

Review

Transmembrane Signaling via Phosphatidylinositol 4,5-Bisphosphate Hydrolysis in Plants¹

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ABSTRACT

Recent investigations have confirmed the presence of the polyphosphoinositides, phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate (PIP₂), as well as inositol phospholipid-specific phospholipase C in higher plant and microalgal cells. In addition, it has been shown that stimulation of some photosynthetic cell types by environmental or hormonal challenge is accompanied by degradation of the polyphosphoinositides. The products of phospholipase C-catalyzed PIP₂ hydrolysis, inositol 1,4,5-trisphosphate and diacylglycerol, appear to be capable of releasing organelle-bound Ca²⁺ and stimulating protein kinase C-like activity *in vitro*. However, a direct cause and effect relationship between stimulated PIP₂ breakdown and changes in intracellular calcium, protein phosphorylation, or cell function has not yet been unequivocally established. Despite a number of technical difficulties slowing progress in this field, it is likely that photosynthetic organisms will soon be shown to transmit physiologically significant extracellular signals across their plasma membranes by a PIP₂-mediated transduction mechanism.

Recent advances have confirmed that PLC²-catalyzed hydrolysis of inositol phospholipids constitutes a major avenue for transducing extracellular signals across the plasma membrane to animal cells. The transduction process ultimately leads to increases in cytoplasmic calcium levels and changes in protein phosphorylation patterns. Because calcium and protein phosphorylation also play critical roles in regulating plant cell function, this PLC-inositol phospholipid transmembrane signal cascade is receiving increasing attention in a number of laboratories as a potentially important regulator of cellular processes in photosynthetic organisms.

Reports describing transmembrane signaling in plants via

¹ Studies cited from the authors' laboratory were supported by: National Science Foundation grant DMB-8802838, Robert Welch Foundation grant F-350, and the Texas Advanced Technology Research Program.

² Abbreviations: PLC, phospholipase C; PIP₂, phosphatidylinositol 4,5-bisphosphate; IP₂, inositol bisphosphate; IP₃, inositol trisphosphate; PIP, phosphatidylinositol 4-phosphate; PA, phosphatidic acid; G protein, GTP-binding protein; GTP γ S, guanosine 5'-0(3-thiotriphosphate); GDP β S, guanosine 5'-0(2-thiodiphosphate); PI, phosphatidylinositol; [Ca²⁺]_i, cytoplasmic calcium concentration; DG, diacylglycerol; PKC, protein kinase C.

inositol phospholipids have until recently been scattered sparsely throughout the scientific literature. As in most developing areas of biology, these reports did not always convey an entirely consistent and unequivocal story. The early, often conflicting findings in this field have been reviewed by Boss (3) and by Morse *et al.* (24). In the limited space available, this short review will (a) highlight selected new developments during the past 2 years, (b) identify possible sources of confusion and areas in need of clarification, and (c) preview potentially significant research areas of the future.

The basic process of transmembrane signaling via the inositol phospholipids as it is understood in nonphotosynthetic cells is outlined in Figure 1. Recent evidence suggesting an involvement of this signaling pathway in plants is summarized below.

EVIDENCE FOR COMPONENTS OF THE SIGNALING PATHWAY IN PLANTS

Stimulation by Extracellular Signals

Despite the expected participation of a plasma membrane-associated receptor in plant PIP₂-mediated signal transduction, no such receptor has been identified. In view of our ignorance of plant plasma membrane receptors in general, this is hardly surprising. So far, data on this early stage of the signaling process merely correlate effects that imposition of an extracellular signal has on elements of the signal transducing mechanism beyond the putative receptor stage without identifying the receptor *per se*.

One of the best documented examples involves auxin action. Ettliger and Lehle (14) studied the ability of 2,4-D or auxin to trigger the resumption of cell division by cultured *Catharanthus roseus* cells arrested in the G₁ phase. Addition of 2,4-D to [³H]inositol-prelabeled cells caused a transient rise in IP₂ and IP₃, peaking after 1 min. These changes were accompanied by a fall in the labeling of PIP and PIP₂ followed by a subsequent rise. Experiments not reported in detail were said to show a release of Ca²⁺ from *C. roseus* microsomes upon addition of IP₃ but not inositol 1-P.

A different approach was used by Zbell and Walter-Back (30) with suspension cultures of *Daucus carota*. Microsomal preparations rapidly incorporated ³²P from [γ -³²P]ATP into the PA and, to a lesser extent, PIP and PIP₂ zones of thin layer chromatographic plates. Addition of 1 μ M IAA acid to the incubation mixture reduced the degree of labeling and

stimulated the release of compounds eluted from anion exchange columns at positions expected for IP_2 and IP_3 . These findings, while implying the auxin-induced hydrolysis *in vitro* of PIP and PIP_2 by PLC, will need to be confirmed by additional studies showing that other potential ^{32}P -labeled metabolites which may form in incubations with $[^{32}P]ATP$ do not complicate the interpretations by contaminating the recovered IP_2 and IP_3 .

Extensive research has been focused on PIP_2 metabolism as a transducing mechanism for the control of leaf movement by light in the legume *Samanea saman*. The effects of light on phosphoinositide turnover in the *S. saman* motor organ (pulvinus) responsible for leaf movement were assessed in $[^3H]$ inositol labeled tissue (22). Following a period of darkness, individual pulvini were exposed to white light ($300 \mu E \cdot m^{-2} \cdot s^{-1}$) for 5 to 30 s before extraction for comparison to pulvini harvested as a dark control. Light caused an increase in the tissue level of IP_2 , first detected after 15 s of exposure, and a parallel but smaller rise in IP_3 , as measured by HPLC. By 30 s PIP radioactivity had dropped to 72% of controls and PIP_2 radioactivity to 40% of controls. In a related study (see below), Morse *et al.* (23) observed that the white light induced rise in IP_2 plus IP_3 was matched by an equivalent rise in DG. Because folding of the pulvinus is influenced specifically by red light and blue light, it will be highly pertinent to learn whether light of one or both of these wavelengths can substitute for the white light that the authors have used so far in their research on phosphoinositide turnover. Other effects of light, namely, on the properties of G proteins, are described below.

In the unicellular green alga, *Dunaliella salina*, hypoosmotic shock induced a rapid degradation of PIP and PIP_2 with a concomitant rise in PA (9). IP_3 and DG levels were not measured. The physiological significance of these results is not clear since neither changes in cytosolic $[Ca^{2+}]$ nor altered protein kinase activity were assessed as potential means of linking PIP_2 turnover to the pronounced metabolic response of these cells to osmotic stress.

G Proteins

Evidence for the involvement of G proteins in polyphosphoinositide-mediated signal transduction of plants has developed along three lines: (a) detection of G proteins, (b) comparison of these proteins with their more thoroughly characterized equivalents in animal systems, and (c) demonstration of their involvement in inositol phospholipid turnover.

Hasunuma *et al.* (15) exploited a filter binding system to show the association of the nonhydrolyzable GTP analog $[^{32}S]GTP\gamma S$ with certain protein fractions of *Lemna*, *Pisum*, and *Anabaena* eluted from Sephadex columns. Based on this criterion and on the extent to which various Sephadex fractions were $[^{32}P]ADP$ -ribosylated by pertussin toxin, it was proposed that four distinct G proteins were present in *Lemna paucicostata* preparations. $[^{35}S]GTP\gamma S$ binding was inhibited by red or far-red light. Competition studies revealed that ATP could prevent the binding of $[^{32}S]GTP\gamma S$ in the filter assay. This is uncharacteristic of animal G-proteins, which display little tendency to bind nucleotides not containing guanine.

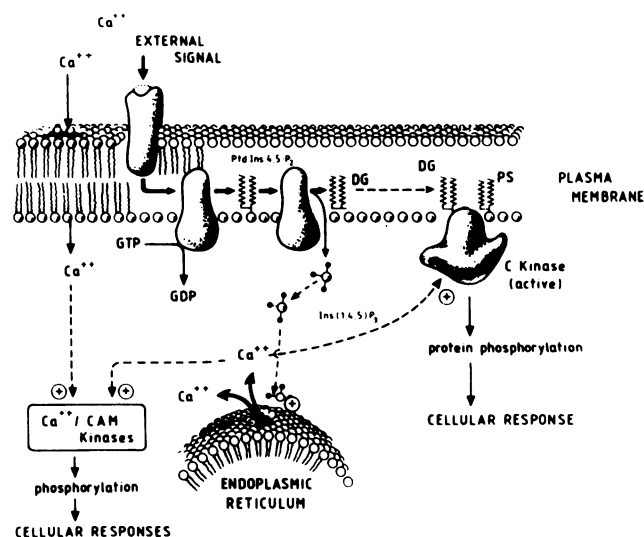


Figure 1. Interaction of an external signal or agonist with a cell surface receptor stimulates the activation of an inositol phospholipid-specific phospholipase C (PI-PLC), with the activation being coupled by a G protein. PI-PLC hydrolysis of PIP_2 , which is highly localized in the plasma membrane, gives rise to DG and IP_3 . DG serves as a potent activator of protein kinase C (C kinase or PKC), while IP_3 stimulates the release of Ca^{2+} from intracellular pools and the subsequent activation of Ca^{2+} -sensitive protein kinases. Drawing modified from Guy GR, Gordon J, Michell RH, *In* RH Mitchell, AH Drummond, CP Downes, eds (1989) *Inositol Lipids in Cell Signaling*, Academic Press, New York, pp 433–458.

Drøbak *et al.* (7) located four distinct $[^{32}P]GTP$ -binding proteins on nitrocellulose blots of SDS-PAGE gels prepared from zucchini (*Cucurbita pepo* L.) hypocotyl microsomes. These proteins, having molecular masses of 23.4, 24.8, 26.6, and 28.5 kD, did not bind $[^{32}P]GTP$ if the blots had been preincubated with GTP or $GTP\gamma S$.

A similar zucchini preparation examined by Jacobs *et al.* (18) contained G proteins from which $[^{32}S]GTP\gamma S$ binding could be blocked by preincubating the membranes in the presence of nonradioactive GTP or $GTP\gamma S$ but not other nucleotide triphosphates. Nitrocellulose immunoblots using antibodies to the α -subunit or rabbit G-protein revealed cross-reacting material having apparent molecular masses of 50 and 33 kD.

Blum *et al.* (2) extended these findings by observing proteins capable of cross-reacting with antibodies to animal G proteins in plasma membranes of several higher plants, including *Vicia faba*, *Arabidopsis thaliana*, and *Commelina communis*. These three species each had two immunoreactive proteins of molecular masses 37 and 31 kD, 36 and 31 kD, and 38 and 34 kD, respectively. The *A. thaliana* proteins were partially purified and shown to bind $GTP\gamma S$ and GTP but not other nucleotide triphosphates.

In none of the above-mentioned cases were the putative G protein α -subunits actually shown to participate in PIP_2 -mediated signal transduction, although indirect evidence for a role in the regulation of PLC has been obtained (see the following section). In several instances, the apparent molecular masses were not close to the 39 to 41 kD range most

commonly associated with the phosphoinositide pathway in animal cells. However, smaller (20–30 kD) G proteins such as the *ras* proto-oncogene family have been implicated in PIP₂ turnover.

Phospholipase C

The presence in photosynthetic cells of a PLC which preferentially hydrolyzes inositol phospholipids has been demonstrated in a number of laboratories. For example, Melin *et al.* (20) reported the presence of inositol phospholipid specific PLC in higher plant tissues. Under their assay conditions, the plasma membrane was highly enriched over cytosol in PLC specific activity. The plasmalemma activity showed a preference for PIP and PIP₂ over PI in contrast to the cytosolic fraction, in which the preference was for PI. Optimal activity was obtained at 10 μM Ca²⁺. Similar results were reported by McMurray and Irvine (19), who found that PLC activity from the cytosolic fraction showed greater preference for PI while the membrane or particulate fraction contained greater activity toward polyphosphoinositides. The PLC specific activity was found to vary greatly among plant tissues, with the specific activity being approximately 40-fold higher in celery stems than in spinach leaves! One must consider such differences in PLC activity as a potentially significant contributing factor to differences in polyphosphoinositide content of different plant tissues. These investigators also found the enzyme to be sensitive to low calcium concentrations but most active at a rather high (100 μM) Ca²⁺ level. Likewise, we have found plasmalemma PLC activity towards PIP₂ in *D. salina* to be maximal at 100 μM free calcium (10). In the latter cell type, PIP₂-PLC activity is highly enriched in the plasma membrane compared to other membrane fractions. Although Melin *et al.* (20) and McMurray and Irvine (19) reported no evidence of G protein involvement in regulating the somewhat different PLC activities in their respective plant systems, Dillenschneider *et al.* (6) demonstrated that guanine nucleotides stimulate PLC activity in membranes isolated from sycamore. Incubation of crude membranes derived from these cells prelabeled *in vivo* with [³H]inositol released inositol phosphates in a reaction that was stimulated by GTP or GTP γ S but not by UTP, CTP, ADP, or nonhydrolyzable ATP analogs. The ability of GDP β S to prevent activation of the PLC activity by GTP further characterized the reaction as a G protein-mediated one. In a separate study the plasmalemma PIP₂-specific PLC of *D. salina* was found to be stimulated by the nonhydrolyzable GTP analog, GTP γ S, over a wide range of calcium concentrations (10).

Polyphosphoinositides

The first and most basic step in the investigation of plant polyphosphoinositides is their identification, quantification, and localization. Though this would appear to be a simple matter, it has been a continuing source of confusion. As a component of plant cell membrane phospholipids, PI generally comprises 8 to 15 mol %, about twice the proportion found in animal cell membranes. Phosphorylated forms of PI (PIP and PIP₂) have escaped close scrutiny in plant cells until the last 5 years or so. Because of the low quantities of PIP

and PIP₂ in most cells, a routine method for identifying and quantifying them is based on radiolabeling (with [³H]- or [¹⁴C] *myo*-inositol or ³²P_i) followed by cochromatography with authentic standards using TLC. Using such techniques, the identification of PIP and PIP₂ has been claimed in a variety of plant material, including several types of cultured cells (reviewed in Boss [3]), leaf discs (29), and microalgae (10). However, other investigators (4, 8) have failed to identify PIP₂ in certain cultured plant cells using similar analytical techniques. A complicating factor is the presence in some cell types of inositol-containing compounds which may be extracted with organic solvents but which differ in structure from PIP and PIP₂ and yet chromatograph near these lipids upon TLC analysis in common solvent systems (8).

Thus, these analytical methods are, by themselves, of limited utility for the unequivocal identification of PIP and PIP₂. Using more definitive chemical analyses, Coté *et al.* (5) studying *S. saman* pulvini and Irvine *et al.* (17) studying pea leaf discs and cells of the microalga *Chlamydomonas eugametos*, have fully documented the presence of PIP and PIP₂ in these photosynthetic cells.

As has been observed in animal cells, the metabolism of the inositol phospholipids, based on ³²P radiolabeling data from the microalgae *D. salina* (9) and *Chlamydomonas eugametos* (17), is very rapid relative to that of other lipids. Less is known about the radiolabeling kinetics of inositol phospholipids in higher plant tissues.

Quantifying polyphosphoinositides on the basis of their incorporation of radioactive precursors assumes that some semblance of isotopic equilibrium has been reached prior to analysis. This state has seldom been confirmed in studies with photosynthetic cells. The incorporation of exogenous inositol into plant cells can be particularly slow (17). Thus, quantitation of the polyphosphoinositides relative to other cellular phospholipids has rarely been satisfactory. A frequent penchant of investigators quantifying the inositol phospholipids in photosynthetic cells is to express the quantity of radiolabeled PIP and PIP₂ as a ratio relative to PI. However, this leaves something to be desired since the molar proportion of total phospholipids accounted for by PI can itself vary considerably from one cell type to another.

By direct analysis of PIP and PIP₂ phosphorus, Coté *et al.* (5) found 0.6% of total lipid phosphorus in PIP and less than 0.02% as PIP₂ in pulvini of *S. saman*. We have found that PIP and PIP₂ comprise 1.2% and 0.3%, respectively, of cellular phospholipids in *D. salina*, based on ³²P radiolabeling, phosphorus analysis, and radiolabeling via fatty acid incorporation (9). Because these phospholipids are particularly sensitive to phospholipase action, homogenization or even much milder manipulations of cells and tissues prior to lipid extraction must be considered critically as sources of polyphosphoinositide loss during sample preparation.

If the polyphosphoinositides play a role in transmembrane signaling, one might expect them to be found in abundance in the plasma membrane. The plasma membrane of photosynthetic cells has indeed been found to be enriched in polyphosphoinositide content (early evidence reviewed by Boss, [3]), comprising as much as 9.5 mol % of plasmalemma phospholipids in *D. salina* (9). It is not known whether other

organelle membranes contain PIP or PIP₂ in photosynthetic cells. It appears that the nuclear envelope of some animal cells contains the polyphosphoinositides as well as the enzymatic machinery necessary for their synthesis. PI kinase activity has been reported in chloroplast membranes (27), although at a lower specific activity than in the plasmalemma. It is currently unknown whether plant cells contain more highly phosphorylated inositol lipids (*i.e.* PIP₃) as recently demonstrated in other cell types.

IP₃ and Its Control of [Ca²⁺]_i

The presence in plants of PIP₂ and phospholipases specific for its hydrolysis has led to the conclusion that IP₃ is also present in the cells. However, when aqueous extracts of cultured wild carrot cells labeled with ³H-inositol were passed through the anion exchange columns routinely used for separating inositol phosphate classes, the radioactivity recovered in the 'inositol phosphate' fractions did not comigrate with authentic 1,4-IP₂ or 1,4,5-IP₃ upon subsequent paper electrophoresis (26). While in this instance the failure to detect these key intermediates could have been due to their low degree of radiolabeling and to their observed rapid degradation by endogenous phosphatases (21), the findings generally underscore the need for exercising greater caution in establishing the presence of inositol lipid metabolites in plants.

In contrast to the above-mentioned findings, Heim and Wagner (16) employed anion exchange chromatography in conjunction with HPLC to identify 1,4-IP₂ and smaller but reproducible amounts of 1,4,5-IP₃ in cultured *C. roseus* and *Nicotiana tabacum* cells grown in the presence of [³H]inositol. Other radiolabeled metabolites had chromatographic properties of glycerophosphorylinositol as well as its mono- and diphosphorylated derivatives.

Early findings that exogenous IP₃ releases Ca²⁺ from plant microsomal preparations have been confirmed for considerably lower IP₃ levels with corn coleoptile microsomes (25) and with intact vacuoles from *Avena* seedling roots (28). The latter report illustrates a growing conviction that vacuoles, as a major (up to 90% of cell volume) plant cell organelle normally having 0.1 to 10 mM Ca²⁺, constitute the physiologically most significant Ca²⁺ depot. The observed release of vacuolar Ca²⁺ by IP₃ was quite specific; no effect was seen when IP₂, IP, or inositol were substituted.

The precise mechanism by which IP₃ releases Ca²⁺ from intracellular stores is not completely understood, although a specific IP₃ receptor has recently been identified in animal cells. While GTP appears to play a role in stimulating Ca²⁺ release from plant (1) as well as animal microsomes, the relationship between GTP and IP₃ in the process is not known.

DG and DG Kinase

Another key second messenger produced by PLC hydrolysis of PIP₂ and other susceptible phospholipids is DG. Evaluating the signaling role of DG can be especially frustrating because of its participation in multiple metabolic pathways, including those supplying the substantial lipid needs of the expanding plastid membranes. Typifying the problems involved is the analysis of DG in *S. saman* pulvini by Morse *et al.* (23).

Under the same conditions found to promote a rise in inositol phosphates, namely exposure of 6 h dark-adapted pulvini to white light, DG increased in amount by 19% during the first 30 s and then declined to 90% of dark control values after 10 min of exposure to light. Although the transient rise in DG almost exactly equaled the concurrent rise in IP₂ and IP₃, this could have been coincidental because the DG so produced was not shown to arise in the plasma membrane. The photosynthetically active white light employed in the experiment might well be capable of enhancing DG synthesis in chloroplast membranes for use there. As the *S. saman* system becomes better characterized, only the most effective red or blue wavelengths of light will be employed to trigger PIP₂ hydrolysis, thereby minimizing photosynthetic stimulation of lipid synthesis.

The problem of dealing with multiple pools of metabolically active DG can be further clarified by employing more discriminating analytical techniques. Measurement of whole tissue DG levels by the use of DG kinase and [³²P]ATP, as employed by Morse *et al.* (23) and many other workers, cannot distinguish between metabolic pools. In many cases it might be more appropriate to use one of the recently developed reverse phase HPLC techniques to separate and quantify individual DG molecular species. The molecular species of DG arising from PIP₂ turnover in the plasma membrane are likely to be quite different from those of the larger chloroplast pool and may also be different from the majority of DG generated in the endoplasmic reticulum. If this is so, even small changes in PIP₂-derived DG would be detectable in whole cell extracts. Another option for identifying the origin of newly released DG is direct analysis of specific cell fractions, particularly the plasma membrane, if the fractionation can be accomplished without artifactual DG production.

Once formed, DG are rapidly hydrolyzed by lipases or are converted to PA by DG kinase. The latter reaction constitutes the first step in resynthesis of PIP₂ as well as certain other phospholipids. In *D. salina* an immediate striking increase in PA has been observed following hypoosmotic shock (9). Because this rise correlates with a drop in PIP and PIP₂ levels, it may be due to the rapid conversion of DG to PA.

Protein Kinase C

DG are potent activators of certain PKC isoforms. Protein kinases having PKC-like characteristics, most particularly a requirement for Ca²⁺, phospholipid, and DG for maximal activity, have been reported from plants. The best documented example is an 84.5 kD protein kinase partially purified from *Amaranthus tricolor* seedlings (12). In the presence of 1 μM Ca²⁺, shown to be required for activity, 40 μg phosphatidylserine/mL and 8 μg diolein/mL greatly enhanced the ability of the kinase to phosphorylate histone. The enzyme's sensitivity to activation by phosphatidylserine was lost if it was isolated from elution from membranes (derived from Ca²⁺-treated homogenates) rather than from cytosol (from EDTA and EGTA-treated homogenates) (13). It was concluded that the lipid necessary for achieving full activity became bound to the PKC during its brief Ca²⁺-induced attachment to membranes. Western blots prepared using antibodies to bovine brain PKC gave bands suspected to be the

intact PKC (84.5 kD), a proteolytic product (65 kD), and the regulatory fragment (40 kD) (11). These and other properties are in general agreement with what we know of animal PKC enzymes.

Most of the other reports of plant protein kinases that resemble PKC are based only on their Ca^{2+} requirement and a stimulatory effect of lipids. No studies have dealt meaningfully with a possible DG- or phorbol ester-induced translocation from cytosol to membranes, with down regulation, or with other properties controlling kinase activity.

FUTURE DIRECTIONS

It is now clear that many, perhaps even most, of the elements involved in the PIP_2 signaling paradigm as established in animal systems are also present in plants. A number of studies have shown that PIP_2 breakdown accompanies cellular stimulation of various sorts in plant cells. Yet the role of specific membrane receptors in initiating the PIP_2 signal cascade has not been satisfactorily addressed. It is premature to assume that the receptors must be integral membrane proteins in the plasmalemma. They might, like phytochrome, be translocated to membranes following activation.

Rapid advances may be anticipated in the characterization of plant G proteins since these may be studied independently of the system in which they function. Progress here may provide the definitive clue as to whether PIP_2 -mediated signaling in plants does closely resemble the animal pathway. The unexpectedly low molecular weights of most putative plant G proteins analyzed to date suggests that these are not members of the same trimeric family operative in animal cell plasma membranes.

Knowledge of PLC, the effector enzyme presumably coupled to a G protein, has advanced well in recent months, but the role in plants of the phospholipase products, DG and IP_3 , is still in need of much attention. It will be especially important to develop a reliable protocol for loading the cytoplasm of plant cells with Ca^{2+} -sensing dyes so that the dynamics of $[\text{Ca}^{2+}]$ change can be monitored under physiological conditions.

Above all, if the concept of PIP_2 -mediated signal transduction in plants is to become credible we must find at least one plant model system in which the various components are clearly shown to function in concert to achieve some recognizable end. Achieving this surprisingly elusive goal has been fraught with ambiguity due to technical difficulties in manipulating plant cells. Success could provide the long sought breakthrough in understanding plant hormone action.

ACKNOWLEDGMENTS

We are grateful to Drs. W. F. Boss, R. C. Crain, B. K. Drøbak, K. Hasunuma, D. E. Hanke, D. J. Morrè, K. G. Wagner, and B. A. Zbell for providing data prior to publication.

LITERATURE CITED

- Allan E, Dawson A, Drøbak B, Roberts K (1989) GTP causes calcium release from a plant microsomal fraction. *Cell Signaling* **1**: 23–29
- Blum W, Hinsch K-D, Schultz G, Weiler EW (1988) Identification of GTP-binding proteins in the plasma membrane of higher plants. *Biochem Biophys Res Commun* **156**: 954–959
- Boss WF (1989) Phosphoinositide metabolism: its relation to signal transduction in plants. In WF Boss, DJ Morrè, eds, *Second Messengers in Plant Growth and Development*. Alan R Liss, Inc., New York, pp 29–56
- Connett RJA, Hanke DE (1987) Changes in the pattern of phospholipid synthesis during the induction by cytokinin of cell division in soybean suspension cultures. *Planta* **170**: 161–167
- Coté GG, Depass AL, Quarmby LM, Tate BF, Morse MJ, Satter RL, Crain RC (1989) Separation and characterization of inositol phospholipids from the pulvini of *Samanea saman*. *Plant Physiol* **90**: 1422–1428
- Dillenschneider M, Hetherington A, Graziana A, Alibert G, Haiech J, Ranjeva R (1986) The formation of inositol phosphate derivatives by isolated membranes from *Acer pseudoplatanus* is stimulated by guanine nucleotides. *FEBS Lett* **208**: 413–417
- Drøbak BK, Allan EF, Comerford JG, Roberts K, Dawson AP (1988) Presence of guanine nucleotide-binding proteins in a plant hypocotyl microsomal fraction. *Biochem Biophys Res Commun* **150**: 899–903
- Drøbak BK, Ferguson IB, Dawson AP, Irvine RF (1988) Inositol-containing lipids in suspension cultured plant cells. *Plant Physiol* **87**: 217–222
- Einspahr KJ, Peeler TC, Thompson GA Jr (1988) Rapid changes in polyphosphoinositide metabolism associated with the response of *Dunaliella salina* to hypoosmotic shock. *J Biol Chem* **263**: 5775–5779
- Einspahr KJ, Peeler TC, Thompson GA Jr (1989) Phosphatidylinositol 4,5-bisphosphate phospholipase C and phosphomonoesterase in *Dunaliella salina* membranes. *Plant Physiol* **90**: 1115–1120
- Elliott DC, Kokke YS (1987) Cross-reaction of a plant protein kinase with antiserum raised against a sequence from bovine brain protein kinase C regulatory sub-unit. *Biochem Biophys Res Commun* **145**: 1043–1047
- Elliott DC, Kokke YS (1987) Partial purification and properties of a protein kinase C type enzyme from plants. *Phytochemistry* **26**: 2929–2935
- Elliott DC, Fournier A, Kokke YS (1988) Phosphatidylserine activation of plant protein kinase C. *Phytochemistry* **27**: 3725–3730
- Ettlinger C, Lehle L (1988) Auxin induces rapid changes in phosphatidylinositol metabolites. *Nature* **331**: 176–178
- Hasunuma K, Furukawa K, Funadera K, Kubota M, Watanabe M (1987) Partial characterization and light-induced regulation of GTP-binding proteins in *Lemna paucicostata*. *Photochem Photobiol* **46**: 531–535
- Heim S, Wagner KG (1989) Inositol phosphates in the growth cycle of suspended cultured plant cells. *Plant Science* **63**: 159–165
- Irvine RF, Letcher AJ, Lander DJ, Drøbak BK, Dawson AP, Musgrave A (1989) Phosphatidylinositol(4,5)bisphosphate and phosphatidylinositol(4)-phosphate in plant tissues. *Plant Physiol* **89**: 888–892
- Jacobs M, Thelen MP, Farndale RW, Astle MC, Rubery PH (1988) Specific guanine nucleotide binding by membranes from *Cucurbita pepo* seedlings. *Biochem Biophys Res Commun* **155**: 1478–1484
- McMurray WC, Irvine RF (1988) Phosphatidylinositol 4,5-bisphosphate phosphodiesterase in higher plants. *Biochem J* **249**: 877–881
- Melin PM, Sommarin M, Sandelius AS, Jergil B (1987) Identification of Ca^{2+} -stimulated polyphosphoinositide phospholipase C in isolated plant plasma membranes. *FEBS Lett* **223**: 87–91
- Memon AR, Rincón M, Boss WF (1989) Inositol trisphosphate metabolism in carrot (*Daucus carota* L.) cells. *Plant Physiol* **91**: 477–480
- Morse MJ, Crain RC, Satter RL (1987) Light-stimulated inosi-

- tolphospholipid turnover in *Samanea saman* leaf pulvini. Proc Natl Acad Sci USA **84**: 7075-7078
23. **Morse MJ, Crain RC, Coté GG, Satter RL** (1989) Light-stimulated inositol phospholipid turnover in *Samanea saman* pulvini. Plant Physiol **89**: 724-727
 24. **Morse MJ, Satter RL, Crain RC, Coté GG** (1989) Signal transduction and phosphatidylinositol turnover in plants. Physiol Plant **76**: 118-121
 25. **Reddy ASN, Poovaiah BW** (1987) Inositol 1,4,5-trisphosphate induced calcium release from corn coleoptile microsomes. J Biochem **101**: 569-573
 26. **Rincón M, Chen Q, Boss WF** (1989) Characterization of inositol phosphates in carrot (*Daucus carota* L.) cells. Plant Physiol **89**: 126-132
 27. **Sandelius AS, Sommarin M** (1986) Phosphorylation of phosphatidylinositols in isolated plant membranes. FEBS Lett **201**: 282-286
 28. **Schumaker KS, Sze H** (1987) Inositol 1,4,5-trisphosphate releases Ca^{2+} from vacuolar membrane vesicles of oat roots. J Biol Chem **262**: 3944-3946
 29. **Wagh SS, Menon KKG, Natarajan V** (1988) Evidence for the incorporation of [^{32}P] orthophosphate into leaf inositol phospholipids. Biochim Biophys Acta **962**: 178-185
 30. **Zbell B, Walter-Back C** (1988) Signal transduction of auxin on isolated plant cell membranes: indications for a rapid polyphosphoinositide response stimulated by indoleacetic acid. J Plant Physiol **133**: 353-360