Molecular regulation of plant developmental transitions and plant architecture via PEPB family proteins – an update on mechanism of action

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Highlight: Recent genomic investigations have opened the door to understanding how PEBP proteins such FLOWERING LOCUS T and its antagonist TERMINAL FLOWER 1 modulate plant form in response to environmental signals.

Abstract: This year marks the 100th anniversary of the experiments by Garner and Allard (Garner and Allard, 1920) that showed that plants measure the duration of the night and day (the photoperiod) to time flowering. This discovery led to the identification of Flowering Locus T (FT) in Arabidopsis and Heading Date 3a (Hd3a) in rice as a mobile signal that promotes flowering in tissues distal to the site of cue perception. FT/Hd3a belong to the family of phosphatidylethanolamine binding proteins (PEBPs). Collectively, these proteins control plant developmental transitions and plant architecture. Several excellent recent reviews have focused on the roles of PEBP proteins in diverse plant species; here we will primarily highlight recent advances that enhance our understanding of the mechanism of action of PEBP proteins and discuss critical open questions.

Keywords

Florigen

FLOWERING LOCUS T (FT)

Genomics and Gene regulation

Hormone signalling

Plant architecture

Photoperiod

Sugar signalling

Seasonal response

TERMINAL FLOWER 1 (TFL1)

TFL1/FT competition

Introduction

The discovery of the PEBP proteins as interpreters of seasonal environmental cues has two major chapters, physiological-biochemical and molecular-genetic investigations (Fig. 1). In 1920, physiological approaches linked dynamic plant flowering responses to the relative length of day and night, also known as the photoperiod (Garner and Allard, 1920). In 1934, plant leaves were shown to perceive the favourable photoperiod stimulus (Knott, 1934). Grafting an induced leaf to a unstimulated plant was sufficient for flowering, pointing to a mobile floral stimulus able to translocate from leaves to the shoot apex where flowers form (Chailakhyan, 1936). The concept of florigen as the mobile stimulus was introduced in 1936 (Chailakhyan, 1936) and the florigenic signal was found to be conserved in diverse plant species (Zeevaart, 1976). The nature of florigen was proposed to be a balance of phytohormones and metabolites (Bernier, 1988; Bernier *et al.*, 1993). Molecular and genetic analyses by many laboratories and in many plant species in last 30 years identified the PEBP protein FLOWERING LOCUS T (FT) and its homologs as a conserved florigenic signal (Putterill and Varkonyi-Gasic, 2016). They also uncovered the components that aid FT long-distance movement (Jackson and Hong, 2012; Liu *et al.*, 2013) and identified PEBP proteins with roles antagonistic to that of FT (Jin *et al.*, 2020).

A brief summary of PEBP family protein function

Evolutionarily, PEBP proteins can be grouped into three classes, the ancestral of which is MOTHER OF FT AND TFL1 (MFT), see (Jin *et al.*, 2020) and references therein. In Arabidopsis, *MFT* is strongly expressed in seeds and roots and promotes seed dormancy (Vaistij *et al.*, 2013). Two additional classes or PEBP proteins comprise FT-like and TERMINAL FLOWER 1 (TFL1)-like proteins, which in Arabidopsis have three and two members, respectively (Jin *et al.*, 2020). FT and TFL1 and their homologs are best known for their opposite roles in flowering time and inflorescence architecture, which underlie important crop traits like day neutrality, determinacy, time to flower formation and number of flowers and seeds produced and may contribute to reproductive barrier formation in wild species (Eshed and Lippman, 2019; Kinoshita and Richter, 2020; Périlleux *et al.*, 2019; Song *et al.*, 2015; Todesco *et al.*, 2020) (Fig. 2 and Fig. 3A). However, activities of *FT*-like and *TFL1*-like genes regulate many other processes, among these are tuberization in potato, bulb formation in onion, bud set and flush in trees [reviewed in (Jin *et al.*, 2020; Périlleux *et al.*, 2019)]. More recently, TFL1 has been implicated in repression of bud outgrowth both in hybrid aspen and in Arabidopsis (Maurya *et al.*, 2020a; Zhu *et al.*, 2020) and in seed development in Arabidopsis(Zhang *et al.*, 2020a) (Fig. 4A). Common to all processes is that they are seasonally controlled life history traits.

Identification of targets of TFL1 and FD - a repressive hub for reproductive development

Although non-nuclear roles have been identified for both TFL1 and FT family proteins (Abelenda *et al.*, 2019; Sohn *et al.*, 2007; Zhang *et al.*, 2020a), studies in Arabidopsis, rice, and potato tubers have suggested nuclear functions of TFL1 and FT as a co-repressor and a co-activator, respectively, by acting in a complex with the bZIP transcription factor FD via 14-3-3 proteins (Goretti *et al.*, 2020; Hanano and Goto, 2011; Kaneko-Suzuki *et al.*, 2018; Navarro *et al.*, 2011; Taoka *et al.*, 2011; Teo *et al.*, 2016; Zhang *et al.*, 2020b; Zhu *et al.*, 2020). FD proteins have a conserved S/TAP motif, phosphorylation of which is thought to mediate 14-3-3 interaction. FD phosphorylation is likely of biological importance. While TFL1 can readily interact with *E. coli* produced FD via 14-3-3 *in vitro* (Collani *et al.*, 2019; Kaneko-Suzuki *et al.*, 2018), FT prefers phosphomimic recombinant FD in vitro and in vivo (Collani *et al.*, 2019; Kawamoto *et al.*, 2015) and potato CENTRORADIALIS (StCEN/TFL1), for example, also cannot readily interact with phosphomutant FD in vivo (Zhang *et al.*, 2020b). FT binds phosphatidyl choline (PC) and a recent high-resolution crystal structure reveals how PC associates with FT (Nakamura *et al.*, 2019).

The nuclear roles of TFL1-FD and FT-FD complexes in vivo have remained poorly understood because of low protein abundance and presence in only very few cell types. Recently, three labs including our own reported identification of FD and/or TFL1 bound loci by ChIP-seq (Collani *et al.*, 2019; Goretti *et al.*, 2020; Romera-Branchat *et al.*, 2020; Zhu *et al.*, 2020). Despite having been conducted at different stages of development and in different photoperiod regimes, there is a strong overlap of binding peaks and target genes between all studies, when using one data analysis pipeline (Zhu *et al.*, 2020) on all datasets (Fig. 5). In the following discussions we will focus on (Zhu *et al.*, 2020), which uncovered 604 immediate early TFL1-FD repressed targets. These targets identify TFL1-FD as a repressive hub for onset of reproduction and inflorescence architecture (Fig. 3B). TFL1-FD repress expression of key flowering time regulators that promote entry into the reproductive phase including *CONSTANS* (*CO*), and *GIGANTEA* (*GI*), and of meristem identity regulators that cause the switch from branch to flower fate in axillary meristems like *FRUITFULL* (*FUL*), *LEAFY* (*LFY*), *APETALA1* (*AP1*) and *LATE MERISTEM IDENTITY 1* (*LMI1*) (Collani *et al.*, 2019; Fornara *et al.*, 2010; Wagner, 2017; Zhu *et al.*, 2020). *LFY* in particular had not been expected to be a PEBP/FD target based on promoter:reporter studies, however the critical regulatory element for photoperiod regulation of *LFY* reside in its second exon (Blázquez and Weigel, 2000; Zhu *et al.*, 2020). The link between TFL1-FD and FT-FD and their direct targets *AP1* and *LFY* is conserved during short-day triggered growth cessation in hybrid aspen (Tylewicz *et al.*, 2015), long-day photoperiod induced opening of guard cells (Kinoshita *et al.*, 2011) and possibly onion bulb formation (Rashid *et al.*, 2019); while that between TFL1-FD and FT-FD and *FUL* is conserved during secondary growth in tomato (Shalit-Kaneh *et al.*, 2019).

Other critical regulators of reproductive competency and the switch from branch to flower identity are members of SQUAMOSA PROMOTER BINDING PROTEIN LIKE (SPL) family of proteins (Hyun *et al.*, 2016; Wang *et al.*, 2009; Yamaguchi *et al.*, 2009; Yamaguchi *et al.*, 2014) which also act upstream of the *AP1/FUL* family of MADS box genes. The combined recent FD and TFL1 ChIP-seq datasets, which collectively sampled diverse developmental stages and growth conditions (Collani *et al.*, 2019; Goretti *et al.*, 2020; Romera-Branchat *et al.*, 2020; Zhu *et al.*, 2020), reveal that the *SPL* genes are not immediately downstream targets of TFL1-FD, in agreement with prior genetic investigations (Wang *et al.*, 2009; Yamaguchi *et al.*, 2009; Yamaguchi *et al.*, 2014).

A role for PEBP proteins in transcriptional control of hormone signalling and response?

In addition, the 604 identified immediate early TFL1-FD repressed genes link to diverse hormonal pathways, including the strigolactone, cytokinin, auxin, brassinosteroid, jasmonic acid and abscisic acid pathways (Collani *et al.*, 2019; Goretti *et al.*, 2020; Romera-Branchat *et al.*, 2020; Zhu *et al.*, 2020) (Fig. 3B). The combined data suggest that – perhaps in a manner analogous to hormonal regulation of bud outgrowth (Barbier *et al.*, 2019) – multiple hormonal signals and response pathways together provide permissive or prohibitive environments for given developmental trajectories in the axillary meristems.

A direct link between PEBP proteins and the above-mentioned hormone signalling pathways is in agreement with independent investigations. Strigolactone sensitive SUPPRESSOR OF MAX2-LIKE 6, 7 and 8 (SMXL6-8) proteins enhance shoot branching by promoting axillary bud outgrowth (Wang *et al.*, 2015). Interestingly, *SMXL6* and *SMXL8* are not only among the immediate early TFL1-FD repressed targets identified, but wild type plants and *tfl1* mutants also display enhanced branch outgrowth in response to photoperiod-mediated upregulation of FT in Arabidopsis (Zhu *et al.*, 2020). The combined data suggest a role for TFL1 in preventing bud outgrowth (Fig. 3B and Fig. 4A). Likewise, in aspen, bud outgrowth is blocked by TFL1 and promoted by the aspen AP1/CAL/FUL homolog Like-AP1 (LAP1), which acts genetically downstream of TFL1 (Maurya *et al.*, 2020a) (Fig. 4A). This block of bud outgrowth occurs at the end of the growing season in response to low temperature or short day which trigger elevated *TFL1* and reduced *FT2* accumulation (Maurya *et al.*, 2020a; Maurya *et al.*, 2020b). Analyses of direct and indirect TFL-FD repressed targets also revealed that TFL1 represses cytokinin response (Zhu *et al.*, 2020) (Fig. 3B). Like SMXL6 and 8, cytokinin promotes branch outgrowth (Barbier *et al.*, 2019; Zhu and Wagner, 2020).

Likewise, direct and indirect targets of TFL1-FD in Arabidopsis suggest that TFL1-FD blocks auxin signalling and response (Zhu *et al.*, 2020) (Fig. 3B). Prior studies have implicated auxin in promotion of flower fate in axillary meristems of the Arabidopsis inflorescence (Yamaguchi *et al.*, 2013). In tomato, loss-of-function mutants of the ortholog of *TFL1* in tomato, *SELF PRUNING* (*SP*), cause pleiotropic changes, such as shoot determinacy, early flowering and simultaneous fruit ripening (Pnueli *et al.*, 1998). Polar auxin transport and auxin responses are altered in *sp* mutants, suggesting that SP may act -at least in part- via auxin (Silva *et al.*, 2018). Finally, DNA affinity purification sequencing (DAP-seq) and expression analysis in mutants linked rice FD homologs to the *OsARF19* auxin responsive transcription factor, supporting the link from PEBP proteins to auxin response (Cerise *et al.*, 2020).

Another group of TFL1-FD repressed genes are components of brassinosteroid (BR) signaling (Zhu 2020) (Fig. 3B). In agreement with the genomic studies, BR biosynthesis mutants in *Setaria* cause formation of additional spikelets, suggesting BR blocks inflorescence branching (Yang *et al.*, 2018). The BR steroid hormone has also been linked to onset of reproductive development (Nolan *et al.*, 2020). In blue light, the BR early response regulator and bHLH transcription factor BR ENHANCED EXPRESSION 1 (BEE1) accumulates and promotes flowering by directly upregulating *FT* expression (Wang *et al.*, 2019). Thus, the BR pathway promotes onset of the reproductive phase and inflorescence branching.

Multiple genes in the abscisic acid (ABA) pathway have been identified as shared targets of TFL1, FD and the FD homolog FDP (Romera-Branchat *et al.*, 2020; Zhu *et al.*, 2020) (Fig. 3B and Fig. 5B). ABA has been linked to upregulation of *FT* accumulation by several studies (Chang *et al.*, 2019; Riboni *et al.*, 2016), yet the strongest link to PEBPs is via MFT during seed germination (Vaistij *et al.*, 2018; Vaistij *et al.*, 2013; Xi *et al.*, 2010) (Fig. 4C). Loss-of *MFT* and *FD* or *FDP* function reveals ABA dependent phenotypes during cotyledon greening and seed germination, respectively (Romera-Branchat *et al.*, 2020; Vaistij *et al.*, 2018; Vaistij *et al.*, 2013; Xi *et al.*, 2010). In addition, TFL1 promotes endosperm cellularization during seed development by stabilizing the ABI5 transcription factor in the cytoplasm surrounding syncytial peripheral endosperm (Zhang *et al.*, 2020a) (Fig. 4C). ABA is furthermore known to prevent bud outgrowth in Arabidopsis and aspen (Gonzalez-Grandio and Cubas, 2014; Gonzalez-Grandio *et al.*, 2017; Tylewicz *et al.*, 2018). Whether and how ABA impacts the axillary meristem identity switch from branch to floral fate is not known.

PEBP proteins and sugar transport and signalling

Sugar is transported from the source (photosynthesis in leaves) to various sink organs including shoot apices, flowers, fruits and seeds as well as roots and storage organs like tubers and bulbs (Baena-Gonzalez and Lunn, 2020; Martin-Fontecha *et al.*, 2018). Tuberization in potato is promoted by the FT-like SELF-PRUNING 6A (StSP6A) protein, expression of which is induced by high sucrose concentration and closely associated with the number of tubers formed (Abelenda *et al.*, 2019). StSP6A physically interacts with the sucrose efflux transporter StSWEET11 at the cell membrane (Fig. 4B). This prevents sucrose from leaking to the apoplast and promotes symplastic sugar unloading into the tuber (Abelenda *et al.*, 2019). Coordinated expression of StSP6A and StSWEET11 promotes potato tuber formation, supporting cross talk between sucrose source-sink partitioning and photoperiodic pathways (Abelenda *et al.*, 2019).

Sugar transport is also linked to onset of sexual reproductive development. Overexpressing *FT* in phloem companion cells of minor leaf veins identified the sucrose transporter encoding *SWEET10* gene as a differentially expressed gene (Andrés *et al.*, 2020). Both overexpression of *FT* and long-day inductive conditions promote *SWEET10* mRNA accumulation and 35S:SWEET10 causes early flowering irrespective of photoperiod (Andrés *et al.*, 2020). In addition, the trehalose-6-phosphate (T6P) synthase TPS1 promotes flowering upstream of *FT* (Wahl *et al.*, 2013). T6P is linked to sucrose signaling and homeostasis (Baena-Gonzalez and Lunn, 2020). Trehalose-6-phosphatases (TPPs) were identified as TFL1-FD repressed targets during the switch from branch to flower fate in axillary meristems (Collani *et al.*, 2019; Goretti *et al.*, 2020; Romera-Branchat *et al.*, 2020; Zhu *et al.*, 2020) (Fig. 3B). In agreement with this finding, multiple TPPs were recently implicated in suppression of branching in the maize inflorescence (Claeys *et al.*, 2019). This role of the TPPs in maize apparently does not rely on their enzymatic activity (Claeys *et al.*, 2019).

Antagonistic activities of FT- and TFL1-like proteins

The relative dose of FT- and TFL1-like activities is critical for flowering and inflorescence traits in many species as well as for tuberization in potato (Kim *et al.*, 2013; Krieger *et al.*, 2010; Maurya *et al.*, 2020a; Zhang *et al.*, 2020b) (Fig. 3A). A large expansion of the PEBP gene family followed by sub- or neo-functionalization occurred in many crop species (Jin *et al.*, 2020; Zheng *et al.*, 2016). For example, members of the eight-member cotton TFL1 family display diverse and frequently non-overlapping expression patterns (Prewitt *et al.*, 2018) and non-overlapping roles were reported for the five TFL1 homologs in tetraploid *Brassica napus*, an important oil and biofuel crop (Sriboon *et al.*, 2020). Moreover, mutations that change the balance between FT- and TFL1-like activities, such as loss-of-function of an FT or TFL1 paralog, have been selected for numerous times during domestication (Blackman *et al.*, 2010; Comadran *et al.*, 2012; Iwata *et al.*, 2012; Liu *et al.*, 2018; Pin *et al.*, 2010; Zheng *et al.*, 2016). Even when acting in concert, the FT or TFL1 paralogs do not contribute equally. For example, in Brachypodium -as in Arabidopsis- weak and strong FT activators have been identified, which act in different photoperiods (Jin *et al.*, 2020; Lv *et al.*, 2014; Shaw *et al.*, 2019).

In most species one ‘central’ TFL1- and FT-like factor dominates, and null mutations in these have pleiotropic and often deleterious effects (Kim *et al.*, 2013; Krieger *et al.*, 2010) (Fig. 3C). Thus, subtle modulation of the relative dose of the opposing ‘central’ FT- and TFL1-like activities are generally most beneficial for yield and other desirable crop traits; these can be caused by weak loss-of-function mutations or by cis motif variation (Eshed and Lippman, 2019; Jiang *et al.*, 2013; Park *et al.*, 2014; Soyk *et al.*, 2017). Indeed, modulation of the relative PEBP activity is being exploited by CRISPR-Cas9-mediated rapid breeding of desirable inflorescence architectures and for flowering on demand; this can be applied to leaf crops to block flowering or to tree crops to accelerate flowering/breeding, it can be used to expand the geographic growth range (day neutrality) or to enhance crop harvestability (determinate cultivars) in addition to yield (Eguen *et al.*, 2020; Eshed and Lippman, 2019; Kwon *et al.*, 2020; Okada *et al.*, 2017).

Underpinning the relative dose of FT- and TFL1-like activities are spatiotemporal control of mRNA and protein accumulation, as well as protein movement. The former includes seasonally controlled transcriptional upregulation of *FT* and increases in *TFL1* expression in inductive long-day photoperiod in Arabidopsis (Luccioni *et al.*, 2019; Moraes *et al.*, 2019; Périlleux *et al.*, 2019; Zhu *et al.*, 2020) and of *FT* homologs like *Hd3a* and *RFT1* in inductive short day photoperiod in rice (Shen *et al.*, 2020; Tsuji *et al.*, 2011). Fine tuning of the Arabidopsis *FT* gene expression occurs, as recently uncovered via elegant approaches, through redundantly acting enhancers at the *FT* locus (Zicola *et al.*, 2019). *FT* accumulation in short days is prevented, at least in part, by a DELLA-MYC3 repressor complex (Bao *et al.*, 2019). This explains the promotion of flowering in short days by gibberellin treatment. Interestingly the MYC3 bound element is not present in early flowering ecotypes such as L*er* and Ws (Bao *et al.*, 2019).

In addition, cross-regulation between PEBP proteins has been described. In long days, the repressive FT-like homolog StSP5G of certain potato cultivars represses expression of the tuberization activating FT *StSP6A* in leaves (Abelenda *et al.*, 2016). Cultivated tomato, which displays day-neutral flowering, has lost both short-day and long-day control of *SINGLE FLOWER TRUSS* (*SFT*) expression by FT-like proteins (Song *et al.*, 2020; Soyk *et al.*, 2017). In the wild tomato, *SFT* expression is repressed by the repressive FT homolog SlSP5G in long-day and activated by FT-LIKE 1 (FTL1) in short-day (Song *et al.*, 2020; Soyk *et al.*, 2017). In Brachypodium, wheat and barley, FT1 activates expression of *FT2* and both proteins act sequentially to promote reproductive development (Digel *et al.*, 2015; Lv *et al.*, 2014; Shaw *et al.*, 2019). In contrast to regulation of *FT* accumulation, that of *TFL1* is underexplored (Serrano-Mislata *et al.*, 2016). Finally, the available FT pool is modulated by interactions with the TCP transcription factor BRANCHED1 (BRC1), which blocks FT activity in Arabidopsis and hybrid aspen (Maurya *et al.*, 2020b; Niwa *et al.*, 2013).

Both FT and TFL1 move from their sites of production to their sites of activity, with FT generally traveling longer distances than TFL1 (Goretti *et al.*, 2020; Putterill and Varkonyi-Gasic, 2016). As in Arabidopsis, FT1 moves from the leaves to the shoot apex to promote seasonal growth in aspen (Miskolczi *et al.*, 2019). TFL1, by contrast only moves within the shoot apex (Conti and Bradley, 2007; Goretti *et al.*, 2020; Miskolczi *et al.*, 2019), while other TFL1 homologs in Arabidopsis can move long-distance (Huang *et al.*, 2012). Studies by the Yu group have uncovered proteins that help FT loading from leaf companion cells to sieve elements via endosomal vesicles in the leaf vasculature and promote long distance transport (Liu *et al.*, 2019; Liu *et al.*, 2012; Zhu *et al.*, 2016). This transport machinery is apparently conserved in rice (Song *et al.*, 2017), yet does not operate on the FT homolog in Arabidopsis (Zhu *et al.*, 2016). Moreover, in the developing seed, Ras-related nuclear (RAN) GTPases directed TFL1 movement from the chalazal endosperm to the syncytial peripheral endosperm; in a manner distinct from the role of RAN GTPases in nucleo-cytoplasmic protein transport (Zhang *et al.*, 2020a). In agreement with possible different transport mechanisms for FT and TFL1, residues critical for FT movement in Arabidopsis are not conserved in TFL1 (Endo *et al.*, 2018).

Competition of FT and TFL1 for chromatin bound FD

What is the mechanism by which different combined levels of FT- and TFL1-like activities modulate biological processes? Zhu et al. recently showed that endogenous TFL1 recruitment to the chromatin depends on FD (Zhu *et al.*, 2020) (Fig. 3C). Moreover, a photoperiod-mediated or estradiol-controlled inducible increase in the nuclear accumulation of FT resulted in FT recruitment to the genomic locations occupied by TFL1 and FD, accompanied by a simultaneous reduction of TFL1 binding to these sites (Zhu *et al.*, 2020). This competition was observed at all flowering and meristem identity targets tested and suggests that the antagonism between FT and TFL1 relies in large part on competition for FD mediated access to shared target loci (Zhu *et al.*, 2020) (Fig. 3C). Further support for this model comes from in vitro studies (Collani *et al.*, 2019; Kaneko-Suzuki *et al.*, 2018). This model provides a mechanistic explanation for the observed modulation of plant phase transitions and architecture by the relative dose of TFL1- and FT-like activities (Eshed and Lippman, 2019; Jiang *et al.*, 2013; Lifschitz *et al.*, 2014).

The above-mentioned model also fits with prior data indicating that TFL1 and FT proteins, which are very similar to each other in their primary amino acid sequence, can be converted into the opposite activity by single amino-acid changes, yielding FT-like proteins with TFL1-like activity and vice versa (Ahn *et al.*, 2006; Hanzawa *et al.*, 2005; Ho and Weigel, 2014). Of note, such FT mutations can be antimorphic or neomorphic (Blackman *et al.*, 2010; Ho and Weigel, 2014; Pin *et al.*, 2010). Neomorphic FT mutations likely confer ability to repress gene expression onto FT, as recently ascribed to TFL1 (Zhu *et al.*, 2020). Antimorphic mutations, by contrast, likely block activation of gene expression by FT by competing for FD binding (Kaneko-Suzuki *et al.*, 2018).

The model suggests a central role of the bZIP transcription factor FD in the FT/TFL1 competition. While FD activity is absolutely required for TFL1 recruitment to the chromatin (Zhu *et al.*, 2020), epistasis analyses using certain gain-of-FT and loss-of-FD function mutants suggests that other FT interaction partners may exist (Jaeger *et al.*, 2013; Romera-Branchat *et al.*, 2020). One candidate for such an interactor is FD PARALOGUE (FDP) (Romera-Branchat *et al.*, 2020). Although FDP bound similar target loci as FD (Fig. 5), CRISPR alleles of FDP were neither late flowering, nor enhanced the late flowering phenotype of *fd* mutants (Romera-Branchat *et al.*, 2020). However, when expressed in the FD domain, FDP partially rescued the *fd* mutant phenotype (Romera-Branchat *et al.*, 2020), suggesting that FDP is able to interact with FD partners such as FT or TFL1, albeit with lower affinity. Of note, FD levels drop in the shoot apex after the first flowers develop (Abe *et al.*, 2019), however FD is not completely absent as evidenced by FD ChIP-seq conducted several days after the first flowers develop (Romera-Branchat *et al.*, 2020). Likewise, two FD paralogs of hybrid aspen have different function; FD-like 1 (FDL1) but not FDL2 upregulates LAP1 together with FT1 to antagonize growth cessation in long-day photoperiod (Tylewicz *et al.*, 2015). Functionally, FDL2 is more divergent from FDL1 than FD is from FDP; since overexpression of *FDL2*, unlike overexpression of *FDL1*, did not delay growth cessation (Tylewicz *et al.*, 2015). Both *FDP* and *FD* overexpression triggers early flowering in Arabidopsis (Romera-Branchat *et al.*, 2020). In rice, OsFD1 and OsFD4 promote flowering in short days. Only *OsFD1* is expressed in leaves, while both *OsFD1* and *OsFD4* are expressed in the shoot apex (Cerise *et al.*, 2020). On the basis of DAP-seq, OsFD1 and OsFD4 bind similar DNA regions and both bZIP proteins are required for upregulation of MADS box transcription factors from the AP1/FUL clade (OsMADS14) or the SEPALLATA clade (OsMADS34) (Cerise *et al.*, 2020). Future studies will reveal whether additional transcription factors contribute to chromatin recruitment of PEBP proteins.

Conclusion

In summary, recent years have uncovered both nuclear and non-nuclear interaction partners for FT-like and TFL1-like PEBP proteins in diverse plant species. Genomic investigations from different laboratories have now linked the plant PEBP proteins, which control plant developmental transitions and architecture in response to seasonal cues, to diverse endogenous signalling pathways (Collani *et al.*, 2019; Goretti *et al.*, 2020; Romera-Branchat *et al.*, 2020; Zhu *et al.*, 2020). Further investigation of these pathways may enhance our understanding how these small proteins integrate information across the entire plant. These recent findings also bring us back to the beginnings of photoperiod studies, which had already invoked the critical importance of hormones and metabolites in mediating photoperiod responses (Bernier, 1988; Bernier *et al.*, 1993). Finally, recent and prior studies have uncovered a mechanism for the dose-dependent opposite activities PEBP proteins that relies on competition for FD bound to shared target loci (Zhu *et al.*, 2020).

Despite these and additional advances (Eshed and Lippman, 2019; Jin *et al.*, 2020; Kinoshita and Richter, 2020; Moraes *et al.*, 2019; Périlleux *et al.*, 2019) many open questions remain. One concerns the mechanism for the TFL1 and FT antagonism in the nucleus. Zhu et al showed that photoperiod upregulation of FT can remove TFL1 from shared target loci, while FD apparently remains bound (Zhu *et al.*, 2020) (Fig. 3C). We posit that additional components likely contribute to the replacement of TFL1 by FT. This is because overall FT protein levels in the inflorescence are lower than those of TFL1 (Conti and Bradley, 2007; Jaeger and Wigge, 2007) and FT - at least in *vitro* - has lower affinity for 14-3-3/FD (Collani *et al.*, 2019; Kaneko-Suzuki *et al.*, 2018).

A second question centers on the mechanism of action of TFL1 and FT. How do complexes that contain either of these two opposite acting PEBP proteins activate or repress transcription? Thus far, very few proteins have been identified that discriminate between TFL1 and FT (Ho and Weigel, 2014; Li *et al.*, 2019; Niwa *et al.*, 2013), and none of these explain transcriptional activation and transcription repression by the FT and TFL1 co-regulators, respectively.

Finally, it is remarkable how conserved the immediate early targets of TFL1-FD and FT-FD are in different species and in different developmental pathways (Kinoshita *et al.*, 2011; Shalit-Kaneh *et al.*, 2019; Tylewicz *et al.*, 2015; Zhu *et al.*, 2020). This begs the question how the gene regulatory networks downstream of PEBP proteins are rewired to control such diverse processes as seed development, growth cessation in trees, bud outgrowth and bolting, switch form branch to flower fate, bulb formation and tuberization. Some of the gene regulatory interaction uncovered during studies of the onset of flower formation might be operational in these different developmental pathways as well, as common to many of the above-mentioned phenomena is a switch from growth and carbon production to slow growth in carbon sinks (Lifschitz *et al.*, 2014; Martin-Fontecha *et al.*, 2018).

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Figure legends

Fig. 1. Identification of PEBP proteins as factors that interpret seasonal cues to promote flowering and that modulate additional life history traits in response to environmental signals. See text for details.

Fig. 2. Inflorescence architecture is shaped by alternative fates adopted by the axillary meristems.

(**A)** The architecture of the racemose inflorescence of Arabidopsis is shaped by the identity and positioning of the lateral organs, such as (i) sessile buds that can be induced to grow out as branches and (ii) indeterminate branches and (iii) determinate flowers. Inflorescence stages (I1, I2) based on (Ratcliffe *et al.*, 1998). (**B**) Alternative fates of axillary meristems, adoption of which depends on a combination of intrinsic and extrinsic cues. If an axillary branch meristem is converted to flower fate by photoperiod shift, the resulting flower is subtended by a cauline leaf (flower node) (Hempel *et al.*, 1998; Zhu *et al.*, 2020). (**C**) Terminal flowers form in *tfl1* mutant inflorescences because the FT-FD activation complex upregulates expression of the floral initiation and identity genes such as *LFY* and *AP1* in the shoot apex when TFL1 activity is absent (Lee *et al.*, 2019; Zhu *et al.*, 2020). Likewise, all branches are converted to flowers. (**D**) I1 phase: Inflorescence meristem and axillary branch meristems prior to formation of the first flower. Left: gTFL1-GFP protein accumulation in 42-day-old short-day-grown *tfl1* plants immediately prior to formation of the first flower. Right: Diagram: before FT starts to be produced in the rosette leaves, TFL1 accumulates in the axillary meristems but is barely detectable in the inflorescence meristem. (**E**) I2 phase: Inflorescence meristem and axillary branch meristems after formation of the first flower. FT protein moves into the inflorescence meristem where TFL1 accumulates to prevent inflorescence meristem termination by FT-FD. In the incipient flower meristems, FT-FD induce *LFY* and both LFY and FD-FT induce *AP1* (Wagner *et al.*, 1999; Winter *et al.*, 2011; Zhu *et al.*, 2020).

Fig. 3. Mechanism by which TFL1- and FT-like PEBP proteins antagonistically tune plant architecture. (**A**) Antagonistic roles of FT and TFL1. Top diagram: Inflorescence architecture. Right: Immediate flower formation (no branches; TFL1<<FT) reduces the total number of flowers and seeds per plant. It also shortens the time to seed set, which is advantageous in geographic regions with a short growing season. Middle: A slight delay in onset of flower formation (TFL1 = FT) allows production of branches of more flowers and seeds per plant. Time to seed set is delayed. Left: Extremely late onset of flower formation (TFL1>>FT) promotes strong branching and can prevent annual plants from completing their life cycle. Center diagram: The relative accumulation or balance of TFL1-like (purple triangle) and FT-like (blue triangle) PEBP proteins defines not only the inflorescence architecture (above) but also tuber formation (below) and other life history traits in response to seasonal cues (Eshed and Lippman, 2019; Jin *et al.*, 2020; Périlleux *et al.*, 2019; Zhu and Wagner, 2020). Bottom diagram: StSP6A (FT-like protein) and StTFL1 in potato promote and antagonize tuberization in inductive photoperiod (Navarro *et al.*, 2011; Zhang *et al.*, 2020b). (**B**) Direct TFL1-FD and FT-FD regulated target genes reveal a prominent role in cell

signaling. Immediate early TFL1-FD repressed and FT-FD activated targets include flowering time genes (*PRR7*, *CO* and *GI*) and floral identity genes (*LFY*, *AP1*, *FUL* and *LMI2*) as expected. They also include genes linked to phytohormone biosynthesis, signaling and response (auxin, abscisic acid, brassinosteroid, cytokinin, jasmonic acid and strigolactone) as well as genes linked to sugar signaling (*TPPs*) (Collani *et al.*, 2019; Goretti *et al.*, 2020; Romera-Branchat *et al.*, 2020; Zhu *et al.*, 2020). (**C**) Molecular mechanism for the antagonistic roles of TFL1- and FT-like proteins (Kaneko-Suzuki *et al.*, 2018; Zhu *et al.*, 2020). TFL1 (large purple circle) and FT (large orange circle) compete for access to shared targets via the FD bZIP transcription factor (orange triangle) and 14-3-3 (orange line). In Arabidopsis, FT and TFL1 are the 'central' activating and repressive PEBP co-regulators (Kim *et al.*, 2013). The final developmental readout depends not only on the balance of these two proteins but also on additional 'minor' TFL1-like co-repressors and FT-like co-activators. We propose that the 'minor' repressive PEBPs (which includes neo- or anti-morph FT as well as TFL1 paralogs) assist TFL1 in competing FT from genome associated FD. In Arabidopsis, these PEBPs include BROTHER OF FT AND TFL1 and ARABIDOPSIS THALIANA (small overlapping purple circles). Likewise, 'minor' activating PEBS help FT compete TFL1 from FD, such as TWIN SISTER OF FT (TSF) in Arabidopsis (small overlapping blue circle).

Fig. 4. New developmental roles for PEBP family proteins.

(**A**) Sessile/dormant bud versus outgrowing branch fate in Arabidopsis (left) or aspen (right). In response to inductive photoperiod, TFL1-FD opposition of branch outgrowth is overcome by FT-FD perhaps via upregulation of *SMXL* gene expression (Zhu *et al.*, 2020). Cold temperature and short-day mediate cessation of growth and onset of dormancy by TFL1 repressing, in opposition to FT2-FDL1, an aspen AP1/FUL/CAL homolog called *LAP1* (Azeez *et al.*, 2014; Maurya *et al.*, 2020a; Maurya *et al.*, 2020b). (**B**) The tuberigenic mobile FT homolog StSP6A synthesized in leaves promotes symplastic sugar unloading into potato tubers (Abelenda *et al.*, 2019). (**C**) A role for TFL1 and MFT in seed development and seed germination, respectively, is linked to abscisic acid pathway response regulator ABI5 (Vaistij *et al.*, 2018; Vaistij *et al.*, 2013; Xi *et al.*, 2010; Zhang *et al.*, 2020a). TFL1-RAN, StSP6A-StSWEET11 and FT2-FDL1 are direct protein-protein interactions (Abelenda *et al.*, 2019; Maurya *et al.*, 2020a; Zhang *et al.*, 2020a).

Fig. 5. Strong overlap between FD, FDP, and TFL1 ChIP-seq peaks and targets at different developmental stages.

(**A**) FD, TFL1 and FDP binding peak overlap based on ChIP-seq analyses from three research groups (yellow gradient) conducted at different developmental stages: 16-day-old long-day-grown plants (Collani *et al.*, 2019; Goretti *et al.*, 2020), 24-day-old long-day-grown plants (Romera-Branchat *et al.*, 2020) and 42-day-old short-day-grown plants (Zhu *et al.*, 2020). Data analysis as in (Zhu *et al.*, 2020). Row shows the peak overlap of the ChIP-seq experiments indicated on the left compared to all other ChIP-seq experiments (columns). The rows focus on ChIP-seq datasets reliant on endogenous promoter driven transgenes. Green color saturation indicates peak number overlap. For Zhu et al. datasets, two normalizations were conducted: one over mock samples (M; as in (Collani *et al.*, 2019; Goretti *et al.*, 2020) and one over inputs (I; as in (Romera-Branchat *et al.*, 2020)). (**B**) Strong overlap between FD, FDP and TFL1 regulated targets at different developmental stages. Proportional Venn diagram of FT-dependent photoperiod induced genes in 42-day-old short-day-grown plants (circle with diagonal green stripes (Zhu *et al.*, 2020)), TFL1-FD bound genes at the same stage (pink circle (Zhu *et al.*, 2020)) and uniquely FDP bound and regulated genes given gene expression changes in the *fdp* mutant in three-day-old long day grown plants (grey circle; identified in (Romera-Branchat *et al.*, 2020))

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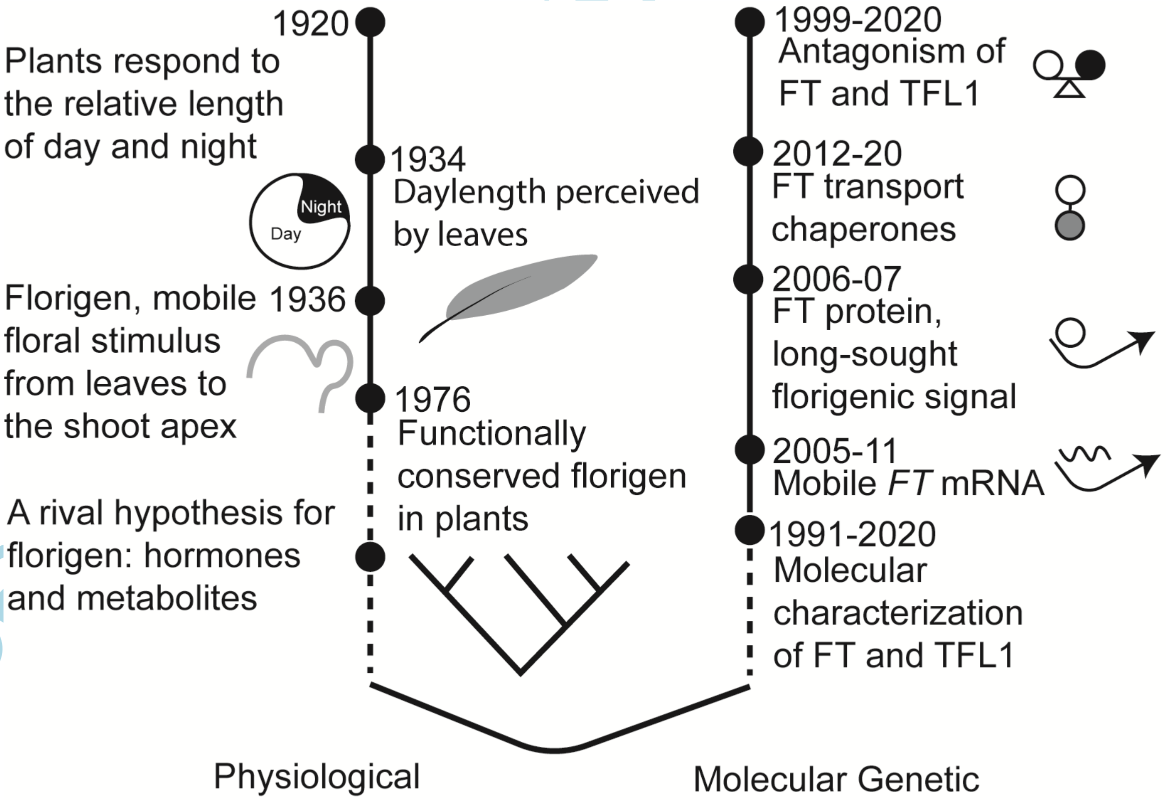


Fig. 1 Identification of PEBP proteins as factors that interpret seasonal cues to promote flowering and modulate additional life history traits in response to environmental signals.
See text for details.

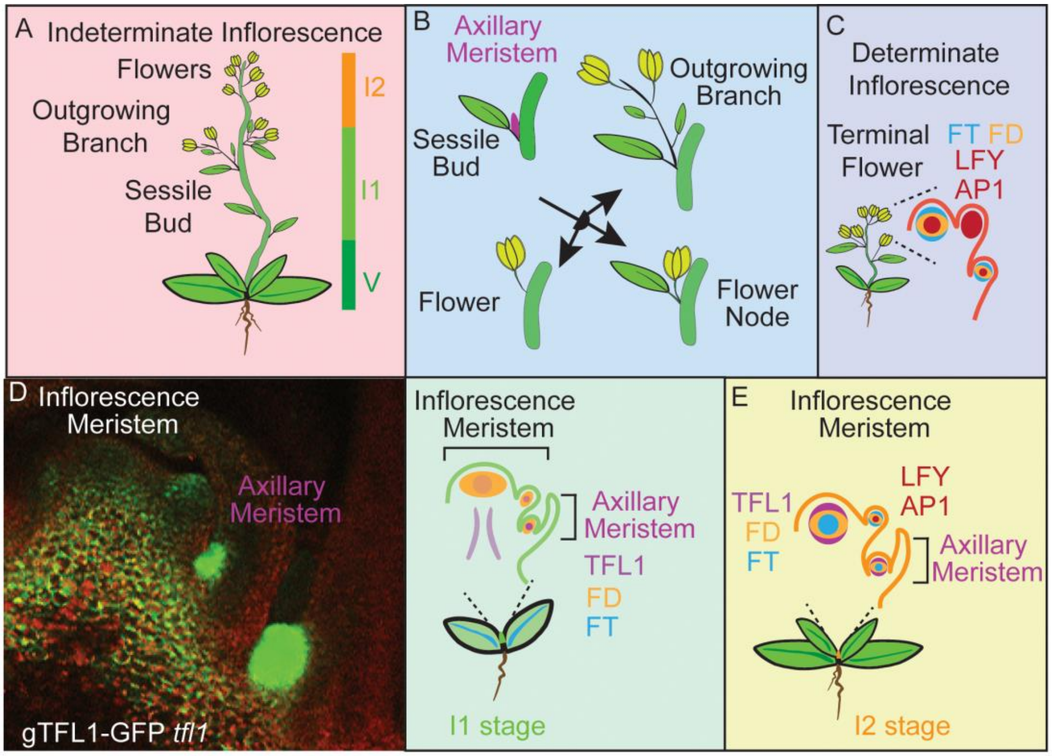


Fig. 2. Inflorescence architecture is shaped by alternative fates adopted by the axillary meristems.

(**A**) The architecture of the racemose inflorescence of Arabidopsis is shaped by the identity and positioning of the lateral organs, such as (i) sessile buds that can be induced to grow out as branches and (ii) indeterminate branches and (iii) determinate flowers. Inflorescence stages (I1, I2) based on (Ratcliffe *et al.*, 1998). (**B**) Alternative fates of axillary meristems, adoption of which depends on a combination of intrinsic and extrinsic cues. If an axillary branch meristem is converted to flower fate by photoperiod shift, the resulting flower is subtended by a cauline leaf (flower node) (Hempel *et al.*, 1998; Zhu *et al.*, 2020). (**C**) Terminal flowers form in *tfl1* mutant inflorescences because the FT-FD activation complex upregulates expression of the floral initiation and identity genes such as *LFY* and *AP1* in the shoot apex when TFL1 activity is absent (Lee *et al.*, 2019; Zhu *et al.*, 2020). Likewise, all branches are converted to flowers. (**D**) I1 phase: Inflorescence meristem and axillary branch meristems prior to formation of the first flower. Left: gTFL1-GFP protein accumulation in 42-day-old short-day-grown *tfl1* plants immediately prior to formation of the first flower. Right: Diagram: before FT starts to be produced in the rosette leaves, TFL1 accumulates in the axillary meristems but is barely detectable in the inflorescence meristem. (**E**) I2 phase: Inflorescence meristem and axillary branch meristems after formation of the first flower. FT protein moves into the inflorescence meristem where TFL1 accumulates to prevent inflorescence meristem termination by FT-FD. In the incipient flower meristems, FT-FD induce *LFY* and both LFY and FD-FT induce *AP1* (Wagner *et al.*, 1999; Winter *et al.*, 2011; Zhu *et al.*, 2020).

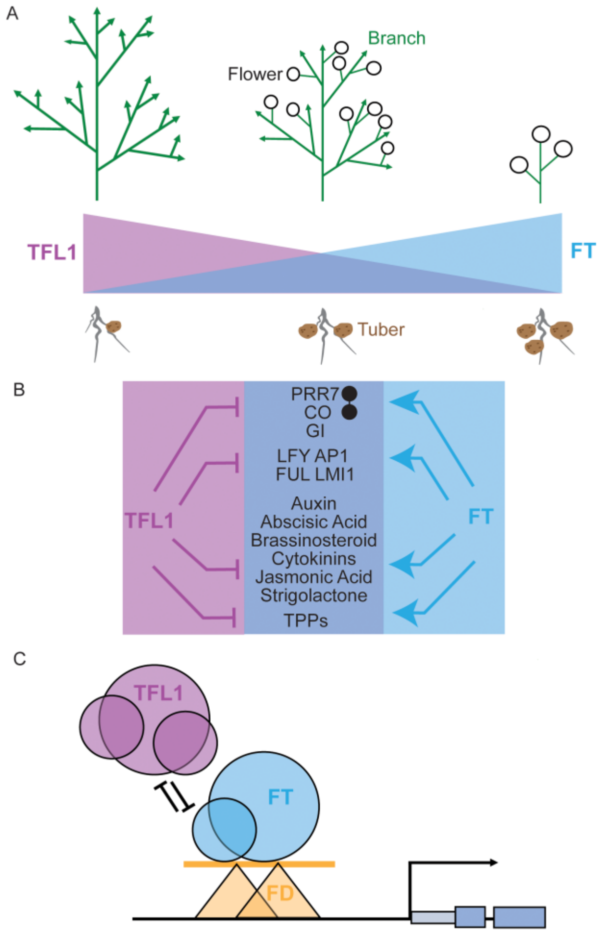


Fig. 3. Mechanism by which TFL1- and FT-like PEBP proteins antagonistically tune plant architecture. (**A**) Antagonistic roles of FT and TFL1. Top diagram: Inflorescence architecture. Right: Immediate flower formation (no branches; TFL1<<FT) reduces the total number of flowers and seeds per plant. It also shortens the time to seed set, which is advantageous in geographic regions with a short growing season. Middle: A slight delay in onset of flower formation (TFL1 = FT) allows production of branches of more flowers and seeds per plant. Time to seed set is delayed. Left: Extremely late onset of flower formation (TFL1>>FT) promotes strong branching and can prevent annual plants from completing their life cycle. Center diagram: The relative accumulation or balance of TFL1-like (purple triangle) and FT-like (blue triangle) PEBP proteins defines not only the inflorescence architecture (above) but also tuber formation (below) and other life history traits in response to seasonal cues (Eshed and Lippman, 2019; Jin *et al.*, 2020; Périlleux *et al.*, 2019; Zhu and Wagner, 2020). Bottom diagram: StSP6A (FT-like protein) and StTFL1 in potato promote and antagonize tuberization in inductive photoperiod (Navarro *et al.*, 2011; Zhang *et al.*, 2020b). (**B**) Direct TFL1-FD and FT-FD regulated target genes reveal a prominent role in cell signaling. Immediate early TFL1-FD repressed and FT-FD activated targets include flowering time genes (*PRR7*, *CO* and *GI*) and floral identity genes (*LFY*, *AP1*, *FUL* and *LMI2*) as expected. They also include genes linked to phytohormone biosynthesis, signaling and response (auxin, abscisic acid, brassinosteroid, cytokinin, jasmonic acid and strigolactone) as well as genes linked to sugar signaling (*TPPs*) (Collani *et al.*, 2019; Goretti *et al.*, 2020; Romera-Branchat *et al.*, 2020; Zhu *et al.*, 2020). (**C**) Molecular mechanism for the antagonistic roles of TFL1- and FT-like proteins (Kaneko-Suzuki *et al.*, 2018; Zhu *et al.*, 2020). TFL1 (large purple circle) and FT (large orange circle) compete for access to shared targets via the FD bZIP transcription factor (orange triangle) and 14-3-3 (orange line). In Arabidopsis, FT and TFL1 are the 'central' activating and repressive PEBP co-regulators (Kim *et al.*, 2013). The final developmental readout depends not only on the balance of these two proteins but also on additional 'minor' TFL1-like co-repressors and FT-like co-activators. We propose that the 'minor' repressive PEBPs (which includes neo- or anti-morph FT as well as TFL1 paralogs) assist TFL1 in competing FT from genome associated FD. In Arabidopsis, these PEBPs include BROTHER OF FT AND TFL1 and ARABIDOPSIS THALIANA (small overlapping purple circles). Likewise, 'minor' activating PEBS help FT compete TFL1 from FD, such as TWIN SISTER OF FT (TSF) in Arabidopsis (small overlapping blue circle).

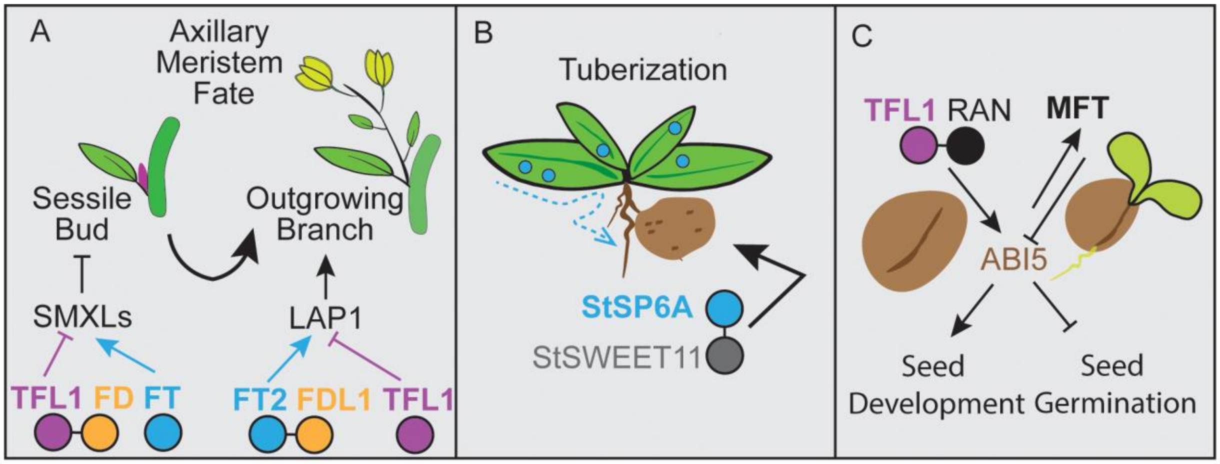
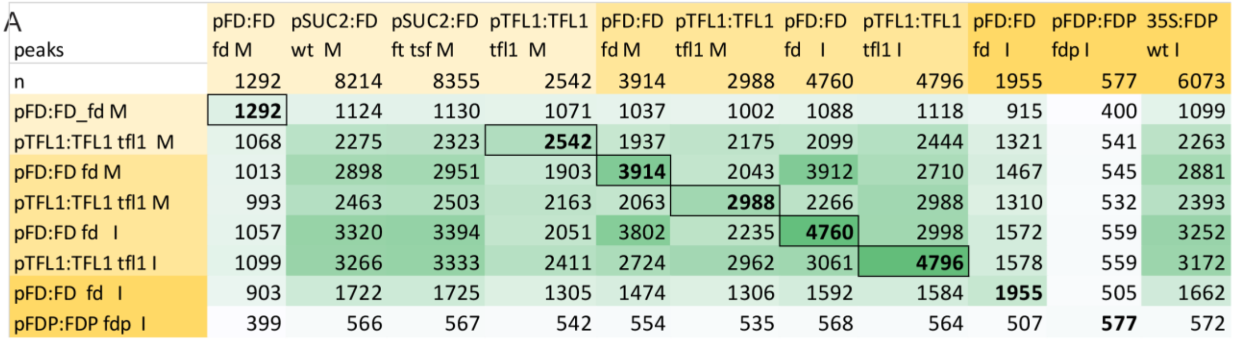


Fig. 4. New developmental roles for PEBP family proteins.
(**A**) Sessile/dormant bud versus outgrowing branch fate in Arabidopsis (left) or aspen (right). In response to inductive photoperiod, TFL1-FD opposition of branch outgrowth is overcome by FT-FD perhaps via upregulation of *SMXL* gene expression (Zhu *et al.*, 2020). Cold temperature and short-day mediate cessation of growth and onset of dormancy by TFL1 repressing, in opposition to FT2-FDL1, an aspen AP1/FUL/CAL homolog called *LAP1* (Azeez *et al.*, 2014; Maurya *et al.*, 2020a; Maurya *et al.*, 2020b). (**B**) The tuberigenic mobile FT homolog StSP6A synthesized in leaves promotes symplastic sugar unloading into potato tubers (Abelenda *et al.*, 2019). (**C**) A role for TFL1 and MFT in seed development and seed germination, respectively, is linked to abscisic acid pathway response regulator ABI5 (Vaistij *et al.*, 2018; Vaistij *et al.*, 2013; Xi *et al.*, 2010; Zhang *et al.*, 2020a). TFL1-RAN, StSP6A-StSWEET11 and FT2-FDL1 are direct protein-protein interactions (Abelenda *et al.*, 2019; Maurya *et al.*, 2020a; Zhang *et al.*, 2020a).



A peaks	pFD:FD fd M	pSUC2:FD wt M	pSUC2:FD ft tsf M	pTFL1:TFL1 tfl1 M	pFD:FD fd M	pTFL1:TFL1 tfl1 M	pFD:FD fd I	pTFL1:TFL1 tfl1 I	pFD:FD fd I	pFDP:FDP fdp I	35S:FDP wt I
n	1292	8214	8355	2542	3914	2988	4760	4796	1955	577	6073
pFD:FD_fd M	**1292**	1124	1130	1071	1037	1002	1088	1118	915	400	1099
pTFL1:TFL1 tfl1 M	1068	2275	2323	**2542**	1937	2175	2099	2444	1321	541	2263
pFD:FD fd M	1013	2898	2951	1903	**3914**	2043	3912	2710	1467	545	2881
pTFL1:TFL1 tfl1 M	993	2463	2503	2163	2063	**2988**	2266	2988	1310	532	2393
pFD:FD fd I	1057	3320	3394	2051	3802	2235	**4760**	2998	1572	559	3252
pTFL1:TFL1 tfl1 I	1099	3266	3333	2411	2724	2962	3061	**4796**	1578	559	3172
pFD:FD fd I	903	1722	1725	1305	1474	1306	1592	1584	**1955**	505	1662
pFDP:FDP fdp I	399	566	567	542	554	535	568	564	507	**577**	572

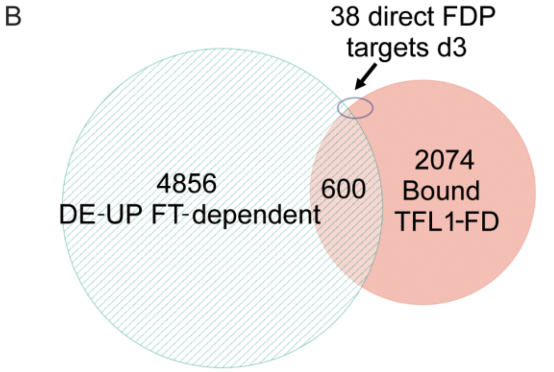


Fig. 5. Strong overlap between FD, FDP, and TFL1 ChIP-seq peaks and targets at different developmental stages.

(**A**) FD, TFL1 and FDP binding peak overlap based on ChIP-seq analyses from three research groups (yellow gradient) conducted at different developmental stages: 16-day-old long-day-grown plants (Collani *et al.*, 2019; Goretti *et al.*, 2020), 24-day-old long-day-grown plants (Romera-Branchat *et al.*, 2020) and 42-day-old short-day-grown plants (Zhu *et al.*, 2020). Data analysis as in (Zhu *et al.*, 2020). Row shows the peak overlap of the ChIP-seq experiments indicated on the left compared to all other ChIP-seq experiments (columns). The rows focus on ChIP-seq datasets reliant on endogenous promoter driven transgenes. Green color saturation indicates peak number overlap. For Zhu et al. datasets, two normalizations were conducted: one over mock samples (M; as in (Collani *et al.*, 2019; Goretti *et al.*, 2020) and one over inputs (I; as in (Romera-Branchat *et al.*, 2020)). (**B**) Strong overlap between FD, FDP and TFL1 regulated targets at different developmental stages. Proportional Venn diagram of FT-dependent photoperiod induced genes in 42-day-old short-day-grown plants (circle with diagonal green stripes (Zhu *et al.*, 2020)), TFL1-FD bound genes at the same stage (pink circle (Zhu *et al.*, 2020)) and uniquely FDP bound and regulated genes given gene expression changes in the *fdp* mutant in three-day-old long day grown plants (grey circle; identified in (Romera-Branchat *et al.*, 2020))