Signal transduction mechanisms in plants: An overview

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This article provides an overview on recent advances in some of the basic signalling mechanisms that participate in a wide variety of stimulus–response pathways. The mechanisms include calcium-based signalling, G-protein-mediated-signalling and signalling involving inositol phospholipids, with discussion on the role of protein kinases and phosphatases interspersed. As a further defining feature, the article highlights recent exciting findings on three extracellular components that have not been given coverage in previous reviews of signal transduction in plants, extracellular calmodulin, extracellular ATP, and integrin-like receptors, all of which affect plant growth and development.

As judged by the recent published record, studies of signal transduction in higher plants are thriving. In the last three years alone over 600 papers dealing with topics in this field were published. Clearly, this relatively short review will not be able to summarize even just these most recent findings. Fortunately, in this issue of *Current Science* other articles will deal with signalling studies related to plant hormones, to phytochrome and blue-light responses, and to stress responses, so this review will not attempt to duplicate that coverage. Instead it will provide an overview that will focus on recent advances in basic signalling mechanisms that participate in a wide variety of stimulus–response pathways. As a further defining feature, it will also highlight recent exciting findings on three extracellular components that have not been given much coverage in previous reviews of signal transduction in plants, extracellular calmodulin (xCaM), extracellular ATP (xATP) and integrin-like receptors, all of which affect plant growth and development.

Signalling agents that are common to many different pathways include Ca^{2+}, inositol phospholipids, G-proteins, cyclic nucleotides, protein kinases and protein phosphatases. All of them will be discussed in this review, with particular attention given to the first three of these agents. As pointed out by Chrispeels *et al.*[1], any single transduction pathway usually involves the coordination of multiple signalling agents operating in multiple cellular structures. Although significant sections of this review will be dedicated to major players in signal transduction, we note here that these agents never work in isolation, but always in networks that intersect multiple signalling pathways. Because of the enormous number of insightful papers in this field, our coverage will necessarily be selective, with apologies to authors of papers not discussed.

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Calcium-based signalling

Two recent reviews[2,3] have provided particularly valuable discussions on topics related to calcium-based signalling in plants. Their discussions on calcium transport highlight recent advances on calcium channels and pumps, and they both discuss methods of visualizing changes in $[Ca^{2+}]_{cyt}$. The problem of specificity of the calcium signal is cogently addressed in the review by Sanders *et al.*[2], who propose that the magnitude and duration of Ca^{2+} transients, the oscillations of these transients, and the subcellular localization of changes in the $[Ca^{2+}]_{cyt}$, all contribute to specifying which Ca^{2+} signals will participate in which signalling pathways.

In this section we will focus on topics not covered in the 1999 reviews, discuss more in depth topics that were only touched on in those reviews, and/or cover more recent papers that have been published on Ca^{2+}-based signalling since those reviews appeared.

Measuring and regulating Ca^{2+} in cells

As reviewed by Rudd and Franklin-Tong[3], changes in $[Ca^{2+}]_{cyt}$ in plant cells are typically visualized by either Ca^{2+}-sensitive fluorescent dyes or the Ca^{2+}-sensitive photoprotein, aequorin. A recent improvement in this technology was reported by Allen *et al.*[4], who showed that GFP-based cameleon Ca^{2+} indicators accurately report both changes in $[Ca^{2+}]_{cyt}$ in guard cells and patterns in these changes.

Many different environmental (e.g. light, hormones, drought, hypoosmotic stress, touch, temperature stress) and chemical (e.g. hormones, cyclic nucleotides, fungal elicitors, NOD factors) signals induce changes in $[Ca^{2+}]_{cyt}$. However, such changes in themselves would only be an indication that they may be important for

inducing a given response downstream of the signal. Additional supporting evidence that Ca^{2+} helps to link a specific stimulus with a specific response would include demonstrations that blocking the Ca^{2+} changes blocks the response and that specific components needed to amplify the Ca^{2+} signal are present and are activated by the stimulus.

Manipulation of [Ca^{2+}] in sub-cellular domains by selective release of caged Ca^{2+} or caged ionophores has been used effectively to demonstrate the effects of locally changing [Ca^{2+}] on pollen tube growth, as reviewed by Franklin-Tong[5]. Selective release of caged Ca^{2+} ionophores has also been used to generate a new Ca^{2+} gradient in root hairs pre-treated with microtubule antagonists and to induce the formation of a new growth point at the site of elevated Ca^{2+} influx in these cells[6]. These studies elegantly demonstrate a key role for Ca^{2+} in the regulation of polarized growth in single cells.

Another recent study also highlights the importance of Ca^{2+} for polarized growth. More interestingly, this report, by Chatterjee *et al.*[7], shows that the subtle force of gravity can orient the direction of a Ca^{2+} current needed for polarized growth and thereby orient the direction of that growth. This investigation used a self-referencing Ca^{2+}-selective electrode to document that germinating spores of the fern *Ceratopteris richardii* developed a Ca^{2+} current within 1 h after they were induced to germinate by light. The current predominately moved in at the cell bottom and out from the top. The current was specific, polarized and strongest in a direction that opposed the vector of gravity. Turning the spores upside down reversed the current in less than the 5 minutes it took to get a new measurement. Treatment with nifedipine, a Ca^{2+} channel blocker, dramatically diminished the Ca^{2+} current and caused the cell to lose its responsiveness to the orienting influence of gravity. The inhibitor also blocked polarity development in over one-third of the cells. These results strongly indicate that Ca^{2+} plays a crucial role in the ability of a single cell to respond to gravity and in the subsequent establishment of its polarity.

Calcium-binding proteins

In order for Ca^{2+} transients to be converted into downstream transduction steps, the ions must bind to Ca^{2+}-binding proteins. Recent findings on several of these targets of Ca^{2+} signals, including CDPKs and the CaM-binding phosphatase, calcineurin, were reviewed well by Sanders *et al.*[2]. In this section we will review and discuss recent findings on other Ca^{2+}-activated proteins, with particular attention to investigations of calmodulin (CaM), annexins and protein kinase C.

Calmodulin: By far the best studied of all the Ca^{2+}-binding proteins is CaM. The insightful and thorough review by Zielinski[8] comprehensively summarizes the literature on CaM through 1997. As pointed out by that article, progress on clarifying CaM functions in plants will depend heavily on identifying more of its targets and where it localizes. In this regard three recent papers merit special mention.

Hong *et al.*[9] have documented that the *Arabidopsis* Ca^{2+}-ATPase, isoform 2 protein resides in the endoplasmic reticulum, making it distinct from all other CaM-regulated pumps found in plants or animals. Since in animals CaM-regulated pumps are believed to be found exclusively in the plasma membrane, this finding indicates a fundamental difference between how plant and animal cells use these pumps.

Rodriguez-Concepcion *et al.*[10] have identified a novel CaM in *Petunia*, CaM53, that can be prenylated. The prenylation status of this CaM directs its localization: the prenylated version goes to the plasma membrane; the non-prenylated version goes to the nucleus. Transfer of plants to darkness (sugar depletion) or blocking mevalonic acid synthesis in the plants directs CaM53 to the nucleus, indicating that its subcellular localization is regulated by changes in metabolism. Sugar-mediated signal transduction is a major emerging area in plant biology, and there is evidence for a role of Ca^{2+} in the mechanism of sugar sensing[11]. The fact that the sugar status of *Petunia* leaves can alter the localization of CaM53, and that this alteration has metabolic consequences[10], suggests a role for this protein in sugar sensing.

The conventional expectation is that all the roles of CaM would be exclusively intracellular. Thus the report of Ma *et al.*[12] describing a role for extracellular CaM in pollen germination and growth is surprising and reveals previously unsuspected roles for CaM in plants. These authors showed that anti-CaM antiserum inhibited pollen germination and tube growth, but that this effect could be reversed by treatment with cholera toxin, a membrane permeable G-protein agonist. Conversely, addition of purified CaM stimulated pollen tube growth, and, when added to a medium containing plasma membrane vesicles, strongly promoted GTPase activity in these vesicles. Both of these effects were reversed by the membrane-permeable antagonist of heterotrimeric G-proteins, pertussis toxin. Previous work from Sun's laboratory showed both that CaM was present in plant cell walls[13] and that extracellular CaM could stimulate the proliferation of cells in culture and cell wall regeneration and cell division in protoplasts[14].

The concentration of Ca^{2+} in plant cell walls is higher than that in the cytoplasm, although how much higher is not entirely clear[15]; it is unlikely that the concentration would be below the threshold for CaM activation. This raises the question of how could extracellular CaM be a regulator if it is constitutively active? Ma *et al.*[12] propose two potential solutions. One is that the [Ca^{2+}] re-

quired for CaM activation is more than 10 times higher in the low pH environment of the wall, and microenvironments of the wall may have free [Ca^{2+}] lower than μM[15]. The other is that extracellular CaM-binding proteins, rather than strictly Ca^{2+}, may also play a key role in the activation and deactivation of extracellular CaM.

Ecto-apyrase as one potential target of extracellular CaM: To resolve how extracellular CaM can affect plant cell growth it will be crucial to identify CaM-binding proteins in the ECM and to identify the function of these proteins. Some progress along these lines is reported by Thomas *et al.*[16,17], who report that a CaM-binding NTPase previously reported to have a nuclear locale in etiolated plants has an ECM locale in light-grown plants. This NTPase is a member of the ecto-apyrase family of NTPases. In plants, this enzyme plays a role in phosphate scavenging[16] and in toxin resistance[17]. In animals, ecto-apyrases are critical for turning off the hormonal activity of extracellular ATP (xATP) or extracellular ADP (xADP) by hydrolysing these molecules. The signalling events induced by xATP and xADP in animals are mediated by G-protein-linked purinergic receptors. Evidence that such receptors may be present in plants was recently reported by Lew and Dearnaley[18], who described the effects of extracellular nucleotides on the electrical properties of growing root hairs of *Arabidopsis*.

That ATP is present and functional in the ECM of plant cells has been shown by Thomas *et al.*[17], who also described evidence that MDR proteins help transport ATP from the inside to the outside of plant cells, just as they do in animal cells. Surely, if it can be shown that plants have purinergic receptors that are responsive to xATP and mechanisms for transducing the activation of such receptors into physiological responses can be discovered, this will represent a novel development in our understanding of signalling pathways that are operative in plants.

Protein kinase C

In mid-summer of 1998 two papers related to protein kinase C (PKC) in plants were published almost simultaneously, one, a review stating that no functional plant homologues of protein kinase C had yet been found[19], and the other an original report describing the full purification and partial characterization of a plant PKC-type kinase[20]. The review paper[19] accurately concluded that there was a lack of rigorous evidence for the existence of a PKC-like enzyme in plants through mid-1998, despite the effort of many laboratories to find such a protein kinase. The original report described the purification of an enzyme with all the biochemical characteristics needed to qualify it as a conventional PKC: it was activated by a phorbol ester, phorbol myristate acetate (PMA), it showed a [Ca^{2+}] dependence for its activation, the addition of Ca^{2+} in the presence of phosphatidyl serine and PMA lowered its K_m, it phosphorylated histone H1 at serine residue(s). Additional evidence for its PKC character were that it had only a single binding site for PMA and it was inhibited by two general PKC inhibitors, staurosporine and H-7 (ref. 20). A potential role for PKC in signalling was demonstrated by showing that PMA, and not an inactive analogue of PMA, could increase transcript levels of nitrate reductase, and that this increase could be inhibited by the PKC inhibitor H-7 (ref. 20).

Annexins

The annexin family of Ca^{2+}-dependent membrane-binding proteins has emerged as another important target for changes in cytosolic [Ca^{2+}] in plant cells. Annexins are a diverse and multifunctional gene family in animal cells, where they are involved in a wide variety of essential cellular processes such as membrane ion channel activity, membrane–cytoskeletal linkage, membrane trafficking, apical exocytosis, mitotic signalling, and DNA replication (for reviews see refs 21 and 22). The annexin fold is one of three main types of Ca^{2+} regulatory motifs found in plants[23], and interest in plant annexins is growing (for reviews see refs 24 and 25). Early studies suggested that annexins were involved in Golgi-mediated secretion in plant cells[26,27]. More recently, direct evidence for annexin participation in exocytosis of polysaccharides in maize root cap cells was demonstrated by Carroll *et al.*[28]. In the past several years, there have been reports linking annexin signalling to many different physiological processes in plants. For example, a role for annexins has been implicated in an early stage of nodulation signalling in *Medicago truncatula*[29], in the touch response of *Bryonia dioica* stems[30], and in responding to H_2O_2 stress in *E. coli*[31]. Annexin gene expression in plants also appears to be regulated by developmental and environmental signals. Changes in expression of plant annexins have been observed during fruit ripening and cell cycle progression and in response to stress and abscisic acid[32–34].

Several studies have indicated that plant annexins are able to bind to membranes at Ca^{2+} concentrations in the micromolar range[27,35] and there is a celery 42 kDa annexin in which the half-maximal binding to membranes occurred in the nanomolar range[36]. In plant cells, cytosolic [Ca^{2+}] in the nanomolar range is clearly of physiological relevance. The Ca^{2+} levels associated with membranes may reach micromolar levels.

Plant annexins may be regulated by more than just changes in cytosolic [Ca^{2+}]. Cotton annexin is a

substrate for phosphorylation[37] and maize and tomato annexins have intrinsic ATPase activity[38-40]. There is a cotton fiber annexin which binds to and hydrolyses GTP more efficiently than ATP, and this activity was mapped to the fourth structural repeat[41]. Interestingly, GTP was shown to strongly inhibit the annexin/Ca^{2+}-dependent exocytosis in the maize root cap protoplast system[28]. Certain animal annexins also bind to ATP[42], and the membrane fusion activity of annexin VII is mediated by Ca^{2+} and GTP-binding[43].

In animal cells, annexins are distributed throughout the entire endomembrane system as well as associated with the cytoskeleton, the ECM and the nucleus. Similarly, plant annexins are associated with the plasma membrane, Golgi membranes and the ECM[26]. They are also in nuclei[44], and there is a vacuolar-specific annexin in celery associated with cell expansion[45]. Plant annexins also have F-actin binding ability[39], suggesting that they may be linked to the actin cytoskeleton. Plant annexins appear to be widely distributed subcellularly, so it would be expected that they might exhibit different functions depending on their cellular locales.

One intriguing area of research on animal annexins is testing the hypothesis that annexins can act as a new kind of Ca^{2+} channel. Crystallography studies have indicated that certain annexins have a hydrophilic pore which could act as a channel for Ca^{2+} ions, and annexins do have *in vitro* Ca^{2+} channel activity. However, so far, annexin channel activity has not directly been shown to be an *in vivo* property of any annexin. Plant annexins are structurally similar to their animal counterparts and thus it is possible that plant annexins may also exhibit Ca^{2+} channel activity. Recently, structural similarities between Ca^{2+} channels in the *Characeae* alga *Nitellopsis* and those formed by annexin proteins have been discussed[46]. Due to the localization of annexins at the extreme tips of polarly growing plant cells[47,48], it is tempting to speculate that they may be doing more than just delivering Golgi-derived vesicles there and may play a direct role in creating the Ca^{2+} gradient observed in these cells.

Early Southern data suggested that annexins were a relatively simple gene family in several plant species. The presence of two annexin cDNAs in a single plant species has been observed in tomato, corn and cotton. However, the progress of the *Arabidopsis* genome sequencing effort has helped to establish that annexins are a multigene family in plant cells. In *Arabidopsis*, there appear to be five different annexin cDNAs, which range from 30 to 67% in deduced amino acid sequence identity[49,50]. These *Arabidopsis* annexin cDNAs as well as all other plant annexin cDNAs obtained thus far have very short N-terminal regions preceding the first structural repeat. Variation in the sequence and length of the N-terminal region in animal annexins is one source of functional differences among the individual members of this gene family. Thus whatever functional differences there are among plant annexins would have to be due to variation found within their structural repeats. Genetic studies will be needed to clarify whether the different annexins have overlapping or distinct functions. Research on annexin knock-outs, currently in progress, will help reveal just how important a role these proteins play in plant Ca^{2+} signalling.

Signalling involving inositol phospholipids

Phospholipase C-mediated signalling

It is clear that most, if not all, plants have the well-known signal transduction pathway involving the hydrolysis of phosphatidylinositol 4,5-*bis*-phosphate [PtdIns(4,5)P_2] by plasmalemma-associated phospholipase C (Figure 1) (refs 51 and 52). Although PtdIns(4,5)P_2 is itself localized in the plasmalemma, it constitutes only 2 mol% or less of the lipids in that membrane, while its immediate precursor, PtdIns4P, is 10–20 times more abundant.

PtdIns(4,5)P_2 is cleaved by one of several phospholipase C (PLC) enzymes identified in plants. Properties of the plant PLC, which most resemble the PLC-subfamily of animals, have been summarized by Munnik *et al.*[51]. Certain physiological signals can cause the green alga *Dunaliella salina* to lose more than 30% of its cellular PtdIns(4,5)P_2 within 2 minutes (ref. 53), and higher plants can also sustain a pronounced and rapid PtdIns(4,5)P_2 turnover.

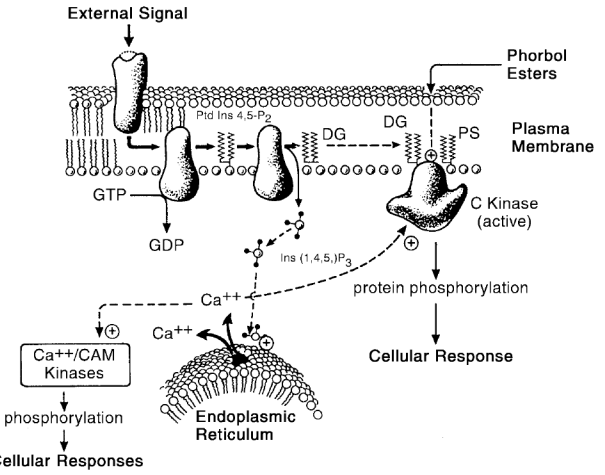

Figure 1. Interaction of activated receptors through G proteins to specific effector molecules. Adapted from figure 2 of ref. 52, after a model proposed by Y. Nishizuka. Model illustrates interaction of heterotrimeric G-proteins with receptors, the metabolism of inositol phospholipids and the role of Ins(1,4,5)P_3 in the release of Ca^{2+} from internal stores, and the role of diacylglycerol (DG), phosphatidyl serine (PS) and phorbol esters in activating PKC.

The hydrolysis of PtdIns(4,5)P_2 by PLC produces two second messengers – diacylglycerol and inositol 1,4,5-trisphosphate($InsP_3$). Signal transduction via diacylglycerol is by activation of PKC, which has been considered earlier in this review. $InsP_3$ is a major regulator of cytosolic free Ca^{2+} in animals, and there is strong evidence for a similar role in plants. Plant $InsP_3$ binds to high-affinity receptors which appear to be located in the tonoplast[54], as opposed to the endoplasmic reticulum in animal cells. The animal $InsP_3$ receptor is a 250 kDa highly regulated mediator of Ca^{2+} release, whereas its analogue in plants is much smaller (~110 kDa)[54] and may therefore interact with fewer ligands than the animal receptor.

Free $InsP_3$ is quickly dephosphorylated or, in some cases, further phosphorylated to Ins(1,3,4,5)P_4 or to $InsP_6$. The signalling capabilities of these compounds in plants is unclear.

PtdIns4P functions

In addition to the PLC/PtdIns(4,5)P_2 signalling pathway, the somewhat more abundant inositide PtdIns4P appears to play a critical regulatory role in plants. It associates with profilin and other actin-binding proteins[55], regulates P-type ATPases[56], and activates phospholipase D[57]. More recently, PtdIns4P has been found to bind to a pleckstrin homology domain of PtdIns4K, the enzyme catalysing its formation[58]. This may serve as a feedback regulatory point for the formation of both PtdIns4P and its downstream product PtdIns(4,5)P_2.

PtdIns3K products

Today there is much interest in a phosphoinositide pathway that is independent of the PLC/PtdIns(4,5)P_2 signalling sequence discussed above. This involves inositol phospholipids phosphorylated on position D3 of the inositol ring. Many plant species have been shown to contain PtdIns3P and lesser amounts of PtdIns(3,4)P_2 (ref. 51). A signalling role for these lipids and their biosynthetic enzyme PtdIns3K is not yet confirmed in plants, but their widespread involvement in animal signal transduction[59] suggests that plants also utilize this system.

G Protein-mediated signalling

G proteins in animals and plants

Heteromeric GTP-binding proteins (G proteins) are involved in coupling many hundreds of animal plasma membrane receptors with intracellular signalling pathways. A similar broad pattern of involvement is currently developing in plants. A typical interaction of activated receptors through G proteins to specific effector molecules is shown in Figure 1. Activation of the G protein occurs when the binding of GTP to the trimer triggers its dissociation into a GTP-associated G_α subunit and a $G_{\beta\gamma}$ subunit. It is now recognized that both the GTP-G_α and the $G_{\beta\gamma}$ subunits can transmit signals to downstream elements of the signal pathway. And it is clear that certain additional proteins termed 'regulators of G protein signalling' (RGS) catalyse the cycle of G protein activation/inactivation[60].

Evidence for G proteins has been found in a variety of algae and higher plants[61]. A G_α subunit gene was first cloned from *Arabidopsis* in 1990 (ref. 61), and additional G_α and G_β genes have been reported from several plant species, reviewed by Hooley[62]. All of the plant G_α proteins appear to belong to the same G protein family, with the exception of an unusually large (99 kDa vs a more typical ~28–38 kDa size) G_α protein[63]. This new type provides evidence for the diversity expected on the basis of known functions. Genes for the G_β subunit have been found in several plant species[62]. G_γ has not yet been characterized in plants.

G protein-mediated signalling in plant

Whereas thousands of G protein-coupled receptors have been authenticated in animal and yeast cells, their positive identification in plants has been more difficult. The cloning of a putative G protein-coupled receptors from *Arabidopsis*[64] effectively assured a signalling role for G proteins there and heralds a period of progress in this area.

There is direct evidence for G protein participation in several diverse physiological processes. They have a role in the gibberellin-induced expression of α-amylase in wild oat aleurone[65]. They also are important in the regulation of pollen germination and tube growth[12]. G proteins are involved as well in the rhodopsin-based signal transduction in the eyespot of green algae[66].

Through the use of G protein-specific activators and inhibitors, indirect but convincing evidence has been presented for their action in many other plant signalling processes. Recent examples of this include phytochrome signalling, reviewed by Nagy and Schäfer[67], and protein-mediated nod factor signalling in legume nitrogen fixation[68]. G proteins also have an apparent function in regulating phospholipase D activity in various plants[69]. In addition, a strong case has been made for their regulation of the inwardly-rectifying K^+ channels controlling stomatal opening[70].

G proteins may have multiple functions in plant defence responses. When activated by GTPγS or mastoparan, a ~45 kDa putative G protein of cultured soybean cells induced a defense-related oxidative burst[71]. A ho-

mologue of conventional PKC of potato mediates an elicitor-induced defence response, suggesting G protein action earlier in this process[72]. And microinjection of GTPγS or overexpression of cholera toxin in tobacco induced increased synthesis of pathogenesis-related proteins[73].

Integrins: New evidence for receptor and signalling functions in plants

Integrins are a multigene family of transmembrane glycoproteins that act as ECM-specific cell surface receptors in animal cells (for recent reviews see refs 74 and 75). Integrins are composed of two different subunits, β and α subunit, and there are up to 20 different combinations of these subunits found in different cell types. They are made up of a large N-terminal extracellular domain followed by a transmembrane domain and a short cytoplasmic tail and have extracellular and cytoplasmic binding partners, thus connecting the inside of a cell with the outside. Inside the cell they are connected to intermediate filaments, or, more usually, to the actin cytoskeleton through binding to actin and actin attatchment proteins such as talin, vinculin and α-actinin. They are linked with the extracellular matrix through binding to fibronectin, vitronectin, collagen, laminin and other proteins through an arg–gly–asp (RGD) motif found on these ECM adhesion proteins. Integrin activation results in dimerization and clustering of integrins, and downstream signalling is mediated by a wide variety of molecules including MAP kinases, G-proteins and Ca^{2+}-binding proteins. Integrins act as receptors for mechanical signals in animal cells and are involved a wide array of cellular processes such as cell proliferation, cell motility, cell adhesion, cell survival, gene expression and cell differentiation and morphogenesis.

Plants, like animals, have a number of physiological responses that are initiated through mechanical signals such as touch, wind and gravity. The importance of the ECM–plasma membrane–cytoskeleton continuum in plant cells for the perception and transduction of positional information and environmental signals has previously been discussed[76,77]. This continuum, which consists of cell adhesion sites where groups of signalling molecules may be clustered together, appears to be functionally similar to the ones found in animal cells and has been called plasmalemmal control centres in plant cells[78]. Plants are very sensitive to mechanical signals, which are perceived as tension and compression forces in appropriate cells through ECM–cytoskeletal connections. Changes in cytosolic $[Ca^{2+}]$ may be involved in transduction of these signals, and stretch-activated Ca^{2+} channels in onion epidermal cells appear to cluster when activated and have been suggested to be closely associated with plant integrin-like proteins[79].

Although during most of the past ten years there has been no compelling sequence evidence for plant integrins, researchers have found considerable experimental support for the existence of an extracellular RGD-receptor in plant cells. The first evidence for this came from a study where the addition of GRGDSP peptides to a soybean root cell suspension significantly increased growth rate and led to aberrant cell wall/plasma membrane interactions and organization[80]. Further data supporting the idea that plants have RGD-binding proteins was obtained using *Chara* internode cells, where the polarity of gravity regulated cytoplasmic streaming was blocked by application of RGDS peptides to the ends of the cells[81]. Some other examples of processes inhibited by addition of RGD peptides are cell adhesion in NaCl-adapted tobacco protoplasts[82], the defence response in peas[83], and embryo formation in sunflower protoplasts[84]. RGD receptors located in adhesion sites are also thought to be required for establishment of polarity in *Fucus* embryos and for cell plate assembly in plant cells[85,86]. Consistent with the ability of RGD to affect plant cells is the finding that there are high affinity protein RGD-binding sites in the plasma membrane of *Arabidopsis*[87]. The number of different RGD-mediated processes in plants further emphasizes the important role cell adhesion sites play in plant growth and development.

There is now biochemical and immunological evidence to support the hypothesis that RGD receptors in plants are integrin-like proteins. In several plant species, proteins ranging from 55 to 120 kDa have been found that cross-react with animal anti-β_1 integrin antibodies[88–92]. There is also immunological evidence for the existence of several ECM integrin-binding proteins in plants, such as vitronectin-like and fibronectin-like proteins[88,93,94], and there are several plant vitronectin-like cDNA sequences in the database[95].

There have been a number of immunolocalization studies done in plants using anti-β_1 integrin antibodies. In one study, *Arabidopsis* root cap cells and *Chara* rhizoid tips showed the highest levels of anti-integrin immunostaining[96]. This finding that plant integrin-like proteins are enriched at two cellular sites essential for gravity perception/transduction provides further support for the hypothesis that these receptors participate in gravity perception. The first evidence for a cell surface distribution of integrin-like proteins comes from an immunolocalization study in onion epidermal cells, which used computational optical-sectioning fluorescence microscopy to colocalize integrin-like, vitronectin-like, and fibronectin-like proteins[88]. These results were further substantiated in a later study where integrin-like proteins showed punctate staining on the cell surface and colocalized with vinculin-like and talin-

like proteins[97]. However, conflicting immunolocalization results have been obtained concerning the subcellular locale of these integrin-like proteins. One study using two different anti-β_1 integrin antibodies at the electron microscope level provided evidence for localization of integrin-like antigen on amyloplast membranes[90]. Whereas another study found that this antigen was localized specifically on the plasma membrane and that there were possible problems using anti-animal integrin antibodies for immunolocalization in plant tissue[92].

The search for integrin-like cDNA sequences in fungi and plants has been challenging because integrin sequences are not highly conserved. Recently, an *Arabidopsis* full-length cDNA, *At14a*, was obtained by screening an expression library with a vertebrate anti-β_1 integrin antibody. This cDNA codes for a 43 kDa protein that is partially localized to the plasma membrane and shows very limited overall sequence homology to integrins[98]. However, the *At14a* cDNA does have two predicted transmembrane domains and shows higher levels of homology to integrins in several smaller regions. More recently, another *Arabidopsis* cDNA clone, *AtELP1*, containing β-integrin domains was obtained[99]. The *AtELP1* cDNA codes for a 70 kDa protein that contains cysteine-rich domains specific to β-integrin, a potential integrin-binding motif (DxSxS), a potential transmembrane domain, and is mainly localized at the plasma membrane. *AtELP1* is a member of a multigene family in *Arabidopsis*, so there are at least 6 other closely related members containing β-integrin-like domains. For each plant integrin-like protein candidate, it will be important to demonstrate the functional ability to bind to the RGD motif and to bind the actin cytoskeleton. In the last several years, extraordinary progress has been made in this research area and it has become clear that plant integrin-like proteins are going to be major players in plant signal transduction. In the next several years it will be exciting to watch as the molecular details of plant integrin-like receptor signalling unravel.

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