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Molecules of Interest

myo-Inositol-1,2,3,4,5,6-hexa*kis*phosphate

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Abstract

myo-Inositol-1,2,3,4,5,6-hexa*kis*phosphate (Ins P_6) was first described as an abundant form of phosphorus in plant seeds and other plant tissues and dubbed "phytic acid". Subsequently it was found to be a common constituent in eukaryotic cells, its metabolism a basic component of cellular housekeeping. In addition to phosphate, *myo*-inositol (Ins) and mineral storage and retrieval in plant organs and tissues, other roles for Ins P_6 include service as a major metabolic pool in Ins phosphate and pyrophosphate pathways involved in signaling and regulation; possibly as an effector or ligand in these processes; as a form of energy currency and in ATP regeneration; in RNA export and DNA repair; and as an anti-oxidant. The relatively recent demonstration that pyrophosphate-containing derivatives of Ins P_6 can function as phosphate donors in the regeneration of ATP is reminiscent of the proposal, made four decades ago in studies of seed development, that Ins P_6 itself may serve in this function. Studies of Ins P_6 in non-plant systems rarely include the consideration that this compound might represent a significant fraction of cellular P; cellular phosphate nutrition has been viewed as either not interesting or of little importance. However, there may be few fundamental differences among diverse eukaryotes in both the metabolic pathways involving Ins P_6 and the spectrum of possible roles for it and its metabolites.
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1. Introduction

myo-Inositol (Ins; **1**) is the cyclic alcohol (cyclitol) derivative of glucose (Loewus and Murthy, 2000) utilized by several pathways in plant cells including IAA metabolism, cell-wall polysaccharide and cyclitol synthesis, and the phosphatidylinositol (PtdIns)/Ins phosphate pathways. Of its numerous phosphorylated derivatives (**2–19**; see Fig. 1), *myo*-inositol-1,2,3,4,5,6-hexa*kis*phosphate (Ins P_6; 660 mw.; **16**) is the most abundant. First known as "phytic acid", Ins P_6 (**16**) is the major form of phosphorus in seeds, and accumulates in other plant tissues and organs as well, such as pollen, roots, tubers and turions (Cosgrove, 1980; Raboy, 1997). One clear function for Ins P_6 (**16**) metabolism in these tissues is storage and retrieval phosphorus (P), mineral and Ins (**1**) during development and germination.

It became clear in the late 1980s and 1990s that Ins P_6 (**16**) is ubiquitous in eukaryotic species, and is typically the most abundant Ins phosphate in cells (Sasakawa et al., 1995). These studies have shown that Ins P_6 (**16**)and its derivatives, including the more highly phosphorylated pyrophosphate-containing ("PP-Ins phosphate") derivatives such as the 5-PP-Ins(1,2,3,4,6)P_5 (**18**), an Ins phosphate containing 7 moles of phosphorus per mole of Ins, and 5,6-bis-PP-Ins(1,2,3,4)P_4 (**19**) containing 8 moles of phosphorus per mole of Ins, serve in a number of functions/processes other than nutrient storage. Ins P_6 (**16**) represents a major metabolic pool in the Ins phosphate and PP-Ins phosphate pathways involved in signal transduction and regulation, and possibly in energy transduction and ATP regeneration (Safrany et al., 1999). It may serve as a ligand in various signaling or regulatory functions, although care must be taken to demonstrate that in any given case the truly functional ligand is Ins P_6 (**16**) itself and not one of its rapidly metabolized derivatives (Shears, 2001). Ins P_6 (**16**) and its derivatives function in RNA export, DNA repair, and DNA recombination (Hanakahi and West, 2002;

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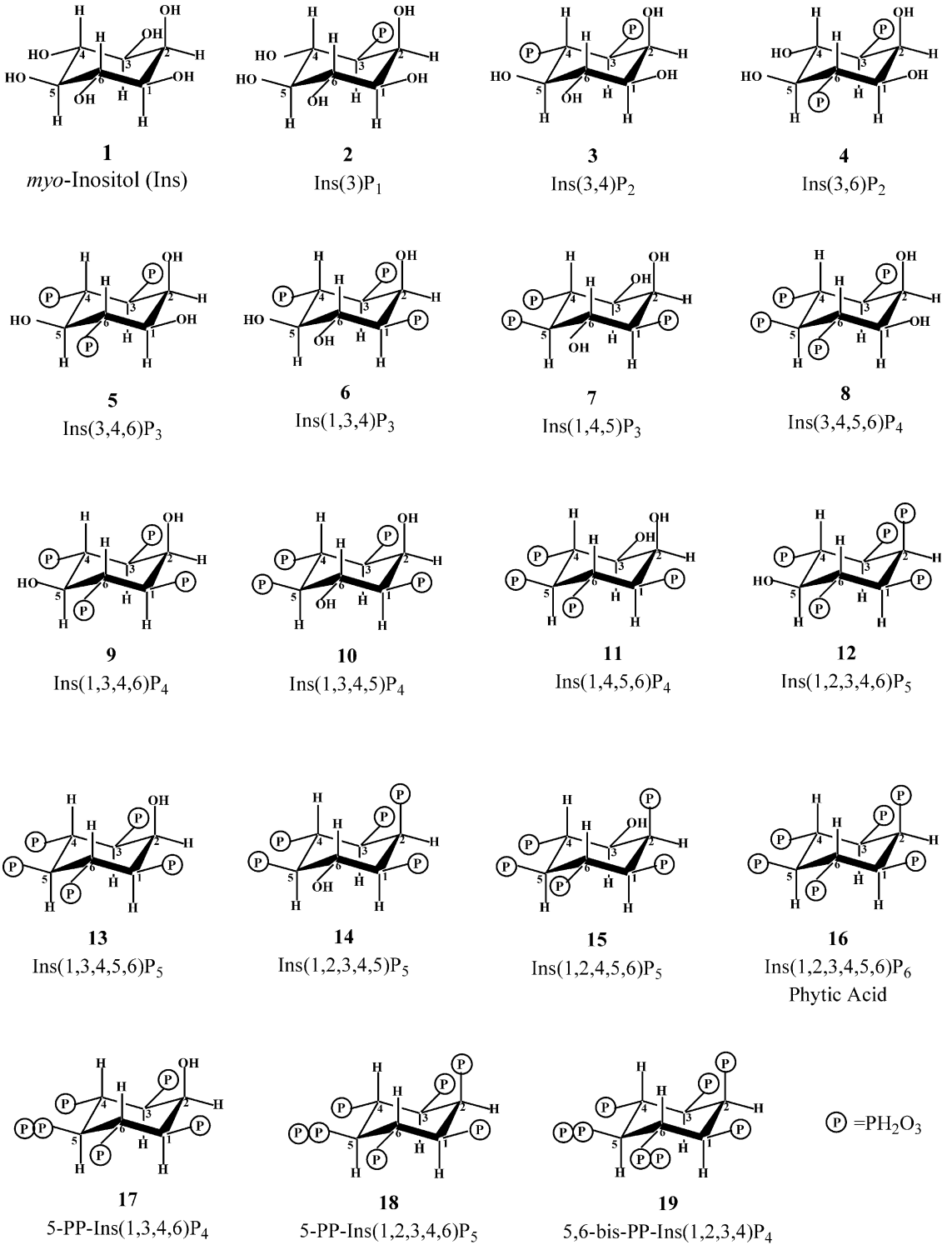

Fig. 1. Structures of *myo*-inositol or "Ins" (**1**) and various Ins phosphates (**2** through **19**) including Ins(1,2,3,4,5,6)P_6 or "phytic acid" (**16**). The numbering of the carbon atoms follows the "D-Convention" (Loewus and Murthy, 2000). "PP" indicates a pyrophosphate moiety.

Luo et al., 2002; York et al., 1999), in endocytosis and vesicular trafficking (Saiardi et al., 2002), and as an antioxidant (Graf et al., 1987).

The pathways to Ins P_6 (**16**) and its more highly phosphorylated derivatives are widespread in eukaryotic cells and thus ancient in the evolution of the eukaryotic species. The sequence of developments that led to this understanding is in itself interesting. Following the discovery of the role of Ins(1,4,5)P_3 (**7**) in signal transduction, a growing number of laboratories started studying the metabolism of this compound (produced via the PtdIns phosphate early intermediate pathway,

Fig. 2), and its ever growing series of derivatives (Berridge and Irvine, 1989). With a growing number of laboratories using chromatographic methods sensitive enough to detect transient, nano-molar levels of Ins(1,4,5)P_3, some inevitably would notice the relatively massive and more polar micro-molar Ins P_6 (**16**) peak typical of many cells. Once enough laboratories started focusing on Ins P_6, (**16**) the same process then led to the discovery of the even more polar PP-Ins phosphates, such as 5-PP-Ins(1,2,3,4,6)P_5 (**18**) and 5,6-bis-PP-Ins(1,2,3,4)P_4

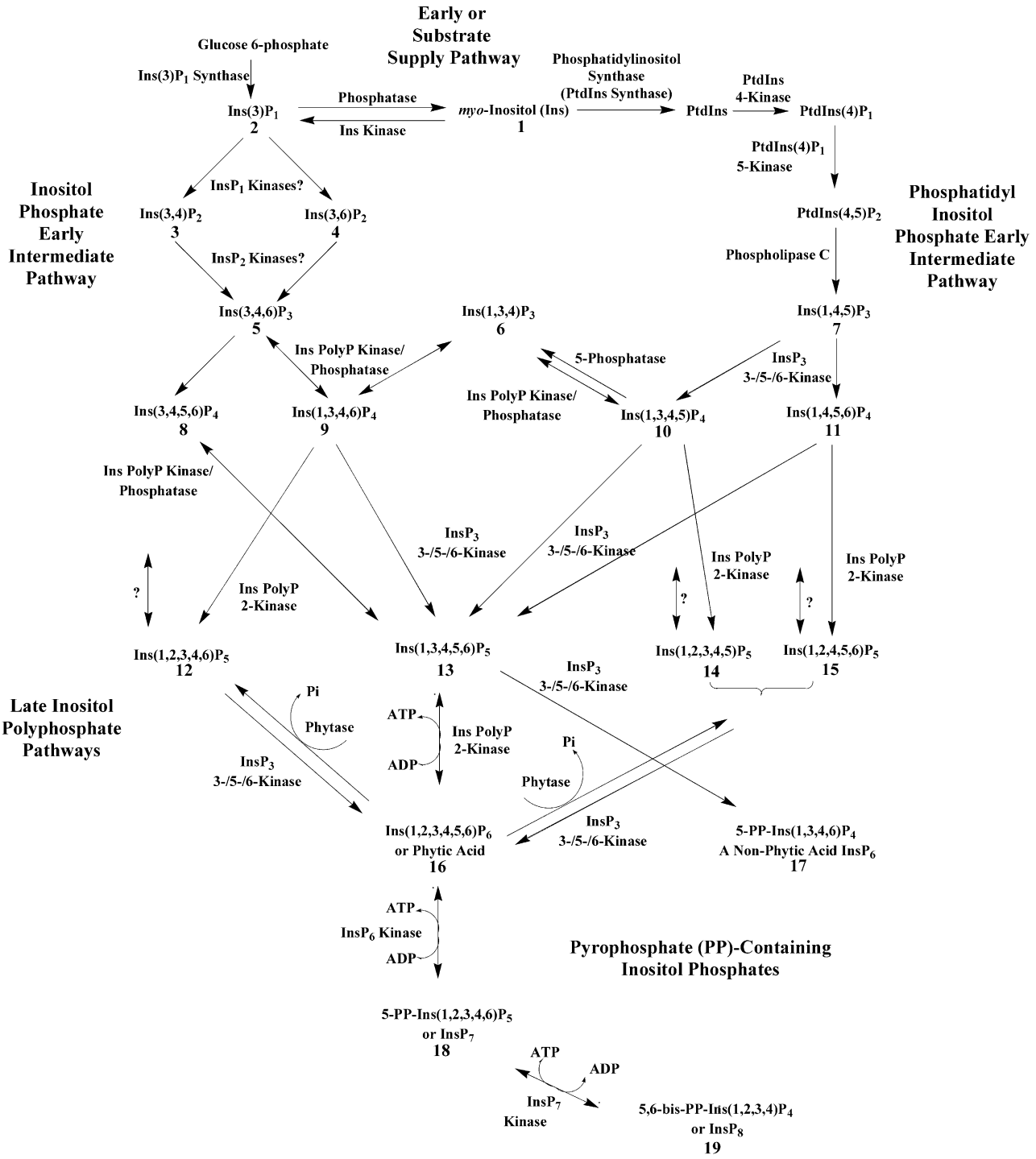

Fig. 2. Biochemical pathways from glucose 6-P to *myo*-inositol (3)P_1 (Ins (3)P_1), and from inositol (3)P_1 to Ins(1,2,3,4,5,6)P_6 (**16**) or "phytic acid", and beyond, in the eukaryotic cell. Enzymes catalyzing critical steps in these pathways are illustrated. The "Early or Substrate Supply Pathway" proceeds from glucose 6-P to Ins (**1**), Ins(3)P_1 and phosphatidylinositol (Ptd Ins). In the center left is the "Inositol phosphate early intermediate" pathway, proceeding solely via soluble inositol phosphates, whereas in the center right is the "phosphatidylinositol phosphate early intermediate" pathway. The "Late Inositol Polyphosphate Pathways" basically involve the conversion of Ins trisphosphates to Ins P_6 (**16**) and the PP-Ins phosphates.

(19). This process subsequently led to the discovery that cells may also contain a non-phytic acid Ins phosphate that, like phytic (16) acid contains 6 moles of phosphate per mole of Ins, such as 5-PP-Ins(1,3,4,6)P_4 (17). Since this step-wise process of discovery began with studies of Ins phosphates as second messenger metabolites, signal transduction has remained the main paradigm for the function of Ins P_6 (16) metabolism in studies of non-plant systems.

There are intriguing parallels in the biology and enzymology of Ins P_6 and its derivatives in organisms as diverse as *Dictyostelium discoideum*, yeast (*Saccharomyces cerevisiae*), endo-parasites such as *Echinococcus granulosus*, mammals and plants. While it is often stated that Ins P_6 (16) represents the most abundant Ins phosphate in the eukaryotic cell, that it also probably represents a major fraction of phosphorus in these cells is less often noted. Despite some recent evidence, most discussion of Ins P_6 (16) and its roles in non-plant systems either ignore a possible role as a cellular phosphate pool, or view it as either lacking in biological interest or importance. But there may be few fundamental differences in either the metabolic pathways involving Ins P_6 (16), or its spectrum of possible roles, throughout the eukaryotes.

2. The background; Ins P_6 in the plant seed

Ins P_6 can represent from 1.0 to several percent of a typical seed's dry weight, about 75.0±10% of a seed's total P, and normally greater than 90% of a mature seed's total, acid-extractable Ins phosphates (Raboy, 1997). As an example, a typical mature dry seed produced by a cultivated species grown under standard conditions might contain about 4.0 mg total P g^{-1} (expressed as its elemental P content, atomic weight 31). If so, about 3.0 mg would be found as "phytic acid P" and less than 0.5 mg as other Ins phosphates. These other Ins phosphates typically consist of four Ins pentaphosphates [the "1-OH/3-OH" (15), "5-OH" (12), "4-OH/6-OH" (14) and "2-OH" (13) pentaphosphates], low levels of less phosphorylated Ins phosphates such as Ins(1,3,4,5)P_4 (10), and trace levels of more highly phosphorylated PP-Ins phosphate (Dorsch et al., 2003). While the relative contribution of these other Ins phosphates to the total may vary in seed produced by different species, the basic pattern does appear to be largely conserved across species. Thus there is an "Ins phosphate" phenotype of seeds that appears to be similar in non-mutant seed of numerous species, if one looks carefully enough.

Since annual plants pack much of the P they take up from the soil into developing seed, and since seed synthesize most of this P into Ins P_6 (16), it is not surprising that a recent estimate of net seed Ins P_6 (16) production annually world-wide by seed crops represents a sum equivalent to more than 50% of all P fertilizer applied annually, world-wide (Lott et al., 2000). Thus seed Ins P_6 (16) represents a major pool in P flux through the world's agricultural ecology. The production of Ins P_6 (16) in plant tissues such as seed, pollen and roots probably is why Ins P_6 and its breakdown products often are the most abundant form of organic P in soils.

Early studies showed that Ins P_6 (16) accumulates during seed development and is broken down during germination in such a way as to appear to maintain a relatively constant level of Pi and cellular P (Strother, 1980). Sequestering Pi as Ins P_6 (16) represents a chemical approach to the problem of P homeostasis and Pi regulation. However, studies of viable *low phytic acid* (*lpa*) mutations indicate that the ability to synthesize Ins P_6 (16) is not essential to P homeostasis (Dorsch et al., 2003; Raboy et al., 2000). Mature seeds homozygous for the barley M 955 *lpa* mutation contain less than 10% of normal or wild-type Ins P_6 (16) levels yet are viable (Dorsch et al., 2003). In M 955 seed essentially all the P that would be found as Ins P_6 (16), typically about 3 mg P gm^{-1}, accumulates as Pi. The viability and relatively normal appearance of seed produced by many plant *lpa* genotypes, including M 955, suggests that Ins P_6 (16) metabolism may play only a minor role in seed P homeostasis and that some other mechanism like compartmentalization probably plays the major role. Therefore, accumulation of Ins P_6 (16) in seeds, pollen and other tissues probably has more to do with storage processes of possible importance to survival in non-cultivated plants, than homeostasis. The primary negative effect of these mutations might be due to perturbations in Ins and Ins phosphate metabolism, rather than in P metabolism.

In mature seeds Ins P_6 (16) is commonly found as deposits of mixed "phytate" salts of mineral cations K, Mg, Ca, Mn and Zn (Lott, 1984). Electron-dense phytate inclusions, referred to as globoids, are found within protein storage vacuoles (PSVs, originally referred to as protein bodies). There is some debate concerning whether or not globoids are membrane-bound. One recent study (Jiang et al., 2001) indicated that globoids are contained within a "vacuole-within-a vacuole" (a membrane-bound compartment within a PSV). Either way, deposition within a PSV or sub-PSV compartment might provide a physical mechanism for the regulation and storage of excess cellular P. Thus while homozygosity for an *lpa* allele might perturb the cell's ability to store Pi as Ins P_6 (16), it might not perturb the cell's ability to pack either Pi or Ins P_6 (16) into PSVs. The fraction of mature seed total Ins P_6 (16) that is compartmentalized in PSVs versus that found in other compartments or in the cytoplasm may vary greatly between species. The cellular sites for the various steps in the synthesis of Ins P_6 (16) prior to its deposition are

not well known. If one assumes that Ins P_6 (**16**) synthesis is largely cytoplasmic, and that once synthesized it's mostly compartmentalized in mature seeds, then there must be some form of presently unknown transport mechanism.

PSVs might primarily contain storage protein, or phytate deposits, or both, and distribution of these different types of storage products may be tissue-specific (Lott, 1984). For example, in the cereal grain Ins P_6 (**16**) accumulates within the diploid tissues of the embryo and scutellum, and within the triploid tissue of the aleurone, but not within the triploid tissue of the central endosperm. Tissue-specific patterns of Ins P_6 (**16**) deposition in the seed are not restricted to the cereal grain, or monocots, and probably are the rule rather than the exception. For example, the sub-epidermal parenchyma tissue of the legume cotyledon is not morphologically differentiated from the cotyledon's central parenchyma, as is the triploid aleurone layer of cereal grains compared with the sub-aleurone endosperm. But in yellow lupin (*Lupinus luteus* L.) seed the sub-epidermal parenchyma is clearly functionally differentiated from the central parenchyma, in that phytate inclusions are localized in this layer of five to ten cell rows, a functional differentiation perhaps similar to that of the cereal aleurone versus central endosperm (Sobolev et al., 1976).

Ins P_6 (**16**) serves as a counter-ion for mineral storage and retrieval processes that are mineral-, tissue- and stage-specific during seed development. For example, developing *Arabidopsis thaliana* (L.) seeds store Mg, K, and Ca as phytates in PSV globoids in the embryo, Mn in phytates in the chalazal endoplasmic reticulum, and Zn in phytates in the chalazal vacuolar compartment (Otegui et al., 2002). The Mn and Zn stores are transient, with subsequent mobilization, at different times during development, to phytate stores in the embryo. This differential storage and redistribution during seed development appears to reflect differing physiological or metabolic demands for Mn and Zn. This is reminiscent of an earlier study of rice grain development (Ogawa et al., 1979). This earlier study described P, K and Mg distribution during rice central, starchy endosperm and aleurone development. Early during development these three minerals are fairly evenly distributed throughout these tissues. P and Mg accumulation in the aleurone layer, presumably as phytate salt, precedes K accumulation. This may reflect the fact that K is important to the enzymology of starch synthesis, which ceases late in endosperm development, only then releasing the K for storage. Thus both studies indicate that "distribution is related to metabolic function" (Ogawa et al., 1979), and that Ins P_6 (**16**) functions as the counter-ion in these processes.

In the small grains (barley, wheat, rice), it has been estimated that more than 80% of the whole grain's Ins P_6 (**16**) is sequestered in the aleurone layer. The aleurone layer might represent 5% of a small grain seed's dry weight. Thus Ins P_6 (**16**) might represent 20% or more of these tissues' mature dry weight. Following imbibition, the initial stage of seed germination, if the water content of the aleurone layer were 80% of its fresh weight, then initial Ins P_6 (**16**) concentration would be in the order of 50 mM.

3. From seeds to all eukaryotic cells

To what extent is there conservation in the biochemistry, genetics and physiology of Ins P_6 across diverse eukaryotes? If the main paradigm for the function of Ins phosphates in non-plant cells has been in signal transduction, studies like that of Xiong et al. (2001) and Lemtiri-Chlieh et al. (2000) confirm that the metabolism of Ins(1,4,5)P_3 (**7**) and Ins P_6 (**16**) also function in signal transduction in plant cells. Xiong et al. (2001) showed that the *fiery1* mutation of *A. thaliana* results in "super-induction" of abscisic acid (ABA)- and stress-response. This mutation was shown to be in a gene encoding an Ins polyphosphate 1-phosphatase, an activity critical to the breakdown of Ins(1,4,5)P_3 (**7**) necessary to maintain the transient nature of the signal. Lemtiri-Chlieh et al. (2000) demonstrated that ABA elicited rapid changes in Ins P_6 (**16**) in intact *Solanum tuberosum* stomatal guard cells. They further found that submicromolar amounts of Ins P_6 (**16**), delivered through a patch electrode to *S. tuberosum* and *Vicia faba* protoplasts, "mimicked the inhibitory effects of ABA and internal calcium" on the inward rectifying K^+ current, a component of ion flux important to stomatal guard cell function and a well characterized target of ABA response. These and other studies have demonstrated a role for Ins P_6 (**16**) as a downstream component of ABA response.

In mammalian cells the interconversion of Ins P_6 (**16**) and the more highly phosphorylated PP-Ins phosphates can be rapid. Up to 50% of a mammalian cell's steady-state level of Ins P_6 (**16**), from 10 to 100 μM, can cycle through PP-Ins phosphates every hour, representing a considerable expenditure of cellular energy (reviewed in Safrany et al., 1999). One function for this relatively expensive PP-Ins phosphate metabolism appears to be in the regulation of endocytic protein trafficking (Saiardi et al., 2002). Yeast (*S. cerevisiae*) mutations that block PP-Ins phosphate metabolism also have very specific abnormalities in the endocytic pathways but not in other components of membrane trafficking. While the mechanistic details are not clearly known yet, it was proposed (Saiardi et al., 2002) that the PP-Ins phosphates may bind to or interact with clathrin, clathrin adapter proteins, and plasma membrane phospholipids, their metabolism functioning as a switching mechanism. This proposal follows the "signaling paradigm" for the function of Ins phosphates.

In these studies rapid metabolism of PP-Ins phosphates is often linked with low steady state levels as compared with Ins P_6 (**16**). In contrast, the pattern and levels of Ins P_6 (**16**) and PP-Ins phosphates during the life cycle of the cellular slime molds *Dictyostelium* and *Polysphondylium pallidum* (Laussmann et al., 2000) appears similar to that observed during the life cycle of higher plants. These organisms can exist in a "vegetative" stage as amoeba, and upon starvation (transfer to bacteria-free phosphate-buffered media) either form multicellular aggregates that differentiate into spores supported on stalks (*Dictyostelium*), or form microcysts (*Polysphondylium*) . During the amoeba cellular stage, at relatively low cellular densities, the levels of Ins P_6 and PP-Ins phosphates remain low, from ~10 μM for PP-Ins phosphates up to ~250 μM for Ins P_6. At higher cell densities Ins P_6 increases to ~650 μM and the PP-Ins phosphates to ~250 μM. Upon starvation and spore formation both Ins P_6 and PP-Ins phosphates can increase up to ~2.0 mM. During subsequent germination cellular Ins P_6 (**16**) and PP-Ins phosphate levels return to basal, "vegetative" levels. This pattern of accumulation during development of reproductive tissues and organs and "breakdown" during subsequent germination is very similar to that observed in tissues and organs involved in reproduction of higher plants, the only difference being that PP-Ins phosphates appear to represent a greater proportion of the "total Ins phosphate" in the slime molds. However, a role in phosphorus storage or cellular nutrition, the main role for Ins P_6 (**16**) accumulation and breakdown during seed development and germination, is not one of the proposed roles for Ins polyphosphate accumulation and breakdown in the slime mold. Suggested roles (Laussmann, et al. 2000) include molecular switching and regulation of cellular processes, perhaps via service as substrate for protein phosphorylation, roles again fitting the signal-transduction paradigm.

A third possible role for the rapid and energetically expensive metabolism of Ins P_6 (**16**) and PP-Ins phosphates, beyond signal transduction or mineral nutrition and homeostasis, is in ATP regeneration or energy transduction per se. Perhaps the accumulation of PP-Ins phosphates in slime mold spores serves as "bond-energy storage" for subsequent use during germination. Studies published in the 1960s (Morton and Raison, 1963) first suggested a link between Ins P_6 (**16**) and ATP regeneration, in this case of importance to seed development and germination. This hypothesis was dismissed at the time, based on the observation that the bond energies of the phosphomonoesters in Ins P_6 (**16**), 3.3–4 kcal mol^{-1}, were insufficient for conversion of ADP to ATP. However, the bond energies of the diphosphate groups of PP-Ins phosphates, 6.6 kcal mol^{-1}, may be sufficient to serve in ATP regeneration. Perhaps the original conjecture of Morton and Raison (1963) was closer to the truth than originally thought.

The work of Biswas et al. (1978a, b) was the first to provide an enzymatic mechanism for a link between Ins P_6 (**16**) and the conversion of ADP to ATP. It also described an enzymology of Ins phosphates reminiscent of that established in more recent studies discussed below. Biswas et al. (1978a, b) described two enzymes in developing mung bean (*Phaseolus aureus* L.) seeds; a "phosphoinositol kinase" that could convert Ins(3)P_1 (**2**) to Ins(1,3,4,5,6)P_5 (**13**) and an "InsP_6-ADP phosphotransferase" that converts Ins(1,3,4,5,6)P_5 (**13**) to Ins P_6 (**16**). The "phosphoinositol kinase", of which two electrophoretic variants were identified, could utilize a series of Ins phosphates as substrates, presumably beginning with Ins(3)P_1 (**2**) in vivo, converting each to a more highly phosphorylated derivative, with the affinity of the enzyme for substrate increasing with increasing phosphorylation. The "Ins P_6-ADP phosphotransferase" described by Biswas et al. (1978b) could also catalyze the reverse reaction and use Ins P_6 (**16**) as a phosphate donor for the conversion of ADP to ATP. A subsequent study of a similar soybean Ins(1,3,4,5,6) 2-kinase (Phillippy et al., 1994) indicated that in tissues with sufficiently high Ins P_6 (**16**) concentrations, such as the cereal grain aleurone layer or the yellow lupine cotyledon's sub-epidermal layer during the initial stage of germination, this type of enzyme might catalyze the conversion of ADP to ATP.

4. Metabolic pathways

The structural metabolic pathways centering on Ins P_6 (**16**) can be thought of as consisting of two parts (Fig. 2); early pathways having to do with Ins (**1**) synthesis and supply, and the later Ins phosphate pathways. The later Ins phosphate pathways centering on Ins P_6 (**16**) can be thought of as consisting of four parts: two alternative early intermediate pathways that proceed either via soluble Ins phosphates to Ins(3,4,6)P_3 (**5**) or via PtdIns phosphate intermediates and the production of Ins(1,4,5)P_3 (**7**); the conversion of Ins *tris*phosphates to Ins(1,3,4,5,6)P_5 (**13**) or other Ins pentaphosphates followed by their conversion to Ins P_6 (**16**); pathways that utilize Ins P_6 (**16**) or other Ins polyphosphates to produce PP-Ins phosphates.

The sole synthetic source of the Ins backbone is the activity of the enzyme *myo*-inositol(3)P_1 synthase ("MIPS"), that converts glucose-6P to Ins(3)P_1 (Loewus and Murthy, 2000). In yeast the regulation of expression of the *Ino1* gene encoding yeast MIPS is central to phospholipid metabolism (Carman and Henry, 1989). The molecular biology of this gene's regulation in yeast, via DNA binding factors such as SNF1 and INO80, is well developed (for example see Shirra et al., 2001). In

plant cells Ins (**1**) is utilized in a number of pathways in addition to phospholipid or Ins P_6 (**16**) synthesis, including pathways to cell wall polysaccharides and cyclitols (for review see Loewus and Murthy, 2000). Plant biology studies have also shown that MIPS is regulated in response to stress (Nelson et al., 1998), and induced in response to ABA (Flores and Smart, 2000). Ins (**1**) synthesis via induction of MIPS by ABA was found to mostly contribute to Ins P_6 (**16**) accumulation (Flores and Smart, 2000). MIPS is also induced at sites proximal to Ins P_6 (**16**) accumulation in seeds (Yoshida et al., 1999), and reduced MIPS expression is believed to be the cause of the reduced Ins P_6 (**16**) in seeds of the maize *lpa*1-1 mutation (Raboy et al., 2000).

Thus the regulation of MIPS expression in yeast and plants, whether or not it was accomplished via similar mechanisms such as conserved DNA binding proteins, might appear at least in part to serve different physiological processes. However two recent studies (Shen et al., 2003; Steger et al., 2003) tie together Ins (**1**) and Ins P_6 (**16**) synthesis and phosphate metabolism in yeast. The Ins P_4 and Ins P_5 precursors to Ins P_6 (**16**) were found to be involved in chromatin remodeling in response to phosphate starvation, and the targets for induced gene expression via chromatin remodeling may include both the *INO1*/MIPS gene and the *PHO5* gene, a phosphate-response gene whose expression is induced in response to phosphate starvation. The DNA binding machinery implicated in the P response include the *SNF1* and *INO80* gene products that regulate the yeast MIPS-encoding gene *INO1*. The conclusion that the Ins P_4 and Ins P_5 precursors to Ins P_6 (**16**) are involved in chromatin remodeling and induction of *PHO5* was derived from the fact that *PHO5* expression is not induced by phosphate starvation in a genetic background, *arg*82, null for Ins(1,4,5)P_3 3-/6-kinase activity, the enzyme in yeast that converts Ins(1,4,5)P_3 (**7**) to Ins(1,3,4,5,6)P_5 (**13**), a step critical to Ins P_6 (**16**) synthesis in yeast (see below). One possible interpretation of these yeast results, not suggested by the authors, is that phosphate metabolism, Ins (**1**) and Ins P_6 (**16**) are all interlinked, as they are in seed development.

The first description of a pathway to Ins P_6 (**16**) consisting completely of a sequence of defined soluble Ins phosphate intermediates—Ins(3)P_1 (**2**), Ins(3,6)P_2 (**4**), Ins(3,4,6)P_3 (**5**), Ins(1,3,4,6)P_4 (**9**), Ins(1,3,4,5,6)P_5 (**13**)—came from studies of Ins P_6 (**16**) synthesis in *Dictyostelium* (Stephens and Irvine, 1990). A similar pathway differing in only two intermediates—Ins(3,4)P_2 (**3**) and Ins(3,4,5,6)P_4 (**8**)—was described in studies of the duckweed *Spriodela polyrhiza* L. (Brearley and Hanke, 1996). These pathways are clearly more similar than they are different. The *Dictyostelium* pathway began with Ins (**1**), presumably produced either directly from the product of MIPS activity, Ins(3)P_1, via a monophosphatase activity, or recycled from the cellular Ins pool, with the initial phosphorylation of Ins at the D-3 position catalyzed by an Ins 3-kinase. Thus, in enzymatic terms the *Dictyostelium* pathway proceeds via a 3-kinase, 6-kinase, 4-kinase, 5-kinase, 1-kinase, and finally a 2-kinase.

Does a pathway to Ins P_6 (**16**) entirely via sequential phosphorylation of soluble Ins phosphates actually exist? There is no genetic evidence for genes encoding an Ins 3-kinase or for an Ins(3)P_1 kinase, the later function essential to such a pathway. In contrast, pathways to Ins P_6 (**16**) that proceed via PtdIns phosphate early intermediates and the production of Ins(1,4,5)P_3 (**7**) are well established, both biochemically and genetically, in yeasts such as *Schizosaccharomyces pombe* (Ongusaha et al., 1998) and *Saccharomyces cerevisiae* (York et al., 1999) and mammalian cells (Chang et al., 2002), and are becoming more established in studies of the model plant species *A. thaliana* (Stevenson-Paulik et al., 2002) and maize (Shi et al., 2003). In each case Ins(1,4,5)P_3 (**7**) is first produced by the action of a specific phospholipase C on PtdIns(4,5)P_2. In yeast, Ins(1,4,5)P_3 (**7**) is first converted to Ins(1,3,4,5,6)P_5 (**13**) by the two-step action of an 6-/3-kinase, encoded by the yeast *Ipk2* gene (York et al., 1999). The yeast version of this enzyme is thought to first convert Ins(1,4,5)P_3 (**7**) to Ins(1,4,5,6)P_3 (**11**) via a "6-kinase" activity, then to convert Ins(1,4,5,6)P_4 (**11**) to Ins(1,3,4,5,6)P_5 (**13**) via a 3-kinase activity. A rat version of this enzyme is thought to first phosphorylate the 3-OH position, then to phosphorylate the 6-OH position (Saiardi et al., 2001). Interestingly, this rat enzyme can also convert Ins(4,5)P_2 to Ins(1,4,5)P_3 (**7**), and Ins(1,3,4,5,6)P_5 (**13**) to an unidentified PP-InsP_4 similar to (**17**), a non-phytic acid Ins phosphate with 6 moles of phosphate per mole Ins. In both cases Ins(1,3,4,5,6)P_5 (**13**) is then converted to InsP_6 (**16**) via a 2-kinase, such as that encoded by the yeast *Ipk1* gene (York et al., 1999).

The Ins(1,4,5)P_3 6-/3-kinase encoded by the yeast IPK2 gene also has 5-kinase activity, as do two *A. thialiana* homologs, thus these enzymes are properly referred to as 3-/5-/6-kinases, as indicated in Fig. 2 (Stevenson-Paulik et al., 2002). The significance of the fact these enzymes also can phosphorylate the 5-OH position is that they therefore may also be able to process "5-OH" Ins trisphosphates like Ins(1,3,4)P_3 (**6**) (see below) or Ins(3,4,6)P_3 (**5**) or their 5-OH derivatives such as Ins(1,3,4,6)P_4 (**9**). While a human version of this enzyme can act on Ins(1,4,5)P_3 (**7**) as substrate, it has even higher affinity for Ins(1,3,4,6)P_4 (**9**), and therefore has been defined as an Ins(1,3,4,6)P_4 5-kinase (Chang et al., 2002). Mutations of the IPK2 gene in yeast blocks Ins P_6 (**16**) accumulation, clearly indicating that this kinase is essential to Ins P_6 (**16**) synthesis in this organism (York et al., 1999). While genome data base searches indicated that all plant species contained expressed genes encoding these types of Ins phosphate kinases,

genetic evidence for this type of kinase's role in Ins P_6 (**16**) synthesis in plant or seed tissues has not been reported yet.

Ins(1,4,5)P_3 kinases were the subject of a third study in non-plant cell systems that stumbled upon an unanticipated link between Ins (**1**) or Ins P_6 (**16**) metabolism and phosphate metabolism (Schell et al., 1999). Rabbit cDNA's were injected into *Xenopus* oocytes, and one was found to stimulate Pi uptake, thus called *Pi Uptake Stimulator*. The *PiUS* sequence appeared to encode an Ins(1,4,5)P_3 3-kinase, but the encoded protein in fact was found to have little activity against Ins(1,4,5)P_3 (**7**) but rather to have substantial Ins P_6 kinase activity, producing PP-Ins phosphates. It turns out that there is a family of genes that encode proteins with either Ins(1,4,5)P_3 and/or Ins P_6 kinase activities. Which type of activity evolved "first" remains the subject of some discussion. Whatever the physiological role or evolutionary significance, these enzymes represent one type of Ins phosphate "multifunctional kinase" or "multikinase" (Saiardi et al., 2001), in that most have been found to recognize several different substrates, phosphorylating each to its more highly phosphorylated derivative.

A second pathway to Ins P_6 (**16**), described first in studies of mammalian cells, also utilizes lipid-derived Ins(1,4,5)P_3 (**7**) but in an indirect manner that first requires its conversion to Ins(1,3,4)P_3 (**6**). This pathway differs from the "yeast pathway" in that 3-kinase action on Ins(1,4,5)P_3 (**7**) first produces Ins(1,3,4,5)P_4 (**10**) which is then hydrolyzed by a 5-phosphatase to produce Ins(1,3,4)P_3. Ins(1,3,4)P_3 in turn is converted to Ins(1,3,4,5,6)P_5 (**13**) via what was first called an Ins(1,3,4)P_3 5-/6-kinase (Wilson and Majerus, 1996, 1997). This second type of Ins trisphosphate kinases encode enzymes that do not recognize Ins(1,4,5)P_3 as substrate and represents a family of sequences in plants and animals that is clearly different from those encoding the Ins(1,4,5)P_3/Ins P_6 kinases discussed above. Like the Ins(1,4,5)P_3 multifunctional kinases, the Ins(1,3,4)P_3 kinases also appear to be multifunctional, but in this case appear to have both kinase and phosphatase activity against a number of Ins phosphates at the 1-OH, 5-OH and 6-OH positions (Ho et al., 2002). Thus these are referred to as Ins PolyP kinase/phosphatase in Fig. 2.

5. Maize and barley *low phytic acid 2* mutants, Ins polyphosphate kinase and the Ins polyphosphate metabolic complex

The maize *low phytic acid* 2 (*lpa*2) gene was recently shown to encode a member of the Ins(1,3,4)P_3 5-/6-kinase or Ins polyphosphate kinase/phosphatase family, representing the first Ins phosphate kinase for which there is genetic evidence that its activity contributes to seed Ins P_6 (**16**) synthesis (Shi et al., 2003). An independent study of barley low phytate mutations (Hatzack et al., 2001) identified an "A-type" mutation phenotypically similar to the maize and barley *lpa*2 mutations, in that reductions in seed Ins P_6 (**16**) were accompanied by increases in "lower" Ins phosphates, in particular Ins(1,3,4,5)P_4 (**10**), and it was hypothesized that the gene perturbed in this mutant encoded an Ins(1,3,4)P_3 5-/6-kinase. Since in seed homozygous for null alleles of maize *lpa*2 Ins P_6 (**16**) is only reduced by ~50%, the maize genome either contains additional copies of genes encoding this activity or genes encoding other types of Ins phosphate kinases like the Ins(1,4,5)P_3 kinases that also contribute to the conversion of lower Ins phosphates to Ins P_6 (**16**). One conclusion drawn from the observation that maize *lpa*2 encodes an Ins(1,3,4)P_3 kinase is that the PtdIns phosphate early–intermediate pathway at a minimum probably produces a substantial amount of the Ins phosphate substrate that is converted to Ins P_6 (**16**) in maize seed, via its initial product Ins(1,4,5)P_3 (**7**). However, since this type of Ins PolyP multi-functional kinase/phosphatase enzyme might also has 1-OH and 5-OH kinase activity, the potential contribution of the soluble Ins phosphate early intermediate pathway, possibly producing Ins(3,4,6)P_3 (**5**), cannot be ruled out at present.

The paradigm followed in many studies of Ins P_6 (**16**) is that its synthesis proceeds via a linear, sequential addition of Ins phosphates. This might reflect the signal transduction model, where specific Ins phosphates, produced transiently, have specific effects as ligands requiring isomer-specific binding proteins (Berridge and Irvine, 1989). While the main or "central" synthetic pathway to Ins P_6 (**16**) may be linear, proceeding via a sequence of soluble Ins phosphates or via PtdIns phosphate intermediates, Ins P_6 (**16**) may actually only be a constituent, albeit the major constituent, of a complex metabolic pool consisting of Ins pentaphosphates, Ins P_6 (**16**) and PP-Ins phosphates. Studies of *Dictyostelium* revealed that two Ins penta*kis*phosphates, D-Ins(1,2,4,5,6)P_5 (**15**) and Ins(1,2,3,4,6)P_5 (**12**) have low turnover rates, accumulate at low steady state levels and interconvert with Ins P_6 in what appears to be futile cycles (Stephens et al., 1991). Seed produced by maize and barley *low phytic acid*2 (*lpa2*) mutants accumulate these same two Ins pentaphosphates, and they are also observed at lower steady-state levels in non-mutant seed (Raboy et al., 2000; Dorsch et al., 2003). Studies of seed Ins phosphates in normal and *lpa* barley genotypes also revealed the presence of an Ins phosphate more polar than Ins P_6 (**16**), a putative PP-Ins phosphate, perhaps an Ins P_7. Thus the pattern of Ins phosphates in normal and *lpa* maize and barley seed, their "Ins phosphate phenotype", is similar to the Ins phosphate "phenotype" of *Dictyostelium* cells.

This review indicates that up until recently a pattern of thinking about these Ins phosphate kinases, a paradigmatic approach, was apparent in the numerous biochemical and genetic studies involving a wide variety of organisms. Most reports have defined a given enzyme's activity as specific to a single substrate, and viewed a particular pathway or function as proceeding via a very specific, linear sequence of intermediates. However, the most recent studies of the Ins(1,4,5)P_3 (**7**) and Ins(1,3,4)P_3 (**6**) multifunctional kinases and kinase/phosphatases tend to find that each of the canonical types of enzymes are in fact "multi-functional". The final example of this concerns the Ins phosphate 2-kinase, first defined as Ins(1,3,4,5,6)P_5 2-kinase. All pathways to Ins P_6 (**16**) where thought to proceed via this single penultimate Ins pentaphosphate. However, even in this case it is clear that Ins phosphate 2-kinases can phosphorylate the 2-OH position of other Ins phosphates. For example maize *lpa*2 seed lacks Ins phosphate 1-/3-kinase activity, but the 1-OH/3-OH Ins tetraphosphates that do accumulate are phosphorylated at the 2-OH position to yield Ins(1,2,4,5,6)P_5 (**15**) or Ins(2,3,4,5,6)P_4 (Raboy et al., 2000). Similarly, the *A. thaliana* Ins phosphate 2-kinase can convert Ins(1,3,4,6)P_4 (**9**) to Ins(1,2,3,4,6)P_5 (**12**), in addition to catalyzing the canonical conversion of Ins(1,3,4,5,6)P_5 (**13**) to InsP_6 (**16**) and thus this enzyme was referred to as an InsP_4/InsP_5 2-kinase (Stevenson-Paulik et al., 2002).

6. Concluding comments

While each of the Ins phosphate kinases typically is defined by the substrate for which it has highest affinity, many can phosphorylate a variety of substrates. As is clear from Fig. 2, if they are expressed together in a cell, these Ins phosphate kinases might function together to convert a large number of lower Ins phosphates to Ins P_6 (**16**). When viewed this way, this most recent work has described a pathway similar to that described by Biswas et al. (1978a); perhaps the Ins(1,3,4)P_3 (**6**) and Ins(1,4,5)P_3 (**7**) multifunctional kinases are in fact the two electrophoretic forms of "phosphinositol kinase". When just two types of kinases appear to recognize such a large number of substrates, then defining a given enzyme by the single substrate it has highest affinity for seems to be a case of "missing the forest for the trees".

The pattern of Ins phosphates in both *Dictyostelium* and seeds indicates that the there is a complex buffer consisting of several Ins penta*kis*phosphates, Ins P_6 (**16**), and more highly phosphorylated PP-Ins phosphates. Ins P_6 (**16**) has been viewed as a substrate for the synthesis of PP-Ins phosphates. Is it possible that all components of this complex buffer, including the more highly phosphorylated PP-containing Ins phosphates, are involved in net Ins P_6 (**16**) synthesis and accumulation in seeds? Perhaps the pathway to Ins P_6 (**16**) in seeds proceeds first to PP-Ins phosphates in the cytoplasm, with the pyrophosphate moiety being hydrolyzed to provide the energy for transport of the resulting Ins P_6 (**16**) into its subcellular storage compartment. Until recently the only organisms in which Ins P_6 (**16**) deposits were observed were plant tissues. However, recently it was shown that Ins P_6 (**16**) is a major component of the extracellular hydatid cyst wall produced by the parasitic cestode *Echinococcus granulosus*, and represents >90% of the total phosphorus of this cell wall (Irigoin et al., 2002). This study also showed that the cell wall Ins P_6 (**16**) is synthesized intracellularly, and secreted into the cell wall, where it occurs as a calcium salt. Thus in organisms as diverse as this parasitic cestode and plant seeds a major portion of a tissue's or organ's total P is synthesized into Ins P_6 (**16**), and this Ins P_6 (**16**) then appears to be transported or secreted via a presently unknown mechanism for subsequent deposition as a mineral salt.

The recent studies in non-plant systems bring this discussion and field full-circle, since they tie together Ins (**1**), Ins P_6 (**16**) and PP-Ins phosphate metabolism and phosphorus metabolism. Perhaps the differences between the slime mold spores and cysts, yeasts, parasites and plant seeds, are relatively trivial, and the major functions of this metabolism include signal transduction, nutrient storage and retrieval, phosphorus homeostasis, and bond-energy storage. If one looks beyond the paradigm of signal transduction, there are clear links between both Ins (**1**) and Ins P_6 (**16**) metabolism and cellular phosphorus metabolism. In the light of both the historical and recent studies of Ins phosphates and Ins P_6 (**16**) in signal transduction and ATP metabolism, or the studies of their biochemistry and physiology, the conservation in the biochemistry, genetics and physiology of Ins P_6 (**16**) across diverse eukaryotes does appear significant.

References

Berridge, M.J., Irvine, R.F., 1989. Inositol phosphates and cell signalling. Nature 341, 388–389.

Biswas, B.B., Biswas, S., Chakrabarti, S., De, B.P., 1978a. A novel metabolic cycle involving *myo*-inositol phosphates during formation and germination of seeds. In: Wells, W.W., Eisenberg Jr., R. (Eds.), Cyclitols and Phosphoinositides. Academic Press, New York, pp. 57–68.

Biswas, S., Maity, I.B., Chakrabarti, S., Biswas, B.B., 1978b. Purification and characterization of myo-inositol hexaphosphate–adenosine diphosphate phosphotransferase from *Phaseolus aureus*. Arch. Biochem. Biophys. 185, 557–566.

Brearley, C.A., Hanke, D.E., 1996. Metabolic evidence for the order of addition of individual phosphate esters to the *myo*-inositol moiety of inositol hexakisphosphate in the duckweed *Spirodela polyrhiza*. L. Biochem. J. 314, 227–233.

Carman, G.M., Henry, S.A., 1989. Phospholipid biosynthesis in yeast. Annu. Rev. Biochem. 58, 635–669.

Chang, S.-H., Miller, A.L., Feng, Y., Wente, S.R., Majerus, P.W., 2002. The human homolog of the rat inositol phosphate multikinase is an inositol 1,3,4,6-tetraphosphate 5-kinase. J. Biol. Chem. 277, 43836–43843.

Cosgrove, D.J., 1980. Inositolhexakisphosphates. In: Cosgrove, D.J. (Ed.), Inositol phosphates. Their Chemistry, Biochemistry and Physiology. Elsevier Scientific Publishing Company, Netherlands, pp. 26–43.

Dorsch, J.A., Cook, A., Young, K.A., Anderson, J.M., Bauman, A.T., Volkmann, C.J., Murthy, P.P.N., Raboy, V., 2003. Seed phosphorus and inositol phosphate phenotype of barley *low phytic acid* genotypes. Phytochemistry 62, 691–706.

Flores, S., Smart, C.C., 2000. Abscisic acid-induced changes in inositol metabolism in *Spirodela polyrrhiza*. Planta 211, 823–832.

Graf, E., Epson, K.L., Eaton, J.W., 1987. Phytic acid: a natural antioxidant. J. Biol. Chem. 262, 11647–11650.

Hanakahi, L.A., West, S.C., 2002. Specific interaction of IP6 with human Ku70/80, the DNA-binding subunit of DNA-PK. EMBO J. 21, 2038–2044.

Hatzack, F., Hubel, F., Zhang, W., Hansen, P.E., Rasmussen, S.K., 2001. Inositol phosphates from barley low-phytate grain mutants analyzed by metal-dye detection HPLC and NMR. Biochem. J. 354, 473–480.

Ho, M.W.Y., Yang, X., Carew, M.A., Zhang, T., Hua, L., Kwon, Y.-U., Chung, S.-K., Adelt, S., Vogel, G., Riley, A.M., Potter, B.V.L., Shears, S.B., 2002. Regulation of Ins(3,4,5,6)P_4 signaling by a reversible kinase/phosphatase. Current Biol. 12, 477–482.

Irigoin, F., Ferreira, F., Fernandez, C., Sim, R.B., Diaz, A., 2002. *myo*-Inositol hexakisphosphate is a major component of an extracellular structure in the parasitic cestode *Echinococcus granulosus*. Biochem. J. 362, 297–304.

Jiang, L., Phillips, T.E., Hamm, C.A., Drozdowicz, Y.M., Rea, P.A., Maeshima, M., Rogers, S.W., Rogers, J.C., 2001. The protein storage vacuole: a unique compound organelle. J. Cell Biol. 155, 991–1002.

Laussmann, T., Pikzack, C., Thiel, U., Mayr, G.W., Vogel, G., 2000. Diphospho-*myo*-inositol phosphates during the life cycle of *Dictyostelium* and *Polysphondylium*. Eur. J. Biochem. 267, 2447–2451.

Lemtiri-Chlieh, F., MacRobbie, E.A.C., Brearley, C.A., 2000. Inositol hexakisphosphate is a physiological signal regulating the K^+-inward rectifying conductance in guard cells. Proc. Nat. Acad. Sci. (USA) 97, 8687–8692.

Loewus, F.A., Murthy, P.P.N., 2000. *myo*-Inositol metabolism in plants. Plant Sci. 150, 1–19.

Lott, J.N.A., 1984. Accumulation of seed reserves of phosphorus and other minerals. In: Murray, D.R. (Ed.), Seed Physiology. Academic Press, New York, pp. 139–166.

Lott, J.N.A., Ockenden, I., Raboy, V., Batten, G.D., 2000. Phytic acid and phosphorus in crop seeds and fruits: a global estimate. Seed Sci. Res. 10, 11–33.

Luo, H.R., Saiardi, A., Yu, H., Nagata, E., Ye, K., Snyder, S.H., 2002. Inositol pyrophosphates are required for DNA hyperrecombination in protein kinase C1 mutant yeast. Biochem. 41, 2509–2515.

Morton, R.K., Raison, J.K., 1963. A complete intracellular unit for incorporation of amino-acid into storage protein utilizing adenosine triphosphate generated from phytate. Nature 200, 429–433.

Nelson, D.E., Rammesmayer, G., Bohnert, H.J., 1998. Regulation of cell-specific inositol metabolism and transport in plant salinity response. Plant Cell 10, 753–764.

Ogawa, M., Tanaka, K., Kasai, Z., 1979. Accumulation of phosphorus, magnesium, and potassium in developing rice grains: followed by electron microprobe X-ray analysis focusing on the aleurone layer. Plant Cell Physiol. 20, 19–27.

Ongusaha, P.P., Hughes, P.J., Davey, J., Michell, R.H., 1998. Inositol hexakisphosphate in *Schizosaccharomyces pombe*: synthesis from Ins(1,4,5)P_3 and osmotic regulation. Biochem. J. 335, 671–679.

Otegui, M.S., Capp, R., Staehelin, L.A., 2002. Developing seeds of *Arabidopsis* store different minerals in two types of vacuoles and in the endosplasmic reticulum. Plant Cell 14, 1311–1327.

Phillippy, B.Q., Ullah, A.H.J., Ehrlich, K.C., 1994. Purification and some properties of inositol 1,3,4,5,6-penta*kis*phosphate 2-kinase from immature soybean seeds. J. Biol. Chem. 269, 28393–28399.

Raboy, V., 1997. Accumulation and storage of phosphate and minerals. In: Larkins, B.A., Vasil, I.K. (Eds.), Cellular and Molecular Biology of Plant Seed Development. Kluwer Academic Publishers, pp. 441–477.

Raboy, V., Gerbasi, P.F., Young, K.A., Stoneberg, S.D., Pickett, S.G., Bauman, A.T., Murthy, P.P.N., Sheridan, W.F., Ertl, D.S., 2000. Origin and seed phenotype of maize *low phytic acid 1-1* and *low phytic acid 2-1*. Plant Physiol. 124, 355–368.

Safrany, S.T., Caffrey, J.J., Yang, X., Shears, S.B., 1999. Diphosphoinositol polyphosphates: the final frontier for inositide research? Biol. Chem. 380, 945–951.

Sasakawa, N., Sharif, M., Hanley, M.R., 1995. Metabolism and biological activities of inositol pentakisphosphate and inositol hexakisphosphate. Biochem. Pharmacology 50, 137–146.

Saiardi, A., Nagata, E., Luo, H.R., Sawa, A., Luo, X., Snowman, A.M., Snyder, S.H., 2001. Mammalian inositol polyphosphate multikinase synthesizes inositol 1,4,5-trisphosphate and an inositol pyrophosphate. Proc. Natl. Acad. Sci. (USA) 98, 2306–2311.

Saiardi, A., Sciambi, C., McCaffery, J.M., Wendland, B., Snyder, S.H., 2002. Inositol polyphosphates regulate endocytic trafficking. Proc. Natl. Acad. Sci. (USA) 99, 14206–14211.

Schell, M.J., Letcher, A.J., Brearley, C.A., Biber, J., Murer, H., Irvine, R.F., 1999. PiUS (Pi uptake stimulator) is an inositol hexaphosphate kinase. FEBS Letters 461, 169–172.

Shears, S.B., 2001. Assessing the omnipotence of inositol hexakisphosphate. Cellular Signaling 13, 151–158.

Shen, X., Xiao, H., Ranallo, R., Wu, W.-H., Wu, C., 2003. Modulation of ATP-dependent chromatin-remodeling complexes by inositol phosphates. Science 299, 112–114.

Shi, J., Wang, H., Wu, Y., Hazebroek, J., Meeley, R.B., Ertl, D.S., 2003. The maize low phytic acid mutant *lpa2* is caused by mutation in an inositol phosphate kinase gene. Plant Physiol. 131, 507–515.

Shirra, M.K., Patton-Vogt, J., Ulrich, A., Liuta-Tehlivets, O., Kohlwein, S.D., Henry, S.A., Arndt, K.M., 2001. Inhibition of acetyl coenzyme A carboxylase activity restores expression of the *INO1* gene in a *snf1* mutant strain of *Saccharomyces cerevisiae*. Mol. Cell. Biol. 21, 5710–5722.

Sobolev, A.M., Buzulukova, N.P., Dmitrieva, M.I., Barbashova, A.K., 1976. Structural-biochemical organization of aleurone grains in yellow lupin. Soviet Plant Physiol. 23, 739–746.

Steger, E.J., Haswell, E.S., Miller, A.L., Wente, S.R., O'Shea, E.K., 2003. Regulation of chromatin remodeling by inositol polyphosphates. Science 299, 114–116.

Stephens, L.R., Irvine, R.F., 1990. Stepwise phosphorylation of *myo*-inositol leading to *myo*-inositol hexakisphosphate in *Dictyostelium*. Nature 346, 580–583.

Stephens, L.R., Hawkins, P.T., Stanley, A.F., Moore, T., Poyner, D.R., Morris, P.J., Hanley, M.R., Kay, R.R., Irvine, R.F., 1991. *myo*-Inositol pentakisphosphates: structure, biological occurrence and phosphorylation to *myo*-inositol hexakisphosphate. Biochem. J. 275, 485–499.

Stevenson-Paulik, J., Odom, A.R., York, J.D., 2002. Molecular and biochemical characterization of two plant inositol polyphosphate 6-/3-/5-kinases. J. Biol. Chem. 277, 42711–42718.

Strother, S., 1980. Homeostasis in germinating seeds. Ann. Bot. 45, 217–218.

Wilson, M.P, Majerus, P.W., 1996. Isolation of inositol 1,3,4-trisphosphate 5/6-kinase, cDNA cloning and expression of the recombinant enzyme. J. Biol. Chem. 271, 11904–11910.

Wilson, M.P., Majerus, P.W., 1997. Characterization of a cDNA encoding *Arabidopsis thaliana* Inositol 1,3,4-trisphosphate 5/6-kinase. Biochem. Biophys. Res. Comm. 232, 678–681.

Xiong, L., Lee, B., Ishitani, M., Lee, H., Zhang, C., Zhu, J.-K., 2001. *Fiery1* encoding an inositol polyphosphate 1-phosphatase is a negative regulator of abscisic acid and stress signaling in *Arabidopsis*. Genes Devel. 15, 1971–1984.

York, J.D., Odom, A.R., Murphy, R., Ives, E.B., Wente, S.R., 1999. A phospholipase C-dependent inositol polyphosphate kinase pathway required for efficient messenger RNA export. Science 285, 96–100.

Yoshida, K.T., Wada, T., Koyama, H., Mizobuchi-Fukuoka, R., Naito, S., 1999. Temporal and spatial patterns of accumulation of the transcript of *myo*-inositol-1-phosphate synthase and phytin-containing particles during seed development in rice. Plant Physiol. 119, 65–72.