Journal of Experimental Botany, Vol. 65, No. 7, pp. 1699–1712, 2014
doi:10.1093/jxb/ert413 Advance Access publication 24 December, 2013

Journal of Experimental Botany
www.jxb.oxfordjournals.org

REVIEW PAPER

The embryonic shoot: a lifeline through winter

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Received 25 July 2013; Revised 18 October 2013; Accepted 11 November 2013

Abstract

The tiny vascular axis of the embryo emerges post-embryonically as an elaborate and critical infrastructure, pervading the entire plant system. Its expansive nature is especially impressive in trees, where growth and development continue for extended periods. While the shoot apical meristem (SAM) orchestrates primary morphogenesis, the vascular system is mapped out in its wake in the provascular cylinder, situated just below the emerging leaf primordia and surrounding the rib meristem. Formation of leaf primordia and provascular tissues is incompatible with the harsh conditions of winter. Deciduous trees of boreal and temperate climates therefore enter a survival mode at the end of the season. However, to be competitive, they need to maximize their growth period while avoiding cellular frost damage. Trees achieve this by monitoring photoperiod, and by timely implementation of a survival strategy that schedules downstream events, including growth cessation, terminal bud formation, dormancy assumption, acquisition of freezing tolerance, and shedding of leaves. Of central importance are buds, which contain an embryonic shoot that allows shoot development and elongation in spring. The genetic and molecular processes that drive the cycle in synchrony with the seasons are largely elusive. Here, we review what is known about the signals and signal conduits that are involved, the processes that are initiated, and the developmental transitions that ensue in a terminal bud. We propose that addressing dormancy as a property of the SAM and the bud as a unique shoot type will facilitate our understanding of winter dormancy.

Key words: CENTRORADIALIS-LIKE1, deciduous perennial, dormancy cycle, FLOWERING LOCUS T, gibberellic acid, GH17 family proteins, lipid body, poplar, rib meristem, shoot apical meristem, terminal bud.

Introduction

Growth and development of plants are buffered against short-term fluctuations in the environment. Importantly, however, plants have evolved sensory-response mechanisms that register longer-lasting alterations in specific environmental cues to schedule developmental transitions and to reprioritize physiology in anticipation of seasonal changes. Particularly important cues are temperature and photoperiod. Of these, photoperiod provides the most reliable information, as it changes incrementally and in a completely predictable fashion. Not surprisingly, many different plant systems depend on photoperiod to schedule crucial developmental events at the apex, like the transition to flowering, and the transition to terminal bud formation and dormancy in perennials (Vince-Prue, 1994; Yanovsky and Kay, 2002; Arora *et al.*, 2003; Cooke *et al.*, 2012). Some developmental transitions can also be triggered by low temperatures, but the site of perception is not the same. While photoperiod is perceived at the leaves, chilling temperatures are perceived directly at the apex (Coville, 1920; Metzger, 1988; Arora *et al.*, 2003). For example, *Arabidopsis* and its perennial relative *Arabis alpina* can flower as a result of the perception of low temperature at the shoot apical meristem (SAM), a process referred to as vernalization (Finnegan *et al.*, 1998; Bastow *et al.*, 2004; De Lucia *et al.*, 2008; Wang *et al.*, 2011).

Abbreviations: ABA, abscisic acid; DAC, dormancy sphincter complex; ES, embryonic shoot; GA, gibberellic acid; GH17, glycosyl-hydrolase 17; GPI, glycosylphosphatidylinositol; LB, lipid body; LD, long day; PD, plasmodesmata; RM, rib meristem; RZ, rib zone; SAM, shoot apical meristem; SD, short day; TAG, triacylglyceride; wt, wild type.

Similarly, release from dormancy in deciduous perennials requires chilling of the SAM inside the winter bud (Vegis, 1964; Rinne *et al.*, 2001; Arora *et al.*, 2003; Horvath *et al.*, 2003; Rinne and van der Schoot, 2003; Rohde and Bhalerao, 2007).

In this review, we describe what is currently known about the processes that constitute the seasonal activity–dormancy cycle in deciduous woody perennials, with emphasis on juvenile hybrid poplar (Rinne *et al.*, 2010; van der Schoot and Rinne, 2011). Herein, we regard the terminal bud as a unique shoot type that can resume a normal mode of growth and development after having passed through dormancy and quiescence. This so-called dormancy cycle is triggered by photoperiod- and temperature-driven alterations in vascular leaf-to-apex signalling and results in a series of events that unfold sequentially and in parallel. Visible changes include the transformation of leaf primordia into bud scales (cataphylls), the development of an embryonic shoot (ES), the assumption of a dormant and freezing tolerant state, the abscission of leaves, the release from dormancy by chilling, the opening of bud scales in spring, and the emergence of a morphogenetically active and elongating shoot system.

To understand the complex choreography of this survival strategy, it will be necessary to identify relevant signals, assess how their production is regulated, map the production and the responder sites, and establish how the conduits are regulated that channel the signals to their targets. Interestingly, *Arabidopsis* responds to a lengthening of the photoperiod by switching from a rosette to a caulescent growth habit, whereas caulescent perennials respond to a shortening of the photoperiod with the production of a rosette-like ES inside a bud. The extensive knowledge about the molecular mechanisms that underlie photoperiodic signalling in *Arabidopsis* may therefore provide an important reference framework for the investigation of photoperiod-regulated transitions in perennials.

Photoperiod-induced floral transition

In *Arabidopsis*, a facultative long-day (LD) plant, the perception of LD by the leaves triggers the formation of an elongating inflorescence that produces flowers at the peripheral part of the inflorescence meristem (Yanovsky and Kay, 2002, 2003). The classic external coincidence model, attributed to Bünning (1936), proposed the existence of a circadian oscillator in the leaves with a light-insensitive day phase and a light-inducible night phase. Coincidence of an extended light period with the photosensitive part of the oscillator or clock triggers a photoperiodic response (Mouradov *et al.*, 2002; Yanovsky and Kay, 2002, 2003; Más, 2008). Inputs to the clock include phytochromes and cryptochromes, the effects of which are mediated by clock-regulated genes like *EARLY FLOWERING 3* (McWatters *et al.*, 2000). *CONSTANS* (*CO*) is a major output gene of the clock. CO protein is reduced in the morning of a LD by phytochrome B (PHYB), but stabilized at the end of a LD by phytochrome A (PHYA) and cryptochromes (Hayama and Coupland, 2004). As a consequence, only LDs that are sufficiently long yield enough CO to effectively upregulate the gene *FLOWERING LOCUS T* (*FT*) in the companion cells (Kardailsky *et al.*, 1999; Kobayashi *et al.*, 1999; Samach *et al.*, 2000; Yanovsky and Kay, 2002; Takada and Goto, 2003; Ayre and Turgeon, 2004). FT is a small (23 kDa) graft-transmissible signalling peptide that moves via plasmodesmal pore complexes into the sieve tubes, a process that requires the endoplasmic reticulum membrane protein FT-INTERACTING PROTEIN1 (FTIP1) (Liu *et al.*, 2012). In the phloem stream, FT reaches the sinks, including the shoot apex. In *Arabidopsis*, as well as in rice, green fluorescent protein-tagged FT/Hd3a is delivered to the rib meristem (RM) but appears not to enter the SAM itself in this form (Corbesier *et al.*, 2007; Tamaki *et al.*, 2007). FT interacts with the basic leucine zipper (bZIP) protein FD in a complex with 14-3-3 proteins at the RM to promote floral transition at the SAM through transcriptional activation of the floral meristem identity gene *APETALA1* (*AP1*) (Abe *et al.*, 2005; Wigge *et al.*, 2005). Coincident with the arrival of FT at the RM, the gene *TERMINAL FLOWER 1* (*TFL1*) is upregulated in a patch below the SAM (Bradley *et al.*, 1997), an area that corresponds to the RM. The TFL1 protein is very similar to FT, has shared binding motifs, and can also bind FD (Yeung *et al.*, 1999; Banfield and Brady, 2000). Nonetheless, TFL1 is regarded as a floral suppressor, as mutants flower early (Kobayashi *et al.*, 1999). As the TFL1 protein can bind FD in a complex with 14-3-3 receptor proteins, like FT, it may compete with FT for access to FD and delay floral transition (Bradley *et al.*, 1997; Hanano and Goto, 2011; Jaeger *et al.*, 2013). In inflorescence branches delay of flowering is due to the expression of the gene *BRANCHED1* (*BRC1*), which is specific for axillary buds (Niwa *et al.*, 2013). The BRC1 protein directly binds FT (as well as its homologue TSF) without the assistance of 14-3-3-proteins, rendering it ineffective (Niwa *et al.*, 2013). TFL1, just like FT, moves symplasmically via plasmodesmata (PD) in the post-phloem tissues. In the inflorescence meristem, TFL1 moves from the RM into the central zone of the SAM to safeguard resident cells against specification by the floral identity genes *LEAFY* (*LFY*) and *AP1* (Conti and Bradley, 2007; Jaeger *et al.*, 2013). The emerging picture is that, in *Arabidopsis*, TFL1 functions together with FT and FD in a signalling hub, integrating various signals to regulate *AP1* expression and restricting it to the peripheral SAM parts (Jaeger *et al.*, 2013).

Although photoperiodic responses are widespread (Vince-Prue, 1994), there might be considerable variation in the precise molecular mechanisms that operate among plants, or in the way they are implemented to serve a specific developmental goal. It seems plausible, however, that photoperiod-sensitive detection and transduction mechanisms resembling those of *Arabidopsis* and rice might have been adopted by perennial species to regulate developmental transitions in different seasonal contexts (Horvath *et al.*, 2003; Rinne and van der Schoot, 2003; Böhlenius *et al.*, 2006; Chao *et al.*, 2007; Rohde and Bhalerao, 2007; Ruonala *et al.*, 2008; Horvath, 2009; Hsu *et al.*, 2011; Cooke *et al.*, 2012; Mimida *et al.*, 2013).

Photoperiod-induced perennial bud formation

The transition to flowering in the rosette plant *Arabidopsis* and the transition to dormancy in deciduous trees are significantly different events. However, the similarity in their regulation and signalling may stem from the fact that both are survival strategies that follow the basic rules of photoperiodism. In *Arabidopsis*, the transition serves the survival in the form of seeds that are left for the next season, while for trees in temperate and boreal climates the apical transition creates a life line through an inhospitable winter. The events that prepare the perennial apex for survival are largely elusive, but significant changes take place in the various tissues that eventually constitute the terminal bud. To emphasize the importance of this, we first describe the morphogenetic events at the apex and explain why they are important for survival.

The vascular system of woody perennials, like that of herbaceous species, is initiated and mapped out at the shoot apex in a meristematic region of incipient vascular tissue just below the peripheral zone of the SAM. For descriptive reasons, this region has been called the residual meristem (Esau, 1965; Larson, 1975) or provascular tissue (Steeves and Sussex, 1989). The topographical organization of the emerging vasculature represents a small replica of the mature system (Esau, 1965). It is simply scaled up in the subapical region and elaborated further during phytomer development. Expansion, in caulescent plants, is mostly due to internode elongation, which results from cell division and cell enlargement in both the provascular tissue ring and in the enclosed, column-like area. The latter is composed of the RM and the subtending rib zone (RZ) (Sachs *et al.*, 1960; Romberger, 1963). These primary morphogenetic processes are highly vulnerable to subzero temperatures and therefore perennials have evolved a strategy to arrest development and protect their morphogenetic tissues during overwintering.

The formation of an overwintering terminal bud is an intriguing event, as under short-day (SD) conditions, the apex ceases its normal morphogenetic behaviour and resorts to morphogenetic processes that normally occur under LDs in axillary buds. Thus, the terminal bud morphologically closely mimics the axillary buds by producing an extremely dwarfed shoot system within the confines of the bud scales (Romberger, 1963; Fig. 1). This dwarfed shoot system, also referred to as an ES, pre-formed shoot, or primordial shoot (Romberger, 1963; Steeves and Sussex, 1989; Rohde *et al.*, 2002; Yuceer *et al.*, 2003; Rinne *et al.*, 2011), is an unextended and only partly developed shoot system with stipule pairs as bud scales at its base and at its summit a SAM that produced it (Romberger, 1963). Although the ES is morphologically undeveloped, the axils of the older embryonic leaves already contain axillary meristems (Romberger, 1963; Yuceer *et al.*, 2003). The SAM forms the ES by continuing morphogenesis for a restricted time period, while internode and leaf expansion are postponed until spring. In *Populus*, the ES may produce a restricted number of embryonic leaves and leaf primordia (Rohde *et al.*, 2002), before the SAM of the ES arrests itself in a dormant state and the bud acquires freezing tolerance (Rinne *et al.*, 2001). Although dormancy at the SAM is incompatible with the production of new stem-leaf units (Rinne and van der Schoot, 1998; Rinne *et al.*, 2001; Ruonala *et al.*, 2008), existing embryonic leaves may still develop further to fill the entire bud space.

Preservation of the embryonic leaves and dwarfed vasculature within the terminal bud are necessary to allow rapid growth and development once favourable environmental conditions return. This dwarfed vasculature of the ES is of crucial importance for bridging the gap between two sequential growth seasons, as it provides the only vascular connection

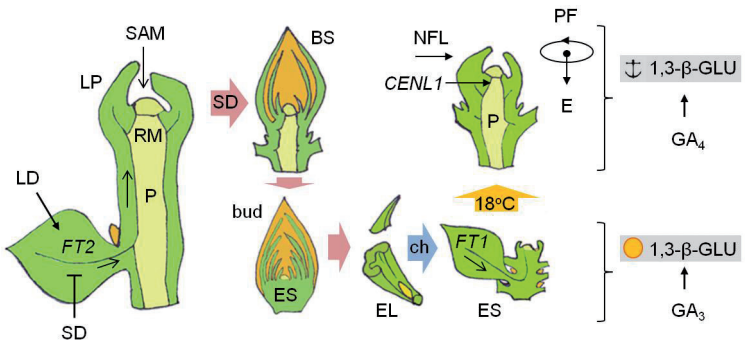

Fig. 1. Sequence of main morphological and signalling events during the dormancy cycle. Perception of LDs is accompanied by a basic level of *FT2* expression in leaves, and probably by FT2 transport to the RM subjacent to the SAM. In poplar, a symplasmic boundary separates RM and SAM. SDs rapidly downregulate *FT2* to undetectable levels, resulting in the initiation of bud scales (BS), arrest of cell divisions in the RM and suppressed internode elongation. In about 6 weeks, a mature terminal bud is formed with an ES inside. The embryonic leaves (ELs) of the ES are folded or rolled up along the longitudinal axis, and the older ELs already possess an axillary meristem. Prolonged chilling (ch) hyperinduces *FT1* in the ES, presumably in the ELs, and upregulates GA_3-induced LB-associated 1,3-β-glucanases (GLU; orange circle). This releases dormancy, restores PD conductivity, and allows symplasmic movement of FT1 from the ELs to the RM. Return of growth-supporting temperatures (18 °C) induces growth-related and GA_4-induced glycosylphosphatidylinositol-anchored 1,3-β-glucanases (GLU; anchor symbol), upregulates *CENL1* at the RM, and promotes the production of novel (neo-) formed leaves (NFL) by the SAM. PF, leaf primordia formation; E, internode elongation; LP, leaf primordia; P, pith.

between the emerging shoot and the supply lines of the mature shoot system. In a literal sense, this little-investigated vasculature is an umbilical that is crucial for shoot emergence during bud burst.

In order to be competitive when the growing season is short, trees are faced with the challenge of maximizing the growing period and at the same time avoiding the risk of damage to their meristems by imminent freezing temperatures (Frewen *et al.*, 2000; Rinne *et al.*, 2010; van der Schoot and Rinne, 2011). Deciduous trees time these crucial events by monitoring the shortening of the photoperiod, the most reliable parameter for seasonal progression (Howe *et al.*, 1995; Rinne and van der Schoot, 1998; Rinne *et al.*, 2001, 2010; Welling *et al.*, 2002; Böhlenius *et al.*, 2006; Rohde and Bhalerao, 2007). Once a critical daylength is perceived at the shoot apex, the photoperiod-guided timing mechanism initiates a series of morphogenetic processes, culminating in the formation of a terminal bud (Horvath *et al.*, 2003; Rinne and van der Schoot, 2003; Rohde and Bhalerao, 2007; Cooke *et al.*, 2012; Figs 1 and 2). While in *Arabidopsis* bolting and flowering is due to a clock-regulated upregulation of *FT* in the leaves (see above) and is correlated with the upregulation of *TFL1* in the RM zone (Bradley *et al.*, 1997), in trees the situation is more complex. An increase in *FT1* expression can similarly regulate flowering in mature trees, as shown in field-grown trees, and overexpression of *FT1* can induce flowering even in juvenile trees (Böhlenius *et al.*, 2006; Hsu *et al.*, 2011). In contrast, reduced expression of *FT* functions in growth cessation and bud formation at the end of the growing season (Böhlenius *et al.*, 2006). The perception of SD leads to a rapid clock-mediated downregulation of *FT* in the leaves (Böhlenius *et al.*, 2006; Hsu *et al.*, 2006; Ruonala *et al.*, 2008). In its wake, the poplar orthologue of TFL1, *CENTRORADIALIS-LIKE1* (*CENL1*), is upregulated in the RM (Ruonala *et al.*, 2008), a domain comparable to that of *TFL1* in *Arabidopsis* flowering (Conti and Bradley, 2007).

Overexpression of *Avena sativa PHYA* (*P35S*:*As PHYA*) in poplar results in a diminished stem elongation while the plastochron remains unchanged under LDs (Ruonala *et al.*, 2008). As PHYA is able to protect CO from degradation (Hayama and Coupland, 2004), these overexpressors have lost the ability to respond to SDs (Olsen *et al.*, 1997; Welling *et al.*, 2002) and instead show an enhanced stem elongation under SDs (Ruonala *et al.*, 2008). The loss of response may relate to the fact that *FT2* expression is only transiently lowered in *PHYA* overexpressors under SDs and rapidly recovered to a level somewhat higher than in leaves of wild-type (wt) poplars exposed to LDs (Ruonala *et al.*, 2008). The fact that SAM activity remains unchanged under SDs as judged from the leaf plastochron, while elongation is enhanced in *PHYA* overexpressors, indicates that the morphogenetic units of RM and SAM can be regulated independently (Ruonala *et al.*, 2008; van der Schoot and Rinne, 2011).

Heterograft systems have shown that overexpression of *PHYA* in the stock is insufficient to prevent the formation of dormant terminal buds in the wt scion, while the reverse grafts, with a wt stock and *PHYA* scion, were able to form buds without being able to enter dormancy as they repeatedly flushed (Ruonala *et al.*, 2008). This suggests that, for the production of a dormant terminal bud, SD perception may be required in the leaves as well as the apex. Indeed, literature reports have suggested that apices can respond directly to photoperiod (Wareing, 1956; Romberger, 1963), supporting the idea that not only temperature but also light responses may occur in apices.

Again, these observations indicate that the RM may respond to leaf-derived and endogenously generated signals to redirect morphogenetic events at the apex (van der Schoot and Rinne, 2011). In *Arabidopsis*, the RM is inactive during SDs, as reflected by a rosette form, but exposure to LDs activates the RM and initiates the formation of an elongating inflorescence stem. Similarly, in poplar, SD exposure deactivates the RM (Fig. 2), whereas under LDs the RM is active in extending the shoot. Indeed, it has been recognized in classic studies that important problems in the developmental morphology of plants are fundamentally only different aspects of the problem of how cell division and elongation are controlled in the subapical meristem (Romberger, 1963) and the area that includes the RM. Despite this, dormancy cannot be explained as resulting from deactivation of the RM alone, as axillary buds that develop under LD enter quiescence but not dormancy.

The role of the RM

Shoot-derived vascular signals destined for the apex are supplied by the source leaves via the phloem. At close proximity to the apex, they exit the sieve tubes and then travel, presumably via PD, to their destination at the RM. The RM may act as a relay station that shuttles signal or signal complexes to various destinations at the shoot apex, like leaf primordia and SAM (van der Schoot and Rinne, 2011). This might require gating of PD between the RM and the overlying SAM. Indeed, microinjection studies have shown that small fluorescent dyes do not immediately transit from the RM to the SAM (Ruonala *et al.*, 2008), suggesting that there is a symplasmic boundary between the RM and the SAM (Fig. 1).

The question is, what are the signals delivered to the RM that promote dormancy transition, and in what form and how are they relayed to their final destinations? Furthermore, which genes are involved in the regulation of these events? Under LDs, the leaf-born signalling peptide FT2 is likely to be delivered to the RM of poplar in a manner analogous to FT1 delivery in LD-exposed *Arabidopsis* (Corbesier *et al.*, 2007). In contrast, under SDs, *FT2* is completely downregulated and RM activity is arrested by either lack of FT2 import or by the arrival of as-yet-uncharacterized leaf signals (van der Schoot and Rinne, 2011).

Remarkably, the *As PHYA* mRNA of the *PHYA*-overexpressing poplar (see above) was localized among others to the RM/RZ while it was absent from the SAM, thereby strongly influencing RM activity and elongation growth (Ruonala *et al.*, 2008). *PHYA* overexpression modulated the expression levels of the *CENL1* gene, an orthologue of the *TFL1* gene, which selectively accumulates in the RM area in both poplar and *Arabidopsis* (Conti and Bradley, 2007;

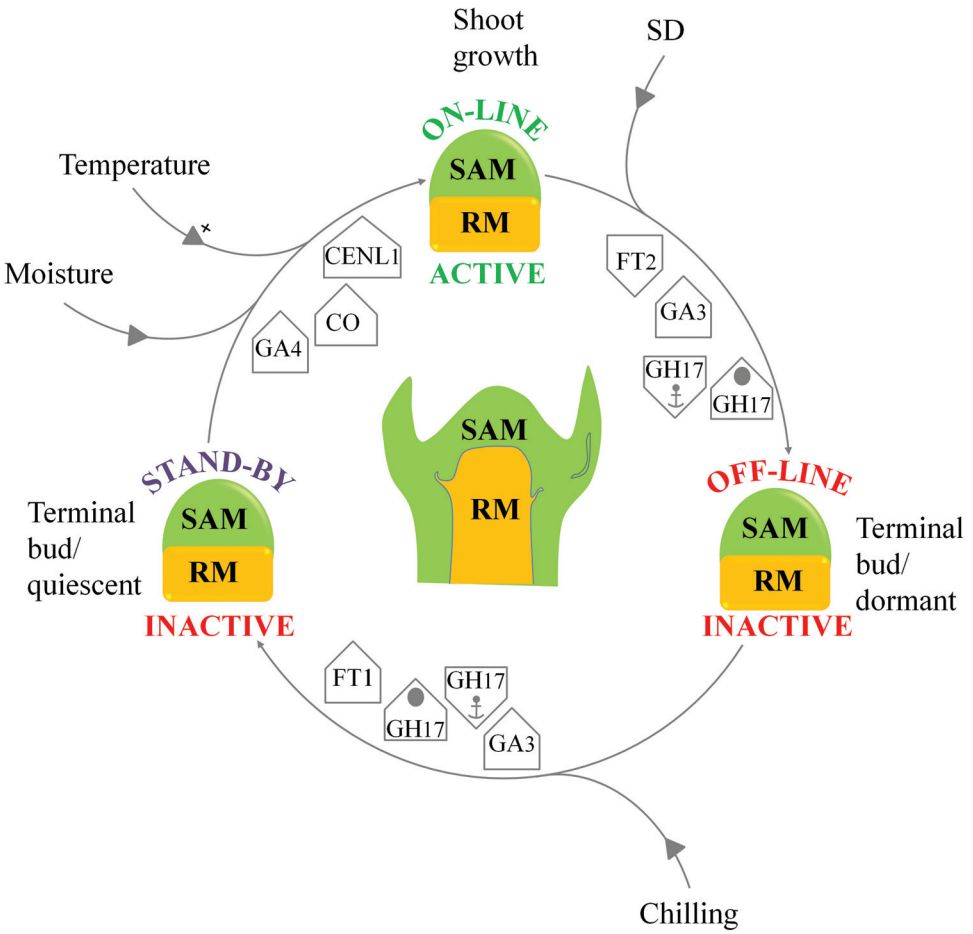

Fig. 2. Simplified heuristic model of bud formation and dormancy cycling at the shoot apex of hybrid poplar. The apex is composed of two distinct morphogenetic units, the SAM and the RM, which respond individually to environmental signals. The SAM passes through three distinct states of intrinsic symplasmic communication dubbed ON-LINE, OFF-LINE, and STAND-BY, each with its characteristic responsiveness to specific environmental cues. The ON-LINE SAM and active RM cooperatively produce phytomers in response to mobile signals that arrive via the post-phloem PD network. During SD exposure, the RM is deactivated and the SAM enters an OFF-LINE state in which symplasmic communication is disrupted by callosic circuit breakers at the PD. Exposure of the resulting terminal bud to sufficient chilling removes PD callose and reconnects SAM cells, resulting in a quiescent STAND-BY state. The SAM and the RM remain quiescent until the ambient temperature rises (+). Environmental factors that promote developmental transitions are marked as inputs at the outside of the circle, and selected genetic and physiological factors are placed on the inside. The arrow shape of the factors indicates their increase or decrease, in quantity or effect (after Rinne PLH, Kaikuranta PM, van der Schoot C. 2001. The shoot apical meristem restores its symplasmic organization during chilling-induced release from dormancy. *The Plant Journal* **26,** 249–264).

Ruonala *et al.*, 2008). Elongation growth, which in poplar requires a basic level of *CENL1* expression (Mohamed *et al.*, 2010), was strongly reduced in *PHYA* overexpressors, and *CENL1* expression levels were only half of those in wt apices (Ruonala *et al.*, 2008). Under SDs, not only did the *FT* levels bounce back to wt levels, but *CENL1* expression levels gradually rose to a level double that of wt poplars and, consequently, elongation growth was enhanced in these overexpressors (Ruonala *et al.*, 2008). This implies that *PHYA* overexpression in the RM/RZ helped the apex to successfully resist the SD-induced inhibitory influences on elongation growth and morphogenesis. Interestingly, also in wt, *CENL1* is initially upregulated during terminal bud formation, before it is completely downregulated at dormancy (Ruonala *et al.*, 2008). We propose that lack of CENL1 function during bud formation is probably due to inhibitory influences. These could include restrictions in vascular and symplasmic connectivity, as well as *BRC*-like genes that are known to inhibit axillary bud activation in Arabidopsis (Niwa *et al.*, 2013).

The role of symplasmic signalling within the apex

Starting with the early initiation of bud scales (cataphylls), the above events play themselves out in a timeframe of about 6 weeks, at which point the ES has entered a dormant state. So far, the sequence of these events is mapped out only roughly, while the molecular pathways that bring about these transitions are almost completely unknown. Within the confines of

the terminal bud, a developing ES emerges with a dwarfed vascular system that provides continuity with the vascular system of the main shoot (Fig. 1). *FT2* is completely downregulated within a week of SDs, while *CENL1* is initially upregulated and subsequently downregulated between week 3 and 6 (Ruonala *et al.*, 2008). Therefore, it seems likely that CENL1 protein liberated from competition for FD with FT, supplied by source leaves, may interact with the bZIP factor FD in a complex with 14-3-3 receptor proteins, as described for *Arabidopsis* (Bradley *et al.*, 1997; Hanano and Goto, 2011; Taoka *et al.*, 2011, 2013; Jaeger *et al.*, 2013). This function could safeguard the SAM from determination during dormancy, in line with the role of *TFL1* in the inflorescence meristem in *Arabidopsis* (Conti and Bradley, 2007). In brief, it can be speculated that, in poplar, ES maturation requires *CENL1* but not *FT2*, although *FT2* downregulation might be a prerequisite.

The precise timing of bud scale formation and its regulation is not known. It is observed that shortly after the downregulation of *FT2* in the source leaves, the SAM starts to produce bud scales (Ruttink *et al.*, 2007; Ruonala *et al.*, 2008). Significantly, when a certain number of bud scales are produced, the developmental programme of primordia is again altered and the SAM commences production of leaves. However, these leaves are small and embryonic, and they arise on a severely dwarfed shoot that completely lacks internode elongation.

That after scale formation leaf initiation resumes suggests that scale regulation is a transient event. As basic levels of *FT2* expression in source leaves are required during normal vegetative development (Böhlenius *et al.*, 2006; Ruonala *et al.*, 2008), it seems possible that FT2 is, directly or indirectly, involved in the formation of leaf primordia. Consequently, withholding the apex FT2 would influence primordia fate and development. Alternatively, or additionally, a positive signal is generated within the source leaves to instruct remote leaf primordia to execute a transient developmental programme.

ES emergence

The ES inside the enlarging bud scales is formed by the activity of the SAM, which produces a restricted number of embryonic leaves with secondary axillary meristems developing in the older leaf axils (Fig. 1). The processes that regulate growth of the extremely dwarfed ES are largely unknown. Early SD-induced changes in gene expression, additional to *FT2* and *CENL1*, which potentially relate to bud scale initiation and ES formation, are: *PICKLE*, a gene that in *Arabidopsis* represses the gene *AINTEGUMENTA* (Mizukami and Fischer, 2000); *GA-INSENSITIVE* (*GAI*), which encodes a nuclear protein of the DELLA subfamily of GRAS transcription factors (Ruttink *et al.*, 2007); genes involved in GA biosynthesis and signalling (Rinne *et al.*, 2011; Cooke *et al.*, 2012); genes controlling the signalling conduits (Rinne *et al.*, 2011); a diverse cluster of MADS box genes (Bielenberg *et al.*, 2004, 2008; Yamane *et al.*, 2011). Interestingly, the mRNA of *GAI*, a systemic signalling molecule that requires specific RNA motifs for its trafficking through the phloem (Huang and Yu, 2009), influences leaf formation (Haywood *et al.*, 2005) and could be involved in changing the fate of primordia. While bud scales are initiated early, they might not become consolidated as bud scales until after 2–3 weeks of SDs, as, up to that point, return to LDs will reverse lamina development, albeit incompletely (Ruonala *et al.*, 2008). *CENL1* expression might be needed for the maturation of the dwarfed ES system, even if leaf expansion and internode elongation are prevented. In contrast, FT2 might be necessary for normal non-dwarfed growth and development, and perhaps also for elongation, and its complete downregulation may contribute to the dwarfing of the ES. It is not known if the plastochron of the ES is different from that of the apex before SD exposure. It appears, however, that the PD that interconnect SAM cells into symplasmic fields are significantly narrowed after 10–15 d of SD exposure in poplar (Ruonala *et al.*, 2008). It was speculated that diminished symplasmic connectivity at this point in time might contribute to a decreased sink function of apical tissues, reducing not only the intake of building materials but potentially also signalling molecules, including peptides, hormones, mRNAs, and microRNAs that are provided via the sieve tubes and the symplasmic extension network (Lough and Lucas, 2006; Kehr and Buhtz, 2008).

Somewhat later in the developmental trajectory of the terminal bud, at around SD week 3–4, major shifts occur in the expression of genes that are involved in cell division, metabolism, and acclimation (Ruttink *et al.*, 2007). Changes also occur in the expression of genes involved in light-, ethylene-, and abscisic acid (ABA) signalling (Ruttink *et al.*, 2007). Ethylene is thought to be involved in the timing of dormancy (Ruonala *et al.*, 2006; Ruttink *et al.*, 2007), while, in its wake, genes for ABA production are upregulated (Ruttink *et al.*, 2007). ABA has various roles in the developing bud, and although its role in dormancy is not clear, it has a prominent function in facilitation acclimation and dehydration tolerance (Welling *et al.*, 1997, 2002; Rinne *et al.*, 1998; Welling and Palva, 2006).

During exposure to SDs, large numbers of minute lipid bodies (LBs) emerge in the cytoplasm of apex cells, mostly in the SAM and RM/RZ (Rinne *et al.*, 2001, 2011). In terminal buds, LB accumulation is particularly high after a few weeks of SDs, coinciding with ES maturation. LBs are of universal occurrence and, although originally viewed as simple storage bags for triglycerides (TAGs), they are emerging as novel organelles with important signalling functions (Murphy, 2001). They are pinched off from specialized sites at the endoplasmic reticulum, where the ethylene receptor is also localized (Grefen *et al.*, 2008), and possess a half-membrane that separates their lipid core of TAGs from the aqueous environment of the cytoplasm (Murphy, 2001; van der Schoot *et al.*, 2011). A possible link exists between ethylene and LB formation, as ethylene biosynthesis genes (Ruttink *et al.*, 2007) are co-expressed with a LB marker in birch (van der Schoot *et al.*, 2011). The specific mechanism by which LBs form is under debate and might vary from tissue to tissue in different organisms. The LBs of seeds, called oil bodies, are decorated by a number of specific proteins, among

which are oleosins, caleosins, and steroleosins, that function to stabilize the single lipid layer, prevent fusion of individual LBs, and provide docking sites for enzymes that function in TAG hydrolysis (Murphy, 2001; van der Schoot *et al.*, 2011). In *Arabidopsis* seeds, oleosins improve freezing tolerance (Shimada *et al.*, 2008), and in their absence LBs fuse, resulting in a delay in seed germination (Siloto *et al.*, 2006). In the *Populus trichocarpa* genome, eight oleosins have been identified (Huang *et al.*, 2009), and three of them are differentially regulated during the dormancy cycle in the poplar apex (P.L.H. Rinne, L.K. Paul and C. van der Schoot, unpublished results). The presence of oleosins in *Arabidopsis* seeds and in the buds of trees suggests that they are intimately related to survival strategies in plants (van der Schoot *et al.*, 2011).

ABA might play a role in LB production and LB maintenance by promoting oleosin production, as *abi3-4* mutants in *Arabidopsis* have delayed oleosin expression (Parcy *et al.*, 1994) and fused LBs. In addition to their role in freezing tolerance, as in *Arabidopsis* seeds (Shimada *et al.*, 2008), LBs may be involved in the chilling-induced release from dormancy at the SAM by virtue of the presence of enzymes associated with the LB protein coat. Although many proteins may associate with LBs (Murphy, 2001; van der Schoot *et al.*, 2011), an important group of proteins are the 1,3-β-glucanases (Rinne *et al.*, 2011). These enzymes [glycosyl-hydrolase17 (GH17) family proteins; Fig. 1)] are of interest as they are regulated by ABA (Leubner-Metzger *et al.*, 1998; Leubner-Metzger and Meins, 2000), the biosynthesis of which increases during the development of dormancy and the production of 1,3-β-glucanase-decorated LBs.

Cessation and self-arrest of the SAM

As bud development proceeds in a precisely scheduled fashion, it is clear that the completion of the ES is mainly regulated by physiological and genetic factors. However, it is plausible that additional mechanisms, such as physical constraints imposed by closed hardy bud scales, play a role in halting ES development during bud development (Couturier *et al.*, 2011). Mechanical forces might directly influence gene expression, as illustrated by for example the expression of so-called touch genes (Lee *et al.*, 2005). Regardless of the precise mechanism, it is true to say that the differentiation of leaves in the terminal buds under SD ceases when the available space inside the bud is filled. Unlike the axillary buds, which may be subjected to similar 'filling laws' (Couturier *et al.*, 2011) under LD conditions, the SD-induced terminal buds (winter buds) are subjected to additional self-imposed constraints. The end station of the SD-induced train of events is the self-arrest of the SAM in a state of dormancy (van der Schoot and Rinne, 2011). This is achieved via an intrinsic mechanism that obstructs return of the SAM to a morphogenetically active state. Although axillary buds form under LDs in the axils of leaves, these buds are merely paradormant, a condition of inhibition imposed by a proliferating apex, in which polar auxin transport and/or the subtending leaves play a major role (Crawford *et al.*, 2010; Kohlen *et al.*, 2011). Removal of the sources of suppression, such as an auxin-producing shoot apex, will release paradormancy and result in bud burst and branch formation, while dormant buds will remain in a dormant state even when the apex is removed (van der Schoot and Rinne, 2011).

The SD-induced dormant state is a transient affair that serves to arrest all morphogenetic patterning at the SAM. In both poplar and birch, SAM cells assume this state of self-arrest by shutting down all forms of intercellular communication (Rinne and van der Schoot, 1998; Rinne *et al.*, 2001; Ruonala *et al.*, 2008). Symplasmic signalling is prevented by the production of dormancy sphincter complexes (DSCs) at PD entrances. DSCs are composed of a callose-containing ring external to the PD neck and a callosic plug inside the PD channel (Rinne and van der Schoot, 1998; Rinne *et al.*, 2001; Ruonala *et al.*, 2008). Callose deposition results from a shift in balance of two enzymes, 1,3-β-glucanase and 1,3-β-glucansynthase, also called callose synthase (Rinne *et al.*, 2001, 2005; Levy *et al.*, 2007*a*,*b*; Levy and Epel, 2009; Xu and Jackson, 2010; Maule *et al.*, 2011). DSCs function as circuit breakers in the symplasmic circuitry of the SAM (van der Schoot and Rinne, 2011), the installation of which ensures that SAM cells can no longer exchange the morphogens, signalling molecules and transcription factors that drive and coordinate primary morphogenesis (Rinne and van der Schoot, 1998; Rinne *et al.*, 2001; Ruonala *et al.*, 2008). As a direct consequence, the symplasmically integrated SAM (Rinne and van der Schoot, 1998; Rinne *et al.*, 2001) becomes a mere collection of disconnected cells where communication lines are silenced and all cells are 'off-line' (Rinne *et al.*, 2001; van der Schoot and Rinne, 2011; Fig. 2). This state of isolation effectively prevents the ES from resuming morphogenesis. Disconnected from their shared signalling network, and deprived from the stabilizing condition of electric and metabolic coupling, individual cells may rely exclusively on cell autonomous processes and may reprioritize metabolism in order to enhance their freezing tolerance (Rinne *et al.*, 2001). Cut off from the supply routes of the stem, the heterotrophic SAM strongly reduces its metabolism. The membrane potentials of individual cells are reduced to values below the diffusion potential, indicating absence of ATP-driven proton-mediated uptake processes and lack of substrate availability (Rinne and van der Schoot, 1998; Rinne *et al.*, 2001; Ruonala *et al.*, 2008). Reserve stores in the young stem subtending the terminal bud are filled with materials that are retrieved from the senescent leaves before they are abscised (Fracheboud *et al.*, 2009), to proactively support the fast growth of the young shoot during bud break in spring.

Temperature effects on vegetative and floral meristems

The arrested dormant state of the SAM is both the end point of the SD-initiated trajectory as well as the turning point and the start for regrowth in spring. The first requirement for regrowth is that the SAM is released from dormancy. The universal mechanism for this is a sufficiently long exposure to

low chilling temperatures (Coville, 1920; Chouard, 1960; Vegis, 1964; Fig. 2) that by convention is expressed as a chilling requirement, which can be species and ecotype specific. Nonetheless, a 6–8-week exposure to a temperature of approximately 5 °C is adequate for most species (Rinne *et al.*, 2001, 2011). In addition to its indispensable role in dormancy release, chilling is vital for the development of freezing tolerance (Weiser, 1970; Welling *et al.*, 1997; Rinne *et al.*, 2001). In juvenile trees, development of freezing tolerance is a two-step process, the first level of which is initiated by SD exposure, whereas the second, deeper level requires chilling but not light (Rinne *et al.*, 2001; Welling *et al.*, 2002). For example, in juvenile birch, SDs at 18 °C bring freezing tolerance to a level of approximately –20 °C, but subsequent additional and prolonged chilling releases the SAM from dormancy and deepens freezing tolerance of the quiescent 'stand-by' state to –70 °C (Rinne *et al.*, 2001; Fig. 2).

The effects of chilling are cell autonomous. They cannot be imported from other parts of the plant and the SAM itself has to perceive chilling (Vegis, 1964; Rinne *et al.*, 2001). Import is also physically impossible, as both the phloem supply routes and the PD of the SAM are dysfunctional and physically obstructed by callose deposits (Aloni *et al.*, 1991; Aloni and Peterson, 1991, 1997; Rinne and van der Schoot, 1998; Rinne *et al.*, 2001; Ruonala *et al.*, 2008). In addition, however, a substantial part of the effect of SDs and chilling might be at the level of chromatin modifications, a situation reminiscent of vernalization (Chouard, 1960; Rinne and van der Schoot, 2003; Horvath *et al.*, 2003; Horvath, 2009; Cooke *et al.*, 2012). Both chilling-induced dormancy release and vernalization have a quantitative aspect, and in both cases the chilling requirements are only slowly fulfilled. In both cases, it is also the SAM itself that has to perceive the chilling. It remains to be seen whether there are any commonalities at the molecular level. In vernalization of *Arabidopsis* winter annuals, chilling epigenetically silences *FLOWERING LOCUS C* (*FLC*), thereby releasing the repression of flowering (Gendall *et al.*, 2001; Bastow *et al.*, 2004; De Lucia *et al.*, 2008). This *FLC*-based mechanism is not operating in dormancy release.

Chromatin modifications might also underlie the chilling-induced hyperinduction of *FT1* in poplar buds (Hsu *et al.*, 2011), up to a level 800–900 times compared with non-chilled bud (Rinne *et al.*, 2011). Such chilling-induced modifications also occur in gibberellin (GA) biosynthesis genes, the upregulation of which has been related to gene methylation (Finnegan *et al.*, 1996, 1998). Our unpublished results show that applied GA_3 could also upregulate *FT* in LD apices of juvenile poplar but not in dormant buds (L.K. Paul and C. van der Schoot, unpublished results). Thus, it seems plausible that chilling-induced changes in *FT1* methylation are a prerequisite for GA-enhanced *FT1* expression (Rinne *et al.*, 2011). It is noteworthy that *CENL1* did not undergo such modifications and was responsive to applied GA also under SDs. The effect of chilling on *FT1* expression might not be mediated by a functional circadian clock, as it tends to be disrupted by chilling, as shown in chestnut (Ramos *et al.*, 2005). This direct effect of chilling on *FT1* expression is not dissimilar to the situation in vernalization, where H3K4 demethylation and H3K27 trimethylation of *FT* repressors advance flowering in *Arabidopsis* (Jiang *et al.*, 2008). However, whether the molecular mechanism of *FT1* hyperinduction in *Populus* involves histone modifications like H3K4 trimethylation in FT chromatin remains to be established.

Dormancy release by GA-recruited GH17 proteins

How does chilling release the SAM from dormancy? It is known that application of GA can often release dormancy without chilling, suggesting that chilling might result in accumulation of GA. The literature is somewhat ambiguous about this capacity of GA, and it was suggested that this might be due to the fact that different GA species might have been applied (Rinne *et al.*, 2011). It appears that applied GA_4, which is the GA that functions in shoot elongation in poplar (Eriksson, 2000; Eriksson and Moritz, 2002; Israelsson *et al.*, 2004), results in canonical bud burst, i.e. bud opening and the emergence of a morphogenetically active and elongating shoot (Rinne *et al.*, 2011). In contrast, low concentrations of GA_3 only resulted in bud opening and the protrusion of a few embryonic leaves, a non-canonical form of bud burst, whereas higher concentrations induced cellular proliferation at the bud base and subsequent abscission (Rinne *et al.*, 2011). It is tempting to speculate that this resembles the little-investigated process of terminal bud abscission that takes place in many trees species beyond the juvenile stage (Junttila, 1976; Cooke *et al.*, 2012).

As in many dormant systems callose obstructs sieve tubes (Aloni *et al.*, 1991; Aloni and Peterson, 1991, 1997) as well as PD in the SAM (Rinne *et al.*, 2001; Rinne and van der Schoot, 2003; Mazzitelli *et al.*, 2007), chilling and GA_4 application, which release dormancy, must be able to reconstitute these symplasmic connections through hydrolysis of callose. In birch, extended chilling promotes the production of 1,3-β-glucanases in the dormant SAM. These enzymes (GH17 family proteins) regulate the turnover of callose (Iglesias and Meins, 2000; Rinne *et al.*, 2005; Levy *et al.*, 2007*a*,*b*; Epel, 2009; Chen and Kim, 2009) and are targeted to the PD during chilling, resulting in the restoration of PD conductivity (Rinne *et al.*, 2001; Rinne and van der Schoot, 2003). Localization experiments showed that the LBs that are produced under SDs (see above) in the SAM and RM/RZ of the ES are decorated with 1,3-β-glucanases, while some 1,3-β-glucanase is present in the cytoplasm (Rinne *et al.*, 2001). During chilling, the randomly distributed cytoplasmic LBs become displaced towards the cell wall where they appear to associate with PD, resulting in hydrolysis of PD callose and restoration of PD conductivity (Rinne *et al.*, 2001; Rinne and van der Schoot, 2003) and conversion of the off-line dormant state into a stand-by quiescent state (Fig. 2).

In *Arabidopsis*, the GH17 family is subdivided into three clades (Doxey *et al.*, 2007). To determine which genes are involved in the release from dormancy, 10 orthologues of poplar genes belonging to two of these clades were identified

(Rinne *et al.*, 2011). They included homologues encoding 1,3-β-glucanases, detected in isolated LBs of birch (Rinne *et al.*, 2001), and genes that encode cell-wall 1,3-β-glucanases with a glycosylphosphatidylinositol (GPI) anchor (Bayer *et al.*, 2006; Rinne *et al.*, 2011). One of them lacks the catalytic site of GH17 but it has a C-terminal domain that is similar to that of the other GH17 proteins, and a CBM43 carbohydrate-binding module that targets PD callose (Simpson *et al.*, 2009). Similar modules were present in the two poplar GH17 members that were identified as orthologues of the *Arabidopsis* BG_ppap (Rinne *et al.*, 2011), which also has a GPI anchor, and localizes at PD to modify its conductance (Levy *et al.*, 2007*a*).

Transcript profiling revealed that the poplar GH17 genes were differentially regulated by SDs, chilling, and application of GA_3 and GA_4, showing that they are regulated in different phases of the dormancy cycle (Rinne *et al.*, 2011; Figs 1 and 2). Remarkably, expression analysis showed that their responses to these different treatments could be clustered into groups that correspond to their specific protein domain architecture. For example, the four genes encoding LB-GH17 proteins lacking a GPI anchor are upregulated by GA_3 (Fig. 1) but downregulated by GA_4, and are mostly upregulated towards the end of the dormancy induction period, corresponding to the peak in ethylene, ABA, and LB production, and during chilling-induced release from dormancy (Rinne *et al.*, 2011). In contrast, five genes encoding GH17 proteins that possess a GPI anchor were downregulated by GA_3 but upregulated by GA_4, while two of them were initially upregulated but then downregulated towards the end of the dormancy induction period. Only one of the selected members with a GPI anchor was unaffected by both GA_3 and GA_4, and showed only a slight upregulation by SDs and chilling (Rinne *et al.*, 2011).

Although GA_4 can release dormancy and induce canonical bud burst when applied to a dormant and non-chilled bud, it was concluded that it cannot be responsible for dormancy release in the natural situation, as judged from the gene expression data. In contrast, in case of GA_3, which cannot induce release from dormancy when applied, the transcript pattern was highly similar to the pattern induced by natural chilling during release from dormancy (Rinne *et al.*, 2011). That applied GA_3 could not induce canonical bud burst is thought to be due to the fact that it upregulates genes that encode LB-associated GH17 enzymes (Rinne *et al.*, 2011). These reside on LBs that are randomly scattered in the cytoplasm and can only hydrolyse callose at PD after chilling has displaced them to the plasma membrane, from where they diffuse to the PD entrances (see below).Thus, it is expected that GA_3, together with chilling, might advance natural bud burst. This hypothesis could be tested by analysing the actual GA forms during dormancy cycling.

Another noteworthy difference between GA_4 and GA_3 action is that, when applied to dormant non-chilled buds, GA_4 transiently upregulates *CENL1* while GA_3 upregulates it more constitutively (Rinne *et al.*, 2011). This transient upregulation of *CENL1* by GA_4 is followed by canonical bud burst (Fig. 1), whereas applied GA_3 fails to induce canonical bud burst, possibly because it cannot promote opening of PD with LB-associated GH17s.

GA biosynthesis genes have non-redundant functions as they are differentially expressed during the dormancy cycle. In poplar, chilling specifically upregulated certain members of the GA3 oxidase and GA20 oxidase families, which have very low abundance during growth under LDs, while other members of the same families showed the opposite pattern (Rinne *et al.*, 2011). The GA-catabolizing genes from the GA2 oxidase family, for example *GAox1* (Thomas *et al.*, 1999), were also upregulated towards the end of the chilling period, possibly to counteract increased GA biosynthesis and maintain homeostatic control. The two putative poplar orthologues of the GA receptor *GIBBERELLIN INSENSITIVE DWARF* (*GID*) were upregulated during SD exposure, showing sensitization of the dormant system to GA, while *DELLA-like1*, a negative regulator of growth, was downregulated.

Our studies suggest that chilling-induced release from dormancy is mediated predominantly by LB-associated GH17 enzymes (Figs 1 and 2). Nonetheless, two GH17 enzymes with a GPI anchor are upregulated in the first few weeks of chilling exposure (Rinne *et al.*, 2011). Thus, LB GH17 proteins may act in concert with some GH17 proteins with a GPI-lipid anchor that are regulatable by GA_4 as well as by chilling. The LB delivery system is an ingenious solution for the problem that metabolic activity in the partially dehydrated system might be challenging under low temperatures. The machinery for dormancy release appears to be pre-installed during SD exposure at a time when temperatures still support normal cellular activities. This dormancy-release function is not incompatible with the fact that the multifunctional LB-associated oleosins may facilitate freezing tolerance, as in *Arabidopsis* seeds (Siloto *et al.*, 2006; Shimada *et al.*, 2008), and provide TAGs for membrane restoration in spring. Delivery of the GH17 proteins to the target sites at the plasma membrane and PD takes place in a relatively small window of opportunity, which is during chilling but prior to the dehydration of the system by freezing conditions. By the end of the chilling period dormancy is released, after having primed the bud for a further increase in freezing tolerance (Welling *et al.*, 2002; Rinne *et al.*, 2010).

It is not known precisely how LBs deliver their cargo to the PD, but it appears to involve components of the cytoskeleton (L.K. Paul and C. van der Schoot, unpublished results). Transient expression studies with enhanced green fluorescent protein-labelled GH17 proteins in *Nicotiana benthamiana* showed that LBs deliver the fluorescent fusion protein to the plasma membrane where they localize as distinct sandwich-like patches at both ends of the PD channels, which were indicated by a florescent PD marker (movement protein of tobacco mosaic virus conjugated to red fluorescent protein; Rinne *et al.*, 2011). In contrast, protein fusions of GH17 proteins with a GPI anchor localize in a punctate pattern at the PD or inside the PD channel. A model depicts delivery of LB-associated GH17 proteins resulting from fusion of the single lipid layer of the LB with the cytoplasmic leaflet of the plasma membrane (van der Schoot *et al.*, 2011). GH17 enzymes may be recruited by membrane rafts

and laterally transferred to the intracellular callose plug of the DSC at the PD entrance (Rinne *et al.*, 2011; van der Schoot *et al.*, 2011). GH17 proteins with a GPI anchor are likely to be transferred via the Golgi excretion pathway to the cell membrane, where they are moved to the extracellular space to be collected by membrane rafts at the extracellular leaflet of the plasma membrane and transported to the extracellular sphincter ring of the DSCs (Simpson *et al.*, 2009; Rinne *et al.*, 2011).

The release from dormancy of non-chilled buds by GA_4 application does not utilize the LB delivery system, and GA_4 actually inhibits transcription of LB-associated *GH17* genes. In addition, application of GA_4 prevents alignment of LBs with the plasma membrane and instead may 'dissolve' the LBs, as apparent from transmission electron microscopy investigations (Rinne *et al.*, 2011). Nonetheless, the DSCs at the PD disappear, indicating that applied GA_4 induces hydrolysis of callose in DSCs (Rinne *et al.*, 2011).

Bud break: a time to grow up

The genes *FT2*, *FT1*, and *CENL1* are under complex regulation during the dormancy cycle (Fig. 2). For example, *FT1* in embryonic leaves is hyperinduced by prolonged chilling (Fig. 2), and independently of *CO* (Rinne *et al.*, 2011), but the situation is reversed when, after sufficient chilling, the temperatures rise in spring. At about 18 °C, the expression of *CO* was upregulated while that of *FT1* transcripts was reduced (Rinne *et al.*, 2011). In plants growing in LDs, *CENL1* is expressed at modest levels in the RM, whereas, coinciding with ES development, *CENL1* is transiently upregulated during the first 3–4 weeks of SDs (Ruonala *et al.*, 2008). Throughout the chilling phase, *CENL1* transcripts are at a very low level, but once dormancy is released, the elevation of the ambient temperature to 18 °C upregulates its expression (Figs 1 and 2). Just prior to canonical bud burst *CENL1* expression peaks, suggesting that in mature buds it is a marker for bud burst (Mohamed *et al.*, 2010; Rinne *et al.*, 2011; Fig. 1), The expression of GA biosynthesis and signalling genes is also under complex regulation. Considering how they regulate *FT1*, *FT2*, and *CENL1*, they are likely to have a significant role in the development of the ES and its activation in spring.

To sustain and fuel the initiation of morphogenetic activity of the SAM and the RM/RZ may require a combination of import via the restored symplasmic connections as well as substrate uptake from the apoplasmic space in the ES. The latter requires substrate-specific carriers and proton-extrusion pumps. Plasma membrane-localized H^+-extrusion pumps (H^+-ATPases) are encoded by a small family of genes (Palmgren, 2001) that are upregulated just prior to bud break, as shown in raspberry and peach (Gévaudant *et al.*, 2001; Mazzitelli *et al.*, 2007). As light stimulates substrate uptake by increasing plasma membrane H^+-ATPase activity (van Bel and van der Schoot, 1981; van Bel *et al.*, 1981), this may drive cellular division and growth in both the bud scale bases and the ES (van der Schoot and Rinne, 2011). The initiation of internode elongation, leaf enlargement, and leaf production at the activated SAM subsequently results in bud break and shoot formation. Bud scales have their own dynamics and, although they develop in synchrony with the ES, growth of the scales can be localized and independent of ES activity, as demonstrated by the fact that some bud scales open exposing a dead shoot that did not survive winter (Romberger, 1963). In *Populus*, GA_3 application to dormant buds induces non-canonical bud burst, in which the buds open and a few small leaves may protrude without any signs of internode elongation and new formation of leaves. Although these unexpanded ES are not dead, applied GA_3 may only override constraints on the bud scales and older embryonic leaves but not those of the entire ES. In the natural situation, chilling differentially affects the various parts of the dormant bud. Bud scales and embryonic leaves respond much earlier than the RM and SAM, and only sufficient chilling leads to canonical bud burst (Rinne *et al.*, 2011), which requires collaboration, synchrony, and control of the various parts of this unique shoot system.

Acknowledgements

This work was supported by the Norwegian Research Council (grant numbers 171970 and 192013). We thank the reviewers for helpful comments.

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