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Molecular basis for the integration of inositol phosphate signaling pathways via human ITPK1

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Introduction

Ion channels are the most rapid of all signaling entities. Minor fluctuations in the actions of regulatory signals can switch the conductance state of a single channel so as to influence the transmembrane movement of millions of ions per second (Clapham, 2001). This is a particularly impressive example of signal amplification. The conductance of the particular Cl^- channel that we study, ClC3, is activated by CaMKII (Robinson et al., 2004). ClC3 is also regulated by one member of the inositol phosphate signaling family, Ins(3,4,5,6)P_4 (Fig. 1), which inhibits Cl^- conductance through this channel (Mitchell et al., 2008). The cellular levels of Ins(3,4,5,6)P_4 are dynamically regulated by receptor-initiated control over the activities of a multifunctional kinase/phosphotransferase (ITPK1) that interconverts Ins(3,4,5,6)P_4 with Ins(1,3,4,5,6)P_5 (Fig. 1). It is this enzyme, and its biological significance, that are the focus for this review.

Ins(3,4,5,6)P_4 regulates Cl^- channel conductance

Whole-cell electrophysiological analysis has been used to demonstrate that Ins(3,4,5,6)P_4 is a concentration-dependent inhibitor of a CaMKII-activated Cl^- conductance that is located in the plasma membrane (Ho et al., 2001; Mitchell et al., 2008; Xie et al., 1996, 1998). At least in mammalian cells, the inhibition of Cl^- channel conductance by Ins(3,4,5,6)P_4 is an exquisitely specific regulatory process; it is not imitated by any of the many other inositol phosphates that exist inside cells (Ho et al., 2001; Ho and Shears, 2002; Xie et al., 1996).

In secretory epithelia, the major role for Cl^- channels is to sustain salt and fluid secretion (Petersen, 1992), so Ins(3,4,5,6)P_4 is now viewed as playing a key regulatory role in this important biological process. Ins(3,4,5,6)P_4 has also been reported to regulate cell growth through an effect on Cl^- fluxes in a plant model, namely, the apex of the pollen tube from lily and tobacco (Zonia et al., 2002). The recent identification of ClC3 as the channel that Ins(3,4,5,6)P_4 regulates, at least in mammalian cells (Mitchell et al., 2008), has greatly expanded the biological repertoire of this inositol phosphate. For example, ClC3 is responsible for the Ins(3,4,5,6)P_4-regulated Cl^- conductance in hippocampal neurones (Mitchell et al., 2008), which is thought to contribute to the overall regulation of the synaptic efficacy in generating action potentials (Wang et al., 2006). Long-term changes in synaptic efficacy comprise a cellular basis for information storage and memory formation (Bliss and Collingridge, 1993). Thus, Ins(3,4,5,6)P_4 is a molecule that has the potential to

affect neuronal development. It therefore seems pertinent that Ins(3,4,5,6)P_4 has also previously been suggested to have the characteristics of a “memory molecule”, because its relatively slow rate of metabolism permits its physiological effects to long outlast the duration of the stimulus that initially prompts intracellular Ins(3,4,5,6)P_4 to accumulate (Ho and Shears, 2002). ClC3 is known to have many other roles, including tumor cell migration (Mao et al., 2008), bone remodeling (Okamoto et al., 2008), apoptosis (Claud et al., 2008), insulin secretion (Barg et al., 2001), and inflammatory responses (Moreland et al., 2006). We can now anticipate that Ins(3,4,5,6)P_4 might also regulate these processes.

Some of the newly appreciated functions for Ins(3,4,5,6)P_4 arise because ClC3 is not only present in the plasma membrane, but in addition this ion channel resides in intracellular vesicles such as insulin granules (Barg et al., 2001) and the early endosomal compartment (Gentzsch et al., 2003; Hara-Chikuma et al., 2005; Mitchell et al., 2008; Stobrawa et al., 2001; Zhao et al., 2007). In these intracellular compartments, considerable ClC3 driven Cl^- flux occurs even in the apparent absence of CaMKII activation (Mitchell et al., 2008). It has been proposed that Cl^- influx into these vesicles provides the charge-neutralization without which the electrogenic H^+-ATPase would not be capable of acidifying the vesicle interior (Hara-Chikuma et al., 2005; Weylandt et al., 2007). A recent complication for the latter hypothesis is the determination that two close relatives of ClC3, namely, ClC4 and ClC5, are actually $n Cl^-/H^+$ antiporters ($n > 1$) (Picollo and Pusch, 2005; Scheel et al., 2005). As yet, there is no direct evidence that ClC3 is also a transporter rather than a channel. In fact, a recent study (Lisal and Maduke, 2008) has rationalized how the ClC family might actually include both channels and transporters. It is even possible that a ClC protein might functionally switch between the two modes; this could, for example, be mediated by CaMKII-dependent phosphorylation, or perhaps by the association of regulatory proteins (Wang et al., 2006). Irrespective of the mechanisms involved, there is no doubt that ClC3 contributes to endosomal acidification (Jentsch, 2008). Indeed, when a cell-permeant analogue of Ins(3,4,5,6)P_4 was added to cells so as to inhibit ClC3, the pH of certain vesicular sub-compartments became more alkaline (Mitchell et al., 2008; Renström et al., 2002). What is the biological significance of this regulation of intra-vesicular pH? With regards to insulin granules, it has been proposed that their intraluminal acidification is a priming process, without which they become less competent to fuse with the plasma membrane and release their cargo (Barg et al., 2001). In support of this idea, we have shown that alkalinization of insulin granules by Ins(3,4,5,6)P_4 has the effect of reducing insulin secretion from pancreatic β-cells (Renström et al., 2002). This has previously led us (Renström et al., 2002) to consider what might be the pathological consequences of a persistently activated pancreatic Ins(3,4,5,6)P_4 signal, such as that which would inevitably (see Section 4) accompany sustained glucose-dependent activation of PLC (Trimble et al., 1987). Perhaps in some individuals, such abnormally elevated Ins(3,4,5,6)P_4 levels contribute to the hyperglycemia-dependent refractoriness of β-cells (Meyer et al., 2002) which typifies type 2 diabetes etiology (Kilpatrick and Robertson, 1998).

In many other cell types, the acidification of the intracellular vesicles by H^+-ATPases serves other important functions, including modulation of certain ligand–protein interactions during endocytosis, enzyme targeting, H^+-coupled uptake of small molecules (such as

neurotransmitters), and optimization of proteolytic activities of, for example, prohormone processing enzymes (Nishi and Forgac, 2002). It appears that we have only scratched the surface of our understanding of the biological importance of Ins(3,4,5,6)P_4. Unfortunately, we have not yet determined the mechanism by which Ins(3,4,5,6)P_4 regulates ClC3.

Besides ClC3, are there other species of Cl^- channels that might be inhibited by Ins(3,4,5,6)P_4? The proteins that are most closely related to ClC3 are ClC4 and ClC5, but neither of these appear to be regulated by Ins(3,4,5,6)P_4 (Mitchell et al., 2008). We have to move outside this protein family, to another group of entirely different proteins, the so-called ClCA family, in order to find any evidence for other Cl^- channels being regulated by Ins(3,4,5,6)P_4 (Ismailov et al., 1996). In the latter study, recombinant bovine tracheal CLCA (bCLCA1) was expressed in *Xenopus* oocytes, and then membrane fragments were prepared and incorporated into lipid bilayers. However, it is rather puzzling that Ins(3,4,5,6)P_4 inhibits bCLCA1 at low nanomolar concentrations (Ismailov et al., 1996), far below the micromolar levels observed even in resting cells (Section 4). It is difficult to avoid the conclusion that, if these observations are biologically relevant, bCLCA1 must be constitutively inhibited by the levels of Ins(3,4,5,6)P_4 that prevail even in non-stimulated cells. There is no additional information to indicate that these observations might have any other regulatory context. Another confounding issue is that the CLCA family have been reported to exhibit pharmacological properties (inhibition by dithiothreitol (Fuller et al., 2005)) and electrophysiological parameters (13–30 pS unitary conductance (Fuller et al., 2005)) that do not match those of the native Ins(3,4,5,6)$_4$-inhibited Cl^- current (insensitivity to dithiothreitol and 1–2 pS unitary conductance (Ho et al., 2001)). Moreover, there are good reasons to believe that the CLCA proteins, some of which are clearly secreted from cells, do not form chloride channels *per se*, but instead have other functions (Jentsch and Günther, 1996; Mundhenk et al., 2006). Thus, it is difficult to understand what could be the biological relevance of the apparent interaction of Ins(3,4,5,6)P_4 with bCLCA1.

The pathway of Ins(3,4,5,6)P_4 synthesis

The idea that *de novo* synthesis of Ins(3,4,5,6)P_4 occurs by dephosphorylation of Ins(1,3,4,5,6)P_5 (Fig. 1) was first proposed by our laboratory in 1990 (Menniti et al., 1990), although more than a decade passed before we were able to experimentally validate this hypothesis (Chamberlain et al., 2007; Ho et al., 2002). As for Ins(1,3,4,5,6)P_5 itself, there are two routes by which this molecule is synthesized from Ins(1,4,5)P_3 (Fig. 2). The more protracted of these, Ins(1,4,5)P_3 -> Ins(1,3,4,5)P_4 -> Ins(1,3,4)P_3 -> Ins(1,3,4,6)P_4 -> Ins(1,3,4,5,6)P_5, was the first to be discovered around 20 years ago (Hunyady et al., 1988; McConnell et al., 1991; Menniti et al., 1990; Shears, 1989; Stephens et al., 1988) and this involves ITPK1. Subsequently, a more direct route of Ins(1,3,4,5,6)P_5 synthesis was discovered, following the identification and molecular cloning of an enzyme (IPMK/IPK2) that harbors both 3- and 6-kinase activities and, therefore, can single handedly phosphorylate Ins(1,4,5)P_3 to Ins(1,3,4,5,6)P_5 (Odom et al., 2000; Saiardi et al., 1999, 2000). The latter route to Ins(1,3,4,5,6)P_5 is generally believed to be the more evolutionarily ancient of the two pathways; its origin is now thought to even predate the evolution of Ins(1,4,5)P_3 as a Ca^{2+}-mobilizing signal (Irvine and Schell, 2001; Schell et al., 1999; York, 2006). In fact, some organisms (yeasts and insects) do not possess an *Itpk1* gene, yet they

still synthesize adequate quantities of inositol phosphates. In higher animals, which possess both pathways of Ins(1,3,4,5,6)P_5 (and hence also InsP_6) synthesis, ITPK1 may have a multitasking capability, contributing both to the pathway of InsP_6 synthesis (a metabolic function) and regulation of Ins(3,4,5,6)P_4 levels (a signaling function). However, the relative contributions of ITPK1-dependent and -independent pathways for the synthesis of Ins(1,3,4,5,6)P_5 (Fig. 2) and InsP_6 are still being debated (Alcazar-Roman and Wente, 2008; Leyman et al., 2007; Verbsky et al., 2005). Perhaps this varies between cell types.

Interestingly, plants contain multiple homologues of ITPK1 (Josefsen et al., 2007; Shi et al., 2003; Stiles et al., 2008; Sweetman et al., 2007; Wilson and Majerus, 1997). Rice has six such genes (Suzuki et al., 2007). The catalytic efficiencies of the different plant ITPK isoforms can differ by up to two orders of magnitude (Stiles et al., 2008). There is good evidence of an important and ubiquitous role for at least some of these ITPK isoforms in the synthesis of higher inositol phosphates. For example, a maize mutant has been identified which has a defective ITPK gene which results in decreased levels of InsP_6 (Shi et al., 2003). Perhaps there are tissue specific differences in the expression of the various plant isoforms that might preferentially fulfill either metabolic functions (synthesis of InsP_6) or signaling functions (synthesis of Ins(3,4,5,6)P_4).

The contributions that ITPK makes to Ins(1,3,4,5,6)P_5/InsP_6 synthesis in plants involve metabolic pathways that are independent of both PLC and Ins(1,4,5)P_3; these pathways do not exist in animal cells (Brearley and Hanke,1996; Stiles et al., 2008; Sweetman et al., 2007). For example, the phosphorylation of Ins(3,4,6)P_3 to Ins(1,3,4,6)P_4 is likely a plant-specific step in the Ins(1,3,4,5,6)P_5/InsP_6 synthetic pathway (Brearley and Hanke, 1996; Stiles et al., 2008).

Receptor-dependent regulation of Ins(3,4,5,6)P_4 levels by ITPK1

The inhibition of CaMKII-activated Cl^- conductance by Ins(3,4,5,6)P_4 shows an IC_{50} value of approximately 5 μM (Mitchell et al., 2008; Xie et al., 1996). This is a significant observation because it demonstrates that the actions of Ins(3,4,5,6)P_4 occur within a physiologically relevant concentration range: cellular levels of Ins(3,4,5,6)P_4 are around 1 μM in resting cells, and they increase to the 5–10 μM range whenever PLC is activated (Ho and Shears, 2002). It is worth emphasizing this point: in all animal cells, irrespective of the mechanism by which PLC is activated, there is an accompanying elevation of Ins(3,4,5,6)P_4 concentration (Barker et al., 1992; Li et al., 1992; Menniti et al., 1990; Wong et al., 1992). This obligatory connection between Ins(3,4,5,6)P_4 levels and the PLC-dependent production of Ins(1,4,5)P_3 is a vital component of this entire signaling system. Clearly, mass-action effects can explain why elevations in Ins(1,4,5)P_3 levels inevitably lead to accompanying increases in some of the downstream metabolites (Fig. 2). In some cells, this metabolic domino effect may “knock-on” as far as Ins(3,4,5,6)P_4. However, in most cases we do not believe that this is the major mechanism by which Ins(3,4,5,6)P_4 levels are regulated. We initially came to this conclusion after it was demonstrated that Ins(3,4,5,6)P_4 and Ins(1,3,4,5,6)P_5 belong to a metabolic pool that is separate from that of Ins(1,4,5)P_3 and its more closely related metabolites (Menniti et al., 1990; Wong et al., 1992). In other words, in the short-term, the metabolic pool of Ins(3,4,5,6)P_4 is somewhat insulated from changes in

Ins(1,4,5)P_3 concentrations. This phenomenon is clearly seen during short-term radiolabelling of cells with [^{3}H]inositol; the Ins(1,4,5)P_3 pool becomes saturated with the radiolabel several days faster than does Ins(3,4,5,6)P_4 (Menniti et al., 1990). Furthermore, it has been observed that a receptor-dependent *increase* in Ins(3,4,5,6)P_4 levels can take place in parallel with a *decrease* in Ins(1,3,4,5,6)P_5 levels (Menniti et al., 1990). The latter phenomenon clearly does not reflect a mass-action effect. Instead, there is receptor-dependent activation of Ins(1,3,4,5,6)P_5 dephosphorylation to Ins(3,4,5,6)P_4 by ITPK1.

In 2000, we (Yang and Shears, 2000) determined that ITPK1 is also an active Ins(3,4,5,6)P_4 1-kinase. Later, we discovered that ITPK1 also synthesizes Ins(3,4,5,6)P_4 from Ins(1,3,4,5,6)P_5 through a unique phosphotransferase activity (Chamberlain et al., 2007; Ho et al., 2002). It was this elucidation of the ADP-dependent phosphotransferase activity of mammalian ITPK1, an unprecedented phenomenon in the inositol phosphate field (Chamberlain et al., 2007; Ho et al., 2002), that uncovered the molecular mechanism by which Ins(3,4,5,6)P_4 levels are coupled to receptor-regulated PLC activity. One important factor in this process (Fig. 3) is the tenacity with which ITPK1 binds adenine nucleotide; crystallographic data show that less than 10% of the nucleotide is solvent exposed (Chamberlain et al., 2007; Miller et al., 2005). In its ADP-bound form, ITPK1 dephosphorylates Ins(1,3,4,5,6)P_5 to Ins(3,4,5,6)P_4 (Fig. 3). The Ins(3,4,5,6)P_4 is released to the bulk phase in exchange for Ins(1,3,4)P_3, but the nucleotide – now ATP – remains bound. In other words, the inorganic phosphate that is removed from Ins(1,3,4,5,6)P_5 is not released. Instead, it is fated to be passed on to the newly bound Ins(1,3,4)P_3, thereby phosphorylating it to Ins(1,3,4,6)P_4, which the active site then exchanges for a new molecule of Ins(1,3,4,5,6)P_5 (Fig. 3), and the entire phosphotransferase cycle is repeated. Importantly, the rate at which Ins(1,3,4,5,6)P_5 is dephosphorylated to Ins(3,4,5,6)P_4 has been shown to be stimulated as the concentration of phosphate acceptor – Ins(1,3,4)P_3 – is increased (Ho et al., 2002). In turn, the cellular levels of Ins(1,3,4)P_3 – a metabolite of Ins(1,4,5)P_3 – mirror both the intensity and the duration of receptor-activated PLC activity (Batty et al., 1998; Batty and Downes, 1994). In other words, the degree of PLC activity sets Ins(1,3,4)P_3 levels, which controls Ins(3,4,5,6)P_4 synthesis. This is the molecular basis for the integration of inositol phosphate signaling pathways via human ITPK1.

The reaction mechanisms for these phosphotransferase reactions (Fig. 3) have not yet been established. Crystal structures have been obtained for human ITPK1 (Chamberlain et al., 2007) and an amoeboid homologue of ITPK1 (Miller et al., 2005), but unfortunately substrate-bound crystals have not yet been isolated, which has contributed to uncertainties concerning the reaction mechanisms. It has been proposed (Miller and Hurley, 2004) that the phosphorylation of Ins(1,3,4)P_3 involves “in-line” transfer of the γ-phosphate from ATP directly to the inositol phosphate, i.e., without the participation of a phospho-enzyme intermediate. However, recently obtained preliminary data suggest that a phospho-histidine intermediate may accumulate when ITPK1 phosphorylates substrate (Majerus et al., 2008).

In the absence of structural information on enzyme–ligand interactions, we (Ho et al., 2002; Riley et al., 2006) have put forward a proposal which is based on the long-standing observation (Wilcox et al., 1994) that some inositol phosphates may interact with the binding sites of receptors and enzymes in more than one orientation (i.e. “mode”), enabling

one inositol phosphate to mimic another by presenting to the docking site some key recognition features. Thus, we have proposed that ITPK1 uses three different binding modes (Ho et al., 2002) (Fig. 4): Mode 1 binding (permitting 1-kinase activity) was designated for Ins(3,4,5,6)P_4. We further proposed that Ins(1,3,4)P_3 could itself bind to the active site in two different orientations (Fig. 4), designated mode 2 (permitting 6-kinase activity) and mode 3 (in an effort to explain why Ins(1,3,4)P_3 can also be phosphorylated at the 5-position (Abdullah et al., 1992; Shears, 1989)). Our three mode binding model has the advantage of accounting for the 5-hydroxyl phosphorylation of the non-physiological substrate, Ins(1,2,4)P_3 (Adelt et al., 2001). Note that Miller et al. (2005) also advocate the same three different binding "modes" that we have put forward (Ho et al., 2002). However, the model of Miller et al. (2005) differs from ours in several key respects. First, they propose only a single binding mode for Ins(1,3,4)P_3 (equivalent to our "mode 2") with both the 5- and 6-hydroxyls being close enough to the γ-phosphate of ATP that either can be phosphorylated. A more provocative aspect of the model of Miller et al. (2005) is the proposal that, for the amoeboid ITPK1 at least, ligand specificity is not significantly affected by either the hydroxyl groups, or by their orientation (i.e. axial vs equatorial), or by the stereochemistry at any of the six stereogenic centers of the inositol ring. The amoeboid ITPK1 is certainly the most promiscuous of the enzymes that metabolize inositol phosphates (Field et al., 2000; Miller et al., 2005), but it would be a truly exceptional enzyme if it lacked all stereochemical specificity. In any case, we have shown that this is absolutely not the case for human ITPK1 (Chamberlain et al., 2007; Riley et al., 2006). We have clearly shown that the determinants of ligand binding include the two-dimensional arrangement of phosphates and hydroxyls around the inositol ring, and also the three-dimensional stereochemistry at each position of the ring. This stereochemically based model is, perhaps, most clearly vindicated by the empirical demonstration that Ins(1,4,5,6)P_4 is not a substrate for ITPK1 (Riley et al., 2006), in contrast to the prediction that arose out of the model of Miller et al. (2005).

In our model, three groups around the inositol ring (groups coloured red in Fig. 4), are common to each binding mode, but by themselves, these are insufficient to fully define ligand specificity, since all three groups are also present on Ins(1,4)P_2, which is not a substrate (Ho et al., 2002; Ongusaha et al., 1998). We therefore proposed that ligand recognition was combinatorial in nature, with some groups on the inositol ring only contributing to specificity in just one or two of the three proposed substrate-binding modes (Ho et al., 2002). A possible molecular basis for this model for ligand binding is that there are some rigid regions of the active site, which could then be used in multiple binding modes, whereas others might be more flexible (for mode-specific binding).

The extent to which receptor-activation of PLC leads to an elevation in cellular Ins(3,4,5,6)P_4 levels can be influenced by alterations in the degree of ITPK1 expression. This has been shown using T84 cells in which ITPK1 was over-expressed by about 2-fold, leading to an approximate doubling of the increase in Ins(3,4,5,6)P_4 levels that were observed during PLC activation (Ho et al., 2002). A similar conclusion arose from a comparison of Ins(3,4,5,6)P_4 levels in immortalized murine tracheal cells derived from wild-type and cftr (−/−) mice (Yang et al., 2006). In the latter study, the cells from the cftr (−/−) mice expressed less Itpk1, and so less Ins(3,4,5,6)P_4 was formed in these cells (Yang

et al., 2006). Such observations may be of clinical interest in the treatment of the cystic fibrosis (CF) condition. For example, cell-permeant antagonists of Ins(3,4,5,6)P_4 are being developed for the purpose of enhancing the activity of Ca^{2+}-activated Cl^- channels and thereby improving salt and fluid secretion from CF individuals (Rudolf et al., 2003). We (Yang et al., 2006) have previously proposed that the degree of improvement in Cl^- secretion that can be elicited by Ins(3,4,5,6)P_4 antagonists would be expected to be influenced by the extent to which endogenous Ins(3,4,5,6)P_4 inhibits the Cl^- channels, which in turn depends upon the Ins(3,4,5,6)P_4 concentration (Ho et al., 2001). Therefore, those CF individuals with the higher levels of *ITPK1* expression (and hence higher levels of Ins(3,4,5,6)P_4) potentially stand to benefit the most from a therapy based on Ins(3,4,5,6)P_4 antagonists, should an appropriate drug eventually become available. Conversely, those CF individuals with the lower levels of ITPK1 in airway cells would synthesize less Ins(3,4,5,6)P_4 following activation of PLC. This is a significant point because another potential therapy for CF is the inhalation of purinergic agonists in order to activate airway PLC, mobilize Ca^{2+}, and activate Ca^{2+}-activated Cl^- channels (Ho and Shears, 2002). Unfortunately, PLC activation also elevates Ins(3,4,5,6)P_4 levels, thereby blocking the very channels the therapy is designed to activate. However, patients with less ITPK1, and therefore less Ins(3,4,5,6)P_4, might be expected to receive greater benefit from the inhalation of purinergic agonists. We (Yang et al., 2006) have proposed that clinical trials of these candidate pharmacological approaches to CF should be correlated with *ITPK1* expression profiling in order to detect responsive patient subgroups.

ITPK1 is not a protein kinase

A pair of publications from Majerus' laboratory concluded that human ITPK1 is not just an inositol phosphate kinase, but additionally acts as a protein kinase (Sun et al., 2002; Wilson et al., 2001). Several apparent substrates were identified, including the following transcription factors: IκBα, c-jun and ATF-2. This was an unexpected observation since ITPK1 has no recognizable protein kinase domains (Wilson et al., 2001). In one of their studies, Majerus and colleagues (Sun et al., 2002) also heterologously expressed recombinant ITPK1 in sf21 cells and then incubated the enzyme with [^{32}P]-ATP; ITPK1 became phosphorylated on serine and tyrosine residues (Sun et al., 2002). This phenomenon was interpreted as evidence of autophosphorylation (Sun et al., 2002). Others (Qin et al., 2005) have suggested this itself to be direct evidence that ITPK1 is a protein kinase. However, this is not a strong argument; many ATP-binding proteins that are not protein kinases still undergo autophosphorylation (Hunter, 1995). "Autophosphorylation" of ITPK1 can also be the result of contaminating protein kinase activity. Indeed, we (Qian et al., 2005) subsequently demonstrated that the protein kinase activity that is associated with recombinant ITPK1 produced in insect cells is, in fact, a persistent contaminant; once free of this impurity, ITPK1 showed no protein kinase activity, even though its inositol phosphate kinase activity was near identical to preparations produced by the Majerus laboratory. Furthermore, recombinant ITPK1 that is expressed in *Escherichia coli* does not "autophosphorylate" on serine or tyrosine (Majerus et al., 2008). This particular difference in behaviour in the enzyme, which depends upon which expression system is used, can be explained by phosphorylation (rather than "autophosphorylation") by a protein kinase that

contaminates preparations of ITPK1 that are expressed in the insect cells but not in the bacteria. Finally, when Majerus and colleagues (Miller et al., 2005) described the crystal structure of an amoeboid homologue of ITPK1, they were unable “to model binding of a peptide substrate” into the constricted, “small-molecule modifying” active site. There was no alternative means by which a protein could get close enough to the tightly embedded ATP. A similar conclusion that ITPK1 cannot be a protein kinase can be drawn from crystal structure of the human enzyme (Chamberlain et al., 2007).

Summary

The synthesis and the metabolism of inositol 3,4,5,6-tetrakisphosphate (Ins(3,4,5,6)P_4) are the responsibility of a single multifunctional kinase/phosphotransferase, ITPK1. This enzyme dynamically couples the cellular levels of Ins(3,4,5,6)P_4 to the receptor-dependent hydrolysis of inositol lipids by phospholipase C. This is a biologically significant event because Ins(3,4,5,6)P_4 regulates the conductance of a specialized class of chloride ion channels, which regulate many cellular functions including epithelial salt and fluid secretion, synaptic efficacy, bone remodelling, tumor cell migration, insulin release from pancreatic β-cells, and inflammatory responses. This review assesses the current state of our knowledge of this versatile and ubiquitous signalling cascade.

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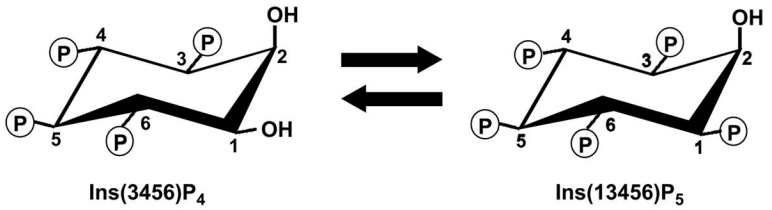

Fig. 1.

The structures of Ins(3,4,5,6)P_4 and Ins(1,3,4,5,6)P_5. The *myo*-inositol building block is a ring structure made from six carbon groups, each of which has a free hydroxyl. The hydroxyl attached to the 2-carbon is axial (perpendicular) to the plane of the ring, and the remaining hydroxyls are equatorial (i.e. approximately in the same plane as the ring). The carbons are numbered in an anticlockwise direction when the ring is viewed from above. Substitution of four of the hydroxyls with phosphates at positions 3, 4, 5 and 6 produces inositol 3,4,5,6-tetrakisphosphate. The standard abbreviation for this polyphosphate – Ins(3,4,5,6)P_4 – therefore reflects the recognition by inositol phosphate nomenclature of the number of phosphate groups (denoted by the subscript), as well as their positions around the inositol ring.

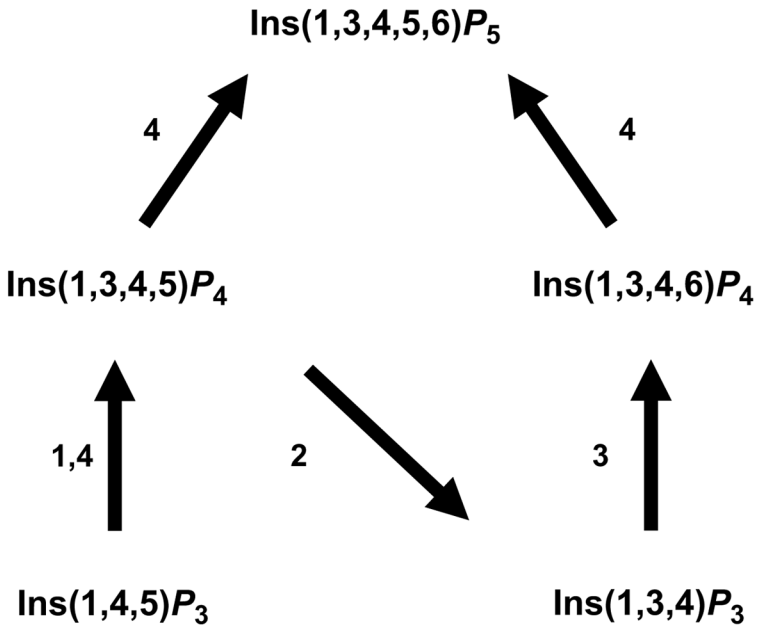

Fig. 2.

The metabolic link between Ins(1,4,5)P_3 and Ins(1,3,4,5,6)P_5 in animal cells. The figure shows the quantitatively most important reactions in animal cells that link Ins(1,4,5)P_3 to Ins(1,3,4,5,6)P_5. Numbers in the figure refer to various enzymes as follows: 1. Ins(1,4,5)P_3 3-kinase (EC 2.7.1.127); 2. Ins(1,4,5)P_3/Ins(1,3,4,5)P_4 5-phosphatase (EC 3.1.3.56); 3. ITPK1 (EC 2.7.1.134); 4. Inositol phosphate multikinase (EC 2.7.1.151); note that kinetic data have led to it being questioned whether IPMK can contribute significantly to Ins(1,3,4,5)P_4 synthesis *de novo* in animal cells (Chang et al., 2002).

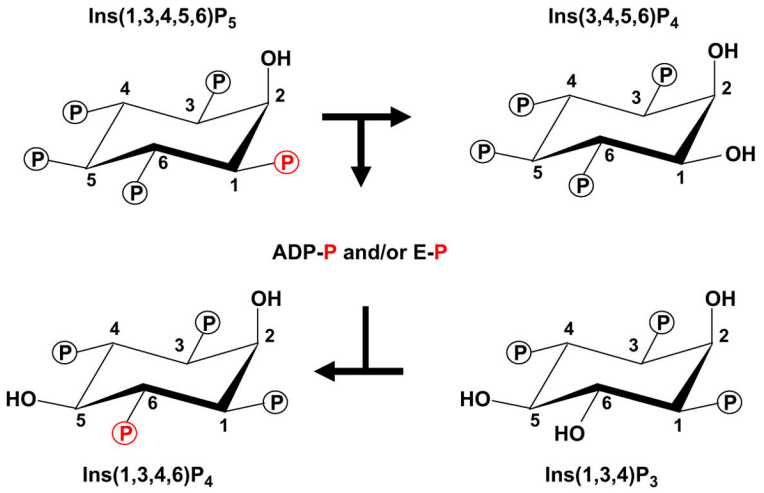

Fig. 3.

The phosphotransferase activity of ITPK1. The graphic illustrates the proposed enzymatic reactions by which the 1-phosphate on Ins(1,3,4,5,6)P_5 (coloured red) is transferred to Ins(1,3,4)P_3. The evidence for this reaction pathway came from HPLC analysis of the reaction products following the metabolism of [1-^{32}P]-Ins(1,3,4,5,6)P_5 by ITPK1 (Chamberlain et al., 2007). It has not yet been established whether or not a phosphoryl-enzyme (E–P) intermediate is involved, but this is a likely possibility.

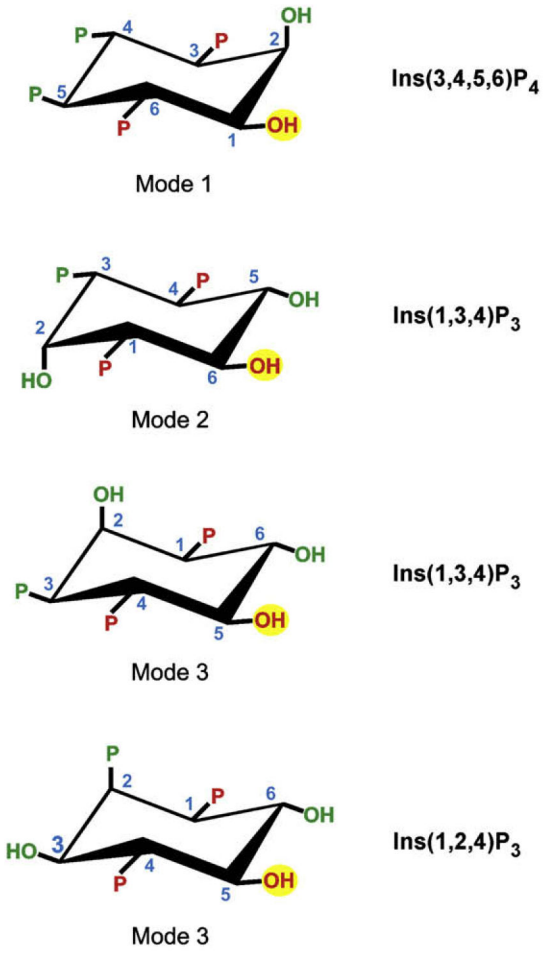

Fig. 4.

A model for the structural determinants of ligand specificity for mamalian ITPK1. The figure depicts our proposal (Ho et al., 2002; Riley et al., 2006) that there are three modes of binding of inositol phosphates to mamalian ITPK1. It can be illuminating to consider these different binding modes (i.e., “1”, “2” and “3”) as permitting 1-kinase, 6-kinase and 5-kinase activities, respectively. These phosphorylation sites are marked with a yellow circle. Three groups in Ins(3,4,5,6)P_4, Ins(1,3,4)P_3 and Ins(1,2,4)P_3 (coloured red) are conserved in all three of these proposed binding modes. We have previously noted that these groups by themselves are insufficient to designate substrate specificity, so we have proposed a combinatorial recognition model in which some of the additional groups (coloured green) contribute to ligand recognition, but in a mode-specific manner.