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REVIEW ARTICLE

The plant phosphoinositide system

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INTRODUCTION

Plant signal perception and transduction

The discovery of the mammalian phosphoinositide signalling system has led to tremendous progress in the understanding of how extracellular signals are perceived by cells and converted into intracellular responses. This advance has naturally led to the question of whether similar types of signalling systems also are present in other eukaryotes.

Three often stated differences between plant and animal cells are: the limited discernable movement of plants, the very wide variety of environmental signals plant cells need to respond to and the continuous organogenesis of plant tissues. The presence of the plant cell wall means that movement of cells within tissues is very limited. The considerable constraint upon plant cells by their walls is likely to influence significantly both the need and ability to perceive, and respond to, extracellular signals. It may also be one of the determining factors for the developmental plasticity offered to plants by the potential for perpetual organogenesis—a feature dominant in sessile organisms. If an overall consideration is made of both the major types of signals that plants need to respond to, and of the responses they display, it nevertheless becomes clear that many features are similar to those found in animals, and current evidence suggests that several of the basic molecular aspects of cellular signal transduction have been highly conserved during evolution and are shared by most, if not all, eukaryotes. One example is the use of Ca^{2+} as a second messenger.

Ca^{2+} as a messenger in plant cells

The role of Ca^{2+} as a second messenger has been discussed in recent reviews (e.g. Hepler & Wayne, 1985; Blowers & Trewavas, 1990; Saunders, 1990; Trewavas & Gilroy, 1991; Johannes *et al.*, 1991) and only a brief description will be given here. Many measurements using both Ca^{2+}-sensitive indicator dyes and microelectrodes have shown that the cytoplasmic Ca^{2+} activity in unstimulated plant cells, like in many other eukaryotes, is in the region of 100–300 nM. A 1000–10000-fold difference exists between the cytosol and the plant cell exterior. The Ca^{2+} activity in the cell wall is likely to be highly variable, and figures ranging from μM to low mM have been obtained with various methods of analysis. As the surface of the plant plasma membrane is negatively charged the Ca^{2+} activity in close proximity to the uptake site(s) is likely to be considerably higher than the mean Ca^{2+} activity in the wall. The concentration difference and the membrane potential across the plasmalemma (approx. −150 mV) result in a very steep electrochemical gradient in favour of Ca^{2+} influx. Considerable electrochemical gradients also exist between various organelles and the cytoplasm. To maintain low levels of free Ca^{2+} in the cytoplasm, active transport systems are required to undertake the task of removing Ca^{2+} ions. The main Ca^{2+} stores and Ca^{2+}-transport systems in plant cells are illustrated in Fig. 1.

Cellular Ca^{2+} transport

Outward transport of Ca^{2+} across the plasma membrane is mediated by a Ca^{2+}-ATPase which energizes Ca^{2+} export in exchange for extracellular H^+. Active Ca^{2+} accumulation systems are also localized in endomembranes. The uptake of Ca^{2+} into the endoplasmic reticulum is mediated by a Ca^{2+}-ATPase, whereas transport across the vacuolar membrane is by Ca^{2+}/H^+ exchange. The vacuolar system is driven by the proton motive force set up by a primary electrogenic tonoplast-associated H^+-ATPase. This H^+-ATPase generates a vacuole-positive membrane potential between +20 and +50 mV and an inside-acid pH gradient (Johannes *et al.*, 1991). Membrane potential-driven Ca^{2+} uptake occurs in both chloroplasts and mitochondria. Whereas mitochondria are not thought to play any significant role in the control of cytoplasmic Ca^{2+} activity in unstimulated plant cells, the Ca^{2+} uptake by chloroplasts is likely to have the dual function of both controlling stromal activity and affecting free cytoplasmic Ca^{2+}. Although there is some evidence for the presence of verapamil-sensitive Ca^{2+}-channels in the plasma membrane of higher plant cells (Graziana *et al.*, 1988), so far little is known about the role of such channels in cellular Ca^{2+} homeostasis.

Until the discovery of the mammalian PtdIns system the focus of attention in plant Ca^{2+} research was on the effector roles of Ca^{2+} via, e.g., Ca^{2+}–calmodulin complex formation and the effects of Ca^{2+} upon kinase activity, and only little attention was given to the possible nature of agonist-sensitive releasable Ca^{2+} pools. The general view at the time was that Ca^{2+} most likely reached the cytosol from the extracellular matrix via plasma

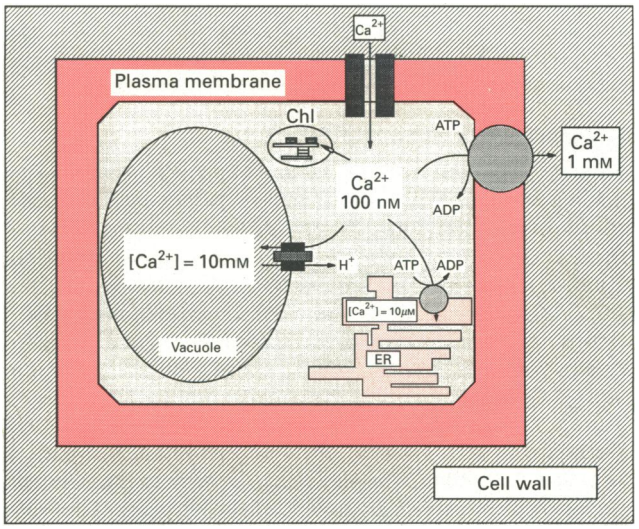

Fig. 1. Outline of Ca^{2+}-transporting systems in plant cells

Abbreviations: Chl, chloroplast; ER, endoplasmic reticulum.

Abbreviations used: PLC, phospholipase C; GTPγS, guanosine 5′-[γ-thio]triphosphate; PKC, protein kinase C.

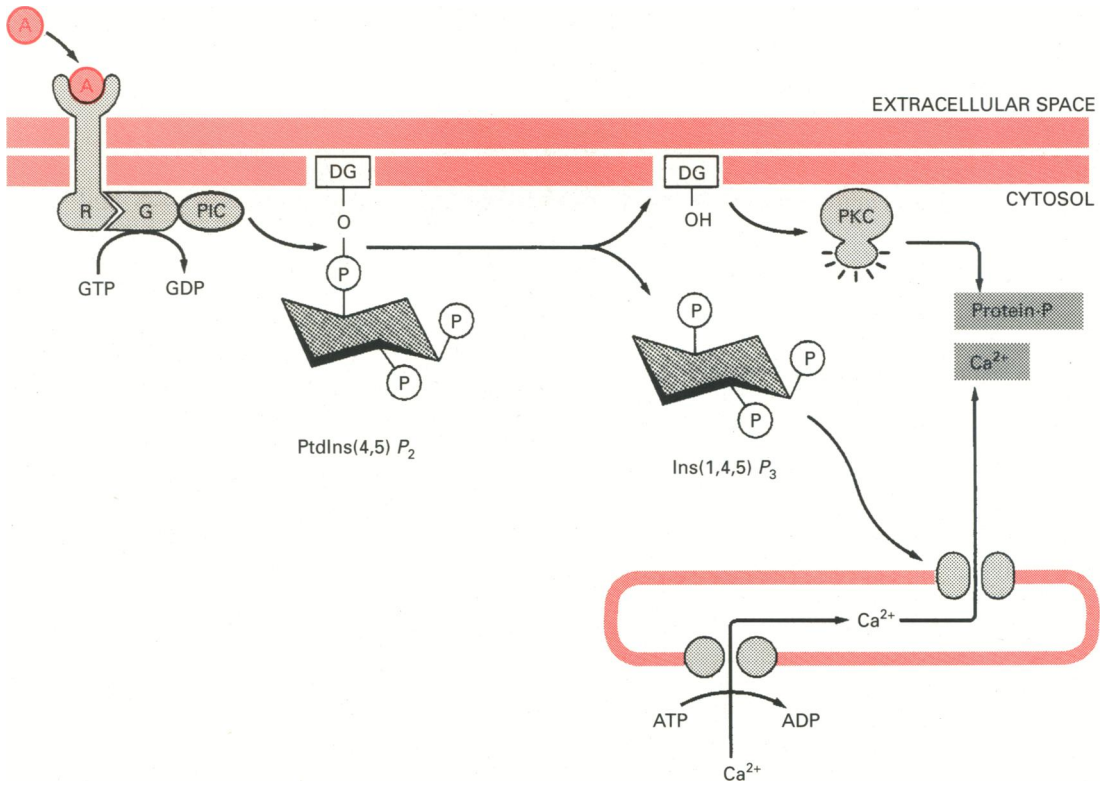

Fig. 2. Simplified and schematic overview of some of the main components of the mammalian phosphoinositide system

Abbreviations: A, agonist; R, receptor; G, heterotrimeric regulatory GTP-binding protein (G_q); PIC, phosphoinositidase C (β-isoform); DG, diacylglycerol.

membrane-associated Ca^{2+} channels. With the discovery of Ins(1,4,5)P_3-induced Ca^{2+} release in mammalian cells, attention rapidly became focused on intracellular stores being the site of agonist induced Ca^{2+} release (e.g. Berridge & Irvine, 1984).

Since Ins(1,4,5)P_3 was found to be able to release Ca^{2+} from intracellular stores in plant cells (Drøbak & Ferguson, 1985), and as it was demonstrated that several components of the mammalian-type phosphoinositide system also were present in plants, the possibility that a phosphoinositide-like system could be involved in plant cell signal transduction began to receive increased attention.

The mammalian phosphoinositide system

Some of the main elements of mammalian phosphoinositide signalling are illustrated in Fig. 2. In brief, occupancy of receptors at the plasma membrane by the appropriate agonist results in the activation of a phospholipase C isoenzyme(s) (phosphoinositidase C) localized on the cytosolic side of the plasma membrane. Activation of the mammalian phosphoinositidase C isoforms is currently thought to proceed via one of several different routes which include activation of one (or more) coupling G protein(s) (G_q), tyrosine phosphorylation and/or elevation of cytosolic Ca^{2+} (for details see Guillon *et al.*, 1992). Phospholipase C cleaves the phosphodiester bond of phosphatidylinositol 4,5-bisphosphate, providing the two messenger molecules Ins(1,4,5)P_3 and 1,2-diacylglycerol. The water-soluble Ins(1,4,5)P_3 diffuses to intracellular Ca^{2+} stores where it induces the release of Ca^{2+} by binding to Ca^{2+}-channel-linked Ins(1,4,5)P_3-specific receptors. Diacylglycerol, being highly lipophilic, remains in the plasma membrane matrix where it activates protein kinase C. Two downstream signal cascades are thus initiated, involving both elevation of the cytosolic Ca^{2+} activity resulting in modulation of Ca^{2+}-sensitive response elements and Ca^{2+}/protein kinase C-promoted protein phosphorylation. Removal of the agonist from the agonist–receptor complex results in the cessation of phospholipase-C-mediated PtdIns(4,5)P_2 hydrolysis. Both Ins(1,4,5)P_3 and diacylglycerol are rapidly metabolized in cells so in the absence of *de novo* synthesis the cellular levels of these two messengers rapidly decline; protein kinase C is inactivated and cytosolic Ca^{2+} levels are brought back to resting levels by the membrane-associated Ca^{2+} transport systems (Nishizuka, 1988; Berridge & Irvine, 1989; Rhee *et al.*, 1989; Shears, 1989; Downes & MacPhee, 1990; Carpenter & Cantley, 1990).

Against this general background the current evidence for the presence of structural and functional elements of the plant phosphoinositide system is now described, and its possible roles in cellular signal perception and transduction discussed. The emphasis will be on higher plants, although selected data from research on other lower eukaryotes will be presented where appropriate.

METABOLISM OF INOSITOL AND INOSITOL-CONTAINING COMPOUNDS IN PLANT CELLS

Occurrence of inositol in plants

There are nine stereoisomers of inositol; seven of which are meso structures and two of which form a chiral pair. Of these nine isomers, seven are known to occur in nature, the exceptions being *epi*- and *allo*-inositol. *myo*-, D/L-*chiro* and *scyllo*-inositol constitute the major stereoisomers of inositol in plants, although both *muco*-inositol and *neo*-inositol have been found to exist in several species (Loewus, 1990). *myo*-Inositol can accumulate to high levels in some plant tissues; for example, it comprises 1.5 %

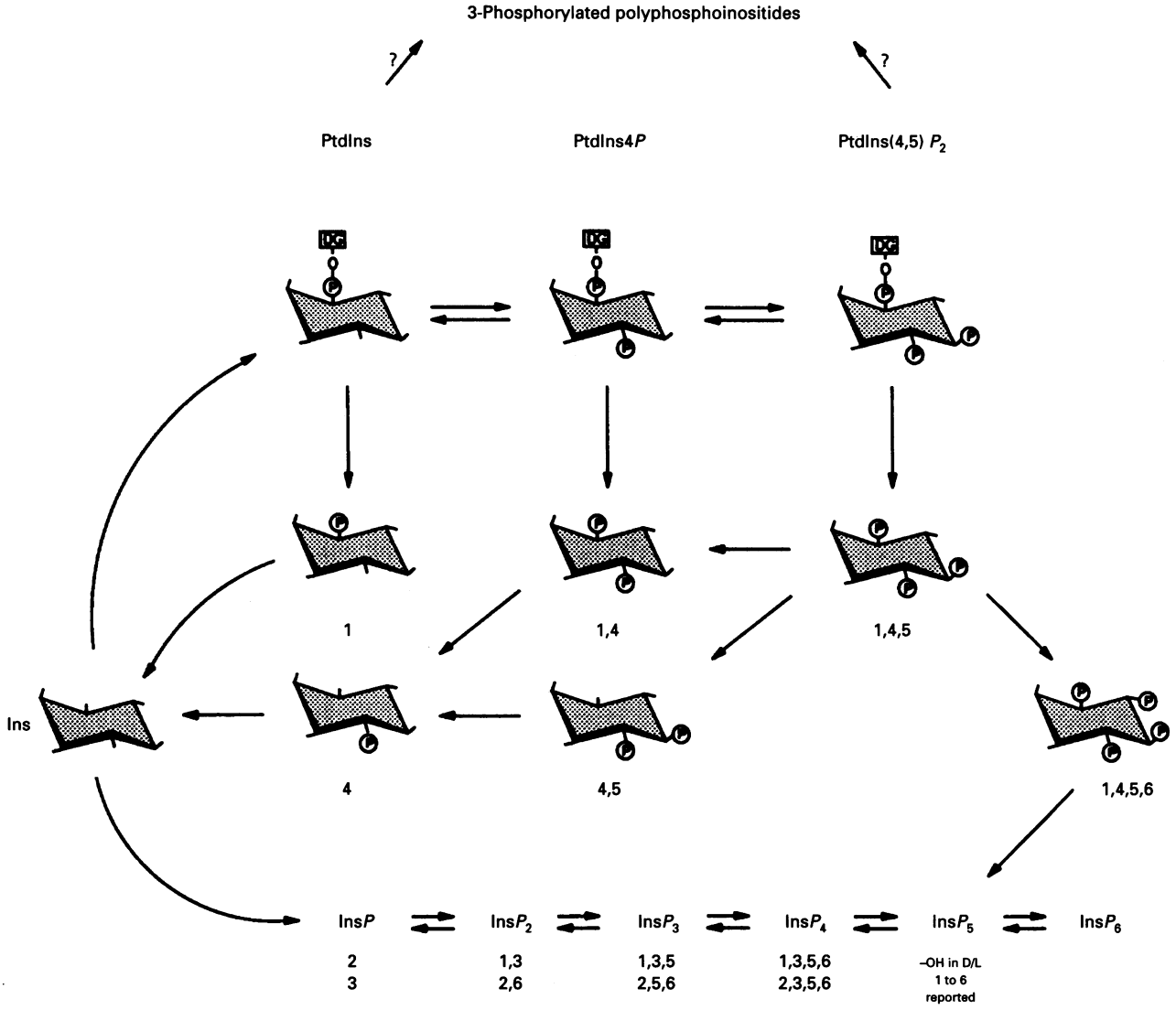

Fig. 3. Likely routes of phosphoinositide and inositol phosphate metabolism in plant cells

Inositol polyphosphate isomers are numbered using the D-configuration.

of the dry matter in the pistil and ovaries of *Digitalis*, and as much as 10% in dry tea leaves (Loewus & Loewus, 1980).

Biosynthesis of *myo*-inositol

Free *myo*-inositol is produced by a three-step conversion of D-glucose by the enzymes hexokinase (EC 2.7.1.1), L-*myo*-inositol 1-phosphate synthase (EC 5.5.1.4) and L-*myo*-inositol 1-phosphate phosphatase (EC 3.1.3.25). It is currently believed that this route of biosynthesis is solely responsible for the *de novo* formation of *myo*-inositol in plant cells (Loewus, 1990; Loewus & Loewus, 1983).

Inositol phosphates

Phosphorylation of *myo*-inositol. Inositol 1,2,3,4,5,6-hexakisphosphate (phytic acid) accumulates in plant seeds and other (storage) tissues in very significant amounts. Phytic acid is present as a complex salt with counterions such as K^+, Mg^{2+} and Ca^{2+}, and may represent as much as 50–80% of total seed phosphorus (Raboy, 1990). In seeds, phytate is commonly deposited as discrete globular inclusions (globoids) in cellular storage microbodies (referred to as protein bodies). It has been suggested that phytate synthesis in seeds takes place in the cisternal endoplasmic reticulum and that following synthesis phytate-bearing vesicles migrate towards the protein body where the vesicular content is discharged (Loewus, 1990).

The biosynthetic route of phytic acid formation has been the cause of controversy for some time and several hypotheses have been put forth. A Mg^{2+}- and ATP-dependent kinase capable of phosphorylating free *myo*-inositol in the D-3 position is present in plant cells (Loewus *et al.*, 1982) and this makes D-*myo*-inositol 3-phosphate (L-*myo*-inositol 1-phosphate), both the precursor for *de novo* synthesis of *myo*-inositol and the product of *myo*-inositol phosphorylation. The physiological role of this apparent futile cycle is not currently clear, but one possibility is that it may act as a controller of free *myo*-inositol levels in plant cells. Further phosphorylation of inositol monophosphates is known to occur but the precise mechanism(s) is still being debated.

Mung beans have been shown to contain a Mg^{2+}-dependent kinase which until recently was thought to be capable of phosphorylating Ins3*P* to InsP_6 (Chakrabarti & Majumder, 1978; Majumder *et al.*, 1972). However, subsequent work by Biswas and co-workers (e.g. Chakrabarti & Biswas, 1981) suggests that the successive phosphorylation of Ins3*P* only proceeds to InsP_5 and is followed by conversion of InsP_5 to InsP_6 in a reaction

mediated by a reversible phytate–ADP phosphotransferase (De & Biswas, 1979). Surprisingly, when Ins2*P* is the initial substrate the phosphorylation seems to proceed all the way to InsP_6. The possibility that Ins1*P* or cyclic Ins(2:3)*P* can be phosphorylated *in vivo* in plant cells has been suggested (e.g. Loewus & Loewus, 1983) but as yet no firm proof exists. In contrast, Ins2*P* has been suggested to be the precursor for InsP_6 formation in rice (Igaue *et al.*, 1980). Igaue *et al.* (1982) determined the isomeric configuration of potential inositol phosphate intermediates by proton-decoupled ^{31}P-n.m.r. and found the following isomers: Ins(1,3)P_2, Ins(2,6)P_2, Ins(1,3,5)P_3, Ins(2,5,6)P_3, Ins(1,3,5,6)P_4, Ins(2,3,5,6)P_4 and Ins(2,3,4,5,6)P_5, Ins(1,2,3,5,6)P_5 and Ins(1,3,4,5,6)P_5. This isomerism suggests two routes of phosphorylation starting with either Ins3*P* or Ins2*P*. It cannot be ruled out that some of the higher inositol phosphates found by Igaue *et al.* (1982) may be products of InsP_6 dephosphorylation or InsP_5/InsP_6 cycling. The possible presence of these isomers in other plant tissues has not been investigated in detail. However, it has been demonstrated that in germinating mung bean seedlings, D- and/or L-Ins(1,2,3,4,5)P_5 and (D)/L-Ins(1,2,4,5,6)P_6 dominate (see Stephens, 1990; Stephens *et al.*, 1991) and in addition to these isomers Phillippy & Bland (1988) also found D- and/or L-Ins(1,2,3,4,6)P_5 and D- and/or L-Ins(1,3,4,5,6)P_5.

A much more comprehensive picture of inositol phosphorylation in *Dictyostelium* has recently emerged from a series of elegant experiments by Stephens & Irvine (1990). In this organism it was demonstrated that InsP_6 was formed as a result of stepwise phosphorylation of *myo*-inositol, catalysed by a series of soluble ATP-dependent kinases, and the following intermediates were identified: Ins3*P*, Ins(3,6)P_2 and Ins(3,4,6)P_3, Ins(1,3,4,6)P_4 and Ins(1,3,4,5,6)P_5. It was further shown that the 3- and 5-phosphates of InsP_6 take part in apparent futile cycles in which Ins(1,2,4,5,6)P_5 and Ins(1,2,3,4,6)P_5 are rapidly formed by dephosphorylation only to be converted back into InsP_6 by phosphorylation. Clearly it would be of some considerable interest to investigate whether the differences in isomeric configuration of inositol phosphates encountered in this study and the isomers reported to be present in (at least some) plant cells reflect real differences in InsP_6 biosynthesis or may have other explanations. A scheme proposed by Asada *et al.* (1969) suggested a phosphorylated inositol derivative X-inositol-*P* as the initial substrate, with phosphates being added sequentially until X-inositol-P_6 was formed and InsP_6 then being released from the 'X' moiety. The likelihood of such a pathway being in operation in plant cells has been questioned in recent years in the light of the large numbers of free Ins*P*–InsP_6 intermediates found in a variety of plant cells. However, it should be pointed out that the first few steps may still be proven valid, especially if 'X' turns out to be diacylglycerol.

Hydrolysis of inositol 1,2,3,4,5,6-hexakisphosphate (phytic acid). Dephosphorylation of phytic acid in plants is mediated by one or more phosphohydrolases (phytases). Two classes of phytases are recognized at present, a 3-phytase (EC 3.1.3.8) which catalyses the removal of the D-3 phosphate from phytic acid, and a 6-phytase (EC 3.1.3.26) which initially attacks the D-6 phosphomonoester (Loewus, 1990; Gibson & Ullah, 1990). Both enzymes are capable of causing successive dephosphorylation of InsP_5 to free inositol. The 3-phytases are typically found in micro-organisms and filamentous fungi, whereas the dominant form found in plant cells is the 6-phytase. Recent studies of the soybean 6-phytase by Gibson & Ullah (1990) have shown a K_m of 48 μM ($V_{max.}$ 145 pkat) for this enzyme and suggest that the apparent sluggishness of this enzyme *in vitro* may be explained, at least partly, by its high sensitivity to orthophosphate (K_i 28 μM). The phytase from wheat bran, a comparatively non-specific acid phosphatase, produces predominantly D-Ins(1,2,3,5,6)P_5 when hydrolysing phytic acid. The further dephosphorylation proceeds via removal of the D-3- or D-5-phosphomonoesters. Hydrolysis of D-Ins(1,2,5,6)P_4 by wheat phytase produces a mixture of D-Ins(1,2,6)P_3 and D-Ins(1,5,6)P_3 whereas hydrolysis of D-Ins(1,2,3,6)P_4 only leads to formation of Ins(1,2,3)P_3 (Phillippy, 1989). Further details of plant phytases can be found in Loewus (1990) and Gibson & Ullah (1990).

Metabolism of inositol(1,4,5)P_3. Little is known about the metabolism of (1,4,5)P_3 *in vivo* but several recent studies using soluble plant extracts and membrane preparations as enzyme source suggest that *in vitro* the metabolism of Ins(1,4,5)P_3 by plant enzymes differs significantly from that of other eukaryotes. Using gel-filtered extracts from cultured tobacco cells, Joseph *et al.* (1989) found that Ins1*P*, Ins4*P*, Ins(1,4)P_2 and Ins(1,4,5)P_3 were dephosphorylated in a Ca^{2+}-dependent manner to a lower inositol phosphate or inositol. Although a detailed study of (1,4,5)P_3 metabolism was not carried out, their results indicate that Ins(1,4,5)P_3 (7 μM) after 2 min is completely converted into inositol bisphosphates. In the absence of Ca^{2+} roughly equal amounts of Ins(1,4)P_2 and Ins(4,5)P_2 are formed, whereas in the presence of 1.8 μM-Ca^{2+} the ratio of Ins(1,4)P_2 to Ins(4,5)P_2 was 1:2. Memon *et al.* (1989*b*) have investigated the possibility of Li^+-sensitivity of Ins(1,4,5)P_3 hydrolysis in soluble and microsomal fractions from cultured carrot cells and shown that 50 mM-Li^+ had no effect upon Ins(1,4,5)P_3 hydrolysis.

Using a soluble enzyme preparation from pea roots, Drøbak *et al.* (1991) found that Ins(4,5)P_2 was the major breakdown product of Ins(1,4,5)P_3 both at comparatively low Ca^{2+} concentrations (400 nM) and micromolar Ca^{2+} concentrations. The formation of Ins(1,4)P_2 by the pea root enzyme(s) was negligible at all Ca^{2+} concentrations. Further dephosphorylation of InsP_2 was found to proceed via Ins4*P* to free inositol. Direct phosphorylation of Ins(1,4,5)P_3 was also observed and the InsP_4 formed was tentatively identified as (1,4,5,X)P_4 (Drøbak *et al.*, 1991). Periodate oxidation/borohydride reduction/dephosphorylation and analysis of the alditol produced has demonstrated that this InsP_4 is Ins(1,4,5,6)P_4 and not the expected D-1,3,4,5 isomer (Chattaway *et al.*, 1992). Further work indicates that small amounts of D/L-Ins(1,2,3,4)P_4 are also formed by action of plant enzymes on Ins(1,4,5)P_3 (B. K. Drøbak & J. A. Chattaway, unpublished work). In summary, these data suggest that the metabolism of Ins(1,4,5)P_3 in higher plants, at least *in vitro*, differs significantly from that of mammalian cells and certain other photosynthetic eukaryotes such as, e.g., *Chlamydomonas* (see Irvine *et al.*, 1992).

Phosphatidylinositol and polyphosphoinositides

Phosphatidylinositol (PtdIns) has long been known to be a common constituent of the plant phospholipid pool. In non-photosynthetic plant tissues PtdIns is the third most abundant phospholipid after phosphatidylcholine and phosphatidylethanolamine, comprising as much as 21 % of total phospholipid in various membrane types including organellar membranes (Harwood, 1980). Two reactions for incorporation of *myo*-inositol into PtdIns have been found to exist in plant cells. One is mediated by the enzyme CDP-diacylglycerol: inositol phosphatidyltransferase (EC 2.7.8.11) and the other, the 'head-group exchange reaction', is carried out by PtdIns: inositol phosphatidyltransferase. It should be noted that of the two reactions only the first is capable of net synthesis of PtdIns. Both reactions for PtdIns synthesis are thought to occur in the endoplasmic reticulum of plant cells, and also possibly in the Golgi complex (Moore, 1990).

Following the first report describing the presence of polyphosphoinositides in plant cells (Boss & Massel, 1985) a number of studies have confirmed these findings, and it is now

accepted that polyphosphoinositides are generally present in plant cells (Strasser *et al.*, 1986; Heim & Wagner, 1986; Drøbak *et al.*, 1988*a*; Boss, 1989; Morse *et al.*, 1987). The reason for the relatively late discovery of polyphosphoinositides in plants is probably two-fold: first, little interest in such lipids has been evident until the discovery of the eukaryotic PtdIns signalling system and second, most plant lipid biochemists have routinely used non-acidic lipid extraction systems that are ineffective in extracting the highly charged polyphosphoinositides.

Direct determination of the chemical quantities of polyphosphoinositides in plant cells has not so far been carried out, so the best estimates come from radiolabelling studies where lipids have been extracted from tissues where isotopic equilibrium is assumed to have been reached. Such experiments have given widely differing results, and ratios of PtdsIns:PtdIns*P*:PtdInsP_2 from 10:1:1 to 300:17:1 have been reported (see Boss, 1989; Sandelius & Sommarin, 1990; Drøbak & Roberts, 1992; Hetherington & Drøbak, 1992). In most experiments phosphoinositides were separated by t.l.c. and identified by co-chromatography of labelled compounds with authentic mammalian standards. Although this approach was considered adequate until recently for the routine analysis of animal polyphosphoinositides it has for some time been known that this is not the case with plant polyphosphoinositides. The reasons for this are several and include: first, the differences in fatty acid composition between mammalian and plant phosphoinositides (see below) which can lead to 'double running' in commonly used t.l.c. systems and second, several reports suggest the presence of considerable amounts of lysophosphoinositides in certain plant tissues (Wheeler & Boss, 1990). Both lysophosphoinositides and inositol-containing phytoglycolipids show chromatographic behaviour similar to that of polyphosphoinositides (Drøbak *et al.*, 1988*a*).

Experiments in which glycerophosphorylinositol or inositol phosphate derivatives of labelled phosphoinositides from equilibrium-labelled plant tissues have been separated by h.p.l.c. (a procedure which largely overcomes problems of co-chromatography of other polyphosphoinositide- and inositol-containing lipid derivatives) indicate that PtdIns(4,5)P_2 in most plant tissues is unlikely to constitute more than 0.05% of total phospholipids or 0.5% of phosphoinositides (Drøbak *et al.* 1988*a*; Irvine *et al.*, 1989; Drøbak, 1991). With the recent advances in the methods for determining the mass of very low chemical levels of phosphoinositides (e.g. Divecha *et al.*, 1991) a clearer picture of true phosphoinositide levels in plant cells should soon emerge.

Structure of plant phosphoinositides. The determination of the structure of the headgroups of plant phosphoinositides was carried out using the approach originally devised by Grado & Ballou (1961) and later refined by Irvine *et al.* (1984, 1989) and (Stephens, 1990). In brief, this method is based on deacylation of the phosphoinositide followed by removal of the glycerol backbone. The resulting inositol phosphate is treated with periodate which cleaves C–C bonds in the inositol ring between carbon atoms if both carry hydroxyls. Reduction of possible aldehydes followed by dephosphorylation yields a non-cyclic alditol if cleavage of the inositol ring occurred during the periodate treatment. The type of alditol produced in this process bears witness to the structure of the original inositol phosphate (phosphoinositide). Investigation of the D/L-isoforms of the alditols can be achieved by the use of stereospecific enzymes (Stephens, 1990). Using this approach Irvine *et al.* (1989) showed that only two polyphosphoinositides were present in pea leaves and their structures to be PtdIns(4)*P* and PtdIns(4,5)P_2.

A number of novel phosphoinositides, e.g. phosphatidylinositol 3-phosphate, phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol 3,4,5-trisphosphate, have recently been discovered in mammalian cells (Whitman *et al.*, 1988). In intact neutrophils activated by the formyl peptide fMet-Leu-Phe a phosphatidylinositol 4,5-bisphosphate 3-kinase seems to be responsible for agonist-stimulated formation of 3-phosphorylated polyphosphoinositides (Stephens *et al.*, 1991) Although Irvine *et al.* (1989) did not find evidence for the presence of such polyphosphoinositides in plant tissues, Brearley & Hanke (1992) have shown that PtdIns(3)*P* and possibly PtdIns(3,4)P_2 are present in the aquatic plant *Spirodela*. It is clearly of considerable interest to investigate how widespread is the occurrence of 3-phosphorylated polyphosphoinositides in the plant kingdom and to determine their cellular localization and physiological functions. In a study on barley aleurone phosphoinositides, Murthy *et al.* (1992) have obtained data which suggest the presence of a possible novel isomer of PtdIns probably with the diester phosphate bond in the D-2 position of the inositol ring. This finding has not so far been confirmed using other plant tissues.

Fatty acyl moieties of plant polyphosphoinositides. Although a detailed survey of the fatty acid composition of polyphosphoinositides has not been carried out, the limited data at hand suggest that the predominant fatty acids in PtdIns4*P* and PtdIns(4,5)P_2 are palmitate and linoleate (Drøbak *et al.*, 1988*a*; Rincon & Boss, 1990). This is consistent with a synthetic pathway involving direct phosphorylation of PtdIns.

Turnover of plant polyphosphoinositides. In most mammalian cells PtdIns4*P* and PtdIns(4,5)P_2 are formed by a stepwise phosphorylation of PtdIns. PtdIns is first phosphorylated in the D-4 position of the inositol ring resulting in the formation of PtdIns4*P* and this lipid is further phosphorylated in the 5-position yielding PtdIns(4,5)P_2. Two phosphohydrolases work concomitantly with the phosphoinositide kinases removing the 4- and 5-phosphomonoesters, allowing constant turnover of PtdIns4P and PtdIns(4,5)P_2. That polyphosphoinositides are synthesized in a similar manner in plants is likely, but as yet is not proven.

The plant phosphoinositide kinases. Using microsomal and purified plasma membrane fractions from wheat as enzyme source, Sandelius & Sommarin (1986) and Sommarin & Sandelius (1988) have demonstrated the presence of membrane-associated PtdIns and PtdIns*P* kinase activity which can utilize both endogenous and exogenous substrates. Both kinases are highly enriched in the plasma membrane and show complete dependency upon ATP for phosphorylation. Phosphorylation of exogenous PtdIns is maximal at approx. 0.75 mM ATP whereas ATP concentrations above 2 mM are inhibitory. Maximum activity against endogenous substrates requires higher ATP concentrations. The PtdIns*P* kinase shows maximum activity at 1.25 mM-ATP (exogenous substrates) and 1.5 mM (endogenous substrates) and the estimated K_m values for ATP for both kinases are around 200 μM. These figures are considerably higher than those reported for the mammalian PtdIns 4-kinase (type 2) and PtdIns4*P* 5-kinase (Carpenter & Cantley, 1990) but agree well with the K_m for ATP found for mammalian type 3 PtdIns 4-kinase (Li *et al.*, 1989) and the yeast PtdIns 4-kinase (Belunis *et al.*, 1988). Recent studies by Kamada & Muto (1991) have shown that PtdIns kinase of tobacco plasma membranes also resembles the mammalian type 3 kinase with respect to both K_m for ATP and sensitivity to adenosine.

The wheat kinases studied by Sommarin & Sandelius (1988) were found to be Mg^{2+}-dependent and have an optimal pH range for both PtdIns and PtdIns*P* kinases of 6.5–7.0 (endogenous substrates) and 7.8–8.2 (exogenous substrates). Under optimal assay conditions the formation of PtdIns*P* and PtdInsP_2 from endogenous substrates was 175 pmol/min per mg of protein and

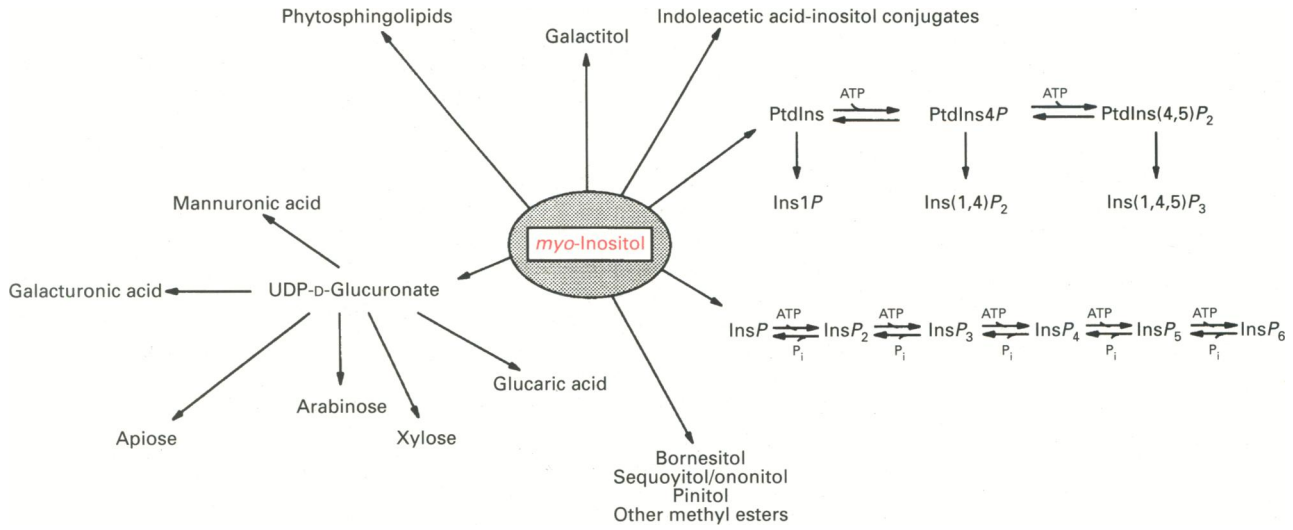

Fig. 4. Metabolism of *myo*-inositol in plant cells

18 pmol/min per mg of protein respectively (Sommarin & Sandelius, 1988). Ca^{2+}, when added alone, did not affect activity but 100 μM-Ca^{2+} added together with optimal amounts of Mg^{2+} reduced the rate of polyphosphoinositide formation. In a study of the effect of Ca^{2+} on the plasma membrane phosphoinositide kinases in tobacco Kamada & Muto (1991) found that both the PtdIns and PtdIns*P* kinases were inhibited by nanomolar Ca^{2+} concentrations, the PtdIns kinase being by far the most sensitive, with a more than 90% reduction in activity in the presence of 100 nM-Ca^{2+}. Reduction in PtdIns*P* kinase activity was less dramatic and this enzyme still exhibited 30–50% of full activity in the interval 1–100 μM-Ca^{2+}.

Plant polyphosphoinositide phosphatases. Little is currently known about the plant (or mammalian) polyphosphoinositide phosphatases. Considering the amount of attention given to the role of phosphatases in the cell cycle this may seem a little surprising. In the original study by Sandelius & Sommarin (1986) the removal of ATP by addition of glucose/hexokinase did not cause any appreciable decrease in the labelling of PtdIns*P* although PtdIns kinase activity was abolished. It is a little difficult to reconcile such data with the apparent rapid turnover of PtdIns*P* *in vivo* (see below). It is however likely that the optimization of the *in vitro* assays for phosphoinositide kinase activity may have led to the suppression of phosphatase activity. The presence of active polyphosphoinositide phosphatases in plasma membranes from bean leaves has been demonstrated (P. Xu & B. K. Drøbak, unpublished work).

As mentioned above, the rate of turnover of PtdIns(4)*P* *in vivo* is very rapid. ^{32}P-labelling studies by Drøbak *et al.* (1988*a*) have shown that more than 30% of label incorporated into phospholipids in tomato suspension cultured cells after short incubation times (i.e. 30 min) is found in PtdIns4*P*. Although the position of the label in the PtdIns4*P* molecule was not determined it is likely that it was predominantly in the 4-phosphomonoester, indicating the presence of a highly active PtdIns 4-kinase. The rapid ^{32}P incorporation when seen in conjunction with the comparatively low chemical levels of PtdIns4*P* suggests that PtdIns4*P* is rapidly metabolized further. Whether phosphomonoesterases/kinases or phospholipases are dominant in this process remains to be clarified. The low levels of PtdIns(4,5)P_2 and the high rate of turnover of PtdIns4*P* has made the task of obtaining reliable data about PtdIns(4,5)P_2 turnover difficult. ^{32}P labelling experiments using brinjal leaves nevertheless suggest that PtdIns(4,5)P_2 turnover, at least in this tissue, may be rapid (Wagh *et al.*, 1988). Confirmation of these observations has not so far been forthcoming, and experiments using other plant tissues do not support the view that rapid PtdIns(4,5)P_2 turnover is a general occurrence in plant tissues.

Subcellular localization of plant polyphosphoinositides. A full picture of the subcellular localization of plant polyphosphoinositides and phosphoinositide metabolizing enzymes has still to emerge. Results from labelling experiments employing both intact cells and membrane fractions enriched in plasma membrane vesicles suggest that the polyphosphoinositides, as in mammalian cells, are predominantly associated with the plasma membrane (Sandelius & Sommarin, 1990) but recent reports suggest that smaller amounts of polyphosphoinositides may be associated with other organelles, such as the nuclei (Hendrix *et al.*, 1989). In isolated membrane fractions from hypocotyls of dark grown soybean, both PtdIns and PtdIns*P* kinases are associated predominantly with the plasma membrane whereas only negligible activity of these enzymes is found in the tonoplast, nuclei, mitochondria and plastid fractions (Sandelius & Sommarin, 1990). It is clear that a more thorough investigation of the subcellular distribution of polyphosphoinositides is needed, in particular in the light of recent findings that PtdIns*P* in mammalian cells can be synthesized via an intracellular route (Lundberg & Jergil, 1988) and that polyphosphoinositides are present in the nucleus (Divecha *et al.*, 1991). Problems facing plant scientists in pursuing this line of work are not only associated with the chemical analysis of small amounts of polyphosphoinositides but also with the production of subcellular membrane fractions of sufficient purity.

Other inositol-containing compounds in plant cells. In addition to the incorporation of *myo*-inositol into phosphoinositides and inositol phosphates, *myo*-inositol acts as a precursor for a bewildering array of compounds in plant cells. The major routes of inositol metabolism in plant cells are illustrated in Fig. 4. It is beyond the scope of this Review to go into any detailed descriptions of all these compounds, but a brief listing of the more prominent pathways is appropriate for later discussions.

Methyl esters of inositol. A variety of methyl esters of *myo*-inositol are known to occur in plant cells. These include the monomethyl esters D/L-bornesitol (1 D- and 1 L-1-*O*-methyl-*myo*-inositol), D-ononitol (1 D-4-*O*-methyl-*myo*-inositol) and sequoyitol (5-*O*-methyl-*myo*-inositol). Dimethyl esters are also

Table 1. Properties of plant phosphoinositide-specific phospholipase C enzymes

Tissue	Localization	Preferred substrate	Ca^{2+} concentration for full activity	Reference
Celery stem	Soluble	PtdIns	2 mM	Irvine *et al.* (1980)
Bean	Plasma membrane	PtdIns (PtdInsP_n not tested)	500 μM	Pfaffmann *et al.* (1987)
Bean	Soluble	PtdIns (PtdInsP_n not tested)	500 μM	Pfaffman *et al.* (1987)
Lillium pollen	Soluble and particulate	PtdIns (PtdInsP_n not tested)	1 mM	Helsper *et al.* (1986, 1987)
Celery stalks	Particulate	PtdIns and PtdIns(4,5)P_2	1 mM	McMurray & Irvine (1988)
Wheat roots	Plasma membrane	PtdIns4P/PtdIns(4,5)P_2	10 μM	Melin *et al.* (1987, 1992)
Oat roots	Plasma membrane	PtdIns4P/PtdIns(4,5)P_2	10–100 μM	Tate *et al.* (1989)
Dunaliella salina	Plasma membrane	PtdIns4P/PtdIns(4,5)P_2	10 μM	Einspahr *et al.* (1989)
Soybean	Membranes	PtdIns4P/PtdIns(4,5)P_2	1 μM	Biffen & Hanke (1990)

known to occur in plant tissues and include dambonitol (1,3-di-*O*-methyl-*myo*-inositol) and D-liriodentritol (1 D-1,4-di-*O*-methyl-*myo*-inositol). Methyl esters of *chiro*- and *scyllo*-inositol have also been found to exist in plants (e.g. pinitol, quebrachitol and pinpollitol) (Loewus & Loewus, 1980).

Indoleacetic acid inositol esters. Several *myo*-inositol conjugates of the plant hormone indoleacetic acid are present in plant cells (e.g. Bandurski, 1978; Cohen & Bandurski, 1982). Four basic classes of conjugates have been described. These include low-molecular-mass esters such as indoleacetic acid *myo*-inositol, higher-molecular-mass esters such as indoleacetic acid-glucans, indoleacetic acid in amide linkage to single amino acids such as aspartate, and indoleacetic acid in amide linkage to small proteins (Cohen *et al.*, 1988; Michalczuk *et al.*, 1990). It is not at present clear which functions these conjugates have in plant cells but Michalczuk *et al.* (1990) have proposed that the non-hormone moiety may be involved in targeting of indoleacetic acid within the plant, or itself act as a messenger. Another possibility is that the conjugation process is involved in the response mechanism to the hormone.

Phytoglycolipids. Carter *et al.* (1958) were the first to isolate and partially characterize a series of complex inositol- and phosphate-containing glycolipids with a phytosphingosine base. These lipids were named phytoglycolipids and at least ten different types are known to be present in various plant tissues (e.g. Kaul & Lester, 1975). Little is currently known about the structure of many of the phytoglycolipids, their route of biosynthesis and possible functions.

***myo*-Inositol as precursor for oligosaccharides and polysaccharides.** *myo*-Inositol has an important role as intermediary in the biosynthesis of oligosaccharides of the raffinose family and as a precursor for cell wall polysaccharide synthesis. The biosynthesis of, e.g., raffinose proceeds via a number of steps, the first being conversion of *myo*-inositol into galactinol by reaction with UDP-D-galactose. Galactinol functions as a galactosyl donor to sucrose to form raffinose (Kandler & Hopf, 1982). In these reactions *myo*-inositol functions as a 'cofactor' as it is consumed during galactinol synthesis but is released again during galactosyl transfer. The steps leading from *myo*-inositol to cell wall polysaccharides follow the *myo*-inositol oxidation pathway. This pathway involves the following enzymes: *myo*-inositol oxygenase (EC 1.13.99.1), glucoronokinase (EC 2.7.1.43) and glucuronate-1-phosphate uridyltransferase (EC 1.7.7.44); the product is UDP-glucuronate (Loewus, 1990). UDP-glucuronate acts as the precursor for other glycosyl units such as UDP-galacturonate, UDP-xylose, UDP-arabinose and UDP-apiose. These nucleotide sugars/sugar acids are widespread in the plant kingdom and are found in non-cellulosic cell wall polysaccharides, in glycoproteins and in gums/mucilages.

Phospholipase C

It is now recognized that mammalian cells contain a number of immunologically distinct phospholipase C enzymes which differ significantly from each other with regard to both molecular mass, amino acid sequence and biochemical characteristics (Rhee *et al.*, 1989; Rhee & Choi, 1992). It is not unlikely that a similar situation exists in plant cells.

The presence of a PtdIns-specific phosphodiesterase (phospholipase C, PLC) in plant tissues was reported by Irvine *et al.* (1980) who found a soluble activity in celery stems capable of hydrolysing PtdIns, the resulting products being Ins1P and cyclic Ins(1:2)P. This enzyme showed no activity against phosphatidylcholine and phosphatidylethanolamine, required a divalent cation for activity (Ca^{2+} being most effective) and had a pH optimum of 5.9–6.6. Pfaffmann *et al.* (1987) found a PtdIns-specific PLC activity in plant stems with 90% of the activity being in a soluble form and the remainder associated with membranes. As with the soluble celery enzyme, relatively high concentrations (500 μM) of Ca^{2+} were needed for full activity. A similar activity has been reported to be present in pollen (Helsper *et al.*, 1986, 1987).

Evidence for PLC activity capable of hydrolysing polyphosphoinositides was reported by Melin *et al.* (1987) who found a plasma-membrane-associated PLC-activity which hydrolysed all three phosphoinositides. This enzyme had a 5–20 times greater activity towards PtdIns4P and PtdIns(4,5)P_2 than to PtdIns. The pH optimum for PLC activity was 5.5–6.0 with PtdIns(4,5)P_2 as substrate whereas the range was much wider for PtdIns4P (pH 5.5–7.5). Optimal PLC activity was found at 10 μM-Ca^{2+} both when PtdIns4P and PtdIns(4,5)P_2 were used as substrate and very little activity was found in the absence of Ca^{2+}. Melin *et al.* (1987) also reported the presence of a soluble PLC activity which showed a preference for PtdIns and had a 10-fold lower activity towards polyphosphoinositides than the plasma membrane associated activity.

Several reports on plant phosphoinositide-specific PLC have emerged in the last few years. Table 1 indicates that a number of different phosphoinositide-specific phospholipase(s) C exist in plant cells. It appears that all the enzymes so far investigated fall in one of two main groups: PLC type I reported by Irvine *et al.* (1980) is predominantly soluble (cytosolic/vacuolar?), has a clear preference for PtdIns over PtdIns4P and PtdIns(4,5)P_2 and requires millimolar concentrations of Ca^{2+} for full activity. PLC type II as described by Melin *et al.* (1987) is predominantly

associated with the plasma membrane, shows a marked preference for polyphosphoinositides versus PtdIns and is fully activated by low μM concentrations of Ca^{2+}. Although both Pfaffmann *et al.* (1987) and McMurray & Irvine (1988) have reported the presence of an apparent membrane-associated PtdIns PLC, it is not clear whether this enzyme is distinct, as the low level of activity could be explained by either PtdIns hydrolysis by PLC type II and/or low level contamination by the soluble type I PLC. Further investigations of both the biochemical and molecular characteristics of the plant PLC isoenzymes will hopefully help to reveal whether the diversity in primary structure, expression, cellular distribution and function is as pronounced in plant tissues as in mammalian cells.

Activation of PLC: involvement of G-proteins?

The possible modes of activation of plasma membrane associated PLC type II is central to any discussion of agonist-induced phosphoinositide turnover in plant cells, especially if the analogy to mammalian systems is maintained. It is known that several different mechanisms exist for coupling of receptor occupancy to PLC activation in mammalian cells (Rhee & Choi, 1992). One much studied mechanism involves the activation of the PLC-β isoforms by a heterotrimeric regulatory GTP-binding protein belonging to the G_q subfamily (Simon *et al.*, 1991). Several attempts have been made to investigate whether a similar phenomenon exists in plant cells but so far the evidence has been conflicting. Neither McMurray & Irvine (1988) nor Biffen & Hanke (1990) found any effects of GTP or GTP analogues on the celery and soy bean PLC. Similar conclusions were reached by Melin *et al.* (1987) and Tate *et al.* (1989), although both groups comment on the possibility of putative G-proteins being lost during the preparation of highly purified plasma membrane vesicles. Another possibility is that regulatory G-proteins are removed from plant membranes during freezing/thawing. That this possibility deserves attention is illustrated by a recent report by Bilushi *et al.* (1991) showing that when frozen plasma membranes isolated from maize seedling roots are thawed, a significant proportion of GTP-binding activity is solubilized. A heterodimeric GTP-protein with a molecular mass of 61 kDa was purified from the soluble fraction from the thawed plasma membranes. As pointed out by Bilushi *et al.* (1991) it is clearly a possibility that such proteins may be involved in transmembrane signalling.

In contrast to the results described above, Einspahr *et al.* (1989) were able to demonstrate a marked activation of the plasma membrane polyphosphoinositide-specific PLC of *Dunaliella salina* by 100 μM-GTPγS over a range of free Ca^{2+} concentrations. Evidence in favour of involvement of G-proteins in PLC activation has also been presented by Dillenschneider *et al.* (1986) who found that guanine nucleotides stimulated the release of inositol phosphates from ^{3}H-inositol-labelled membranes isolated from cultured sycamore cells.

Evidence exists for the presence of proteins in plants that are recognized by antibodies to mammalian heterotrimeric G-protein subunits (Blum *et al.*, 1988; Jacobs *et al.*, 1988) and also for the presence of low-molecular-mass GTP-binding proteins of the *ras*-p21 type (e.g. Drøbak *et al.*, 1988*b*). Whether one or more of these proteins are involved in modulation of PtdIns-cycle enzymes remains to be resolved.

Protein kinase C

Protein kinase C (PKC) was discovered in mammalian cells as a proteolytically activated protein kinase (Nishizuka, 1984). It was later shown that PKC was a Ca^{2+}-activated, phospholipid-dependent enzyme and that its affinity for Ca^{2+} could be greatly increased by 1,2-diacylglycerol formed by PtdIns(4,5)P_2 hydrolysis (Nishizuka, 1988). This finding, together with the discovery that the tumour-promoting phorbol esters were able to activate protein kinase C irreversibly, has led to a dramatic upsurge in interest in this enzyme in the signal-transduction field. It is now known that the protein kinase C family includes a number of isoenzymes with different enzymic properties and tissue distribution (Nishizuka, 1988).

Several attempts have been made to investigate whether a kinase activity resembling mammalian PKC is present in higher plants. Schafer *et al.* (1985) succeeded in partially purifying a protein kinase from zucchini by DEAE-Sephacel chromatography; this enzyme had several biochemical features in common with mammalian PKC. The zucchini enzyme was activated by Ca^{2+} with maximum activity at approximately 10^{-6} M free Ca^{2+}. At this Ca^{2+} concentration the enzyme activity was markedly stimulated by PtdSer, PtdEtn and PtdOH whereas PtdIns and PtdCho were ineffective. In the absence of Ca^{2+} all these phospholipids were found to stimulate kinase activity, with PtdIns being the most effective. The potential role of diacylglycerol/phorbol esters in the regulation of a putative plant PKC was investigated by Olah & Kiss (1986) who partially purified a 50 kDa kinase from wheat. It was found that this kinase was stimulated 2-fold by 100 μM Ca^{2+} and that the activity was further stimulated by the addition of PtdSer plus phorbol ester or PtdSer plus diolein. PtdSer alone was less effective in stimulating kinase activity, whilst phorbol ester or diolein alone were without effect. The stimulatory effects of PtdSer/phorbol ester/diolein were not observed in the absence of Ca^{2+}.

Elliott & Skinner (1986) have reported the presence in a number of plant tissues of a kinase which resembles mammalian protein kinase C with regard to its behaviour on DE-52 cellulose columns. This kinase showed a marked Ca^{2+}-dependency and a strong synergy between Ca^{2+}/diolein/PtdSer was observed. These authors assayed fractions containing the kinase from a number of plant tissues for binding of radio-labelled phorbol esters (phorbol 12,13-dibutyrate) but were unable to find any specific binding and concluded that although the plant kinase in several respects show similarity to mammalian PKC it is unlikely that the enzymes are identical. In later experiments by Elliott and co-workers a protein kinase from *Amaranthus tricolor* was shown to associate reversibly, and in a Ca^{2+}-dependent manner, both with inside-out erythrocyte membranes and plant membranes (Elliott & Kokke, 1987*a*,*b*; Elliott *et al.*, 1988). It was found when this kinase activity was purified further that it lost its phospholipid dependence, thus making it difficult to assess the precise degree of homology to mammalian PKC. Immunological evidence has been presented by Elliott & Kokke (1987*a*,*b*) that antiserum raised against the regulatory sub-unit of bovine brain PKC cross-reacts with at least three protein species from a partly purified calcium-dependent, phospholipid-activated protein kinase from *A. tricolor*, and that two of the protein species were phosphorylated. Lawton *et al.* (1989) cloned plant transcripts encoding protein kinase homologues and used oligonucleotides corresponding to conserved regions of mammalian protein Ser/Thr kinases to isolate cDNA encoding plant homologues in bean and rice. They found that *C*-terminal regions of deduced polypeptides encoded by the bean and rice cDNA (PVPK-1 and G11A respectively) contained features characteristic of the catalytic domains of known eukaryotic protein kinases, indicating that these cDNAs encode plant kinases. The putative catalytic domains were closely related to both cyclic nucleotide-dependent protein kinases and the mammalian protein kinase C family, and Lawton *et al.* (1989) conclude that it is likely that these plant kinases may be involved in signal transduction cascades. In summary, the reports described above provide both biochemical and molecular evidence for the presence of protein kinases in

plant cells which in one or more respects resemble members of the mammalian protein kinase C family. However, current data indicate that the differences between the plant and animal enzymes are significant enough to justify the conclusion that, unlike the other PtdIns-cycle enzymes, PKC does not have a direct functional equivalent in higher plant cells. This conclusion is in agreement with a recent study by Schaller *et al.* (1992), who showed that the predominant calcium- and lipid-dependent protein kinase associated with the plant plasma membrane is not protein kinase C, but instead belongs to a novel class of calcium-dependent but calmodulin- and phosphatidylserine-independent protein kinases.

THE PLANT PHOSPHOINOSITIDE SYSTEM AND SIGNAL TRANSDUCTION

With the discovery that so many of the components of the mammalian PtdIns signalling system also are present and functioning in plant cells the obvious question is: what is the role of this system in plant cell signalling? The central feature of the phosphoinositide system is, as pointed out earlier, the production of the two messenger molecules Ins(1,4,5)P_3 and 1,2-diacylglycerol. Whereas only comparatively little attention has been paid to the possible production and messenger function of diacylglycerol in plant cells (partly due to practical difficulties), Ins(1,4,5)P_3 has since 1985 been the focus of significant interest.

Inositol(1,4,5)P_3 and Ca^{2+} release

The first indication that Ins(1,4,5)P_3 was able to release Ca^{2+} from intracellular stores in plant cells came from studies by Drøbak & Ferguson (1985) who investigated Ca^{2+} fluxes across microsomal membranes isolated from zucchini hypocotyls. These authors found that around 30% of intravesicular Ca^{2+} accumulated in the presence of ATP could be released by micromolar concentrations of Ins(1,4,5)P_3. The Ca^{2+} release was rapid (seconds) and was followed by a slower reuptake (minutes). The release of Ca^{2+} was highly dependent on the extravesicular Ca^{2+} activity, which needed to be in the nanomolar region for the Ins(1,4,5)P_3 effect to be manifest. Although the apparent EC_{50} was low micromolar it is likely that this figure is too high, as the Ins(1,4,5)P_3 used in these experiments was known to contain some of the 2,4,5 isomer. In a subsequent study (Shumaker & Sze, 1987) the vacuole was identified as a likely candidate for the Ins(1,4,5)P_3-sensitive pool. Using vacuolar membrane vesicles from oat roots it was found that Ins(1,4,5)P_3 induced a transient Ca^{2+} release with an apparent EC_{50} of 600 nM. Neither Ins(1,4)P_2 (15 μM), Ins1P (100 μM) nor Inositol (100 μM) had any effect upon Ca^{2+} release. Although these data pointed towards the vacuole being the Ins(1,4,5)P_3-sensitive pool, Shumaker & Sze (1987) left the option open for one or more additional Ins(1,4,5)P_3-sensitive pool(s) to be present in plant cells. The idea of the vacuole as the Ins(1,4,5)P_3-sensitive pool was further supported by Ranjeva *et al.* (1988) who demonstrated that Ins(1,4,5)P_3 could release Ca^{2+} from intact vacuoles isolated from *Acer* cells. The EC_{50} for Ins(1,4,5)P_3 was 200 nM and the Ca^{2+} release was inhibited by the Ca^{2+} antagonist TMB-8.

Several characteristics of the Ins(1,4,5)P_3-induced Ca^{2+} release in plant cells are now known. Alexandre *et al.* (1990), using a patch-clamp technique, found that a Ca^{2+} current in the red beet root vacuole was induced by Ins(1,4,5)P_3 (K_m 220 nM at +80 mV) as well as the voltage across the membrane. Neither Ins(1,4)P_2, Ins(1,3,4)P_3, Ins(2,4,5)P_3 nor Ins(1,3,4,5)P_4 (20 μM) were able to mimic the effect of Ins(1,4,5)P_3 although Ins(2,4,5)P_3 (20 μM) was able to induce a current 15% of that induced by 1 μM-Ins(1,4,5)P_3. The channels which were opened by Ins(1,4,5)P_3 had conductances of 30 pS with 5 mM-Ca^{2+} in the vacuole and 1 mM-Ca^{2+} outside (Alexandre *et al.*, 1990). It was shown that the opening of these channels was voltage dependent; open channel states were observed at positive voltages and closed channel states at negative voltages. As pointed out by the authors, this observation indicates that Ins(1,4,5)P_3-induced opening of vacuolar Ca^{2+} channels may not only depend on 'primer' Ins(1,4,5)P_3 but also on the rate of positive change in the potential across the channel. Subsequently Alexandre & Lassalles (1990) demonstrated that Ins(1,4,5)P_3-induced changes in currents across vacuoles clamped at +30 mV were dependent on both the extravacuolar [Ca^{2+}] but also on the Ca^{2+} buffering strength of the external medium. Thus, it is likely that a close interaction exists between $[Ca^{2+}]_c$, $[Ca^{2+}]_c$-buffering capacity, transmembrane potential and the kinetics of Ins(1,4,5)P_3 production/hydrolysis, and that the ratio of these parameters in a given cell may determine the outcome of receptor-activated Ins(1,4,5)P_3 production.

Brosnan & Sanders (1990) investigated the effect of various Ca^{2+} channel modulators on Ins(1,4,5)P_3-induced Ca^{2+} release in beet microsomes and found that both nifedipine and ryanodine were ineffective as inhibitors whereas the glycosaminoglycan heparin was an effective inhibitor. The IC_{50} for heparin inhibition was 86 nM, which is similar to values reported for heparin inhibition of the Ins(1,4,5)P_3-induced Ca^{2+} release in mammalian cells (Cullen *et al.*, 1988). Heparin, a sulphated polysaccharide, might act as a structural analogue of Ins(1,4,5)P_3, but the difference in sensitivity to heparin displayed by various enzymes all using Ins(1,4,5)P_3 as substrate indicates that other properties of heparin may also play a role. Whatever the precise mode of Ins(1,4,5)P_3 receptor/heparin interaction is, the similar effects of heparin upon Ins(1,4,5)P_3-induced Ca^{2+} release in plant and mammalian cells raise the possibility that the Ins(1,4,5)P_3-sensitive receptors in both type of organism share common Ca^{2+}-release characteristics.

Ins(1,4,5)P_3-induced Ca^{2+} release from sensitive pools *in vitro* is now known to be accompanied by an inward flux of K^+, and the release has been shown to be sensitive to K^+ channel blockers (Canut *et al.*, 1989). Recently the effect of Ins(1,4,5)P_3 on Ca^{2+} and K^+ fluxes in stomatal guard cells has been studied (Blatt *et al.*, 1990; Gilroy *et al.*, 1990). Blatt *et al.* (1990) found that Ins(1,4,5)P_3 released from the inactive photolabile precursor P^5-1-(2-nitrophenyl)ethyl ester of Ins(1,4,5)P_3 [caged Ins(1,4,5)P_3] injected into stomatal guard cells resulted in a reversible inactivation of one type of K^+ channels while simultaneously activating a time-dependent inward current, thought to depolarize the membrane potential and promote K^+ efflux through a different class of K^+ channels. These results are consistent with an Ins(1,4,5)P_3-induced Ca^{2+} release into the cytoplasm and further suggest a direct link between Ins(1,4,5)P_3 action and modulation of K^+ channel activity. Direct evidence for Ins(1,4,5)P_3-induced Ca^{2+} release in stomatal guard cells was presented by Gilroy *et al.* (1990), who used microinjected caged Ins(1,4,5)P_3 as Ins(1,4,5)P_3 precursor and monitored changes in Ca^{2+} concentration by the fluorescent Ca^{2+} indicator Fluo-3. The photoactivation of caged Ins(1,4,5)P_3 led to a rapid rise in cytosolic Ca^{2+} activity that was sustained for 5–10 min and was followed by stomatal closure. Photolysis of microinjected caged Ca^{2+} mimicked the effects of Ins(1,4,5)P_3.

Nature of the Ins(1,4,5)P_3-sensitive Ca^{2+} pool

Although all current data point to the vacuole as the main Ins(1,4,5)P_3-sensitive Ca^{2+} pool it is still far from clear why plant cells, whilst seemingly sharing most of the basic characteristics of Ins(1,4,5)P_3-induced Ca^{2+}-release with mammalian cells, should have developed a releasable Ca^{2+} pool so different from that of higher eukaryotes. The vacuole, which occupies around 90% of

many plant cells, has a luminal Ca^{2+} activity of approx. 5 mM. In comparison, the Ca^{2+} activity of plant cell endoplasmic reticulum is about 5–10 μM. Assuming that the cytoplasmic Ca^{2+} activity is around 200 nM there will be a 225000-fold difference in *total* Ca^{2+} between the vacuole and the cytoplasm and a 25000-fold $[Ca^{2+}]_{free}$ gradient will exist across the tonoplast membrane. Such differences make it clear that the regulation of Ins(1,4,5)P_3-sensitive Ca^{2+} channels in the tonoplast must be under strict control to avoid rapid build-up of lethal Ca^{2+} concentrations in the cytosol. It is important that the possibility of the existence of further Ins(1,4,5)P_3-sensitive Ca^{2+} pools in plant cells is not overlooked. The presence of calsequestrin, a high-capacity low-affinity Ca^{2+}-binding protein thought to be specifically associated with various Ins(1,4,5)P_3-releasable Ca^{2+} pools in mammalian cells (Michalak *et al.*, 1992) has been demonstrated in microsomal membranes isolated from plant cells (Krause *et al.*, 1989). Further studies of the subcellular distribution of this protein may help to shed some light on the possibility of additional Ins(1,4,5)P_3-sensitive Ca^{2+} pools being present in plant cells. Hepler *et al.* (1990) have drawn attention to the presence of cortical endoplasmic reticulum in plant cells. Individual elements of the cortical endoplasmic reticulum network in plants are often positioned extremely close to the plasma membrane and may form appositional contacts, although direct fusion does not occur. The close proximity of this organelle to the plasma membrane, its active Ca^{2+}-accumulating system and its continuity with cortical endoplasmic reticulum networks in adjacent cells makes it ideally suited to participate in trans-plasma-membrane signalling events involving intracellular Ca^{2+}-release (Hepler *et al.*, 1990).

Oscillatory behaviour of intracellular Ca^{2+} in plants?

When agonist-induced cytosolic Ca^{2+} modulation is studied in populations of mammalian cells the observable pattern is often one of relatively slow Ca^{2+} increase and decrease. This pattern should be seen as the total sum of Ca^{2+} transients in all the cells under observation. A quite different pattern does in many cases become evident if Ca^{2+} transients in single cells are monitored. Thus, in response to Ca^{2+} mobilizing agonists, cells display oscillatory behaviour, that is, the resting levels of Ca^{2+} are interrupted by rapid periodic spikes. Much attention has been devoted to this phenomenon after the discovery that both the shape and frequency of spikes depend on both the type of agonist and its concentration (for recent reviews see Berridge, 1990; Meyer & Stryer, 1991). There is some evidence which points to possible oscillatory behaviour of cytosolic Ca^{2+} in plant cells. Using double-barrelled ion-sensitive microelectrodes to investigate the effects of the auxin, indole-3-acetic acid, on cytosolic free Ca^{2+} in maize epidermal cells, Felle (1988) found a distinct pattern of cytosolic Ca^{2+} oscillations. In contrast to Ca^{2+} oscillations in mammalian cells the Ca^{2+} transients in maize cells were extremely slow with around 36 min from beginning to end of each period. The origin of this oscillatory behaviour is not known, but Felle (1988) suggests that a link may exist between Ca^{2+} oscillations and oscillations in membrane potential and/or cytosolic pH. In a study of oscillating opening of Ca^{2+}-dependent potassium channels in the alga *Eremosphaera* Forster *et al.* (1989) showed that addition of caffeine in the presence of 0.1 mM-Ca^{2+} could release prolonged transient polarization of the plasma membrane, indicating reversible opening of K^+-channels. If caffeine was added in the absence of Ca^{2+}, periodic opening and closure of K^+ channels was induced (period approx. 1 min). The oscillatory behaviour could be suppressed instantaneously by the Ca^{2+} channel blocker verapamil. Forster *et al.* (1989) suggest a scheme involving caffeine-induced cytosolic Ca^{2+} increases and feed-back control of cytosolic Ca^{2+} on further Ca^{2+} release, to be responsible for the observed oscillations. The suppression of oscillations by 0.1 mM extracellular Ca^{2+} were suggested to be due to Ca^{2+} influx across the plasma membrane followed by negative feed-back upon cytosolic Ca^{2+}-release mechanisms. The rapid development of technology for monitoring Ca^{2+}-fluxes in single (plant) cells will without much doubt shortly lead to an intensification of the study of possible agonist-induced Ca^{2+}-oscillations in plant cells.

Does Ins(1,4,5)P_3 act as an extracellular messenger?

A potential role for Ins(1,4,5)P_3 as an extracellular messenger has been suggested. Rincon & Boss (1987) investigated the effect of Ins(1,4,5)P_3 on the flux of Ca^{2+} across the plasma membrane of protoplasts isolated from carrot cells, and found, somewhat surprisingly, that 20 μM-Ins(1,4,5)P_3 caused a 17% net loss of accumulated $^{45}Ca^{2+}$ within 4 min. A reaccumulation of Ca^{2+} was observed and the $^{45}Ca^{2+}$ of the protoplasts reached the initial value by 10 min. In addition to Ins(1,4,5)P_3, phytic acid (InsP_6) was also found to induce a Ca^{2+} efflux from protoplasts. Both the Ins(1,4,5)P_3 and InsP_6 effects could be abolished by the calmodulin antagonist, trifluoperazine. Although Rincon & Boss (1987) suggest that both the magnitude and the rate of the Ca^{2+} efflux may depend on the permeability of the plasma membrane to Ins(1,4,5)P_3 (and presumably InsP_6) it is difficult to imagine how two such highly charged, water-soluble, compounds would traverse the plasma membrane at all. However, Zherelova (1989) found that the introduction of Ins(1,4,5)P_3 (concn. 15 nM–1 μM) into the bathing solution of the alga *Nitella syncarpa* resulted in an increase in amplitude of an inward Ca^{2+} current and the appearance of an inward Cl^- current and caused a shift in the threshold for activation of the Cl^- channel on hyperpolarization of the membrane to more positive values. It is now recognized that certain second messengers that function in the cytosol, in one type of cell can be utilized as an extracellular messenger molecule by others. Such an example is cyclicAMP, which in many higher eukaryotes is confined to the cytosol where it modulates cyclicAMP-dependent kinase activity but is used by *Dictyostelium* as an extracellular signal for aggregation. Furthermore, it has been found that Ins(1,4,5)P_3 is secreted from *Dictyostelium* at a rate of about 10% of cellular content per minute (Van Haastert, 1989). The possibility that extracellular functions for Ins(1,4,5)P_3 have evolved in higher plants cannot be categorically refuted, but much more evidence is certainly needed before this idea can win general acceptance.

Other effects of diacylglycerol and Ins(1,4,5)P_3

In addition to the studies of the direct effects of Ins(1,4,5)P_3 on Ca^{2+} release and diacylglycerol-induced modulation of PKC activity, several researchers have investigated the effects of Ins(1,4,5)P_3/diacylglycerol on various physiological processes in plants. Most of the relevant literature in this area of study has been recently reviewed (Tucker, 1990; Allen & O'Connor, 1990). Allen & O'Connor (1990) demonstrated that micromolar concentrations of Ins(1,3,4,5)P_4, Ins(1,4,5)P_3 and Ins(1,4)P_2 (in that order) inhibit cytoplasmic streaming in the algae *Acetabularia* and in *Setcreasea* stamen hair cells. The inhibition of streaming does not seem to be directly correlated to the Ca^{2+}-release properties of the inositol phosphates, so mechanisms other than Ca^{2+} mobilization may operate. Cell-to-cell transport through plasmodesmata has also been found to be inhibited by Ins(1,4)P_2, Ins(1,4,5)P_3 (Tucker, 1988) and various molecular species of diacylglycerol (see Tucker, 1990). Diacylglycerol has also been suggested to play a role in the control of mitotic progression in *Tradescantia* stamen hair cells (Larsen & Wolniak, 1990; Larsen *et al.*, 1991) and in ion-pumping and opening/closure of stomata (Lee & Assmann, 1991). Whether these latter effects on stomatal physiology induced by

Table 2. Reported effects of agonists on phosphoinositide-cycle components in plants

Target tissue	Effector	Reported effect(s) on PtdIns-cycle components	Reference
Samanea saman pulvini	Light	PtdIns*P* turnover; Ins*P* formation, diacylglycerol formation	Morse *et al.* (1987); Morse *et al.* (1989)
Sunflower hypocotyls	Light	Changes in PtdInsP_n kinase activity	Memon & Boss (1990)
Brassica seedlings	Light	Changes in PtdInsP_n labelling	Acharya *et al.* (1991)
Maize roots	Light	Increase in inositol phosphate formation	Reddy *et al.* (1987)
Catharanthus roseus cells	Auxin	PtdInsP_n turnover; InsP_n formation	Ettlinger & Lehle (1988)
Soybean	Auxin	PtdIns turnover	Morre *et al.* (1984)
Carrot membranes	Auxin	Increased PLC activity	Zbell & Walter-Back (1988)
Maize coleoptiles	Auxin and fusicoccin	Reduced [^{3}H]Ins labelling of PtdIns	Zocchi (1990)
Cultured *Catharanthus roseus* cells	Auxin, cytokinin	Change in [^{3}H]Ins labelling of PtdInsP_n; change in lipid kinase activity	Grabowski *et al.* (1991)
Cultured soybean cells	Cytokinin	Changed ^{32}P labelling of PtdIns	Connett & Hanke (1987)
Cultured *Catharanthus roseus* cells	Cytokinin	Reduced activity of PtdIns/diacylglycerol kinases	Falkenau *et al.* (1987)
Maize roots and coleoptiles	Fusicoccin	Ins(1,4,5)P_3 increase	Aducci & Marra (1990)
Barley aleurone layers	Gibberellic acid	Increased [^{3}H]Ins labelling of PtdIns	Murthy *et al.* (1989)
Cultured carrot cells	Fungal elicitor	Increase in PtdIns-PLC activity	Kurosaki *et al.* (1987)
Cultured parsley and soybean cells	Fungal elicitors	No effects on PtdInsP_n labelling	Strasser *et al.* (1986)
Beet storage tissue	Hypertonic mannitol shock	Increase in Ins(1,4,5)P_3	Srivastava *et al.* (1989)
Cultured carrot cells	Cell wall degrading enzymes	Increased PtdInsP_n turnover	Chen & Boss (1990)
Dunaliella salina	Hypo-osmotic/hyperosmotic shock	Increased PtdInsP_n turnover	Einspahr *et al.* (1988)
Chlamydomonas eugametos	Ethanol	Increased PtdInsP_n turnover; Ins(1,4,5)P_3 production	Musgrave *et al.* (1992)
Chlamydomonas reinhardtii	low pH and mastoparan	Increased PtdInsP_n turnover; Ins(1,4,5)P_3 production	Quarmby *et al.* (1992)

diacylglycerol are mediated by activation of PKC-like enzymes or by other processes remains unresolved. The results clearly point to potential key roles in plant cell physiology for both Ins(1,4,5)P_3 and diacylglycerol and possibly some of their hydrolysis products. The question remains: are these molecules produced in plants as a result of agonist-provoked signal transduction?

Current evidence for the involvement of the plant phosphoinositide system in transmembrane signalling

Table 2 summarizes a number of reports suggesting that the phosphoinositide system is involved in linking a wide variety of signals to cellular responses in plants and algae. It is beyond the scope of this Review to discuss all these reports in detail but some general conclusions deserve to be mentioned. Although almost every PtdIns-linked response observed in mammalian cells has also been reported to occur in plants there are still a number of obstacles which makes it difficult to integrate the various reported data into a general 'plant phosphoinositide system scheme' which is not just a copy of the mammalian paradigm.

First, there is the timing of the responses. In the case of, e.g., light-stimulated *Samanea saman* pulvini the increase in radioactivity in Ins(1,4,5)P_3 was observed already after 30 s (Morse *et al.*, 1987) and the radiolabelled h.p.l.c. peak identified as Ins(1,4,5)P_3 extracted from auxin-treated *Catharanthus* cells showed a maximum after 1 min of stimulation (Ettlinger & Lehle, 1988). However, Srivastava *et al.* (1989) did not find the peak in Ins(1,4,5)P_3 production (measured by a receptor assay) induced by hypertonic mannitol shock of beet slices until 10 min after shock induction and Ins(1,4,5)P_3 production in maize plantlets in response to fusicoccin was found to be equally slow, with increases observable only after 5 min and still evident after 30 min (Aducci & Marra, 1990). The question is whether these differences in the time it takes from the arrival of the signal to the peak of Ins(1,4,5)P_3 production illustrate a genuine slowness in certain phosphoinositide-mediated responses, or whether other explanations exist.

Another problem that needs attention is the identification of D-Ins(1,4,5)P_3. Researchers who have attempted to identify positively Ins(1,4,5)P_3 have generally used either h.p.l.c. of

radiolabelled compounds or receptor assays based on high-affinity Ins(1,4,5)P_3-binding proteins from mammalian sources. The main stumbling block in the use of both these methods is the bewildering array of water-soluble inositol-containing compounds present in plant cells. Co-chromatography of a plant InsP_3 with authentic Ins(1,4,5)P_3 is suggestive of common isomerism, but does not provide a definitive proof. At least five InsP_3 isomers in addition to Ins(1,4,5)P_3 are likely to be prevalent in plant cells (see the section on inositol phosphate metabolism). Similar problems of specificity are associated with the use of the mammalian-type receptor assays for the analysis of plant inositol phosphates. Although the cross-reactivity of *mammalian* inositol phosphates other than Ins(1,4,5)P_3 is small in the Ins(1,4,5)P_3-receptor assay [e.g. Ins(1,3,4)P_3, 0.22%; Ins(1,3,4,5)P_4, 6.4%; Ins(1,3,4,5,6)P_5, 0.11%], competition for binding sites from large quantities of a variety of inositol phosphates with unknown isomeric configuration (and indeed other plant compounds) could well interfere with the assay of Ins(1,4,5)P_3. Whereas the positive identification of 1,2-diacylglycerol *per se* is more straightforward, its structure does not [unlike e.g. Ins(1,4,5)P_3] give definite clues to the nature of its immediate precursor(s). As several phospholipids may act as precursors for diacylglycerol formation it becomes clear that an increase in cellular diacylglycerol levels cannot necessarily be taken as an indication of phosphoinositide hydrolysis.

In contrast to the evidence for agonist-induced Ins(1,4,5)P_3 production in *Chlamydomonas* (Musgrave *et al.*, 1992; Quarmby *et al.*, 1992) an unequivocal demonstration of Ins(1,4,5)P_3 production in higher plants is thus still lacking (see e.g. Trewavas & Gilroy, 1991). It is important in this connection to point out that although the classification of, e.g., *Chlamydomonas* and *Dunaliella* as 'plants' in many cases may be justified, it is a distinct possibility that signal-transducing systems in these organisms have evolved differently to those of higher plants. Ins(1,4,5)P_3 could in higher plants have a role as a 'special occasion messenger' only, or its messenger function could be confined to specialized cell types. However, before such conclusions are reached, several experimental factors need careful consideration as they may help to explain the apparent elusiveness of Ins(1,4,5)P_3 in plant cells.

First, there is the architecture of plant cells. The diameter of a typical plant cell is 10–15 μm; assuming a spherical shape this gives a total cell volume of 1.8 pl. As the vacuole in many plant cells can occupy as much as 90% of the cell, a cytosolic volume of 0.18 pl can be calculated. Assuming an equal distribution of Ins(1,4,5)P_3 throughout the cytosol in stimulated cells (which is an unlikely event given the high activity of InsP_3-metabolizing enzymes and the apparent discreteness of InsP_3-sensitive Ca^{2+} pools) and a sustained peak Ins(1,4,5)P_3 concentration of approx. 1 μM, each plant cell would contain approximately 0.18 amol of Ins(1,4,5)P_3. Even if the newly developed, and highly sensitive, Ins(1,4,5)P_3-binding assays were used for the detection of Ins(1,4,5)P_3 production (these assays are sensitive in the sub-pmol range; Palmer *et al.*, 1989) it is clear that well in excess of 10^6 cells within a given tissue need to respond to perfect synchrony if any Ins(1,4,5)P_3 production above background levels is to be measurable.

The confusion about the possible timing of Ins(1,4,5)P_3 production is, as pointed out earlier, a further obstacle to the search for Ins(1,4,5)P_3 transients. In studies on *Dictyostelium* it has recently been found that cyclicAMP is capable of inducing PtdIns(4,5)P_2 hydrolysis (Europe-Finner *et al.*, 1989; Van Haastert, 1989). The peak of Ins(1,4,5)P_3 production, determined both by assay of radiolabelled compounds and by an Ins(1,4,5)P_3-binding assay, was found at 5 s after cyclicAMP stimulation; after 20 s the Ins(1,4,5)P_3 levels had returned to basal. If Ins(1,4,5)P_3 transients under certain circumstances were to be equally rapid in plant cells it is likely that they may have been missed as the first 'treated' sample in most experimental protocols are taken after at least 30 s. Alternatively, plant cells could, as some data suggest, rely upon a slow, but sustained, low-level elevation of Ins(1,4,5)P_3.

Much of the research on the mammalian phosphoinositide system has been based on the early observations of rapidly stimulated polyphosphoinositide turnover in response to agonists (e.g. Hokin, 1985). It is evident from Table 2 that similar approaches have been taken by plant scientists. Whereas a rapid change in labelling intensity of phosphoinositides in mammalian cells often is indicative of activation of the phosphoinositide system by agonists, similar conclusions need not always be correct in plant research. As several Ca^{2+}- and/or ATP-dependent enzymes are responsible for the turnover rate of phosphoinositides, it is clear that any change in either cellular ATP or Ca^{2+} levels will affect the relative abundance (and radiolabelling intensity) of phosphoinositides. If, for instance, a particular agonist was to induce rapid opening of plasma membrane-associated receptor-operated Ca^{2+}-channels the increase in cytoplasmic Ca^{2+} caused by this event would lead to a modulation of phosphoinositide turnover. Whereas such a modulation may very well be an integral part of an orchestrated response to the agonist, it would be unwise to conclude that the primary signal was transduced by the phosphoinositide system.

The possibility that increased polyphosphoinositide turnover could be the result of an increase in cytosolic Ca^{2+}, rather than being the event preceding, and inducing, the Ca^{2+} increase may well conjure up feelings of *deja vu*, for these were some of the very arguments used in the 1970s and early 1980s by the sceptics of the PtdIns signal-transduction hypothesis. This said, an unbiased approach is of importance and only detailed knowledge of both causality and relative timing of events during signalling cascades can ensure that misinterpretations are avoided.

The incorporation of radiolabelled precursors such as [2-^{3}H]-inositol into polyphosphoinositides is governed by the rate of PtdIns biosynthesis, which is known to be relatively slow (Drøbak *et al.*, 1988*a*). Unless relatively long labelling times are employed, little or no radiolabelled Ins(1,4,5)P_3 is evident in cell extracts even if PtdIns(4,5)P_2 hydrolysis has occurred. It is often only possible to employ short incubation/radiolabelling times when investigating plant model systems as long incubations can cause physiological and metabolic disturbance. If only short radiolabelling times can be employed a strategy involving biosynthetic PtdIns labelling will be inadequate for the demonstration of (1,4,5)P_3 formation. The use of ^{32}P for labelling of intact cells or [^{32}P]ATP in permeabilized cells, or isolated membranes, proves a far better alternative from a radiolabelling point of view, but the task of recovering (and positively identifying) minute amounts of ^{32}P labelled Ins(1,4,5)P_3 amongst the multitude of water-soluble compounds obtained from such experiments presents a considerable challenge even to the dedicated plant biochemist.

One final issue, of a more speculative nature, deserves brief mentioning. It can often be much more difficult to attach the label 'stimulated' or 'unstimulated' to plant cells than to specialized mammalian cells. Comparatively little is known about the precise mode of action of many plant agonists and even less is known about their mutual antagonism or synergy. It is therefore important that consideration is given to the possibility that cells already may have one, or more, signalling pathways 'turned on' at the start of an experiment which may render them much less sensitive to additional stimuli than would otherwise be the case.

Until more efficient techniques for the isolation of increased quantities of higher plant cells with an established responsiveness

to selected agonists become available, or model systems are developed which will lend themselves to long-term radiolabelling, the answer to the question of whether or not rapid transients of Ins(1,4,5)P_3 occur in plant cells may not be answered satisfactorily.

Alternative functions for the plant phosphoinositide system

Although many common features exist between the plant and animal phosphoinositide systems, there are also significant areas of difference. It is important that an open mind is kept to the possibility that the plant system may have different and/or additional functions to those of its mammalian counterpart. One feature of the phosphoinositide system which currently is receiving increased attention in both mammalian and plant research is the likelihood of the polyphosphoinositides themselves being regulatory molecules rather than just precursors for Ins(1,4,5)P_3 and diacylglycerol production.

Polyphosphoinositides as modulators of enzyme activity. The idea that the polyphosphoinositides themselves may be able to modulate the activity of various enzymes in mammalian cells is not new (Choquette *et al.*, 1984; Schafer *et al.*, 1987). Studies by Memon *et al.* (1989*a*) suggest that this also may be the case in plants for both PtdIns4*P* and PtdIns(4,5)P_2 (40 μM) increased the activity of vanadate-sensitive ATPase activity associated with plasma membranes isolated from sunflower and carrot cells. In a subsequent set of experiments the hypothesis of a direct link between polyphosphoinositides and modulation of the plasma membrane H^+-ATPase was strengthened further. Memon & Boss (1990) thus found that 10 s of white light irradiation of sunflower hypocotyls led to a marked decrease in both PtdIns and PtdIns*P* kinase activity (assayed *in vitro*). The decrease in phosphoinositide kinase activity was accompanied by a 50% decrease in plasma membrane H^+-ATPase activity. The addition of exogenous PtdIns4*P* and PtdIns(4,5)P_2 resulted in full restoration of ATPase activity. Although these experiments do not conclusively prove a direct link between polyphosphoinositide turnover and modulation of the plant plasma membrane H^+-ATPase it is obviously a possibility which deserves further attention.

Lysophosphoinositides. Wheeler & Boss (1989) showed that significant amounts of lysophosphoinositides are present in plant cells. Little is currently known about their function, but it has been suggested that they may be involved in processes as diverse as secretion, fertilization, protoplast fusion and regulation of enzyme activity (Wheeler & Boss, 1990).

Phosphoinositides and cytoskeletal dynamics. In mammalian cells an intimate interaction exists between polyphosphoinositides and components of the cytoskeleton. Polyphosphoinositides have been found specifically to interact with a number of different actin-binding proteins such as profilin, gelsolin and villin, an interaction which results in dissociating of actin-binding protein–actin complexes (Lassing & Linberg, 1990; Goldschmidt-Clermont *et al.*, 1990).

A potential role for actin-binding proteins in controlling the availability of polyphosphoinositides for second messenger production is now evident, and an intriguing three-way interaction between polyphosphoinositide pools, components of the cytoskeleton (such as actin-binding proteins) and the phosphorylation status of phosphoinositidase Cγ isozymes appears to exist in many mammalian cells types (Goldschmidt-Clermont *et al.*, 1990, 1991). Furthermore, several phosphoinositide cycle enzymes have been demonstrated to be associated with the detergent-insoluble cytoskeleton isolated from different animal cell lines (e.g. Nahas *et al.*, 1989; Grondin *et al.*, 1991; Payrastre *et al.*, 1991). The recent demonstrations of a close association of phosphoinositide metabolizing enzymes with both the nucleus and the cytoskeleton in plant cells (Hendrix *et al.*, 1989; Xu *et al.*, 1992) and the positive identification of the actin-binding protein, profilin, in plant cells (Valenta *et al.*, 1991) suggest that a further investigation of the interaction between phosphoinositides and components of the plant cytomatrix is timely.

The plant phosphoinositide system and other Ca^{2+}-mobilizing systems: interactions, cross-talk and confusion

Many of the functional aspects of Ca^{2+} handling in plant cells seem to be closely interdependent, and as insight into the details of these mechanisms increases, so, it seems, does the level of complexity. One of the model systems which has attracted considerable attention recently is abscisic acid-induced stomatal closure. Stomatal closure is the result of turgor loss in the guard cells due to efflux of principally K^+ and Cl^- (MacRobbie, 1989). These fluxes are dependent upon the relative activity of ion channels and the plasma membrane H^+-ATPase, both of which can be modulated by changes in Ca^{2+}.

McAinsh *et al.* (1990) demonstrated a direct link between exogenous addition of abscisic acid, an increase in cytosolic Ca^{2+} activity and guard cell closure. Gilroy *et al.* (1990) and Blatt *et al.* (1990) have further demonstrated that photolysis of caged Ins(1,4,5)P_3 microinjected into single guard cells results in rapid increase in cytosolic Ca^{2+} and guard cell closure [see section on Ins(1,4,5)P_3 and Ca^{2+} release for details]. Abscisic acid has variable effect on Ca^{2+} influx across the plasma membrane into guard cells, ranging from stimulation to inhibition (MacRobbie, 1989), and it has been shown that abscisic acid is able to cause shrinkage of guard cell protoplasts (equivalent to closure) in the absence of exogenous Ca^{2+} (Smith & Willmer, 1988). Neither of the latter observations support the requirement of trans-plasma-membrane Ca^{2+} influx as part of the response mechanism to abscisic acid and point to second messenger-induced Ca^{2+}-release from intracellular pools as a likely mechanism.

In contrast, Schröder & Hagiwara (1989) have provided evidence for abscisic acid activation of Ca^{2+}-permeable ion channels in the plasma membrane of guard cells, and have proposed that it is abscisic acid-activated ion-channels in the plasma membrane which are responsible for repetitive elevations in cytosolic Ca^{2+} concentrations and thereby stomatal closure. However, the problems are far from resolved. In recent reviews Trewavas & Gilroy (1991) and Gilroy *et al.* (1991) point out that although both abscisic acid and elevation of intracellular Ca^{2+} *per se* initiates stomatal closure, the changes in cytosolic Ca^{2+} and plasma membrane Ca^{2+}-channel activity observed in *response* to abscisic acid are so variable (ranging from no change to a sustained increase) that they suggest that abscisic acid initiates stomatal closure *both* through Ca^{2+}-dependent and Ca^{2+}-independent pathways.

The exact pinpointing of the pool(s) of Ca^{2+} which are initially released in response to agonists, and the possible timing and mode of interaction between such signal-sensitive pools and other non-sensitive Ca^{2+} pools, is not made easier by the fact that a large number of compartments exist in plant cells (Trewavas, 1986). Another factor is the steady emergence of novel Ca^{2+} release mechanisms in plant cells. A voltage-gated, Ins(1,4,5)P_3-insensitive Ca^{2+} channel in the tonoplast has thus recently been suggested to be involved in the set-up of medium to long term cytosolic Ca^{2+} elevations, induced by small trans-tonoplastic voltage changes (Johannes *et al.*, 1992).

CONCLUSION AND FURTHER RESEARCH

It is only a few years ago that a comprehensive review of the plant phosphoinositide system, based on the mammalian concept, would have been very brief, as only a dozen or so relevant studies

had been carried out. The data presented on the preceding pages clearly reflects the recent upsurge in interest in the plant phosphoinositide system. It is appropriate in this context to draw attention to the fact that research, often of a high calibre, was carried out on a wide range of aspects of *myo*-inositol metabolism in plant cells well before the subject became fashionable (Loewus & Kelly, 1962; Loewus, 1974; Loewus & Loewus, 1980, 1983; Loewus, 1990).

In spite of the fact that many pieces of the plant phosphoinositide system puzzle have been gathered in recent years an overall picture is still lacking. The list of facets which need clarification is long but the priorities seem to be as follows. To establish the extent to which Ins(1,4,5)P_3 and diacylglycerol are used as messengers and to obtain a clearer understanding of both spatial and temporal aspects of their production and action. The nature of the Ins(1,4,5)P_3-sensitive pool(s) also needs to be clarified as do the unique pathways of Ins(1,4,5)P_3 metabolism in plant cells. The apparent plant polyphosphoinositide enigma (i.e. the very low levels of PtdInsP_2, and the extremely active PtdIns 4-kinase) is another subject which deserves scrutiny. The subcellular distribution of PtdIns-cycle enzymes should be re-assessed in the light of the recent findings of both nuclear and cytoskeletal activities. The roles of 4- and 3-phosphorylated polyphosphoinositides both as precursors for second messenger production and as regulatory molecules in their own right is yet another area which calls for attention. Finally, a better understanding of the interactions and cross-talk between various Ca^{2+}-mobilizing systems is necessary before the exact role of the phosphoinositide system in transmembrane signal transduction in plants can be unequivocally defined.

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