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Signaling a Role for Phospholipid-Derived Compounds in Plants

One of the most significant advances in animal cell biology was the recognition, beginning in the early 1980s, that phospholipids are not only structural components of membranes but also sources of second messengers. Many different phospholipids can be cleaved by phospholipases to generate second messengers; perhaps the most well-known such phospholipid is phosphatidylinositol 4,5-bisphosphate (PtdIns[4,5]P2), whose cleavage by a phosphoinositide-specific phospholipase C (PI-PLC) yields two products, each with second messenger activity. One product, inositol 1,4,5-trisphosphate (Ins[1,4,5]P3), is released into the cytosol, where it triggers the release of Ca2+ from internal stores. The other, diacylglycerol (DAG), remains in the membrane, where it activates protein kinase C (PKC). Many other phospholipid-derived molecules have since been found to participate in signaling pathways in animals. These include phosphoinositide derivatives that are phosphorylated on the 3 position—such as PtdIns(3)P, PtdIns(3,4,5)P3, and Ins(1,3,4,5)P4—as well as phosphatidic acid (PtdOH), the product of DAG phosphorylation.

Even as the phospholipid signaling picture in animal cells has become more complex (for review, see Divecha and Irvine, 1995), a definitive demonstration of a signaling role for any plant phosphoinositide has remained elusive. The circumstantial evidence that plants use these molecules in signaling is very strong: Ca2+ is known to be involved in some plant cell signal transduction events, and Ins(1,4,5)P3—but not inositol phosphates that are without biological activity in animal cells—elicits Ca2+ release from the vacuole and possibly other organelles as well (for review, see Coté and Crain, 1993). Release of caged Ins(1,4,5)P3 in stomatal guard cells causes both an increase in cytosolic free Ca2+ levels and stomatal closure (Gilroy et al., 1990), although it has yet to be shown that closing stimuli result in an increase in Ins(1,4,5)P3. The unicellular alga Chlamydomonas, which sheds its flagella in response to environmental stresses, also does so in response to Ca2+ or Ins(1,4,5)P3 treatment (Quarmby et al., 1992). Moreover, stress-induced deflagellation is accompanied by increases in both Ins(1,4,5)P3 and PtdOH. However, it again remains to be demonstrated that natural environmental stresses actually cause PtdIns(4,5)P2 hydrolysis and Ca2+ increases.

Further hints that phosphoinositides are involved in plant cell signaling come from the finding that G protein modulators influence stomatal aperture, deflagellation, and other putative phosphoinositide-mediated processes. These observations are significant because in animal cells, G protein—coupled receptors mediate the activation of some PI-PLC isozymes. G proteins have also been found to regulate the activity in some animal cells of phospholipase D (PLD), which cleaves phosphatidylcholine and phosphatidyl-ethanolamine to generate the second messenger PtdOH (for review, see Boarder, 1994). Because PtdOH and DAG are readily interconverted, distinguishing between a PtdOH increase caused by PI-PLC and one caused by PLD is not necessarily straightforward. In this issue, Munnik and colleagues (pages 2197–2210) show that some of the PtdOH increase in C. eugametos cells treated with G protein activators actually results from the activation of PLD. The biological relevance of this observation is reinforced by the authors’ demonstration that PtdOH induces deflagellation of C. eugametos cells. Thus, to the likely involvement of PI-PLC in plant cell signaling must now be added the likely involvement of PLD.

To investigate whether PLD becomes activated in response to G protein activators, the authors took advantage of the fact that mammalian PLD can transfer a phosphatidyl moiety not only to water (i.e., producing PtdOH) but also to some alcohols (producing phosphatidyl alcohols). When the G protein activator mastoparan was added together with n-butanol to C. eugametos cells, a new lipid was formed that comigrated with a PtdBut standard. More of this lipid was produced as more butanol was added. Other alcohols could also be transphosphatidylation, including methanol, ethanol, and n-propanol. Mastoparan treatment of carnation petals also produced a dose-dependent activation of PLD, suggesting that a G protein–activated PLD is present in higher plants as well.

Although mastoparan’s principal effect is to activate G proteins, this basic amphiphilic peptide can bind to and alter the activity of other proteins as well, so it was important to test the effect of other G protein modulators on PLD activity. Cholera toxin, which activates a subset of G proteins, activated the C. eugametos PLD; by contrast, pertussis toxin, which inhibits a different subset of G proteins, did not prevent mastoparan from activating the PLD. Thus, the putative PLD-regulating G protein is cholera toxin sensitive and pertussis toxin insensitive. In addition, alcohols themselves are known to activate G proteins, and the authors found that alcohols were indeed able to activate the C. eugametos PLD. Some alcohols, such as n-butanol, acted as both substrates and activators, whereas sec-butanol acted as an activator but not a substrate, and tert-butanol acted as neither. The effects of alcohol and mastoparan appear to be
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additive in that far more phosphatidylated reporter alcohol was produced in the presence of mastoparan than in its absence. Thus, although the ability of alcohols to activate PLD potentially complicates the interpretation of the mastoparan results, the effects of mastoparan are clearly independent of the presence of an activating substrate alcohol. The authors point out that differences in the activities of the various alcohols could be useful in assessing whether a process is G protein activated or not: if sec-butanol induces a response but tert-butanol does not, then the response is likely to be mediated by a G protein.

These results suggest that a G protein is involved in the activation of plant PLDs, but does a G protein--coupled receptor activate PLD directly? Or does the G protein activate PLC, with PLD then being activated indirectly, for example, by a protein kinase that is downstream of PLC? Both types of G protein--mediated PLD activation have been observed in animals, with the most common "indirect" type involving PLC-mediated activation of PKC, which then activates PLD. As yet, despite intensive analysis, there is no evidence that plants possess a PKC homolog, and the authors point out that a rise in Ca2+ is unlikely to mediate PLD activation, because treatments that increase the influx of Ca2+, at least, do not stimulate PLD activity.

These results, as intriguing as they are, also do not prove that PLD-mediated production of PtdOH is in fact involved in any plant signal transduction event. The authors show that PtdOH induces both deflagellation and mating structure formation (a response to gamete--gamete recognition in sexual reproduction) in C. eugametos, but as yet the chain of causality linking environmental stimuli to PLD activation, then to PtdOH accumulation, and then to deflagellation, is incomplete. One way to begin to complete the chain would be to examine, and perhaps to block, the expression of a critical participant in the pathway, such as the PLD gene. A PLD gene whose product is specific for phosphatidylcholine was recently cloned from castor bean (Wang et al., 1994), and this gene should be helpful in exploring the potential regulatory role of PLD in intracellular signaling.

Studies to perturb the accumulation of key intermediates would similarly help in assessing the involvement of phosphoinositides in plant cell signaling processes. Several plant genes involved in phosphoinositide metabolism have been cloned; these include two distinct PI-PLC genes from Arabidopsis (Yamamoto et al., 1994; Hirayama et al., 1995) and one from soybean (Shi et al., 1994), an Arabidopsis gene coding for the synthase that catalyzes the first step in inositol biosynthesis, the cyclization of glucose 6-phosphate to inositol 1-phosphate (Johnson and Sussex, 1995), and phosphatidylinositol 3-kinases from Arabidopsis (Welters et al., 1994) and soybean (Hong and Verma, 1995). In this issue, Gillaspy and colleagues (pages 2175–2185) report the cloning of a second biosynthetic gene, inositol 1-monophosphatase (IMP), which catalyzes the conversion of inositol 1-phosphate to inositol. Animal IMPs also catalyze the conversion of phosphate moieties from inositol 4-phosphate, a breakdown product of In(1,4,5)P3, and are therefore involved in inositol recycling as well as its biosynthesis de novo.

In the course of an effort to clone plant proteins involved in signal transduction, the authors found that IMP, which accepts a wide range of monophosphate substrates, is able to dephosphorylate the artificial substrate 5-bromo-4-chloro-3-indolyl phosphate at neutral pH. Of approximately one million cDNA clones screened from a young tomato fruit expression library, one displayed phosphatase activity against this substrate; this clone was then used to obtain a full-length cDNA, LeIMP1, whose predicted protein sequence has significant similarity to known IMPs (over 30% identity and 70% similarity). Two additional IMP genes were isolated based on their similarity to LeIMP1. All of these genes share four domains that are found not only in known IMP proteins but also in several proteins of unknown biochemical function (see Neuwald et al., 1994) as well as in the yeast HAL2 protein, which encodes a bisphosphate nucleotidase that is involved in salt tolerance (Murguia et al., 1995). Expression of the LeIMP genes in Escherichia coli confirmed that these genes indeed encode IMPs: all were able to dephosphorylate 14C-labeled inositol 1-phosphate. Like all known IMPs, the E. coli--expressed tomato IMPs are lithium sensitive, a finding that lays to rest a controversy over the Li+ sensitivity of plant IMPs.

The finding that tomato contains three IMP genes (as does Arabidopsis) is somewhat surprising in light of the fact that animals appear to possess a single IMP gene. The three tomato genes have distinct spatial and temporal expression patterns, as do their encoded proteins, and there is some evidence for post-transcriptional regulation. LeIMP1 transcripts, for example, accumulate to high levels in flowers and mature green fruits and to lower levels in roots (and several other organs), but little LeIMP protein is detected in mature green fruits. Protein expression analysis using an antiserum that recognizes all three LeIMP proteins shows that these proteins are expressed in interesting tissue- and cell type-specific patterns. In particular, although they are expressed in growing tissue (e.g., cotyledons, hypocotyl, root, and young fruit), they are expressed at only low levels in the shoot apical meristem. Instead, LeIMP protein levels are highest in vascular cells.

Why do plants have multiple IMP genes? A possible answer is found in the wide variety of inositol-containing compounds in plants. In animals, inositol is incorporated mainly into phosphoinositides and inositol phosphates, but in plants, inositol serves as a precursor to several cell wall compounds, and it forms conjugates with auxin that may regulate auxin transport or availability. Inositol may also have an osmoregulatory role, and control of inositol production may help plants cope with drought stress (Bohnert et al., 1995). (In this context, it is interesting to note that one of the Arabidopsis PI-PLC genes is induced by salt treatment.
[Hirayama et al., 1995.) The authors caution, however, that although the LeIMP s have strong similarity to other IMPs, it cannot be ruled out that one or all of them are not primarily IMPs but actually "prefer" a substrate other than inositol 1-phosphate. Assuming that, as is likely, one or all of the LeIMP s is a genuine IMP, it will be interesting to determine whether it also dephosphorylates (I4)P (like animal IMPs) or (P2)P (which is the likely breakdown product of (1,2,3,4,5,6)P6 [phytate], a storage form of phosphorous that accumulates to high levels in plant seeds.

With the cloning of the tomato IMPs (along with that of the inositol monophosphate synthase, PI-PLCs, and PI 3-kinases), it is now possible to assess the effects of disrupting inositol metabolism. For example, will plants in which one or other of these genes is suppressed show reduced salt tolerance? Will they be altered in stomatal regulation? Will they have large-scale growth defects? Given the sensitivity of plant IMP to Li+, one would expect Li+ to affect plants, and indeed Li+ treatment has pleiotropic effects on plants, although whether these are due to the inhibition of a signaling pathway or to the loss of an inositol derivative (for example, a wall component) is still unclear. In light of a recent paper in THE PLANT CELL (Jones and Kochian, 1995), it is tempting to speculate that one possible effect of inhibiting inositol recycling in plants, either by treating them with Li+ or by suppressing the expression of one of the phosphoinositide metabolic genes, would be the inhibition of root growth. Aluminum, which has been found to reduce (1,4,5)P3 formation in animal cells (e.g., McDonald and Mamrack, 1995) (an effect that has also been linked with the development of Alzheimer's disease), rapidly inhibits root growth in plants, and Jones and Kochian have now found that this growth inhibition may result from Al3+ inhibition of PtdIns(4,5)P2 cleavage.

Several lines of evidence indicate that Al3+ s target is a phosphoinositide signaling system. First, AlCl3 treatment blocked the fivefold increase in (1,4,5)P3 levels that normally occurs when H2O2 is added to the medium around wheat roots. (Analysis of (1,4,5)P3 hydrolysis rates indicates that this represents a reduction in (1,4,5)P3 formation rather than an increase in its hydrolysis.) Second, Al3+ strongly inhibited cleavage of exogenous labeled PtdIns(4,5)P2 by isolated wheat microsomal membranes. (Li+, by contrast, had no effect on PLC-mediated PtdIns(4,5)P2 hydrolysis.) Interestingly, Al-citrte only partially inhibited PtdIns(4,5)P2 cleavage in this assay, probably because the Al-citrte complex is stable at the pH at which the assay was carried out. Finally, an assortment of soluble and membrane-bound enzymes that are active in wheat roots were tested for their sensitivity to Al3+, and none was affected by either AlCl3 or Al-citrte.

How Al3+ might affect PtdIns(4,5)P2 activity is not clear; as the authors discuss, it is possible that Al3+ inhibits PI-PLC, either directly or by perturbing the lipid environment, but it is also possible that it inhibits the synthesis of PtdIns(4,5)P2 or its access to the PI-PLC. Similarly, the basis for the toxicity of PI-PLC inhibition remains unresolved. The authors point out that PtdIns(4,5)P2 interacts with the cytoskeleton of animal and possibly also plant cells; the altered morphology of Al3+-treated root cells may therefore be the result of an alteration in the function of the cytoskeleton. Phosphoinositide derivatives have also been implicated in animal cell cycle regulation (Berridge, 1993); if the same is true in plants, then inhibition of PI-PLC might well interfere with plant cell division. Plants vary in their tolerance to aluminum (Delhaize and Ryan, 1995; Kochian, 1995), and it will be interesting to test whether the sensitivity of PtdIns(4,5)P2 hydrolysis to Al3+ is increased in any aluminum-tolerant plants.

Although our understanding of the involvement of phospholipid-based signaling systems in plants remains incomplete, the papers in this issue and the last add to the growing evidence that plant cells indeed use such signaling systems. As more and more tools, in particular cloned gene sequences, become available for interfering with such signaling pathways, we should begin to develop a better understanding of which environmental and developmental signals lead to the production of phospholipid-derived second messengers, what these messengers are, and how they actually influence the behavior of plant cells.

Rebecca Chasan

REFERENCES


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A Goodbye

With this issue, my tenure at THE PLANT CELL comes to an end. Over four years ago I had the amazingly good fortune of being hired to oversee the front section of this journal, a job that has been wonderfully challenging and stimulating. Between writing IN THIS ISSUE articles, attending meetings and writing MEETING REPORTS, editing LETTERS and COMMENTARIES, and shepherding the production of special review issues, I have had the chance to immerse myself in some of the most interesting research areas in plant biology and to interact with many wonderful plant biologists. But everything comes to an end, and it is now time for me to move on to new tasks and challenges. I will always be grateful for the support and encouragement I have received from the editors of the journal, first Bob Goldberg and now Brian Larkins; from the coeditors; from my colleagues in the headquarters office of the American Society of Plant Physiologists, especially managing editor Judy Grollman; and most of all from you, the readers of the journal.

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