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The control of shoot branching: an example of plant information processing

OTTOLINE LEYSER

Department of Biology, Area 11, University of York, York YO10 5YW, UK

ABSTRACT

Throughout their life cycle, plants adjust their body plan to suit the environmental conditions in which they are growing. A good example of this is in the regulation of shoot branching. Axillary meristems laid down in each leaf formed from the primary shoot apical meristem can remain dormant, or activate to produce a branch. The decision whether to activate an axillary meristem involves the assessment of a wide range of external environmental, internal physiological and developmental factors. Much of this information is conveyed to the axillary meristem via a network of interacting hormonal signals that can integrate inputs from diverse sources, combining multiple local signals to generate a rich source of systemically transmitted information. Local interpretation of the information provides another layer of control, ensuring that appropriate decisions are made. Rapid progress in molecular biology is uncovering the component parts of this signalling network, and combining this with physiological studies and mathematical modelling will allow the operation of the system to be better understood.

Key-words: auxin; auxin transport; axillary bud; cytokinin; dormancy; strigolactone; TCP.

BEHAVIOUR AND BODY PLAN

Multi-cellular organisms generally start life as a single totipotent cell. Subsequently, the cell divides, and the daughters specialize to assume particular functions. This is development. In most of higher animals, development proceeds in a constant environment directed entirely by the animal's genetic programme. The mechanisms of development are complex and dependent on stochastic events and feedback regulation, so despite a high degree of homeostasis and redundancy in the system, the results for genetically identical animals are not always the same. None the less, the basic body plan of the resulting embryo is essentially invariant. Post-embryonically, released into an inconstant environment, animals cope by altering their behaviour. Thus, the most environmentally responsive part of an animal's anatomy is the wiring in the brain.

In contrast, higher plants end embryogenesis in a very rudimentary state. The basic body axes are established, with the apical–basal axis defined by the establishment of the shoot apical meristem at one end, and the root apical meristem at the other. Post-embryonically, the meristems give rise to the entire shoot and root systems, respectively. Furthermore, the tissues they establish can produce secondary meristems, which if activated can produce entirely new axes of growth with the same developmental potential as the primary root or shoot from which they were derived. Thus, the body plan of a plant is determined continuously throughout its life cycle, allowing it to be exquisitely environmentally responsive. Plants can alter their body plan to suit their environment, and thus the environmentally regulated development of new growth axes is functionally equivalent to environmentally regulated animal behaviours. This review considers the mechanisms regulating shoot branching behaviours. The control of root branching is discussed elsewhere in this issue.

Correspondence: O. Leyser. Fax: +01904-328682; e-mail: hmol1@york.ac.uk

HORMONAL CONTROL OF SHOOT BRANCHING

As described above, central to the regulation of animal behaviour is the nervous system. Sensory organs gather information about the internal and external environment and relay this information to the brain, where it is integrated, resulting in decisions about appropriate actions, which are transmitted back out into the body to direct the chosen response. Plants do not have a brain and they do not have a nervous system. Instead, information from the environment is integrated and processed in a distributed way throughout the plant body using a range of long-distance signals, prominent among which are the phytohormones. With respect to shoot branching, phytohormones play a central role in regulating the activity of secondary shoot meristems. The accumulation of data over many decades is now allowing a more holistic understanding of the network of interacting hormones that controls branching. Although there are still substantial gaps in our knowledge, a framework for understanding environmentally responsive branch regulation is now emerging.

BACKGROUND

Shoot branches are derived from secondary shoot apical meristems laid down in the axil of each leaf produced by the primary shoot apical meristem. Branch number variation

between species and cultivars can be due in part to the establishment of these meristems, but most of the environmental responsiveness and hence, phenotypic plasticity in shoot branching derives from the regulation of the activity of the axillary meristems after they have formed. Axillary meristems frequently initiate a few unexpanded leaves and then arrest their growth, forming a small axillary bud. The bud may subsequently reactivate to produce a branch, which makes leaves, which themselves bear tertiary shoot apical meristems in their axils, and thus genetically identical plants can occupy a huge area of phenotype space ranging from a single unbranched shoot, to a dense bush with high-order branching.

The regulation of axillary bud activation can be influenced by a panoply of environmental factors. Probably the most famous of these is damage to the primary shoot apex. It was the investigation of the response of dormant axillary buds to the removal of the primary shoot apex above them that first suggested a role for mobile hormonal signals in the control of shoot branching. In a classic series of experiments, Thimann and Skoog demonstrated that the removal of the primary apex results in the activation of dormant axillary buds in the subtending leaf axils, a phenomenon dubbed apical dominance (Thimann & Skoog 1933). They showed that bud activation could be prevented by application of the hormone auxin (at the time referred to as 'the growth substance') to the decapitated stump. As it was known that the primary shoot apex is a good source of auxin and that this auxin is transported basipetally down the stem, these results led to the hypothesis that apical dominance is mediated by auxin.

THE ROLES OF AUXIN IN BUD INHIBITION

Even now, after 75 years, the mechanism – or more likely mechanisms of action – of auxin in the inhibition of bud activation are matters of some debate. The most straightforward explanation, that auxin from the primary apex is transported into the buds and directly inhibits their activity, is not consistent with the evidence and was discounted very early in investigations of the phenomenon (e.g. Snow 1937). An important nail in the coffin of this idea came from the two-branched pea or bean systems developed by Snow (Snow 1931). To make a two-branched pea/bean, the primary shoot is removed just above the cotyledonary node, resulting in the activation of buds in the cotyledon axils. These grow out to form two branches, which may continue to grow evenly, but frequently, one will come to dominate the other, with the subordinate shoot stopping growth altogether. Removal of the dominant shoot results in the reactivation of the subordinate shoot.

These results clearly demonstrate an upward transmission of the inhibitory signal in the subordinate shoot, but it is clear that auxin transport in the stem is specifically downward. The specific basipetal direction of auxin transport is due to the basal localization of auxin efflux transporters in the xylem parenchyma cells of the stem (Blakeslee, Peer & Murphy 2005). Auxin, as a weak acid, is often found in the protonated form in the low pH of the apoplast, allowing it to cross the plasma membrane and enter cells passively. In the cytoplasm, the pH is higher and the auxin ionizes, trapping it in the cell unless exported actively, for example, by members of the PIN-FORMED (PIN) family of auxin efflux carrier proteins, which in the stem are basally localized. Consistent with these observations, radiolabelled auxin applied to the stump of the decapitated primary shoot can inhibit the activity of axillary buds below it without the accumulation of radiolabel in the bud (Prasad *et al.* 1993; Booker, Chatfield & Leyser 2003).

THE SECOND MESSENGER HYPOTHESIS

At least two distinct mechanisms have been proposed to account for the indirect inhibitory effect of auxin. The first involves auxin in the primary stem regulating the production of a second mobile signal that can move upward into the bud to regulate its activity. There is good evidence that cytokinin plays such a role in mediating the inhibition of bud growth by auxin. Cytokinin is a potent and direct activator of bud outgrowth (Sachs & Thimann 1967), and auxin has been shown to down-regulate cytokinin synthesis both locally at the node in the main stem (Tanaka *et al.* 2006) as well as in the roots (Bangerth 1994). For example, in pea, decapitation results in an increase in cytokinin export from roots and in increased transcription of cytokinin biosynthetic genes in the stem. Both these responses are prevented by the application of auxin to the decapitated stump. In the absence of an apical auxin supply, both these responses would increase cytokinin availability to buds and promote their activation. The ability of auxin to modulate cytokinin synthesis in *Arabidopsis* is dependent on the *AXR1* gene (Nordström *et al.* 2004), part of the canonical auxin signalling pathway in which the TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALING BOX PROTEIN (TIR1/AFB) family binds auxin and transduces the auxin signal to changes in gene expression by targeting members of the INDOLE-3-ACETIC ACID INDUCIBLE (Aux/IAA) transcriptional repressor protein family for degradation (Leyser 2006). Consistent with a role for this pathway in bud regulation, *AXR1* is necessary for the full inhibition of *Arabidopsis* buds by apical auxin (Chatfield *et al.* 2000). Tissue-specific expression of *AXR1* in an *axr1* mutant background demonstrated that *AXR1* acts in the xylem parenchyma to mediate this effect, which is the main site of polar auxin transport down the shoot (Booker *et al.* 2003).

THE CANALIZATION HYPOTHESIS

In addition to this cytokinin-mediated system, there is good evidence for a second mechanism of auxin action that does not rely on a second signalling compound moving into the bud. The proposed mechanism is not primarily based on auxin signalling via Aux/IAA destabilization, but rather on auxin transport and the canalization of auxin transport pathways from axillary buds into the main stem. Auxin transport is clearly central to apical dominance. The auxin

that inhibits axillary bud activity appears to be specifically that moving in the polar transport stream in the main stem. Auxin transport inhibitors applied with apical auxin can prevent the inhibitory effect of the auxin, and basally applied auxin, which can move through the stem in the transpiration stream, has no effect of bud activity (e.g. Booker *et al.* 2003).

In addition to auxin transport in the main stem, strong correlative evidence suggests that auxin export from the bud is an important factor in bud activation. For example, in the two-branched pea system, the growth of each branch is strongly correlated with its polar auxin transport activity (Morris 1977; Li & Bangerth 1999). This observation led to the suggestion that auxin moving in the main stem could modulate the export of auxin from buds (Li & Bangerth 1999). Furthermore, the canalization of auxin export from the bud is likely to be essential for the formation of vascular connectivity between the bud and the main stem (Sachs 1968).

Auxin transport canalization is a well-documented phenomenon, although its mechanistic basis is very poorly understood. The concept was introduced by Tsvi Sachs, who proposed that the movement of auxin from a source to a sink is gradually canalized into narrow files of cells with high auxin transport activity, highly polarized towards the auxin sink (Sachs 1981). This is achieved by auxin up-regulating its own transporters and by the flux of auxin out of cells further polarizing auxin export in the direction of that flux. These positive feedback loops result in the formation of narrow transport pathways or canals between the source and the sink. In the right developmental context, vascular strands differentiate from the files of cells along which auxin transport was canalized. Because of this close association with vascular development, canalization has been most studied in the context of various vascularization processes, such as leaf venation pattern formation, and the reconnection of stem vasculature strands interrupted by wounding.

The canalization hypothesis was proposed before any of the proteins that mediate polar auxin transport had been identified. Now that there are good markers for PIN protein accumulation and polarization, it has been possible to observe canalization in action at a molecular level. Sachs' predictions of gradually narrowing cell files with gradually increasing levels and polarity of auxin transport are mirrored in the accumulation and polarization of PIN proteins during canalization (Sauer *et al.* 2006). Furthermore, auxin up-regulates PIN gene expression (Heisler *et al.* 2005; Vieten *et al.* 2005) and can also modulate PIN protein localization in the cell, for example, inhibiting its removal from the membrane by endocytosis (Paciorek, Zazimalova & Ruthardt 2005). These data strengthen the phenomenological evidence for canalization, and begin to address the mechanisms that underlie it.

BUD ACTIVATION AND CANALIZATION

In the context of bud activation, as mentioned above, canalization of auxin export from the young expanding leaves of the axillary shoot apex out into the main stem almost certainly underlies the differentiation of vascular strands connecting the growing vascular network of the lateral shoot to the vascular network in the primary stem. Here, an auxin source – the young leaves in the bud, links to an auxin sink – the existing stem transport pathway. The polar transport stream in the stem is a good sink because of its ability to transport auxin away down the stem. This can be seen in a simple experiment where auxin is applied to the side of an isolated pea stem segment (Sachs 1981). A vascular strand will be induced between the site of auxin application and the existing vasculature in the stem. Interestingly, if apical auxin is simultaneously applied to the existing vascular strand in the stem, this dramatically reduces its sink strength, canalization from the lateral auxin source is not initiated and vascular connections between the source and the existing vascular strands no longer form. In a similar way, the geometry of vascularization of lateral branches can be manipulated in pea by the addition of auxin to the vascular strands with which the bud vasculature can connect (Sachs 1968). Specifically, if a pea plant is decapitated, the buds in the axils of the subtending young expanding will activate. Upon activation, their vascular strands could connect either with the leaf trace of the subtending leaf or with vascular bundles in the main stem. The path chosen depends on whether the leaf remains intact, in which case the bud vasculature connects with main stem vascular bundles; or not, in which case the bud vasculature connects with the leaf trace. As expanding leaves are excellent auxin sources, these results are consistent with the idea that the bud's vascular system links to nearby vascular strands with the least auxin and hence the greatest sink strength. Taken together, these data suggest that if there is no strong auxin sink, canalization of auxin transport from a source will not occur, and if there are several possible sinks, canalization will occur towards the strongest sink.

These observations suggest a mechanism by which apical auxin can inhibit axillary bud activation (Bennett *et al.* 2006). If canalization of auxin out of the bud is needed for bud activation, but the auxin sink strength of the stem vasculature is low because of apically derived auxin, then canalization and bud activation will not occur. In contrast, if the apical auxin source is removed, then the sink strength for auxin in the main stem vasculature will increase, canalization from the auxin source in the bud can be initiated and bud activation will ensue. In this way, apical auxin can regulate bud activation indirectly without anything passing upward into the bud. Instead, the system works by competition for auxin sink strength in the main stem. Another interesting feature of this system is that it is not primarily about auxin concentrations, as detected by the TIR1 auxin receptor family, and transduced through Aux/IAA degradation.

REGULATION OF AUXIN CANALIZATION OUT OF AXILLARY BUDS

Further evidence in support of an auxin-transport-based system for the control of bud activation comes from the

Table 1. Current status of SL-pathway-related protein orthologies

	Strigolactone synthesis			Signalling	
	Carotenoid cleavage dioxygenase (CCD8)	Carotenoid cleavage dioxygenase (CCD7)	Cytochrome P450	F-box protein	Unknown g = graft rescuable; n = not graft rescuable
Pea	RMS1	RMS5		RMS4	RMS3 (n), RMS2 (g)
Petunia	DAD1				DAD2 (g), DAD3 (n)
Rice	D10	HTD1/D17		D3	D14, D27
Arabidopsis	MAX4	MAX3	MAX1	MAX2	

analysis of a series of increased shoot branching mutants identified in diverse species including pea, petunia, rice and *Arabidopsis*. In *Arabidopsis*, the mutants are referred to as the *max* mutants for *more axillary growth* (Stirnberg, van de Sande & Leyser 2002; Sorefan *et al.* 2003; Booker *et al.* 2004); in peas they are called *rms* mutants, for *ramosus* (Beveridge, Ross & Murfet 1996; Beveridge *et al.* 1997; Morris *et al.* 2001); in petunia they are called *dad* mutants, for *decreased apical dominance* (Napoli 1996; Snowden & Napoli 2003; Snowden *et al.* 2005); and in rice they are called *d* mutants, for *dwarf*, or *htd*, for *high tillering dwarf* (Ishikawa *et al.* 2005; Zou *et al.* 2006; Arite *et al.* 2007). Table 1 shows the known orthologies of the genes from these different species. The genes fall into two categories, distinguished by their behaviour in grafting experiments. One class is required for the production of an upwardly mobile and therefore probable xylem transported, graft-transmissible signal (Napoli 1996; Beveridge 1997; Morris *et al.* 2001; Turnbull, Booker & Leyser 2002; Booker *et al.* 2005; Snowden *et al.* 2005; Simons *et al.* 2007). The other class acts locally at the node, presumably in the perception or transduction of this signal (Beveridge *et al.* 1996; Booker *et al.* 2005; Simons *et al.* 2007; Stirnberg, Furner & Leyser 2007). For example, of the four known *MAX* genes, *MAX1*, *MAX3* and *MAX4* fall into the signal synthesis class, and consistent with this, they all encode proteins with homology to biosynthetic enzymes. The fourth gene, *MAX2*, encodes a nuclear-localized F-box protein that is required locally in the shoot, consistent with its proposed signalling role. Like other known F-box proteins, MAX2 participates in an Skp1-Cullin-F box protein (SCF) complex and thus presumably is involved in selecting specific protein targets for ubiquitin-mediated degradation (Stirnberg *et al.* 2007).

The ubiquitinylation targets for this pathway are unknown, but the effect of the mutations is an increase in auxin transport in the main stem (Beveridge, Symons & Turnbull 2000; Bennett *et al.* 2006; Lazar & Goodman 2006), characterized by increased PIN1 protein accumulation in the basal membranes of the xylem parenchyma cells (Bennett *et al.* 2006). Stem segments of *max* mutants, and of the *rms1* mutant in pea, transport an increased amount of applied auxin in unit time compared with wild type. The speed of auxin transport is not greatly affected, but more auxin is transported. Very high levels of expression from the auxin-responsive DR5 promoter in *max* mutant stems suggest that in intact *max* plants, more auxin is moving. These data are somewhat paradoxical in terms of traditional thinking about auxin and shoot branching, as here, high auxin levels in the stem are correlated with high levels of branching. However, this result can be explained in terms of the proposed auxin transport canalization-dependent mechanism for bud activation. The effect of the *max* mutants could be to increase main stem auxin sink strength in some way, allowing establishment of auxin canalization out of axillary buds, despite the presence of apically derived auxin moving in the main stem polar transport stream. In this case, one would predict increased auxin transport, increased auxin levels and increased bud activity, as observed in the mutants. Consistent with a transport-based cause for the phenotype, a wild-type shoot branching phenotype can be restored to *max* mutants by reducing PIN-protein function either through *pin1* mutation or using low doses of auxin transport inhibitors that restore wild-type auxin transport levels (Bennett *et al.* 2006). Furthermore, bud vascular connectivity in the max mutants differs from the wild type in a way that suggests increased main stem auxin sink strength (Ongaro *et al.* 2008).

Analysis of the MAX/RMS pathway therefore supports a mechanism for bud inhibition dependent on main stem auxin sink strength and auxin canalization out of the bud. Furthermore, the fact that the MAX/RMS pathway appears to act by modulating auxin transport properties in the plant suggests that main stem auxin sink strength, and hence auxin canalization from the bud may be influenced both by the amount of apically derived auxin moving in the main stem, and by the amount of MAX activity in the plant, which could be dynamically regulated. As the pathway operates by the production of a mobile signal, this adds a third hormone to auxin and cytokinin, interacting to modulate shoot branching.

STRIGOLACTONES AND SHOOT BRANCHING CONTROL

MAX4 and *MAX3,* which as described above are required for the production of a graft-transmissible, upwardly moving branch inhibitor, encode divergent members of the carotenoid cleavage dioxygenase family, and indeed have been shown to have carotenoid cleavage activity in a range of assays (Sorefan *et al.* 2003; Booker *et al.* 2004; Schwartz, Qin & Loewen 2004; Auldridge *et al.* 2006; Alder

et al. 2008). It was this that led to a hypothesis that the signalling compound defined by these mutants might be a strigolactone (SL) or SL-derivative (Gomez-Roldan, Fermas & Brewer 2008; Umehara, Hanada & Yoshida 2008), as evidence suggests a carotenoid origin for these compounds (Matusova *et al.* 2005). SLs were originally identified as the root-derived germination triggers for parasitic plants such as Striga (reviewed in Humphrey & Beale 2006). As seed resources are limited and parasitic plants cannot photosynthesize, it is important that they only germinate in proximity to a host plant. SLs are secreted by many plants, probably primarily to attract mycorrhizal symbionts (Akiyama, Matsuzaki & Hayashi 2005), and Striga exploits the presence of these compounds to detect potential hosts. The addition of SLs to *max3*, *max4* and *max1* was found to rescue their branching phenotype, whereas no effect was observed when *max2* mutants were treated in a similar way (Gomez-Roldan *et al.* 2008; Umehara *et al.* 2008). Similar results were obtained with the relevant *d* mutants from rice and *rms* mutants from pea. Furthermore, in pea and rice, the predicted biosynthetic mutants had reduced SL levels compared with wild type as assessed directly or through various bioassays, whereas the predicted signalling mutants had at least wild-type levels. These data strongly suggest that the mobile branch-inhibiting signal involved in the MAX/RMS/D/DAD pathway is a SL or a SL-derived compound.

This discovery should speed efforts to understand the mechanism of action of this pathway and to test the proposed auxin canalization mechanism for bud activation. For example, one interesting question is the main site of action of the hormone in suppressing bud growth. Thus far, the canalization-based regulatory mechanism has been expressed entirely in terms of main stem sink strength, but bud source strength could also play a role. Addition of auxin directly to buds does not trigger their activation (Sachs & Thimann 1967), demonstrating that simply providing high local auxin is not sufficient to drive auxin canalization out of the bud. However, if the effect of SLs is to restrict auxin canalization potential in some way, then they might be able to operate equally well in the main stem and in the bud. Whether both these sites operate, and if so, what their relative importance might be is unclear. One issue here is that young buds are not vascularly connected to the main stem, and thus if a root source of SL is sufficient for bud inhibition, as suggested by grafting experiments described above, the amount of SL reaching the bud from the stem will be considerably lower than the SL in the stem. In this situation, a stem site of action might be more important. However, interestingly, direct application of SLs to *rms* mutant pea buds can suppress their growth, whereas in *Arabidopsis*, this treatment was rather ineffective in comparison to feeding the compound hydroponically through the roots (Gomez-Roldan *et al.* 2008; Umehara *et al.* 2008). One interpretation of this result is that SL acting directly in the bud can modulate bud growth in pea; however, in *Arabidopsis* stem SLs are required for full bud growth suppression.

THE HORMONE REGULATORY NETWORK

The shoot branching regulatory system, as described so far (Fig. 1), therefore involves three long-range hormonal signals:- auxin, synthesized mainly in young expanding leaves moves down the plant in the polar transport stream; and SLs and cytokinin, synthesized both in the root and shoot, move up the plant most probably in the transpiration stream. Auxin regulates cytokinin synthesis via the canonical auxin signalling pathway, and SLs regulate auxin transport in some as yet unknown way. In addition, there is evidence that auxin can up-regulate SL synthesis through the same AXR1-dependent pathway by which auxin down-regulates cytokinin synthesis (Bainbridge *et al.* 2005; Foo *et al.* 2005; Zou *et al.* 2006; Arite *et al.* 2007). Thus, the system consists of a series of interlocking feedback loops. On top of this, in common with many signalling systems, each hormone can apparently feedback on its own synthesis and/or degradation and/or signalling. For example, the AXR1/Aux/IAA pathway regulates the transcription of auxin-conjugating enzymes, which remove auxin from the free pool, as well as inducing transcription of the Aux/IAAs themselves, down-regulating auxin signalling, and auxin can also down-regulate its own synthesis (reviewed in Leyser 2006). Similarly, transcription of the *CCD* genes involved in SL synthesis is upregulated in the *ccd* and SL-signalling mutant backgrounds (Foo *et al.* 2005; Arite *et al.* 2007), suggesting negative feedback on SL synthesis. An example in Ck biology is that the best known early up-regulated genes

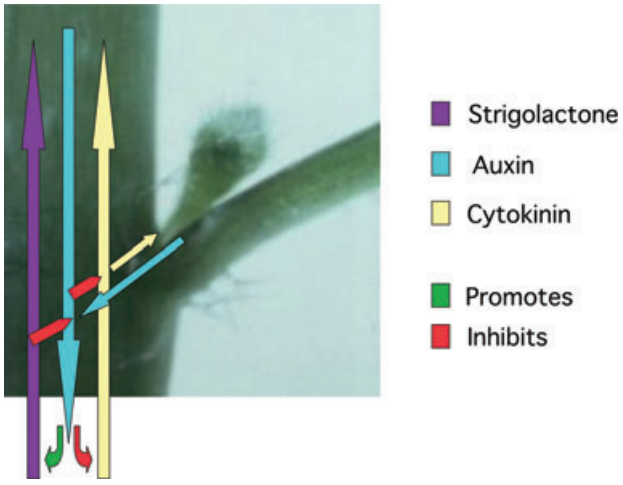

Figure 1. The hormonal network regulating shoot branching. The axillary bud is proposed to activate depending on its ability to export auxin and its cytokinin supply. Strigolactones (purple, SL) move up the plant, presumably in the transpiration stream, and negatively regulate auxin transport, reducing stem sink strength for auxin exported from the bud. Auxin (blue) is actively transported down the plant in the polar transport stream where it negatively regulates cytokinin synthesis and positively regulates SL synthesis. Cytokinin (yellow) moves up the plant in the transpiration stream. Note, the bud itself contributes auxin to the main stem, affecting the status of the network at other nodes. Red arrows indicate inhibition. Green arrows indicate promotion.

in cytokinin signalling are members of the Type-A response regulator family that negatively regulate cytokinin signalling (e.g. To *et al.* 2004).

It is likely that additional signals are also integrated into this network. One obvious example is gibberellin (GA). Several GA-related mutants have shoot branching phenotypes (e.g. Rieu, Ruiz-Rivero & Fernandez-Garcia 2007), and GA has been shown to interact with auxin in the regulation of stem elongation, with apically derived auxin regulating GA synthesis (O'Neill & Ross 2002). Analysis of the *rms* mutants in pea has led to the proposal that there is a novel downwardly mobile signal integral to the network (Beveridge *et al.* 1997, 2000; Foo *et al.* 2005, 2007; Johnson *et al.* 2006). The evidence for this signal comes from analysis of the *rms2* mutant, which has a number of phenotypes that contrast sharply with those of the other *rms* mutants. In particular, while *rms1/max4, rms5/max3* and *rms4/max2* mutants have high expression of the *RMS1/MAX4* and *RMS5/MAX3* genes, the *rms2* mutant has reduced expression of these genes; while *rms1/max4, rms5/max3* and *rms4/max2* mutants have extremely low xylem sap cytokinin levels, the *rms2* mutant has somewhat higher levels than the wild type. Grafting experiments demonstrate that root cytokinin export and *RMS* gene expression phenotypes are governed by the shoot. This suggests that *RMS2* is required for the production of a downwardly mobile signal that is down-regulated by the RMS/MAX pathway and acts as a feedback system to up-regulate *RMS1* and *RMS5* transcription and to down-regulate cytokinin synthesis. This signal shares many features with auxin. It is downwardly mobile, it up-regulates *RMS1* and *RMS5* transcription, and it down-regulates cytokinin synthesis. The suggestion that it is not auxin, but rather a novel signal, comes from the observations that the *rms1/rms5/rms4 max4/max3/max2* mutants do not have increased global auxin levels or an increased speed of auxin transport. However, as described above, they do appear to have increased amounts of auxin moving in the polar transport stream; thus, on these criteria, auxin is still a viable candidate for this signal. Consistent with this view, the *rms2* mutant phenotype is in many respects similar to that conferred by *axr1* mutants.

ENVIRONMENTAL INTEGRATION

Clearly there are still many unanswered questions about the hormonal network described above. Nonetheless, even with current knowledge, it is possible to see how the network could contribute to integrating endogenous developmental programmes with environmental inputs. In terms of apical dominance, it is straightforward to see how information about the health of the primary apex can be transmitted to the buds by changes in auxin levels or auxin transport characteristics in the main stem. In addition, there is good evidence that other environmental signals could act through modulating parameters in the network.

For example, it is well-established that nitrate can up-regulate cytokinin synthesis in the root (Takei *et al.* 2002). This root-derived cytokinin could move up the plant in the transpiration stream and promote branching, shifting the root–shoot ratio in favour of the shoot. There is also some evidence that shoot-derived auxin may be involved in N-status communication. Supernodulating mutants of several legumes fail to suppress nodulation in response to nitrate and in response to existing nodules (e.g. Carroll, McNeil & Gresshoff 1985). This phenotype is mediated by the genotype of the shoot, and is associated with failure to reduce auxin transport to the root (van Noorden *et al.* 2006). Thus, it seems likely that low N may suppress shoot branching by both reducing cytokinin supply from the root and increasing the amount of auxin transported from the shoot apex. In the context of nutritional signals, it is interesting to consider the recent discovery that SLs inhibit shoot branching. The synthesis of these compounds was previously shown to play a major role in signalling between plant roots and mycorrhizal fungi during the early stages of the establishment of a symbiosis in which the fungus improves phosphate acquisition for the plant, in return for fixed carbon (Akiyama *et al.* 2005). It is therefore not surprising that SL synthesis by roots is greatly up-regulated during phosphate starvation. This may serve a dual purpose in suppressing shoot branching, and it will be interesting to determine whether this second function is conserved in non-mycorrhizal plants such as *Arabidopsis*.

Another important environmental signal that regulates branching is light quality. Shoot branching suppression is a characteristic feature of the shade escape response (reviewed in Franklin & Whitelam 2005). In response to low red : far red (R : FR) light ratios, indicative of shading by other plants, shoot elongation is promoted, but leaf expansion and shoot branching are suppressed. There is a close association between shade escape and auxin. For example, recent data suggest that low R : FR ratios increase auxin synthesis in young leaves (Tao, Ferrer & Ljung 2008), resulting in suppression of leaf growth through auxin-induced cytokinin degradation via induction of a cytokinin oxidase (Carabelli *et al.* 2007). Increased amounts of auxin are also exported from young leaves in low R : FR, promoting elongation of the primary axis. Most of this work has been carried out using young *Arabidopsis* seedlings, so the implications for branching control are unclear. However, in older plants, increased auxin export from young leaves would presumably inhibit shoot branching. Consistent with this idea, *Arabidopsis phyB* mutants, which are impaired in the ability to detect R : FR ratios, have reduced branching even in high R : FR ratios, and this phenotype is suppressed in *max2* mutants (Shen, Luong & Huq 2007). Aside from auxin synthesis, there is evidence that auxin response and auxin transport are affected by R : FR ratio, and that they are more generally tightly integrated in light responses, particularly with respect to long range shoot–root communication. For example, in various light signalling mutants such as those affecting the HY5 transcription factor, which plays a central role in light-regulated transcriptional

changes, and in photoreceptor mutants such as *phyA* and *phyB* mutants, there are widespread changes in auxin-regulated gene expression and root system architecture phenotypes indicative of auxin distribution or response defects (Sibout *et al.* 2006; Salisbury *et al.* 2007). Thus, it seems likely that light-regulated effects on shoot branching are mediated at least in part by a combination of changes in auxin fluxes and auxin responses.

REGULATION IN THE BUD

The hormone regulatory network described above can control branching, but how these signals change bud activity, and the extent to which bud-specific factors can override hormonal network status is unknown. We know remarkably little about the events within the buds themselves that determine whether or not they are active.

The apparent requirement for auxin export from the bud for its activity may provide a rather direct mechanism for bud growth control. The production of leaves at an active shoot apex is at least partly driven by the phyllotactic patterning system. Recent advances in our understanding of phyllotaxis suggest that new leaves are initiated at the flanks of the shoot apical meristem in response to the local accumulation of auxin (Reinhardt *et al.* 2003; Heisler *et al.* 2005). Auxin accumulation is driven by the dynamic relocalization of PIN proteins in the meristem epidermal layer (the L1). A critical step in the process is the initiation of a pathway for auxin transport from the newly formed point of auxin accumulation in the L1 into the underlying tissue layers, towards the sink provided by the parastichous vascular strand below. If, as discussed above, there is no such sink because main stem sink strength is low, then this alone might directly block axillary meristem activity, preventing auxin drainage from the site of leaf initiation and thus blocking subsequent events in phyllotactic patterning. Inability to export auxin would also be predicted to stop the expansion of any leaves that had formed, a process that is likely to require removal of considerably larger amounts of auxin than the original leaf initiation process. As auxin synthesis is under negative feedback control (Ljung, Bhalerao & Sandberg 2001), a block in auxin removal would also result in an arrest in auxin synthesis, stabilizing auxin levels in the bud. Bud activation would result not only in auxin removal from the bud, but also in renewed auxin synthesis, such that auxin levels would be at least as high in active as inactive buds, as is observed (e.g. Hillman, Math & Medlow 1977). This is an attractive hypothesis, consistent with current data, but it is yet to be rigorously tested. Nor is it clear as to how cytokinin accumulation in the bud might overcome such a blockage.

Such a mechanism is unlikely to be the only system at work. At this point, it is important to consider that bud inactivity comes in different forms. Thus far, this review has been considering 'paradormancy' in which inactivity is imposed by signals coming from the rest of the plant, or perhaps 'ecodormancy' in which dormancy is imposed by an environmental signal. Given the discussion above, it is clearly not possible to draw a clean distinction between these two dormancy types; however, in many perennial systems, a deeper dormant state – 'endormancy' – is widely recognized in which the internal changes in the bud result in dormancy that cannot be reactivated by the removal of the factors that may have imposed the dormancy in the first place such as short days. Instead, a specific environmental trigger such as prolonged chilling may be required to break the dormancy and allow reactivation of the bud. Here, clearly additional factors over and above the inability to remove auxin, and/or a deficiency of cytokinin, must come into play. Transcriptome studies have shown that the imposition of such dormancy in the apical bud of poplar shares many molecular markers with other deep dormancies, for example, seed dormancy, including protective features such as ABA-dependent desiccation tolerance (Ruttink *et al.* 2007).

Even in paradormant systems, endogenous bud factors are clearly important, because different buds appear to have different activation potentials. For example, in pea, decapitation results in the release of bud inhibition with the classical basipetal progression predicted by auxin depletion. However, in addition, the bud in the second node (number from the base up) activates very rapidly after decapitation, completely out of sequence (Morris *et al.* 2005). The mechanism by which decapitation activates this bud is unclear. It may simply be super-sensitive to changes in auxin or auxin transport properties in the stem that are not detected by bulk measurements. Alternatively, this bud may respond to a different signal. However, as neither node one buds, nor node three buds were reported to activate, it is clear that the bud in node two has an enhanced activation potential with respect to either acropetally or basipetally moving signals.

Transcriptome studies have also been used to identify bud-expressed genes involved in bud activity regulation in non-endodormant buds such as those of *Arabidopsis* (Tatematsu *et al.* 2005). These studies have produced, as expected, lists of genes – the expression of which correlates with bud activity – but the functional significance of these transcriptional changes is largely unknown, although many are predicted to be regulated by TCP family members. Members of the class II subfamily of the TCP transcription factors are the only genes with a really clear role in the bud itself. The class I genes include the rice *PCF* genes which encode proteins that promote the expression of PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA), an important cell cycle regulator (Kosugi & Ohashi 1997). In contrast, class II family members have been shown to be involved in suppressing growth in a range of contexts (Cubas *et al.* 1999). For example, the *Antirrhinum majus CYC* gene is involved in suppressing petal growth during the formation of zygomorphic flowers. The *CYC* gene and the *PCF* genes contribute the C and P to the TCP family name. The T comes from the *TB1* (*teosinte branched1*) gene from maize, which is involved in suppressing bud activity (Doebley, Stec & Hubbard 1997). Fixation of an overexpression allele was a key event in the domestication of maize from an ancestor likely to have resembled its

present-day wild relative Teosinte, which has a highly branched shoot system architecture (Wang *et al.* 1999). Similar roles for closely related genes were demonstrated in rice and Sorghum, and more recently for an *Arabidopsis* orthologue, *BRC1* (Takeda *et al.* 2003; Kebrom, Burson & Finlayson 2006; Aguilar-Martinez, Poza-Carrion & Cubas 2007).

Expression of *TB1* and its orthologues is impressively specific to axillary meristems, and the accumulation of their mRNA is negatively correlated with bud activity (Hubbard *et al.* 2002; Aguilar-Martinez *et al.* 2007). Treatments that repress branching, such as crowding and *PHYB* mutation, increase mRNA levels; however, treatments that increase branching, such as decapitation, reduce mRNA levels. Importantly, loss of function alleles at these loci result in an increased branching phenotype, clearly demonstrating a requirement for this gene function in reducing shoot branching. There are other genes involved in bud activation such as members of the *SPL* family (Schwarz *et al.* 2008; Wang *et al.* 2008), but it is for the *TB1*-like *TCP*s that there is currently the best evidence for a bud-localised role.

The availability of the *brc1* mutant in *Arabidopsis* made it possible to investigate the interactions between *BRC1* and the hormone regulatory network that controls branching (Aguilar-Martinez *et al.* 2007). The data thus far present an interesting picture. *BRC1* expression is significantly down-regulated in the *max* mutants. However, the small reductions in *BRC1* mRNA observed in the *axr1* mutant were not statistically significant. These results support at least partially separable modes of action for auxin signalling via the TIR1/AXR1 pathway in branch suppression, and auxin transport via the MAX pathway. As described above, a likely mode of action for the TIR1/AXR1 pathway is via the down-regulation of cytokinin synthesis, such that increased branching in *axr1* mutants might be due to increased cytokinin. Consistent with the idea that cytokinin might up-regulate bud growth independently of *BRC1* down-regulation, *BRC1* transcript is not significantly lower in *amp1* mutants, in which increased shoot branching is associated with increased cytokinin levels (Aguilar-Martinez *et al.* 2007). These data suggest that *BRC1* levels correlate with bud auxin export status as modulated by the MAX pathway, rather than with bud cytokinin levels. A prediction of this hypothesis is that *brc1* mutants should be SL resistant, while *axr1* mutants should show a more wild-type response to SLs.

CONCLUSIONS

The hormonal control of shoot branching is an area of research with a very long history. An intricate network of hormone signals that move through the plant between the root and shoot systems regulates branching. Multiple feedback loops operate in the network to provide a robust mechanism that co-ordinates and balances information from both root and shoot. Environmental signals influence the network, allowing developmental plasticity, adapting shoot system architecture to the prevailing conditions. This long-range signalling system must interact with local signals in each axillary bud. Internal bud information may reduce or enhance the sensitivity of the bud to the hormonal network. In this way, each bud will activate or not according to locally interpreted output of a distributed information-processing system, resulting in environmentally and developmentally appropriate bud behaviours. The rapidly accelerating rate of discovery in plant biology offers an exciting opportunity to understand the molecular basis for these behaviours, uniting physiology and molecular genetics. The incorporation of computational and mathematical modelling will be an essential tool to develop an understanding of the system.

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