FLOWERING NEWSLETTER REVIEW

Gibberellin as a factor in floral regulatory networks

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Abstract

Gibberellins (GAs) function not only to promote the growth of plant organs, but also to induce phase transitions during development. Their involvement in flower initiation in long-day (LD) and biennial plants is well established and there is growing insight into the mechanisms by which floral induction is achieved. The extent to which GAs mediate the photoperiodic stimulus to flowering in LD plants is, with a few exceptions, less clear. Despite evidence for photoperiod-enhanced GA biosynthesis in leaves of many LD plants, through up-regulation of GA 20-oxidase gene expression, a function for GAs as transmitted signals from leaves to apices in response to LD has been demonstrated only in Lolium species. In Arabidopsis thaliana, as one of four quantitative floral pathways, GA signalling has a relatively minor influence on flowering time in LD, while in SD, in the absence of the photoperiod flowering pathway, the GA pathway assumes a major role and becomes obligatory. Gibberellins promote flowering in Arabidopsis through the activation of genes encoding the floral integrators SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1), LEAFY (LFY), and FLOWERING LOCUS T (FT) in the inflorescence and floral meristems, and in leaves, respectively. Although GA signalling is not required for floral organ specification, it is essential for the normal growth and development of these organs. The sites of GA production and action within flowers, and the signalling pathways involved are beginning to be revealed.

Key words: Arabidopsis, DELLA, floral transcription factors, flower development, flower induction, gibberellin, LEAFY, Lolium, SOC1.

Introduction

The ability of gibberellins (GAs) to promote bolting and flower formation in long-day (LD) and biennial plants under conditions that would not normally permit flowering contributed to the realization that these compounds may function as endogenous growth regulators (Lang, 1957). The bolting response obtained in species such as Hyoscyamus niger (Lang, 1956) is one of the most spectacular effects of applying GAs to plants and contributed to the excitement during the early years of GA research. Lang (1957) distinguished between the promotion of stem extension (bolting), which he considered a direct effect of GAs, and flower initiation, which he thought must be indirect since, in most species, it follows bolting. Lang discounted the possibility that GA could be a universal flowering stimulus (florigen) since he obtained no promotion of flowering by applying GAs to short-day (SD) plants, despite evidence from grafting experiments that LD and SD plants contain a common stimulatory substance.

Almost 40 years after Lang’s publication, promising candidates for florigen were identified as the proteins encoded by FLOWERING LOCUS T (FT) (Corbesier et al., 2007; Jaeger and Wigge, 2007; Lin et al., 2007; Mathieu et al., 2007), and its rice equivalent Hd3a (Tamaki et al., 2007). While the role of GAs in flowering is becoming established for a limited number of species, GA is clearly not a universal flowering stimulus. In some species, such as
discussed at the physiological and molecular levels, drawing involvement of GAs in floral initiation and development are specific, their function in flower development is far more environmental inputs to this response. but it is still unclear to what extent GA mediates the following section.

Although the flowering role for GAs may be species-specific, their function in flower development is far more general and probably universal. In this review, the involvement of GAs in floral initiation and development are discussed at the physiological and molecular levels, drawing heavily from work with Arabidopsis and a limited number of other species. The review will include a consideration of flower development up to the establishment of fertility, but we will not discuss seed or fruit development, in which GAs also have important roles (Ozga and Reinecke, 2003; Serrani et al., 2007). Recent advances in our understanding of the GA-biosynthetic and signal transduction pathways (Ueguchi-Tanaka et al., 2007; Daviere et al., 2008; Hirano et al., 2008a; Itoh et al., 2008; Yamaguchi, 2008) are critical to considering the involvement of GAs in floral initiation and development and will be discussed briefly in the following section.

**Gibberellin signalling**

Gibberellins are required for the normal growth of almost all plant organs through the promotion of cell division and/or cell elongation. In addition, they promote certain developmental switches or phase changes, including seed germination and the juvenile to adult transition, as well as the transition from vegetative to reproductive development in some species. Although 136 different GA structural variants have currently been identified from plants, fungi, and bacteria (http://www.plant-hormones.info/ga1info.htm), only a limited number of compounds have intrinsic biological activity, with GA1 and GA4 being the major endogenous active molecules in most plant species. The GA signalling pathway (Fig. 1), which includes biosynthesis, turnover and signal transduction, is tightly regulated by developmental and environmental cues, with the regulation of GA concentration being of primary importance (Yamaguchi, 2008). The biosynthetic pathway to GA4 from the common diterpene precursor trans-geranylgeranyl diporphosphate comprises 12 steps catalysed by six enzymes, of which the 2-oxoglutaratedependent dioxygenases, GA 20-oxidase (GA20ox) and GA 3-oxidase (GA3ox), that catalyse the final steps, are major sites of regulation. A third group of dioxygenases, the GA 20-oxidases, which by inactivating GAs and their immediate precursors contribute to turnover, are also highly regulated. The three dioxygenase classes are encoded by multiple genes, but there is not complete gene redundancy because the paralogues differ in their expression patterns and regulation.

![Fig. 1. Gibberellin signalling pathway. Regulation of the GA concentration is primarily via the biosynthetic enzymes GA20ox and GA3ox, and the inactivating enzymes GA2ox. Bioactive GAs promote binding of the GID1 receptor to DELLA proteins, which initiates DELLA degradation via the ubiquitination/26S proteasome pathway. DELLA function as transcriptional regulators in combination with transcription factors.](image)

Gibberellin signalling promotes growth by initiating the degradation of DELLA proteins, which are growth-suppressing members of the GRAS family of transcriptional regulators. Perception of GA is by soluble nuclear-localized receptors, known as GID1, which, on binding GAs, undergo a conformational change that allows them to interact with the DELLA N-terminal domain (Murase et al., 2008; Shimada et al., 2008). It is proposed that binding of DELLA to GID1 causes a conformational change in DELLA, allowing them to bind to the F-box component of an SCF E3 ubiquitin ligase (Murase et al., 2008), targeting them for degradation via the ubiquitination/26S proteasome pathway. DELLA have been shown to function by interacting with transcription factors and blocking their activity. This was demonstrated for Arabidopsis hypocotyls, in which DELLA interact with PHYTOCHROME INTERACTING FACTORS (PIFs) and thereby suppress their ability to promote gene expression and growth (Feng et al., 2008; de Lucas et al., 2008). Removal of DELLA as a result of GA action allows PIF function. Since PIFs are also regulated by light through phytochrome, they form a point of convergence between the light and GA growth regulatory pathways. DELLA appear to have many targets (Zentella et al., 2007; Hou et al., 2008) and, although in the above example they suppress gene expression, it is becoming clear that they up-regulate at least as many genes as they repress (Hou et al., 2008). DELLA must, therefore, also function as transcriptional activators, perhaps in partnership with transcription factors or by inactivating transcriptional repressors.

**Floral competence**

Gibberellins may be involved in the developmental events leading to reproductive competence, as well as in floral determinination and commitment. Reproductive competence is often manifested by early visual physiological markers
such as internode elongation (bolting), which, in many monocarpic plants, is widely regarded as indicative of the physiological transition to reproductive growth, even though plants may still be growing vegetatively at this stage. The determination of developmental reprogramming leading to flowering is less visible, eventually leading to the formation of floral meristems. In Arabidopsis this is mediated by the transition of the vegetative meristem to the inflorescence meristem (IM), which then enables a commitment to flowering through the differentiation of cells in the peripheral zone (lateral anlagen) of the IM to produce floral meristems (FM). Distinctions between the different types of meristems exist not only at the morphological level but also at the molecular level through the spatial and temporal expression of gene regulatory networks defining phyllotaxy and organ development at the shoot apex (Doerner, 2003; Sablowski, 2007).

Reproductive competence, when the shoot is capable of responding to the external and endogenous inductive cues for flowering, requires the transition from the juvenile to the adult growth phase (Poethig, 2003). This transition is often associated with changes in leaf morphology, surface structure (waxiness, trichomes) and leaf vein development and has been widely studied in maize and Arabidopsis (Evans and Poethig, 1995; Chien and Sussex, 1996; Dill and Sun, 2001), where GA acts to promote the transition to the adult phase. The role of GAs in developmental transitions reflects their increasingly recognized function as integrators of wide-ranging developmental and environmental signals through DELLA-mediated pathways (Daviere et al., 2008), coupled with their ability to interact with other plant hormones at different levels throughout development (Weiss and Ori, 2007). With the possible exception of the phytochromes, no other floral initiation factors identified so far participate in vegetative and floral transitions to a similar extent as GAs. However, some evidence of overlap between the regulation of vegetative and floral transitions is starting to emerge. For example, miRNA172, which is involved in the regulation of floral homeotic genes (Aukerman and Sakai, 2003; Chen, 2004) and the floral integrator FT (Jung et al., 2007), is now also known to control the juvenile to adult phase change in maize (Lauter et al., 2005).

In some species, competence to flower requires prolonged exposure to low temperature (vernalization). In the LD grass species Lolium perenne, exogenous GA allowed flowering in non-inductive SD conditions only in vernalized plants, whilst non-vernalized plants were unable to respond to GA either by stem elongation or flowering (MacMillan et al., 2005). The lack of response to GA occurred despite an active GA signalling system, because DELLA protein abundance was reduced by GA treatment. The limiting factor in this case could lie down-stream of DELLA or in a non-related pathway; it was not attributed to the action of abscisic acid (ABA), which commonly antagonizes GA signalling. Vernalization-induced bolting is a prerequisite for flowering in the LD sugar beet plant, in which, as in L. perenne, GA can compensate for LD, but not vernalization, with only limited initiation of stem elongation in non-vernalized plants in both LD and SD (ES Mutsasa-Gottgens and P Hedden, unpublished results). Thus, at least in L. perenne and sugar beet, the GA/LD inductive pathway is blocked unless plants are vernalized, although, as reported by Lang (1957), GA can substitute for vernalization in a number of biennial species.

Floral induction

A role for GAs in flower induction in reproductively competent plants has been established primarily for LD and biennial species, in which flowering in non-inductive conditions can be achieved by the application of GAs (Zeevaart, 1983; King et al., 2001). The transition to reproductive development in rosette plants, is frequently signified by bolting of the main axis, a process that involves GA-dependent cell division and elongation (Sachs and Lang, 1957; Silverstone and Sun, 2000), and has been directly correlated with increased bioactive GA in the shoot apex, as, for example, in spinach (Talon et al., 1991), field pennycress (Metzger, 1985) and sugar beet (Debenham, 1999; Sorce et al., 2002). In sugar beet, as in many other biennial species, reproductive competence is achieved only after bolting (Mutsasa-Gottgens et al., 2008), which always precedes flowering.

In Arabidopsis, bolting, unlike flower induction, has an absolute requirement for GA signalling, since the highly GA-deficient mutant ga-3 (Koornneef and van der Veen, 1980) and plants lacking GA receptors (Griffiths et al., 2006) are severely dwarfed, regardless of photoperiod. The number of internodes that elongate from within the rosette as well as their final length is limited by GA content (Rieu et al., 2008a). Bolting is preceded by flower initiation, for which the photoperiod-induced CONSTANS (CO)/FT pathway appears to dominate in LD (Kobayashi and Weigel, 2007; Turck et al., 2008). However, the delayed flowering of ga-3 (Wilson et al., 1992) and the triple gid1 mutant (Griffiths et al., 2006) in continuous light or LD, respectively, indicates that the GA flowering pathway makes some contribution to floral induction even when the CO pathway is active. New data from Arabidopsis now indicate that GAs may act via FT (a key target of CO) as well as independently to induce flowering, as, in LD, GA, was found to promote FT expression in wild-type (Col-0) plants and to be required for FT expression in ga-3 (Col-0) (Hisamatsu and King, 2008). In SD, when expression of FT is low (Wigge et al., 2005), flowering in Arabidopsis is absolutely dependent on GA signalling (Wilson et al., 1992) although this may not directly involve FT, since there is little promotion of FT expression by GA in SD (Moon et al., 2003; Hisamatsu and King, 2008). Indeed, Hisamatsu and King (2008) confirmed the FT-independent role for GA, which rescued the late flowering phenotype in the ft-1 mutant in both LD and SD. Gibberellin and FT have been shown to act independently in Lolium temulentum, in which GA application did not increase FT expression in LD or SD (King et al., 2006). Hisamatsu and King (2008) have
recently reported that *ga*/*3* plants grown in SD for 3 months did not flower after 30 subsequent LD, but could be induced to flower by treatment with GA4. The reason for the discrepancy between these results and those from numerous others showing flowering of this mutant when exposed to LD from germination is unclear, but may be related to the age of the plants when first receiving LD (Hisamatsu and King, 2008). To add further to the confusion, there are reports that triple *gid1* mutants lacking GA receptors fail to flower in LD (Iuchi et al., 2007; Willige et al., 2007) in contrast to the finding of Griffiths et al. (2006). In this case, the explanation could be differences in light quality or intensity: the promotion of flowering by far-red-rich incandescent light, as well as by photosynthetically active radiation, possibly mediated by sucrose, was highlighted recently (King et al., 2008a).

The extent to which GA mediates photoperiod-induction of flowering rather than having a purely permissive role is probably dependent on species. There have been numerous reports showing increased GA biosynthesis when LD plants are transferred from SD to LD, regulation being primarily on the expression of GA20ox genes (Wu et al., 1996; Xu et al., 1997; Hisamatsu et al., 2000; King et al., 2006; Lee and Zeevaart, 2007). In spinach, *SoGA20ox1* transcript and the encoded protein increased in leaves and shoot apices when plants were transferred from SD to LD, although the change was too slow to account for the rapid induction of stem extension under these conditions (Lee and Zeevaart, 2007). Transcript was detected by *in situ* hybridization in the shoot apical meristem, as well as in leaf and flower primordia, but not in the expanding subapical region, indicating that stem extension was dependent on the import of active GAs or precursors to this region or that a different *SoGA2ox* paralogue was expressed there. Leaf expression of the *Arabidopsis* GA20ox GA2ox paralogue *AtGA20ox2* is restricted to the petiole where it is up-regulated by far-red-rich LD via phytochrome B (Hisamatsu et al., 2005; Hisamatsu and King, 2008). Loss of *AtGA20ox2* delays flowering in LD and especially in SD, whereas *AtGA20ox1*, which shows a circadian expression pattern in the leaf blade and petiole, has much less influence on flowering time (Hisamatsu and King, 2008; Rieu et al., 2008a). This latter gene, however, is the predominant GA2ox paralogue expressed in *Arabidopsis* stems and has a major influence on stem extension during bolting (Koornneef and van der Veen, 1980; Rieu et al., 2008a).

A function for GAs as mobile signals for flower induction has been investigated in several studies. In *Arabidopsis* growing in SD, GA4 accumulated in the shoot apex prior to the floral transition (Eriksson et al., 2006). This accumulation was not correlated with changes in expression of GA-biosynthetic genes in the apex, indicating that the GA originated from elsewhere. It was possible to demonstrate movement of exogenous labelled GA4 from leaves to the apex, but the authors did not report on changes in GA4 biosynthesis in leaves before floral initiation. In the LD grass, *Lolium temulentum*, photoperiod induction was shown to be followed rapidly by increased GA20ox expression in the leaf and accumulation of the flurally inductive GA5 at this site (King et al., 2006), and in the shoot apex (King et al., 2001). Treating plants with the GA-biosynthetic inhibitor paclolubranzol prevented LD-induced flowering, but only when applied before induction, indicating an absolute GA requirement for flower initiation (King et al., 2006).

Unlike *Arabidopsis*, in which GA4 is the principal active compound for both floral initiation and stem extension (Eriksson et al., 2006), *L. temulentum* uses different GAs for these functions, with GA5, and possibly GA6, serving as floral inducers, but having weak stem extension activity. On the other hand, GA1 and GA4, which are strongly growth promoting, but less florigenic, accumulate in leaves and shoot apices of LD-induced *L. temulentum* plants more slowly than does GA5 (King et al., 2006). It was suggested that GA1 and GA4 may promote inflorescence differentiation and stem extension once the floral transition has occurred (King et al., 2001). This structural specificity is unlikely to be due to the use of different GA receptors for these functions, but may be related to the rate at which the compounds are inactivated (King et al., 2008b). Gibberellin A5, which on account of its 2,3-double bond is protected from 2β-hydroxylation, would escape inactivation by GA 2-oxidases that are expressed immediately below the shoot apical meristem (SAM). Indeed, this GA was shown to be metabolized more slowly than GA1 and GA4 in isolated shoot apices of *L. temulentum* (King et al., 2008b). Such a role for GA 2-oxidases, in which they restrict access of bioactive GAs to the SAM, was proposed from work with rice, in which GA2ox gene expression, located in the rib meristem below the SAM, was reduced following the floral transition, so allowing GAs to enter the SAM (Sakamoto et al., 2001a). A similar reduction in expression at the shoot apex after floral induction was noted for one of two GA2ox genes monitored in *L. temulentum* (King et al., 2008b). While a pattern of GA2ox gene expression at the shoot apex comparable to that in rice has been reported in *Arabidopsis* (Jasinski et al., 2005), there is no evidence for a change in expression corresponding with floral induction (Eriksson et al., 2006). Nevertheless, loss of GA2ox gene expression in *Arabidopsis* advances flowering time, particularly in SD, with *AtGA2ox4* making the largest contribution to this effect (Rieu et al., 2008b). Expression of this paralogue is mainly restricted to the shoot apex, whereas most other *AtGA2ox* genes have a broader expression pattern (Jasinski et al., 2005; Rieu et al., 2008b). Exclusion of GA from the vegetative SAM is also thought to be necessary for maintaining its indeterminacy; KNOTTED1-type homeobox transcription factors have a prominent role by promoting GA2ox while repressing GA20ox expression (Sakamoto et al., 2001b; Hay et al., 2002; Jasinski et al., 2005).

The evidence discussed above strongly indicates that GAs act directly to induce the floral transition at the shoot apex, although they may also promote formation of other mobile signals, such as FT (Hisamatsu and King, 2008). In *Arabidopsis* the switch from a vegetative meristem to an IM coincides with the expression of molecular markers such as
SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1), SHORT VEGETATIVE PHASE (SVP), and AGAMOUS Like 24 (AGL24), whilst formation of FM is promoted by LEAFY (LFY) and APETALA 1 (API), which have also been shown to repress the activity of the IM genes (Liu et al., 2007). Once established, the IM generally grows indeterminately, continuously generating new lateral meristems, while the FM always commits to flowering and terminates with floral organs (Souer et al., 2008). Gibberellins promote expression of both SOC1 (Bonhomme et al., 2000; Moon et al., 2003) and LFY (Blazquez et al., 1998) by independent DELLA (GAI/RGA)-mediated pathways that directly, or indirectly via GAMYB (Gocal et al., 1999, 2001), modulate the expression of SOC1 and LFY, respectively (Achard et al., 2004) (Fig. 2). LFY integrates the LD and GA pathways, through separate cis elements on its promoter (Blazquez and Weigel, 2000), while SOC1 integrates the autonomous/vernalization and GA pathways (Moon et al., 2003). SOC1 and AGL24 bind to each other’s promoters to create an autoregulatory feedback loop (Liu et al., 2008), and the SOC1/AGL24 heterodimer is required for nuclear localization and transcription of LFY (Lee et al., 2008). Gibberellins therefore have an additional indirect route for up-regulating LFY via SOC1 and probably also control levels of LFY through the DELLA-dependent regulation of miRNA159 which, in Arabidopsis, negatively regulates MYB33 required for LFY transcription (Achard et al., 2004). Different GAs were shown to promote LFY expression at the Arabidopsis shoot apex and to induce flowering in SD with similar activities, supporting the causal relationship between these events (Eriksson et al., 2006). The expression domains of LFY and SOC1 are known to be overlapping within the transitional meristem (Parcy, 2005; Turck et al., 2008), and it is reasonable to assume that GAs, in combination with endogenous (e.g. other hormones) and external (light and temperature) signals, may influence the biological switch that determines cell fate in both the inflorescence and floral meristems.

Flowering in perennial species

The role of GAs in flowering in perennials has mainly been studied in fruit trees (reviewed by Wilkie et al., 2008), in which GAs are generally inhibitory to flowering. In apples, since applied GA and the presence of seeded fruit inhibits floral initiation it has been suggested that seeds, a rich source of GAs, export the hormones to the buds (Hoad, 1978). However, it has been difficult to obtain convincing evidence for the transport of GAs from seeds in sufficient quantities and it was proposed that in fact auxin is the mobile inhibitory factor (Bangerth, 2006). Applied GAs might suppress floral initiation by enhancing the polar transport of IAA from seeds. Alternatively, if GAs are indeed floral inhibitors, IAA may stimulate their synthesis in the bud. It has also been suggested that GAs act

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Fig. 2. Schematic representation of events leading to GA-induced floral transition in Arabidopsis. In the leaf, phytochrome-mediated up-regulation of GA20ox results in increased GA concentration, which may up-regulate FT, also under photoperiod control, via CO. GA and FT protein move from the leaf to the shoot apex. Inactivation of GA by GA2ox in the rib meristem regulates the amount of GA entering the shoot apical meristem, where it activates SOC1 and LFY via repression of the DELLA GAI and RGA. Red arrows (promotion) and T-bars (repression) indicate steps that immediately affect or are affected by GA. Boxed numbers refer to supporting data for the represented scheme as follows: (1) Achard et al., 2004; (2) Liu et al., 2008; (3) Lee et al., 2008; (4) Liu et al., 2007; (5) Gocal et al., 2001; (6) Hisamatsu and King, 2008; (7) Hisamatsu et al., 2005; (8) Jasinski et al., 2005. * Authors presented data to show that these two pathways are independent.
indirectly by delaying bud formation (Bertelsen et al., 2002). A common theme is that GAs promote vegetative growth in perennials at the expense of reproductive development. For example, in grapevine, GA signalling inhibits flower formation and promotes the formation and growth of tendrils (Boss and Thomas, 2002).

There is limited information on the molecular mechanisms involved in the inhibition of floral initiation by GA. In some cases, it could be related to the role of sucrose as a floral stimulus (Corbesier et al., 1998). Flowering in Fuchsia hybrida was promoted by high intensity irradiance, even in non-inductive SD, and this was associated with an accumulation of sucrose at the shoot apex (King and Bent-Tal, 2001). Application of GAs to the shoot tip inhibited the floral response and reduced the sucrose concentration in the apex, presumably by diverting assimilate from the apex to the rapidly growing shoot. In other cases GAs may have a more direct influence on molecular events within the SAM to determine whether shoot apices commit to vegetative or reproductive growth, but as yet there are few clues as to how this is accomplished (Tan and Swain, 2006).

Flower development

Physiological events

Following floral initiation, a functional GA signalling pathway is not required for the specification and differentiation of floral organs, but is essential for the normal development of these organs, with the possible exception of the papillae (Griffiths et al., 2006). Even mildly GA-deficient mutants have impaired male fertility due to abnormal stamen development (Chhun et al., 2007; Hu et al., 2008; Rieu et al., 2008a), while extreme GA deficiency also results in female sterility (Nester and Zeevaart, 1988; Goto and Pharis, 1999). This is consistent with the findings of Goto and Pharis (1999), who showed that Arabidopsis stamens require higher GA concentrations for normal development than do the pistil, petals, and sepals. Gibberellin-deficient or signal transduction mutants typically have short stamens as a result of reduced cell extension within the filament (Cheng et al., 2004) so that self-pollination is compromised. The importance of precise control of GA content for the co-ordinated growth of floral organs was demonstrated recently in an Arabidopsis mutant lacking all five CYP-GA 2-oxidases (Rieu et al., 2008b). In some flowers of this mutant the pistil out-grew the stamens, possibly due to greater sensitivity of the pistil to the elevated GA content, resulting in lower fertility. The accelerated pistil growth sometimes resulted in the organ impacting on the unopened sepals causing the pistil to bend.

In addition to regulating filament length GA signalling is required for anther development, allowing viable pollen formation and dehiscence. In the highly GA-deficient gal-3 Arabidopsis mutant, pollen development does not proceed beyond stage 7/8 (Sanders et al., 1999), with microsporogenesis arrested after meiosis, but prior to mitosis (Cheng et al., 2004), while in GA-deficient mutants of tomato, microsporogenesis arrests before meiosis (Nester and Zeevaart, 1988; Jacobsen and Olszewski, 1991). Development of the tapetum is also impaired in GA biosynthesis/signalling mutants, the premature degeneration of this tissue corresponding temporally with the arrest of microsporogenesis (Izhaki et al., 2002). The tapetum fulfils essential functions for pollen development, including the provision of nutrients and contents of the pollen coat, as well as allowing dehiscence (Goldberg et al., 1993), but it is unclear if its premature degeneration in the absence of GA signalling is causally linked to the arrest of pollen development.

The tapetum appears to be a major site of GA production within the developing anther (Itoh et al., 2001; Kaneko et al., 2003; Hu et al., 2008). Expression of the Arabidopsis GA-biosynthetic genes AtGA3ox3 and AtGA3ox4 reaches a peak in the tapetum just prior to its degradation preceding pollen dehiscence, and decreases thereafter, but remains high in the pollen grains themselves up to and including dehiscence (Hu et al., 2008). This finding is consistent with results from rice indicating that pollen develops the capacity for GA biosynthesis relatively late in development (Chhun et al., 2007; Hirano et al., 2008b). The filament also appears to be a site of GA biosynthesis, based on expression of the AtGA3ox1 gene (Gomez-Mena et al., 2005; Mitchum et al., 2006), notably a different GA 3-oxidase paralogue from those, AtGA3ox3 and AtGA3ox4, expressed in anthers (Hu et al., 2008). However, the early, single-copy GA-biosynthetic gene AtCPS is expressed only in anthers, suggesting that filament growth may require anther-derived GA precursors (Silverstone et al., 1997). In support of this, the presence of intact anthers, rather than filaments, is required for corolla expansion and pigmentation in petunia (Weiss and Halsey, 1989).

The dependence of petals on the stamens as a source of GAs for their development is a clear example of paracrine signalling within P. hybrida flowers. Anthers are an extremely rich source of GAs (Hirano et al., 2008b) and it is interesting to speculate on the extent and distance GAs are exported from this organ. The ELONGATED UPPER INERNODE (EUI) gene of rice encodes a GA 16, 17-epoxidase, which inactivates 13-deoxy GAs, such as GA4, but has no activity against GA1 (Zhu et al., 2006). Loss of EUI function results in the accumulation of GA4 in the upper internode, which becomes hyper-el prolonged. Since vegetative organs of rice produce predominantly 13-hydroxy GAs (Kobayashi et al., 1988; Hirano et al., 2008b), it is reasonable to assume that the GA4 in the upper internode is derived from the panicle, probably the anthers, and may serve to induce elongation of this internode to coincide with pollen maturation. This process would be regulated by EUI, perhaps to restrict GA4 migration. The extent to which floral-derived GAs contribute to stem elongation in Arabidopsis is unknown, but since bolting follows flower initiation in this species, such a role might be anticipated.

Pollen germination and pollen tube growth both require GA signalling (Singh et al., 2002; Chhun et al., 2007). These processes respond to an optimum GA concentration and
are inhibited at super-optimal concentrations. Remarkably, while mutant pollen with defective GA biosynthesis does not germinate and therefore cannot transmit the mutation unless rescued by an exogenous GA source, pollen with lesions in genes encoding components of the GA-signaling transduction pathway are capable of producing mutant zygotes (Chhun et al., 2007). Thus, self-fertilization of rice or *Arabidopsis* plants heterozygous for loss-of-function mutations in *GIDI* GA receptor genes produced homozygous mutant progeny with segregation ratios consistent with complete transmission of the mutant gene (Griffiths et al., 2006; Iuchi et al., 2007; Willige et al., 2007; Feng et al., 2008). This apparent anomaly was explained from work in rice in which it was shown that early GA-biosynthetic genes are expressed in anthers only after meiosis, whereas the signal transduction genes *GIDI* (receptor), *SLRI* (DELLA protein), *GID2* (F-box protein specific for *SLR*), and *GAMYB* were already expressed prior to meiosis so that their mRNA and/or protein products could pass from non-mutant to mutant pollen and rescue the mutation (Chhun et al., 2007).

**Molecular events**

The molecular events underlying the GA regulation of flower development are becoming clearer. In *Arabidopsis*, the GA signal is mediated in flowers primarily via degradation of the DELLAs RGA and RGL2 with a small contribution from RGL1 (Cheng et al., 2004). Thus the flower phenotype of the *Arabidopsis* ga1-3 mutant is essentially rescued when combined with loss-of-function mutations in *RGA*, *RGL1*, and *RGL2* (Cheng et al., 2004; Yu et al., 2004). The specification of floral organs is under the control of homeotic genes, expression of which must be maintained in the organs to allow their normal development. During organ differentiation from the floral meristem, expression of the homeotic genes is promoted by LFY, but not by GA signalling, and, in contrast to the situation during floral induction, LFY expression is not under GA control at this developmental stage (Yu et al., 2004), but is maintained in an activation loop with *AP1* and *CAULIFLOWER* (Bowman et al., 1993; Blazquez et al., 2006). Thus floral organs are initiated under severe GA deficiency (Goto and Pharis, 1999) or in the absence of a functional GA signalling pathway (Griffiths et al., 2006). However, expression of the homeotic gene *AGAMOUS* (*AG*), which is required for stamen and carpel specification, promotes expression of the GA-biosynthetic gene *AtGA3ox1* in floral meristems, indicating that GA signalling may have some role in floral organogenesis (Gomez-Mena et al., 2005), even if it is not essential for this process. At later stages of development, GA promotes the expression of the B and C function homeotic genes *PISTILLATA* (*PI*), *APETALA3* (*AP3*), and *AG* through the DELLA-degradation pathway (Yu et al., 2004). It appears therefore that GA promotion of floral organ expansion and anther development is mediated, in part, by these homeotic transcription factors. Gibberellin-induction of their expression, however, did not occur in the presence of the translation-inhibitor cycloheximide, indicating that it is indirect (Yu et al., 2004). It may be mediated by miRNA159 and MYB33, which were shown to be involved in GA-regulated anther development. Ectopic expression of miRNA159 caused a reduction in MYB33 transcript in *Arabidopsis* flowers and impaired anther development (Achard et al., 2004).

A study of RGA-induced global transcript changes in developing *Arabidopsis* flowers showed that equal numbers of genes were up- and down-regulated by RGA (Hou et al., 2008). As might be anticipated, many down-regulated genes are involved in metabolism, particularly of cell walls. A very high proportion of these RGA-regulated genes are exclusively or predominantly expressed in stamens, indicative of the complexity of the processes regulated by GA signalling in anthers. Interestingly, there was considerable overlap in the RGA-regulated genes in stamens with those responding to jasmonic acid, which is also required for stamen development and pollen maturation, suggesting some convergence of the GA and jasmonate-signalling pathways in the regulation of these processes (Mandaokar et al., 2006; Hou et al., 2008).

**Conclusions**

Reproductive development in plants is dependent on GA signalling to promote the development of floral organs and the stem extension that accompanies flowering in rosette species. Gibberellin involvement in floral initiation is more complex: while GAs promote flowering in some LD and biennial species, their effect in other species is variable or absent and they inhibit flowering of some perennials. Despite the demonstration of a clear role in a number of LD plants, through activation of genes encoding floral pathway integrators, the involvement of GAs in the photoperiod response is established only in a very few cases. In *Arabidopsis*, GAs are most influential for flowering in SD, in the absence of the major photoperiod-inductive pathway via CO. They may act as a gauge of developmental progression, allowing flowering to occur only at the appropriate stage for the environmental conditions.

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**References**


and overlapping roles of two gibberellin 3-oxidases in Arabidopsis development. The Plant Journal 45, 804–818.


