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Additional Signalling Compounds are Required to Orchestrate Plant Development

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Abstract

Plants are necessarily complex systems that require monitoring of multiple environmental signals and, in response to those signals, coordination of differentiation and development of an extensive array of cell types at multiple locations. This coordination must rely on integration of long-distance signals that provide a means of communication among different plant parts. We propose that the relatively well-characterized classical phytohormones must act with several other long-distance signals to achieve this level of organization with dynamic yet measured responses. This is supported by observations that classical phytohormones: (i) operate in complex yet experimentally unresolved networks involving cross-talk and feedback, (ii) are generally multifunctional and nonspecific and hence must rely on other long-distance cues or pre-set conditions to achieve specificity and (iii) are likely to mask roles of other long-distance signals in several experimental contexts. We present evidence for involvement of novel long-distance signals in three developmental processes—branching, flowering and nodulation, and discuss the possible identities of novel signalling molecules.

Key words: Phytohormones; Long-distance signalling; Networks; Master regulators; Branching; Flowering; Nodulation; Novel signals

Introduction

Genes control the growth and differentiation of cells in response to environmental conditions and to local and systemic developmental states. The importance of this dynamic relationship in plants is reflected in enormous phenotypic plasticity such that plants of the same genotype grown under different conditions can appear qualitatively different (for example, see Beveridge and others 2003). To control development, some information about the developmental fate of cells is carried by the state of gene expression in those cells, but a considerable amount of information must also be received from elsewhere. Long-distance signals in plants orchestrate the regulation of development in response to the environment and ensure that growth and differentiation of individual plant parts is commensurate with the activity of other plant parts. Long-distance signals are a well-documented component

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of the regulation of most aspects of development including light and stress responses, flowering, branching, nodulation and root and shoot growth and a multitude of other processes including nutrient uptake and transport (for example, see Curie and Briat 2003; Trewavas 2002a). This is consistent with the observation that plants, like all biological organisms, are complex systems, requiring feedback regulation among their components (Kitano 2002). Consequently, plant development is regulated by complex networks of long-distance signals that involve feed-forward (induction) and feedback (suppression) of both the signals and the processes they control.

The notion of networked control systems in plants has been highlighted by Trewavas (see for example, Trewavas 2002b), who emphasizes problems with attributing control of individual processes to single regulatory substances. The concept of plant hormones as primary limiting factors in development and physiology has rested largely on experiments with biosynthesis mutants and growth regulator applications. There are clear-cut data indicating, for example, that stem elongation correlates with gibberellin content (GA_1) (Ingram and others 1986) or that stomatal aperture can be tightly coupled to incoming xylem abscisic acid flux (ABA) (see Dodd this issue). Trewavas, however, points out that intact plants will have multiple inputs into stem growth rate, including light, nutrient status, and other hormones including auxin (Ross and others this issue) and ethylene; likewise additional factors can influence stomatal aperture, operating via ABA, antagonistically to ABA, or independently of ABA signalling (Dodd this issue). Consistent with the operation of hormones within a complex network, the control of plant processes by hormones is generally relative rather than absolute, with the control strength exerted by any single regulator being modified by other influences existing at that time.

Long-distance signals usually act in target cells via local signalling cascades comprising receptor complexes and several signal transduction proteins (for example, Leyser and Deng 2000). These signal transduction pathways have recently been recognized as networks themselves with feedback and cross-talk among the components and among different transduction pathways and phytohormones (Leyser and Deng 2000 and references therein; Trewavas 2002a). Genetic modification of this control process in target cells may result in altered phenotypes. However, if the modification is in duplicated/redundant genes or in systems where down-stream genes induce feedback regulation of other genes in compensation, a clear mutant phenotype may not eventuate.

Mutant screens for altered response to known phytohormones have revealed several genes critical to normal plant development which preclude or reduce the hormone response. Generally these mutants show highly pleiotropic phenotypes. Some of the most successful screens have yielded genes acting in ethylene responses (for example, Cancel and Larsen 2002). However, involvement of additional components in the signalling network can yield mutants that lack a response to a particular hormone but have a primary lesion in a different part of the signalling network. For example, *lkb* in pea was originally characterized as a GA response mutant but was later found to have a lesion in the brassinosteroid biosynthesis pathway, thereby causing an indirect effect on GA response (Nomura and others 1997). Such cross-talk in hormone response would probably not have been revealed had brassinosteroids not been discovered independently of gibberellins. Defining mutants as either ''hormone deficient'' or ''hormone response'' may therefore be misleading in the case of the latter.

One of the central themes of this special issue is the importance and prevalence of hormone cross-talk and interactions in the regulation of plant development, whether that be at the level of biosynthesis and metabolism or of signal transduction. The interaction of long-distance signals contributes to the pleiotropic effects of plant hormones. Given that relatively complicated networks of interacting components, including classical plant hormones, regulate plant development, it is likely that these networks mask the involvement of additional, novel long-distance signals.

Pleiotropic Regulators Mask Specific Regulators

The role of auxin in plant development is an excellent example of how the pleiotropic effects of one hormone may mask the effects of another. Let us suppose that auxin is a ''master regulator'' (see also Ross and others this issue), disseminating critical information on the growth and status of the shoot tip and young leaves, whereas the induction of individual developmental processes, such as flowering, nodulation and branching, requires one or more long-distance signals specific to that particular process (Figure 1). Modulations in auxin levels would override or regulate the action of these specific pathways. Indeed, auxin appears to be associated with nearly every developmental process

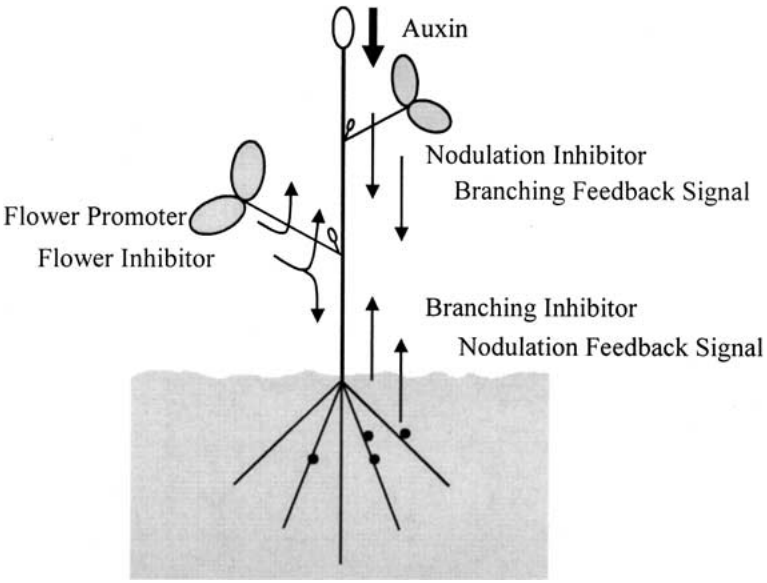

Figure 1. Coordinate regulation of flowering, branching and nodulation. A tiny subset of the signals involved in plant development is shown here including a ''master regulator'', auxin, and six presumably novel, but specific long-distance signals. The central importance of the shoot tip and young leaves is reflected by the influence of auxin on many processes (including nodulation and branching). The specific induction and homeostatic regulation of these developmental processes may in some cases occur independently of auxin, enabling more precise information transfer within the plant.

(Leyser 2001), different signal transduction pathways mediate different effects of auxin (Vogler and Kuhlemeier 2003) and other hormones and signals, known and unknown, are involved in complex networks with auxin (see also Vogler and Kuhlemeier 2003). Moreover, if auxin action over long-distances requires modification by other components in the signalling network, surely these components must also act as long-distance signals in order to achieve specificity in the message transfer from one location to another? The step-wise analysis of branching and other developmental mutants described below shows how grafting studies and hormone analyses can identify roles for known ''master regulators'' and demonstrate involvement of specific, yet novel long-distance regulators.

Case Studies Highlighting Novel Developmental Signals

Branching

Studies on branching, flowering and nodulation have provided evidence for specific roles of novel long-distance signals in plant development. Shoot branching is an enormously plastic component of plant architecture (Beveridge and others 2003). Environmental factors, especially light and soil nutrition, have dramatic effects on shoot architecture and nearly all plants rapidly initiate new branches from lateral buds if the main stem is damaged. Branching therefore represents the product of activity or inactivity of many shoot meristems at positions along every plant stem. Exogenous hormone studies, especially with auxin and cytokinin, but also with ABA, ethylene and gibberellin, indicate multiple responses of such meristems.

Mutants selected on the basis of altered branching phenotype allow focus on branching-specific regulators. Genes such as *RMS1*, *RMS2* and *RMS5* in pea, *MAX1* and *MAX3* in *Arabidopsis*, and *DAD1* in petunia all appear to regulate long-distance signals that can inhibit branching (Morris and others 2001; Turnbull and others 2002; Napoli 1996). In grafting experiments, a whole wild-type rootstock (Figure 2) and/or a few millimeters of wild-type interstock is able to suppress branching of mutant scions that would otherwise be highly branched. Further grafting experiments in pea indicate that the signal moves acropetally, but not basipetally in shoots (Foo and others 2001). Cytokinin and auxin quantification from xylem sap and shoots, respectively, indicate that the signal is not a major cytokinin or an auxin precursor and is therefore probably not a known phytohormone (Figure 2) (Beveridge and others 1997b; Morris and others 2001). Nevertheless, feedback regulation of these hormones is evident in the *rms* mutants, with several exhibiting decreased xylem sap cytokinin content and often increased shoot auxin content (reviewed by Beveridge 2000). The branching signal that moves to the shoot appears to be essential for applied auxin to inhibit branching following decapitation. Decapitated *rms* shoots have a reduced response to exogenous auxin but grafting to WT can restore this response (Beveridge and others 2000). Grafting provided the essential demonstration that the reduced auxin response was not due to a local lesion in auxin signal transduction, but to a lesion in the

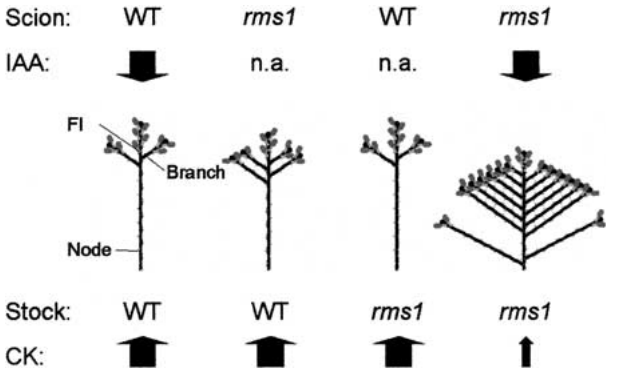

Figure 2. Evidence that *RMS1* in pea regulates a novel branching signal. Reciprocal graft combinations *of rms1* and WT plants are shown. Shoot indole-3-acetic acid (IAA) levels and total root xylem sap cytokinin (CK) concentrations are represented by the thickness of arrows, whereas arrow direction represents direction of transport. The WT self-graft is labelled to show a node, a branch, and the node of flower initiation (FI). Data are mostly from Beveridge and others 1997b; Foo and others 2001 who provide evidence that the signal is an inhibitor. Photoperiod, 18 h. Data not available are shown as n.a.

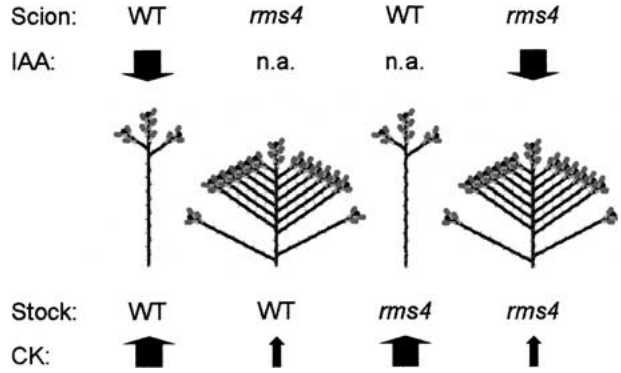

Figure 3. Evidence that a long-distance signal derived in shoots regulates cytokinin export from roots in pea. Reciprocal graft combinations of *rms4* and WT plants are shown and annotated, as described in Figure 2. Data are mostly from Beveridge and others 1997a and Beveridge 2000. Photoperiod, 18 h. Data not available are shown as n.a.

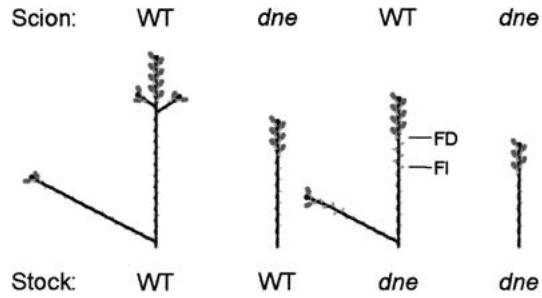

Figure 4. Evidence that *DNE* in pea regulates a long-distance signal required to delay flowering. Reciprocal graft combinations of *dne* and WT plants are shown and annotated, as described in Figure 2. The delay in flowering in *dne* scions grafted to WT rootstocks is about 3.5 nodes, or 125% that of *dne* self-grafts. WT scions grafted to *dne* rootstocks initiate flowers at the node of flower initiation (FI), but do not develop flowers until several nodes later (FD). Data are mostly from Murfet 1971 and King and Murfet 1985. Photoperiod, 8 h.

synthesis, transport or metabolism of a long-distance signal that is part of the branching control network.

Based again on branching mutant grafting, there appears to be a second novel signal in this network, capable of transmission from shoot to root, that down-regulates root cytokinin export (Beveridge and others 1997a). Branching mutant scions (*rms3* and *rms4*) grafted to WT rootstocks cause a decrease in root cytokinin export whereas WT scions can restore cytokinin export from mutant roots (Figure 3). This effect of the scion does not appear to be due to altered indole-3-acetic acid transport or level in mutant shoots (Beveridge and others 1996, 2000).

Flowering

Molecular-genetic analyses of flowering in *Arabidopsis*, the species most thoroughly investigated for this developmental process, indicate four distinct floral signalling pathways: circadian clock/photoperiod response, vernalization response, autonomous and GA response (for example, Mouradov and others 2002). Some genes have been shown to act in more than one pathway and may be involved in integration of responses to multiple environmental stimuli including cold and photoperiod. None of these genes is expressed exclusively in either the shoot apical meristem or in leaves (Aukerman and Amasino 1998) and, despite most of them being cloned, none has provided an obvious clue on the biochemical identities of long-distance flowering signal(s). Moreover, until recently (Turnbull and others 2002), grafting studies have not been available in *Arabidopsis* in order to elucidate which of these genes may regulate long distance signals.

Nevertheless, it is well established via studies in tobacco, *Sinapis* and pea that long-distance signals play an important role in the ontogenetic timing of flowering and the response to photoperiod (for example, Lang 1977; Murfet 1971; Havelange and others 2000). In tobacco and *Sinapis*, inductive light treatments supplied to individual leaves induce flowering in the shoot tip; grafts among different pea genotypes are discussed below. The *INDETER-*

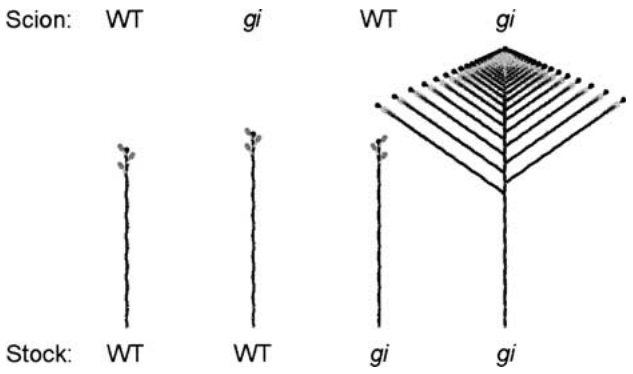

Figure 5. Evidence that *GI* in pea regulates a long-distance signal required to promote flowering. Reciprocal graft combinations of *gi* and WT plants are shown and annotated as described in Figure 2. Data are mostly from Beveridge and others 1996. Photoperiod, 24 h (8 h natural daylight followed by 16 h incandescent light only).

MINATE 1 gene (*ID1*) of maize may regulate a long-distance signal because it controls the transition to flowering at the shoot apical meristem while expressed specifically in immature leaf tissue (Colasanti and others 1998).

Although flowering in potato is not influenced by photoperiod, a photoperiod-dependent pathway and a gibberellin-dependent pathway have been proposed for potato tuber formation (Martinez-Garcia and others 2002a). Interspecific grafts between tobacco and potato indicate that the signal produced in leaves of florally induced tobacco species is similar to or the same as the signal that induces tuberization in potato (Jackson 1999). When the *Arabidopsis* flowering gene *AtCO* (CONSTANS; for example, Suarez-Lopez and others 2001) is overexpressed in potato, it impairs tuberization under SD conditions (Martinez-Garcia and others 2002b) indicating that *CO* may act on a common pathway for these species. Moreover, grafting of *AtCO* overexpressing potato lines and WT plants revealed that *AtCO* exerts its inhibitory effect on tuberization by acting in leaves and not directly at the site of tuberization (Martinez-Garcia and others 2002b). Functional homology of CO may also occur in the short-day plants, rice and *Pharbitis* (Samach and Gover 2001). A rice *CO* homolog corresponds to the QTL *Hd1* which is responsible for the difference in photoperiod sensitivity between rice cultivars (Yano and others 2000) and the *Pharbitis CO* homolog *PnCO* is able to complement the late-flowering *Arabidopsis co* mutant (Liu and others 2001).

Flowering mutants at more than ten loci in pea have been used to elucidate roles of long-distance signals in flowering control in that species (for recent review, see Beveridge and others 2003). Grafting and photoperiod-response experiments have provided evidence for two distinct long-distance signals, an inhibitor acting in a photoperiod response pathway and a floral stimulus acting in a flower-specific (autonomous) pathway. For example, rootstocks of photoperiod-responsive WT seedlings cause a delay in flower initiation in day-neutral mutant (*dne*) scions and *dne* rootstocks cause early flowering in WT scions (Figure 4) (Murfet 1971; King and Murfet 1985). In contrast, WT rootstocks accelerate flower initiation of very late-flowering photoperiod responsive *gigas* (*gi*) scions, although *gi* rootstocks have little effect on the flowering node of WT or *dne* scions (Figure 5; Beveridge and Murfet 1996).

Because of the different approaches used to characterize the mutants, parallels between *Arabidopsis* and pea are difficult to draw, particularly when comparing the photoperiod pathways in which a putative inhibitor is involved in pea but an activator in *Arabidopsis* (Weller and others 1997). Despite the lack of experiments directly testing for long-distance signalling in the vernalization response in *Arabidopsis*, and the observation that several genes acting in the vernalization response pathway are expressed in tissues in addition to the shoot apex (for example, FLC; Michaels and Amasino 1999), the most common hypothesis is that vernalization in that species acts only in the apex. In contrast, while grafting studies with vernalized and control pea seedlings show a vernalization response that is specific to shoots (Beveridge and others 1996), there is an additional vernalization response mediated by a graft-transmissible signal, perhaps that controlled by the photoperiod response pathway (Reid and Murfet 1975). Similar experiments should be conducted in *Arabidopsis*, either by grafting (Turnbull and others 2002) or by transgenic approaches with tissue-specific promoters.

Unlike the flower-specific promotion pathway in pea, the photoperiod response pathway regulates many developmental processes (Beveridge and others 2003). In *Arabidopsis*, the photoperiod response system is associated with the circadian system, known to regulate many genes (Harmer and others 2000). This led Beveridge and others (2003) to hypothesize that the photoperiod response pathway in pea does not simply regulate a single

long-distance signal, but is associated with the circadian system which may control multiple long-distance signals specific to particular developmental processes.

Several hypotheses and candidate molecules have been proposed for the biochemical nature of long-distance signals controlling flowering (for example, Bernier and others 1993). Whereas gibberellins may play a major role in flowering for species such as *Arabidopsis*, *Lolium temulentum* (King and others 2001, 2003; King and Evans 2003) or for tuber development in potato (Fernie and Willmitzer 2001; Martinez-Garcia and others 2002a), it may not do so for species such as maize or pea where the delay in flowering in GA-deficient mutants is only slight, and attributable to indirect effects (Colasanti and Sundaresan 2000; Murfet and Reid 1993). Moreover, exogenous GA can actually cause a delay in flowering in pea, rather than a promotion (Beveridge and Murfet 1996). Similarly, mutant *rms* plants indicate that root-derived cytokinins may not influence flowering in pea. Mutant *rms* lines can differ up to 40-fold in xylem sap cytokinin concentration and yet flower at a similar node to WT (Beveridge and others 1997a, b). Schmülling (2002) also argues that root-derived cytokinins may not be critical regulators of shoot branching, but may play an important role in regulating shoot responses to nutrients. Bernier and others (1993) suggests that flowering is controlled by a complex network involving interplay of several phytohormones, and assimilates. Assimilate partitioning is altered in different pea and sweet pea mutants (Kelly and Davies 1988; Beveridge and others 1992), and some late-flowering *Arabidopsis* mutants can be rescued by culture on sucrose media (Araki and Komeda 1993), but the evidence remains correlative at this stage.

Nodulation

Nodulation involves the formation of a new organ through the induction of centers of new cell division that develop into meristems and subsequent organs. Nodulation provides a unique developmental system as the inducer, target cell type and important parts of the receptor signal cascade are known (Endre and others 2002; Stracke and others 2002), and knock-out mutants can be rescued by growth on an alternative nitrogen source such as nitrate (Carroll and others 1985). Significantly, nodule meristem initiation is induced by rhizobia by elicitation of a new type of phyto-regulatory compound, namely, a lipo-oligosaccharide (Dénarié and others 1996). These N-acetyl-glucosamine oligomers, with lipid, acetyl, fucose and/or sulfate moieties attached, induce cell division as well as membrane associated responses (Oldroyd 2001).

In branching, flowering and nodulation, one has to presume that meristems and pluripotent stem cells, such as pericycle and cambium, must maintain control of their proliferating status. Concurrently meristem-derived cells must be able to leave the cell cycle and differentiate to appropriate organs. Although phytohormone gradients may provide positional information on the type of proliferation, other mechanisms appear to modulate continued meristem development. In the case of nodulation, discussed below, these additional mechanisms are related to a process termed ''autoregulation of nodulation'' (AON) and involve receptor kinases and presumably ligand peptides.

Loss-of-function mutants in AON have extensive nodulation (super- or hypernodulation) over large portions of the root system instead of restricted crown nodulation (Searle and others 2003; Men and others 2003; Gresshoff 2003 and references therein). Although phytohormones are directly implied in the induction and regulation of nodule ontogenesis, it has been impossible to "cross-feed" or complement nodulation-deficient mutants through additions of known phytohormones.

Nodule autoregulation is controlled by a complex root-shoot-root regulatory circuit involving initially *Rhizobium*-induced cell division clusters in the root that send an unknown signal to the leaf, which in turn responds with another unknown signal (shoot derived inhibitor) that blocks further proliferation of nodule meristems (Gresshoff 1993). Thus, ontogenetically advanced meristems (possibly with some type of autonomy) escape the inhibition of nodulation leading to the characteristic crown nodulation pattern, while younger, non-autonomous meristems become arrested. Grafting has demonstrated that supernodulation mutants of several legumes lack this putative leaf signal (Delves and others 1986, 1992; Jiang and Gresshoff 2002). Biochemical analyses of root and leaf tissue from supernodulating mutants revealed significant changes in cytokinin profile compared with wild type plants, but these changes may not be due to transported signals but to responses in specific tissues (Caba and others 2000). Nevertheless, the emerging model of nodule development in legumes has roles for almost all known signalling molecules in plants (see Ferguson and Mathesius this issue) with the roles for as-yet-unidentified signals remaining a real possibility.

Recently it was discovered that the supernodulation genes of soybean and *Lotus japonicus* encode a receptor kinase named NARK, for nodule autoregulation receptor kinase in soybean, and HAR1, for

hypernodulation and aberrant root in *Lotus japonicus*, that is closely related to CLAVATA1 in *Arabidopsis* (CLV1) (Searle and others 2003; Nishimura and others 2002). A mutated supernodulation gene of garden pea, *sym29*, turns out to be the same as those in soybean and *Lotus japonicus* (Krusell and others 2002). Alleles at the *sym29* locus define, like the soybean and *Lotus* mutant alleles, the importance of the protein kinase domain with most missense mutations being located within the ATP binding region or the catalytic site. Non-sense mutations in the autoregulation gene, leading presumably to the truncation of the protein, have severe phenotypes.

An analysis of the function of the *Arabidopsis* CLAVATA complex provides clues as to how the nodule autoregulation receptor kinase might be involved in long-distance signalling. CLV1 controls cell fate at a short distance in the shoot apical meristem of *Arabidopsis*, including the proliferation of cells, and consequently the transition of the vegetative to floral meristem (Clark 2001; Fletcher 2002). The CLAVATA1 protein interacts with several proteins, including CLAVATA2, to regulate cell divisions in meristems (Trotochaud and others 2000). CLV1-CLV2 dimers form the receptor site for a peptide ligand (CLAVATA3) that is transported through several cell layers within the apical meristem of *Arabidopsis* (Fletcher 2002). Cells that have slowed their cell cycle appear to produce the peptide, which then is perceived by progenitor cells, which slow in turn.

The sequence similarity of *CLV* and *NARK* genes could have arisen by ancestral gene duplication, followed by specialization which involved a switch in tissue specificity (*GmNARK* is weakly expressed in the apical meristem but strongly expressed in leaf and root) and a refinement of signal output. Consequently, GmNARK, in contrast to the classical *Arabidopsis* CLV1 model, plays a role in long-distance signalling (Searle and others 2003; Gresshoff 2003). Nevertheless, the basic biological function, namely the arrest of proliferating cells, remains the same. Loss-of-function mutants of *GmNARK* possess structurally normal shoot apical meristems suggesting that GmNARK and GmCLV1A do not cross complement.

The nature of the specificity of these related protein complexes is not understood. However, the discovery of an autoregulation gene closely related to an apical meristem regulation gene suggests functional similarities of the recognition and response pathways. Furthermore, the function of CLV3 indicates that the nodulation system is likely to contain peptide signals.

Isolation of Novel Signalling Molecules

Having generated genetic and/or physiological evidence for existence of novel long-distance signals, the next challenge is to isolate these compounds. Technical limitations may have been one reason why more long-distance signals have not been identified to date, but such hurdles have diminished recently with dramatic advances in sensitivity and throughput of chemical analyses, especially by tandem mass spectrometry. Metabolomic and proteomic approaches taking advantage of inducible gene expression and comparisons of different genotypes will be valuable as will testing of specific bioactivity of candidate molecules by bioassays. Similarly, localized induction systems and/or grafting studies will be essential for demonstrating roles for long-distance signals. Inventories of components of xylem sap and phloem sap, being suites of mobile molecules within major plant transport pathways, can provide new resources for discovery of novel signalling molecules.

What Other Phytohormone-Like Signals Could There Be?

Polypeptides such as insulin have long been established as signalling molecules in animals, but have only recently been discovered in plants (reviewed by Ryan and others 2002; Lindsey and others 2002; see also Hoffmann-Benning and others 2002). An 18 amino acid polypeptide factor that can initiate signal transduction to regulate the synthesis of defensive proteins in plant tissues has been isolated by Pearce and others (1991) and named ''systemin.'' This molecule moves through the phloem and, like animal peptides, acts at low or sub-nanomolar concentrations. Another group of peptide compounds, the phytosulfokines, were discovered following analysis of the proliferation-induction properties of the medium of a high-density asparagus cell suspension culture (Matsubayashi and Sakagami 1996). These compounds are found in several species and have effects relating to cell division and development. The role of signalling peptides CLAVATA3 and ENOD40 are briefly discussed above and by Ferguson and Mathesius (this issue). It is interesting to note that the phytosulfokine receptor is also a protein kinase related to CLAVATA1 and GmNARK (Matsubasyashi and others 2002; see discussion in Gresshoff 2003). The function of plant peptides as phytohormone-like signals in parallel with the role of peptides in animals is yet to be fully established. Moreover, we are yet to determine the number of signalling peptides

in plants, the generality of function across species and specificity within species.

Grafting has been used to show that RNA silencing can induce movement of systemic signals that transmit the silencing state throughout the plant (for example, Mlotshwa and others 2002). The movement of endogenous plant mRNA has also been shown to regulate plant development (Ruiz-Medrano and others 1999; Xoconostle-Cazares and others 1999; see Jorgensen 2002). Such evidence of RNA movement led Jorgensen to suggest that perhaps such small RNAs may selectively regulate developmental processes such as flowering.

Rolland and others (2002) review evidence for the emerging theory that sugars have important hormone-like functions in plants. Unlike the major plant hormones that are usually in the nano-molar range, sugar levels are usually in the millimolar range. Nevertheless, sugars are mobile, moving essentially from source to sink. Recent genetic evidence indicates they interact with signalling pathways for several of the major hormones. For example, *GLUCOSE-INSENSITIVE* (*GIN1*) and *ABSCISIC ACID DEFICIENT2* (*ABA2*) are allelic genes that encode a dehydrogenase/reductase enzyme involved in ABA biosynthesis (Cheng and others 2002). This theory is still under discussion and genetic and other analyses are required to determine if aspects of growth and development such as flowering, branching and nodulation are regulated by sugars acting as long-distance signals.

In addition to macromolecule long-distance signals that may serve hormone-like functions, it is possible that other phytohormones derived from products in the terpenoid pathway (GAs, brassinosteroids and ABA), or adenine or tryptophan metabolism (cytokinin and auxin) might be discovered. Other forms of information transfer that occur at a more physical level, such as hydraulic, mechanical, oxidative and electrical signals, should not be overlooked as they, too, form part of the complex signalling network. The future for our understanding of long-distance signalling in plants therefore lies partly in recognizing that plants are complex networks involving feedback and cross-talk among pleiotropic and specific regulators and a multitude of signal transduction pathways, some complex, and others comparatively simple.

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REFERENCES

Araki T, Komeda Y. 1993. Flowering in darkness in *Arabidopsis thaliana.* Plant J 4:801–811.

Aukerman MJ, Amasino RM. 1998. Floral induction and florigen. Cell 93:491–494.

Bernier G, Havelange A, Houssa C, Petitjean A, Lejeune P. 1993. Physiological signals that induce flowering. Plant Cell 5:1147–1155.

Beveridge CA, Murfet IC. 1996. The *gigas* mutant in pea is deficient in the floral stimulus. Physiol Plant 96:637–645.

Beveridge CA, Murfet IC, Kerhoas L, Sotta B, Miginiac E, Rameau C. 1997a. The shoot controls zeatin riboside export from pea roots. Evidence from the branching mutant *rms4.* Plant J 11:339–345.

Beveridge CA, Ross JJ, Murfet IC. 1992. Mutant *dn* influences dry matter distribution, assimilate partitioning and flowering in *Lathyrus odoratus* L. J Exp Bot 43:55–62.

Beveridge CA, Ross JJ, Murfet IC. 1996. Branching in pea. Action of genes *Rms3* and *Rms4.* Plant Physiol 110:859–865.

Beveridge CA, Symons GM, Murfet IC, Ross JJ, Rameau C. 1997b. The *rms1* mutant of pea has elevated indole-3-acetic acid levels and reduced root-sap zeatin riboside content but increased branching controlled by graft-transmissible signal(s). Plant Physiol 115:1251–1258.

Beveridge CA, Symons GM, Turnbull CGN. 2000. Auxin inhibition of decapitation induced branching is dependent on graft-transmissible signals regulated by genes *Rms1* and *Rms2.* Plant Physiol 123:689–697.

Beveridge CA, Weller JL, Singer SR, Hofer JMI. 2003. Axillary meristem development: budding relationships between networks controlling flowering, branching and photoperiod responsiveness. Plant Physiol (in press).

Beveridge CA. 2000. Long distance signalling and a mutational analysis of branching in pea. Plant Growth Regul 32:193–203.

Caba JM, Luz-Centeno M, Fernández B, Gresshoff PM, Ligero F. 2000. Inoculation and nitrate alter phytohormone levels in soybean roots:differences between a supernodulating mutant and the wild type. Planta 211:98–104.

Cancel JD, Larsen PB. 2002. Loss-of-function mutations in the ethylene receptor ETR1 cause enhanced sensitivity and exaggerated response to ethylene in *Arabidopsis.* Plant Physiol 129:1557–1567.

Carroll BJ, McNeil DL, Gresshoff PM. 1985. Isolation and properties of soybean mutants which nodulate in the presence of high nitrate concentrations. Proc Natl Acad Sci USA 82:4162–4166.

Cheng WH, Endo A, Zhou L, Penney J, Chen HC, Arroyo A, Leon E, Nambara E, Asami T, Seo M, Koshiba T, Sheen J. 2002. A unique short-chain dehydrogenase/reductase in Arabidopsis glucose signaling and abscisic acid biosynthesis and functions. Plant Cell 14:2723–2743.

Clark SE. 2001. Cell signalling at the shoot meristem. Nature Reviews. Mol Cell Biol 2:276–284.

Colasanti J, Sundaresan V. 2000. 'Florigen' enters the molecular age: long-distance signals that cause plants to flower. Trends Biochem Sci 25:236–240.

Colasanti J, Yuan Z, Sundaresan V. 1998. The *indeterminate* gene encodes a zinc finger protein and regulates a leaf-generated signal required for the transition to flowering in maize. Cell 93:593–603.

Curie C, Briat J-F. 2003. Iron transport and signaling in plants. Annu Rev Plant Biol 54:07.1–07.24.

Delves AC, Mathews A, Day D, Carter AS, Carroll BJ, Gresshoff PM. 1986. Regulation of the *soybean-Rhizobium* symbiosis by shoot and root factors. Plant Physiol 82:588–590.

Delves AC, Higgins A, Gresshoff PM. 1992. The shoot apex is not the source of the systemic signal controlling nodule numbers in soybean. Plant Cell Environ 15:249–254.

Dénarié J, Debellé F, Promé J-C. 1996. *Rhizobium* lipo-chitooligosaccharide nodulation factors: signaling molecules mediating recognition and morphogenesis. Ann Rev Biochem 65:503–535.

Dodd IC. 2003. Hormonal interactions and stomatal responses. J Plant Growth Regul 22:32–46.

Endre G, Kereszt A, Kevei Z, Mihacea S, Kalo P, Kiss GB. 2002. A receptor kinase regulating symbiotic nodule development. Nature 417:962–966.

Ferguson BJ, Mathesius U. 2003. Signaling interactions during nodule development. J Plant Growth Regul 22:47–72.

Fernie AR, Willmitzer L. 2001. Molecular and biochemical triggers of potato tuber development. Plant Physiol 127:1459–1465.

Fletcher JC. 2002. Shoot and floral meristem maintenance in *Arabidopsis.* Annu Rev Plant Biol 53:45–66.

Foo E, Turnbull CGN, Beveridge CA. 2001. Long-distance signalling and control of branching in the *rms1* mutant of pea. Plant Physiol 126:203–209.

Gresshoff PM. 1993. Molecular genetic analysis of nodulation genes in soybean. Plant Breeding Rev 11:275–318.

Gresshoff PM. 2003. Post-genomic insights into plant nodulation symbioses. Genome Biol 4:201.

Harmer SL, Hogenesch JB, Straume M, Chang HS, Han B, Zhu T, Wang X, Kreps JA, Kay SA. 2000. Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock. Science 290:2110–2113.

Havelange A, Lejeune P, Bernier G. 2000. Sucrose/cytokinin interaction in *Sinapis alba* at floral induction: a shoot-to-root-to-shoot physiological loop. Physiol Plant 109:343–350.

Hoffmann-Benning S, Gage DA, McIntosh L, Kende H, Zeevaart JA. 2002. Comparison of peptides in the phloem sap of flowering and non-flowering *Perilla* and lupine plants using microbore HPLC followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Planta 216:140–147.

Ingram TJ, Reid JB, MacMillan J. 1986. The quantitative relationship between gibberellin A_1 and internode growth in *Pisum sativum* L. Planta 168:414–420.

Jackson SD. 1999. Multiple signaling pathways control tuber induction in potato. Plant Physiol 119:1–8.

Jiang Q, Gresshoff PM. 2002. Shoot-control and genetic mapping of the *har1-1* (hypernodulation and aberrant root formation) mutant of *Lotus japonicus.* Funct Plant Biol. 29:1371–1376.

Jorgensen RA. 2002. RNA traffics information systemically in plants. Proc Natl Acad Sci USA 99:11561–11563.

Kelly MO, Davies PJ. 1988. Photoperiodic and genetic control of carbon partitioning in peas and its relationship to apical senescence. Plant Physiol 86:978–982.

King RW, Evans LT. 2003. Gibberellins and flowering of grasses and cereals: prizing open the lid of the ''florigen'' black box. Annu Rev Plant Biol 54:12.1–12.22.

King RW, Evans LT, Mander LN, Moritz T, Pharis RP, Twitchin B. 2003. Synthesis of gibberellin GA(6) and its role in flowering of *Lolium temulentum.* Phytochemistry 62:77–82.

King RW, Moritz T, Evans LT, Junttila O, Herlt AJ. 2001. Long-day induction of flowering in *Lolium temulentum* involves sequential increases in specific gibberellins at the shoot apex. Plant Physiol 127:624–632.

King WM, Murfet IC. 1985. Flowering in *Pisum:* a sixth locus, *Dne.* Ann Bot 56:835–846.

Kitano H. 2002. Computational systems biology. Nature 420:206–210.

Krusell L, Madsen LH, Sato S, Aubert G, Genua A, Szczyglowski G, Duc G, Kaneko T, Tabata S, de Bruijn F, Pajuelo E, Sandal N, Stougaard J. 2002. Shoot control of root development is mediated by a receptor-like kinase. Nature 420:422–426.

Lang A. 1977. Promotion and inhibition of flower formation in a day-neutral plant in grafts with a short-day plant and a long-day plant. Proc Natl Acad Sci USA 74:2412–2416.

Leyser HMO. 2001. Auxin. Curr Biol 11:R728.

Leyser O, Deng X-W. 2000. Cell signalling and gene regulation: new directions in plant signalling. Curr Opin Plant Biol 3:351–352.

Lindsey K, Casson S, Chilley P. 2002. Peptides: new signalling molecules in plants. Trends Plant Sci 7:78–83.

Liu J, Yu J, McIntosh L, Kende H, Zeevaart JA. 2001. Isolation of a *CONSTANS* ortholog from *Pharbitis nil* and its role in flowering. Plant Physiol 125:1821–1830.

Martinez-Garcia JF, Garcia-Martinez JL, Bou J, Prat S. 2002a. The interaction of gibberellins and photoperiod in the control of potato tuberization. J Plant Growth Regul 20:377–386.

Martinez-Garcia JF, Virgos-Soler A, Prat S. 2002b. Control of photoperiod-regulated tuberization in potato by the *Arabidopsis* flowering-time gene *CONSTANS.* Proc Natl Acad Sci USA 99: 15211–15216.

Matsubayashi Y, Sakagami Y. 1996. Phytosulfokine, sulfated peptides that induce the proliferation of single mesophyll cells of *Asparagus officinalis* L. Proc Natl Acad Sci USA 93:7623–7627.

Matsubayashi Y, Ogawa M, Morita A, Sakagami Y. 2002. An LRR receptor kinase involved in perception of a peptide plant hormone, phytosulfokine. Science 296:1470–1472.

Men AE, Laniya TS, Searle IR, Iturbe-Ormaetxe I, Gresshoff I, Jiang Q, Carroll BJ, Gresshoff PM. 2003. Fast neutron mutagenesis of soybean. *Glycine soja* L. produces a supernodulating mutant containing a large deletion in Linkage Group H. Genome Lett 1:1–9.

Michaels SD, Amasino RM. 1999. *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a represser of flowering. Plant Cell 11:949–956.

Mlotshwa S, Voinnet O, Mette MF, Matzke M, Vaucheret H, Ding G, Pruss G, Vance VB. 2002. RNA silencing and the mobile silencing signal. Plant Cell 14(Supp):S289–301.

Morris SE, Turnbull CGN, Murfet IC, Beveridge CA. 2001. Mutational analysis of branching in pea, *Pisum sativum* L: evidence that *Rms1* and *Rms5* regulate the same novel signal. Plant Physiol 126:1205–1213.

Mouradov A, Cremer F, Coupland G. 2002. Control of flowering time: interacting pathways as a basis for diversity. Plant Cell 14(Suppl):S111–S130.

Murfet IC. 1971. Flowering in *Pisum.* Reciprocal grafts between known genotypes. Aust J Biol Sci 24:1089–1101.

Murfet IC, Reid JB. 1993. Developmental mutants. In: Casey R, Davies DR, editors. Peas-genetics, molecular biology and biotechnology. Wallingford, UK: CAB International, ISBN 0 85198 863 6, p 165–216.

Napoli CA. 1996. Highly branched phenotype of the petunia *dad1-1* mutant is reversed by grafting. Plant Physiol 111:27–37.

Nishimura R, Hayashi M, Wu G-J, Kouchi H, Imaizumi-Anraku Y, Murakami Y, Kawasaki S, Akao S, Ohmori M, Nagasawa M, Harada K, Kawaguchi M. 2002. *HAR1* mediates systemic regulation of symbiotic organ development. Nature 420:426–429.

Nomura T, Nakayama M, Reid JB, Takeuchi Y, Yokota T. 1997. Blockage of brassinosteriod biosynthesis and sensitivity causes dwarfism in garden pea. Plant Physiol 113:31–37.

Oldroyd GED. 2001. Dissecting symbiosis: developments in nod factor perception. Ann Bot 87:709–718.

Pearce G, Strydom D, Johnson S, Ryan CA. 1991. A polypeptide from tomato leaves induces wound-inducible proteinase inhibitor proteins. Science 253:895–898.

Reid JB, Murfet IC. 1975. Flowering in *Pisum*: the sites and possible mechanisms of the vernalisation responses. J Exp Bot 26:860–867.

Rolland F, Moore B, Sheen J. 2002. Sugar sensing and signaling in plants. Plant Cell 14(suppl):S185–S205.

Ross JJ, O'Neill DP, Rathbone DA. 2003. Auxin-gibberellin interactions in pea: integrating the old with the new. J Plant Growth Regul 22: DOI:10.1007/s00344-003-0021.

Ruiz-Medrano R, Xoconostle-Cazares B, Luca WJ. 1999. Phloem long-distance transport of *CmNACP* mRNA: implications for supracellular regulation in plants. Development 126:4405–4419.

Ryan CA, Pearce G, Scheer J, Moura DS. 2002. Polypeptide hormones. Plant Cell Suppl:S251–S264.

Samach A, Gover A. 2001. Photoperiodism: the consistent use of CONSTANS. Curr Biol. 11(16):R651–4.

Schmülling T. 2002. New insights into the functions of cytokinins in plant development. J Plant Growth Reg 21:40–49.

Searle IR, Men AE, Laniya TS, Buzas DM, Iturbe-Ormaetxe I, Carroll BJ, Gresshoff PM. 2003. Long distance signaling in nodulation directed by a CLAVATA1-like receptor kinase. Science 299:109–112.

Stracke S, Kistner C, Yoshida S, Mulder I, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J, Szczyglowski K, Parniske M. 2002. A plant receptor-like kinase required for both bacterial and fungal symbiosis. Nature 417:959–962.

Suarez-Lopez P, Wheatley K, Robson F, Onouchi H, Valverde F, Coupland G. 2001. CONSTANS mediates between the circadian clock and the control of flowering in *Arabidopsis.* Nature 410:1116–1120.

Trewavas A. 2002a. Plant cell signal transduction: the emerging phenotype. Plant Cell 14(suppl):S3–S4.

Trewavas A. 2002b. Mindless mastery. Nature 415:841.

Trotochaud AE, Jeong S, Clark SE. 2000. CLAVATA3, a multimeric ligand of the CLAVATA1 receptor-kinase. Science 289:613–617.

Turnbull CGN, Booker JP, Leyser HMO. 2002. Micrografting techniques for testing long-distance signaling in *Arabidopsis.* Plant J 32:255–262.

Vogler H, Kuhlemeier C. 2003. Simple hormones but complex signalling. Curr Opin Plant Biol 6:51–56.

Weller JL, Reid JB, Taylor SA, Murfet IC. 1997. The genetic control of flowering in pea. Trends Plant Sci 2:412–418.

Xonconostle-Cazares B, Xiang Y, Ruiz-Medrano R, Wang H-L, Yoo B-C, McFarland KC, Franceschi VR, Lucas WJ. 1999. Plant paralog to viral movement protein potentiates transport of mRNA into the phloem. Science 283:94–98.

Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna L, Fuse T, Baba T, Yamamoto K, Umehara Y, Nagamura Y, Sasaki T. 2000. *Hd1,* a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene *CONSTANS.* Plant Cell 12:2473–2484.