in the fall, triggering growth cessation, bud set, and eventually dormancy induction (Fig. 4). The match between daily *FT2* rhythm and abiotic factors may allow poplar to detect and respond rapidly to such environmental changes. Consequently, *FT2* provides trees with adaptive properties important not only for growth under favorable conditions but also for survival under unfavorable conditions. Thus, temporal separation of reproductive onset and vegetative growth into different seasons via functionally diverged *FT1* and *FT2* appears to be one of the prominent features of poplar perennialism that enable formation of vegetative buds and shoots for future growth and allow trees to accommodate both vegetative and reproductive growth. These findings indicate a mechanism different from that previously reported for the herbaceous perennial *A. alpina*, in which repeated transcriptional repression and activation of *PEP1*, the *Arabidopsis FLC* ortholog, controls recurring seasonal transitions between reproductive and vegetative phases (5).

Unlike a previous report showing that *FT1* expression induces reproductive onset and controls growth cessation and bud set in the growing season (7), our findings clearly differentiate the regulation and function of the paralogs *FT1* and *FT2*. Specifically, we show that *FT1* expression in winter initiates the transition of vegetative meristems to the reproductive phase, whereas *FT2* controls vegetative growth, including growth cessation, bud set,

and dormancy induction, in the growing season. Our data indicate the following four reasons for this discrepancy: First, the *FT1* primer pair used for expression analysis by Böhlenius et al. (7) cross-reacts with *FT2* transcripts in PCR reactions (Fig. S2*B*). Thus, their *FT1* gene expression data during the growing season [e.g., figures 2 I and J, 3 C and F, S6A, and S7 in Böhlenius et al. (7)] probably reflect *FT2* expression. Second, Böhlenius et al. (7) did not conduct an extensive year-round transcript analysis, as we did, to determine the spatial and temporal expression of both *FT1* and *FT2* in normally growing trees (Fig. 1). Thus, their expression analysis missed a piece of information that *FT1* normally is expressed only in winter or in response to cold temperatures. Third, in interpreting their results, Böhlenius et al. (7) relied primarily on Pro_{35S}:*FT1* trees. As our current results show, the CaMV 35S constitutive promoter causes abnormal gene expression, resulting in additional phenotypes (e.g., vegetative growth) not necessarily associated with the primary function of the gene under normal conditions. Furthermore, their RNAi construct was not *FT1* specific and thus would be expected to knockdown both *FT1* and *FT2*. Finally, Böhlenius et al. (7) did not conduct extensive, long-term field tests on their genetically manipulated trees. Moreover, previous findings by Hsu et al. (8) showed that *FT2* induced reproductive onset when both poplar and *Arabidopsis* were transformed with the Pro_{35S}:*FT2* construct. Our current results suggest that induction of reproductive onset is not *FT2*'s primary function. However, we do not dismiss the possibility that *FT2* might be involved in reproductive development, because *FT2* normally is expressed in reproductive buds during the growing season (Fig. 1*C*). As we did in the current study, Hsu et al. (8) should also have used weaker and/or inducible promoters in their constructs along with suppressing the expression of *FT2*. Thus, we suggest that experimental designs concerning the duplicated genes in duplicated genomes should carefully consider all these aspects as appropriate.

Our results imply that changes in both gene expression and protein sequence have contributed to diverged functions of *FT1* and *FT2*. Transcription of *FT1* and *FT2* is temporally and spatially separated and is under the regulation of contrasting environmental and internal factors. Similarly, under the same inducible promoter, different phenotypes resulting from heat treatment of trees harboring constructs overexpressing *FT1* or *FT2* indicate diverged protein functions, which can be attributed to 16 amino acid changes between the two paralogs (Fig. S1*C*). One of the changes (alanine to proline in *FT2*) is located in a C-terminal external loop (residues 128–145) that contributes to antagonistic activity of *FT* and *TERMINAL FLOWER 1* on flowering time in *Arabidopsis* (25). This change makes the *FT2* external loop more hydrophilic based on hyropathy index, potentially affecting protein–protein interactions. A recent report shows that in biennial sugar beet (*Beta* spp.), the *FT* duplication products *BvFT1* and *BvFT2* have diverged in function (26). *BvFT1* and *BvFT2* are expressed mainly in leaves but differ in temporal expression: *BvFT1* is expressed at the juvenile stage, and *BvFT2* is expressed at the reproductive stage. *BvFT1* expression represses reproductive onset and bolting (vernalization response); similar to *Arabidopsis FT*, *BvFT2* function is needed during the growing season for flowering. The functional difference between BvFT1 and BvFT2 proteins results in part from three amino acid changes in the external loop area of BvFT1 (Fig. S1*C*), making this region more hydrophilic. In contrast to these two examples, a single amino acid change (asparagine to glutamine) in TSF does not appear to affect the external loop hydropathicity, thus showing a structure similar to that of *FT* in annual *Arabidopsis*. In addition, *FT* (10, 11) and *TSF* (12) not only show similar temporal and spatial expression patterns and redundantly control reproductive onset under warm-temperature and long-day conditions but also appear to have similar biochemical functions by interacting with the same transcription factors (27). These advances provide a framework for understanding how changes in *FT* genes have contributed to the evolution of plant life forms and adaptation.

In conclusion, our findings in perennial poplar suggest that *FT* duplication and subsequent changes in gene expression patterns, proteins, and molecular networks leading to adaptive functional differentiation between the paralogs appear to have increased phenotypic flexibility for responding to seasonal and yearly environmental variation. Given that divergence in the expression patterns of many other duplicated gene pairs on paralogous chromosomes VIII and X, as well as in the whole genome, is widespread in poplar (Fig. S10), gene duplication followed by expression pattern shifts, adaptive changes to proteins, and divergence in gene regulatory networks appears to be one of the important elements for the evolution of complex perennial life-history traits.

Materials and Methods

Details of year-round transcript analysis, transcriptional regulation, functional studies, molecular network analysis, and growth and stress experiments are described in *SI Materials and Methods*.

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