BACK IN THE WATER: THE RETURN OF THE INOSITOL PHOSPHATES

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Following the discovery of inositol-1,4,5-trisphosphate as a second messenger, many other inositol phosphates were discovered in quick succession, with some understanding of their synthesis pathways and a few guesses at their possible functions. But then it all seemed to go comparatively quiet, with an explosion of interest in the inositol lipids. Now the water-soluble phase is once again becoming a focus of interest. Old and new data point to a new vista of inositol phosphates, with functions in many diverse aspects of cell biology, such as ion-channel physiology, membrane dynamics and nuclear signalling.

The existence of inositol phosphates in biology has been known for over 80 years[1]. However, during the 1980s, a new level of attention was focused on inositol phosphates after the discovery, in 1983, that inositol-1,4,5-trisphosphate (Ins(1,4,5)P_3) is a Ca^{2+}-mobilizing second messenger[2]. Within a short time, it was found that Ins(1,4,5)P_3 and its immediate dephosphorylation products Ins(1,4)P_2 and InsP were not the only inositol phosphates to increase when cells were stimulated — both Ins(1,3,4)P_3 and Ins(1,3,4,5)P_4 emerged as rapidly synthesized metabolites of Ins(1,4,5)P_3 (REFS 3,4). Moreover, InsP_5 and InsP_6, previously thought to be confined to plants and the erythrocytes of a few animals, were found to be components of mammalian (and probably all eukaryotic) cells[5].

By the end of the 1980s, the inositol phosphates comprised a rather intimidating cast of characters, most of which had no known biological function (for example, see REF. 6 for the state of play at that time). The 'proliferation phenomenon' then spread to the lipid phase, when Lew Cantley and his colleagues discovered that the phosphatidylinositol (PtdIns) kinase activity associated with tyrosine kinases such as Src and Middle T antigen was synthesizing PtdIns3P rather than PtdIns4P[7]. At the same time, Alexis Traynor-Kaplan and colleagues discovered PtdIns(3,4,5)P_3 in neutrophils[8]. Since then, there has been an explosion of interest in these lipids and their functions (which were quickly found to include many important cellular processes), and they have dominated research on inositol-containing compounds in the 1990s.

Meanwhile, the interest in the inositol phosphates waned a bit, for three main reasons. First, it is much more difficult to understand how their levels are regulated, due chiefly to the problems in elucidating their pathways of metabolism. Second, there are many more of them (polyphosphoinositol lipids, in which only the 3-, 4- and 5- hydroxyls are involved in generating isomers, have an upper limit of seven, whereas there could be more than 60 inositol phosphates; BOX 1), and synthetic chemistry has only recently begun to address this complexity. And last, the diverse areas of cell biology in which inositol phosphates are suspected to act has precluded their being brought together easily in one place.

Recently, inositol phosphates other than Ins(1,4,5)P_3 have become more prominent in the literature, so this is an excellent moment to bring together what we do and do not know about them. We hope that newcomers to this area, having discovered that they can no longer avoid inositol, will find our treatment user-friendly. Pathways of metabolism can be daunting, so we have dealt with them in FIG. 1 and the text boxes. However, we urge most readers to consult BOX 1 before proceeding further, so that the sometimes confusing nomenclature and isomeric assignments are clear — this is probably another contributory factor that has inhibited a more widespread appreciation of inositol phosphates.

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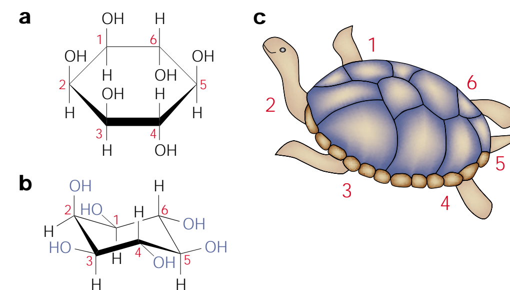
Box 1 | Nomenclature, structure and enantiomers



Nomenclature. **Inositol phosphates contain only inositol and phosphate, and are therefore water-soluble. Inositol lipids also contain inositol and phosphate, but in addition they have a hydrophobic component (usually a diacylglycerol moiety — two fatty acids esterified onto glycerol, which is in turn attached through a diester phosphate to the 1-hydroxyl of the inositol ring; FIG. 1), and are therefore not water-soluble.**

Numbering. **Much of the confusion that surrounds inositol phosphate nomenclature can be circumvented by using Bernie Agranoff's turtle[92]. a shows myo-inositol as a Haworth projection and b shows a more accurate repesentation of the 'chair' structure, probably the most thermodynamically stable conformation of myo-inositol *in vivo*. The 2-hydroxyl is axial, and the other five hydroxyls are equatorial (that is, they are more-or-less in the plane of the ring). Agranoff noted that this structure, if one ignores the hydrogens, superficially resembles a turtle (c). Modern biochemical nomenclature exclusively uses the D-numbering system, so only two facts have to be remembered: first, the turtle is right-flippered, so its number 1 flipper is the front right flipper; and second, the turtle's head is the 2-hydroxyl. The anticlockwise numbering of the rest of the appendages (viewed from on top of the turtle) then follows logically (c).**

Mathematically, there are 63 possible inositol monophosphates, a potential that can be expanded further by attaching pyrophosphate moieties instead of monophosphates (see main text, section on $InsP_7$ and $InsP_8$). FIG. 1 illustrates most of those that have so far been shown to occur in cells, with their known metabolic routes of synthesis and degradation (BOXES 3, 4).

Enantiomers. **This is another source of confusion, where the turtle analogy can also help. A turtle has a plane of symmetry running through its head and tail, so distinguishing a left-flippered from a right-flippered turtle will require using a technique that can discriminate D and L configurations (that is, a chiral analysis or separation method). For example, two prominent $InsP_4$ isomers discussed in this review are inositol-3,4,5,6-tetrakisphosphate (Ins(3,4,5,6)P_4) and Ins(1,4,5,6)P_4, which both have an unphosphorylated hydroxyl in the 2-position (the head), so they differ only in having vacant either the 1- or the 3- positions (the right versus the left front flipper). They are therefore an enantiomeric pair, because they can be converted one to the other by reflection in the plane of symmetry. The standard separation techniques used in inositol phosphate analyses cannot distinguish between enantiomers, so these two $InsP_4$s co-chromatograph exactly. Quantifying Ins(3,4,5,6)P_4 and Ins(1,4,5,6)P_4 separately is possible only using enantiomer-specific enzyme-based analyses[28,93,94].**

Note that a left-flippered (L) turtle would obviously regard its left front flipper as number 1, and so because its head is still number 2, the limbs will be numbered clockwise starting at the front left flipper, which is why the alternative name for Ins(3,4,5,6)P_4 is L-Ins(1,4,5,6)P_4. In biological journals, and in this review, the D numbering is now used universally, but this is not so in chemical journals, where L numbering of the inositol ring is frequently found. This presents no problem because it is always made clear in chemical journals which numbering is being used. However, there is one aspect of nomenclature that could cause confusion, and this is that in the 1970s the official designations of the D and L numberings of the inositol ring were actually swapped over, and so in older papers (for example, REF. 95), what you think should be called D will actually be called L and vice versa.

Inositol-1,3,4,5-tetrakisphosphate

Inositol-1,3,4,5-tetrakisphosphate (Ins(1,3,4,5)P_4), along with its immediate catabolic product Ins(1,3,4)P_3, came in the first wave of inositol phosphates to be discovered[3,4]. As detailed in BOX 2, we now appreciate that Ins(1,4,5)P_3 3-kinase, the enzyme that synthesizes Ins(1,3,4,5)P_4 in animals, is probably quite a late addition to the family of inositol phosphate kinases, having probably evolved from the so-called 'InsP multikinases' — enzymes that can phosphorylate several different inositol phosphates. In mammals, where Ins(1,4,5)P_3 3-kinase has been most comprehensively studied, there are at least three isoforms, all of which are regulated by calmodulin (CaM) and two of which are regulated by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). Ins(1,4,5)P_3 3-kinase is, by far, the most active inositol phosphate kinase detectable in mammals, having a prominent role in rapidly metabolizing the pool of Ins(1,4,5)P_3 that is generated when phospholipase-C-coupled receptors are activated. The rapid accumulation of a new $InsP_4$ isomer in response to agonists did not go unnoticed by evolution, and this has led to several physiological consequences (FIG. 2).

VMAX (V_{max}).
The maximum velocity (that is, the rate) at which an enzyme can catalyse a reaction. This occurs when the substrate is at a concentration that saturates the enzyme, and the concentration of the enzymatic product is low.

What was probably the first function to evolve has only recently been verified[9]. It has been known since shortly after its discovery that Ins(1,3,4,5)P_4 is hydrolysed by the same 5-phosphatase that hydrolyses Ins(1,4,5)P_3, but the enzyme has a 10-fold higher affinity and 100-fold lower VMAX for Ins(1,3,4,5)P_4 than it does for Ins(1,4,5)P_3 (REF. 10). So, Ins(1,3,4,5)P_4 can protect Ins(1,4,5)P_3 against hydrolysis, and therefore increase its effectiveness. Hermosura and colleagues[9] have now shown such an effect of Ins(1,3,4,5)P_4 on Ins(1,4,5)P_3-generated Ca^{2+} entry near the plasma membrane, where the 5-phosphatase is predominantly localized. If cells are challenged with a low dose of an agonist linked to Ins(1,4,5)P_3 generation, and then if the agonist is withdrawn and re-applied a short time later, the cells are

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Figure 1 | **Pathways of metabolism of inositol phosphates.** This figure outlines the pathways that have been reported to exist, using the strategies outlined in BOX 3. For many of them, we do not know whether they are the main pathways (indeed, some are almost certainly not, as discussed in BOX 3). The arrows are colour-coded for the organisms in which they have been shown (note that yeast inositol phosphate metabolism is frequently discussed in the text, but we have no separate colour for this group of organisms, as all the reactions described in them are probably universal).

COINCIDENCE DETECTION
The process whereby a cell responds differently to a signal if another signal is received simultaneously (or just before). This is particularly important in the brain, where different spatial, temporal and chemical inputs to a neuron can alter the neuron's output.

LTP
Long-term potentiation is a specific example of coincidence detection, whereby the high frequency stimulation of a neuron increases the magnitude of subsequent responses, an effect that can last for days. LTP is believed to underlie some kinds of learning and memory.

INS(1,4,5)P_3 RECEPTORS
Ca^{2+} channels located on the endoplasmic reticulum which, when Ins(1,4,5)P_3 binds to them, open to release stored Ca^{2+}. There are three subtypes (I, II and III); all have similar structures and functions.

more sensitive to the second challenge because the Ins(1,3,4,5)P_4 produced during the first stimulation has lasted longer than the Ins(1,4,5)P_3 (because of the lower V_{max} of the phosphatase). So, Ins(1,3,4,5)P_4 levels persist as a 'memory' of an earlier stimulation. If this sounds like COINCIDENCE DETECTION, that is because it might indeed serve such a purpose, especially at postsynaptic sites in the dendritic spines of neurons, where coincidence detection is a central aspect of long-term potentiation (LTP). This is discussed in more detail elsewhere[11], but it is relevant to note that, owing to the presence of the A isoform of Ins(1,4,5)P_3 3-kinase (BOX 2), dendritic spines are probably the most active sites of Ins(1,3,4,5)P_4 synthesis in the whole body.

But Ins(1,3,4,5)P_4 can do more. For example, in endothelial cells there is direct evidence[12], and in neurons direct[13] and indirect[14] evidence, that InsP_4 can activate Ca^{2+} channels in the plasma membrane (FIG. 2). In neurons, the consequence is to enhance LTP[14]. Simplistically, this seems to contradict the enhanced LTP shown by mice in which Ins(1,4,5)P_3 3-kinase has been knocked out[15], but the counterintuitive enhanced LTP seen in, for example, mice that lack type I INS(1,4,5)P_3 RECEPTORS[16] tells us that the situation is complex.

Ins(1,3,4,5)P_4 can also interact with Ins(1,4,5)P_3 receptors on the endoplasmic reticulum membrane, but only at high concentrations, and with effects that seem contradictory. In some cell types, it inhibits the actions of Ins(1,4,5)P_3 (REFS 9,17), whereas in others it mimics it by mobilizing Ca^{2+} (REF. 18). There are three isoforms of the Ins(1,4,5)P_3 receptor in mammals, and it is possible that they respond differently to Ins(1,3,4,5)P_4. We should also note that there is a problem in interpreting many other experiments in which Ins(1,3,4,5)P_4 has been added to cell preparations; we have found (unpublished observations) that most commercial sources of Ins(1,3,4,5)P_4 (both purified from biological sources or synthesized) contain considerable amounts (1–10%) of Ins(1,4,5)P_3.

The first effect of Ins(1,3,4,5)P_4 to be reported (which is definitely not explicable by Ins(1,3,4,5)P_4 'protecting' Ins(1,4,5)P_3) — and certainly the most elusive — is its ability to synergize with Ins(1,4,5)P_3 (or artificial non-metabolizable analogues of Ins(1,4,5)P_3) to mobilize Ca^{2+} and to activate STORE-OPERATED CA^{2+} ENTRY. The marked influence that experimental protocols[19] can have on whether this effect is unmissable[20] or invisible[21] suggests a complex mechanism of action. A similarly

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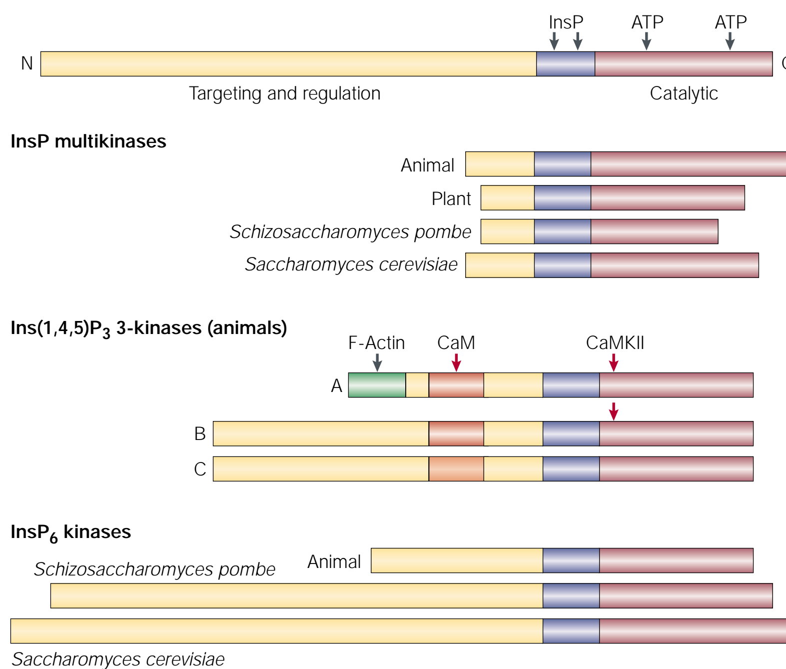
Box 2 | **Inositol phosphate kinases**



By cloning the $InsP_6$ kinase purified from rat brain, Saiardi and colleagues[74] identified a family of inositol phosphate kinases conserved from yeast to humans. Members of the family include yeast and animal $InsP_6$ kinases, animal inositol-1,4,5-trisphosphate (Ins(1,4,5)P_3) 3-kinases, and InsP multikinases, all of which share conserved InsP kinase motifs (blue boxes) involved in recognizing inositide substrates[72,74,76,86,96]. All members of the family are built from a conserved carboxy-terminal catalytic region attached to various amino-terminal regions, which are involved in targeting and regulation. Phylogenetic analyses (M.J.S. and R.F.I., unpublished observations) indicate that the InsP kinases evolved through variation on the basic multikinase structure. InsP multikinases phosphorylate Ins(1,4,5)P_3 twice to make Ins(1,3,4,5,6)P_5 (BOX 4) and they also phosphorylate Ins(4,5)P_2 to Ins(1,4,5)P_3 and Ins(1,3,4,5,6)P_5 to the pyrophosphate-containing species Ins(PP)P_4 (REFS 76,90). Although this might seem surprising — that the same enzyme can catalyse the phosphorylation of both hydroxyl groups and a phosphate group — this observation helps to explain the evolution of $InsP_6$ kinase from the InsP multikinases[74,86].

Ins(1,4,5)P_3 3-kinases (and Ins(1,4,5)P_3 receptors) occur only in animals as far as we know, and Ins(1,4,5)P_3 3-kinases probably evolved for the rapid removal of the Ins(1,4,5)P_3 produced for Ca^{2+} signalling. Ins(1,4,5)P_3 3-kinases are also the only members of the family regulated by Ca^{2+}, through interactions with calmodulin (CaM; red boxes) and phosphorylation by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII; red arrows)[97,98]. The C isoform of Ins(1,4,5)P_3 3-kinase[99] is regulated by Ca^{2+} to a lesser extent (and is not found in brain, a tissue that is rich in inositol phosphate signalling), so either it has lost some Ca^{2+} regulation during evolution, or perhaps it is a more primitive version of the enzyme.

The amino-terminal regions of InsP kinases participate in various protein–protein interactions. For example, the InsP multikinase from *Saccharomyces cerevisiae* is targeted to the nucleus, where it interacts with transcription factors of the MADS-box family[88] (see main text). All Ins(1,4,5)P_3 3-kinases interact with calmodulin at a site that lies amino-terminal to the catalytic region (red boxes). The extreme amino terminus of the Ins(1,4,5)P_3 3-kinase A isoform targets the enzyme to filamentous-actin-rich dendritic spines in neurons (M.J.S., C. Erneux and R.F.I., unpublished observations), whereas the amino-terminal region of the B isoform targets the enzyme to the early secretory pathway, near intracellular Ca^{2+} stores[100].

Two other inositol phosphate kinases have been cloned that have no obvious sequence similarity with the InsP kinase family. York and colleagues[71,77] have cloned two yeast Ins(1,3,4,5,6)P_5 2-kinases implicated in regulating the transport of messenger RNA from the nucleus using their ability to produce $InsP_6$, but no known homologues have yet been found in animal genomes (although animal cell extracts do show Ins(1,3,4,5,6)P_5 2-kinase activity[43]). As discussed above, the enzyme originally cloned as an Ins(1,3,4)P_3 6-kinase[101] is equally likely to be an Ins(3,4,5,6)P_4 1-kinase *in vivo*[102] (see BOX 3 and the main text). Interestingly, the human genome seems to contain only one kinase of this type, whereas the *Arabidopsis thaliana* genome contains at least three, indicating that various eukaryotes have placed emphasis on different inositol phosphate kinase pathways as they evolved.

NCBI accession numbers of genes used to create the schematic depictions of the proteins shown are: Multikinases: animal, AY0114898; plant, BAB10076; *Schizosaccharomyces pombe*, CAB63791; *Saccharomyces cerevisiae*, NP_010458. Ins(1,4,5)P3 3-kinases: isoform A, CAA38700.1; isoform B, CAB65055; isofom C, AJ290975. $InsP_6$ kinases: animal, NP_057375; *S. pombe*, CAA20701; *S. cerevisiae*, NP_010458.

STORE-OPERATED CA^{2+} ENTRY
The activation of a Ca^{2+} channel in the plasma membrane in response to the depletion of Ca^{2+} levels in the endoplasmic reticulum (ER). Decreases in the levels of stored Ca^{2+} inside the ER somehow signal to the plasma membrane channels (store-operated calcium channels, or SOCs).

L-1210 CELLS
A mouse lymphoma cell line that grows readily in suspension, a property useful for studying Ca^{2+} homeostasis in permeabilized cells.

complex molecular mechanism is also implied from the effect of Ins(1,3,4,5)P_4 on Ins(1,4,5)P_3-stimulated Ca^{2+} mobilization in permeabilized L-1210 CELLS, in which merely changing the order of addition of the two inositol phosphates yields different results[22,23]. In this system, there is evidence implicating GAP1^{IP4BP}, cloned as a probable Ins(1,3,4,5)P_4 receptor[24], in the action of Ins(1,3,4,5)P_4 (REF. 23), and this might hint at how this particular function evolved.

GAP1^{IP4BP} is closely related to GAP1^{m}, which is a probable receptor for the lipid phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3)[25]. Most PtdIns(3,4,5)P_3 receptors have been shown to bind Ins(1,3,4,5)P_4 *in vitro*, which is not surprising as Ins(1,3,4,5)P_4 is the head group of PtdIns(3,4,5)P_3. Perhaps the synergistic effects described above stem from a regulation of endomembrane function by PtdIns(3,4,5)P_3 that has been partly taken over by Ins(1,3,4,5)P_4. This possiblity is intriguingly consistent with recent evidence from ligand-binding experiments on GAP1^{IP4BP} and GAP1^{m} reported by Cozier and colleagues[26]. They showed that although both GAP1^{IP4BP} and GAP1^{m} can discriminate between Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 by more than three orders of magnitude, GAP1^{IP4BP} seems to have completely lost (or never had) the ability to distinguish between PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3, an ability that GAP1^{m} has retained.

Inositol-3,4,5,6-tetrakisphosphate

In epithelial cells, inositol-3,4,5,6-tetrakisphosphate (Ins(3,4,5,6)P_4) seems to be a physiologically important

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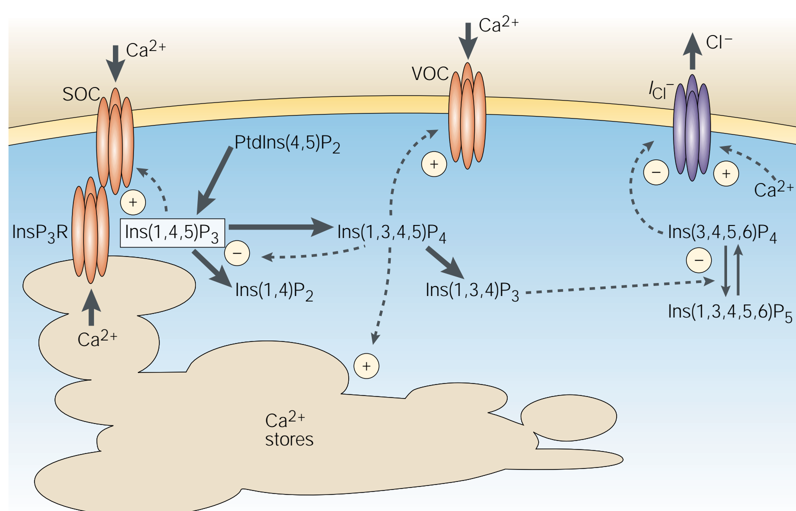

Figure 2 | **Consequences of inositol-1,3,4,5-tetrakisphosphate generation.** Actions (activating or inhibiting) are shown in dashed lines, and chemical conversions are shown in solid lines. Four direct or indirect consequences of inositol-1,3,4,5-tetrakisphosphate (Ins(1,3,4,5)P_4) generation are shown. On the left, Ins(1,3,4,5)P_4 inhibits the 5-dephosphorylation of Ins(1,4,5)P_3 (REF. 9), thus enhancing the mobilization of Ca^{2+} by Ins(1,4,5)P_3. In the middle, Ins(1,3,4,5)P_4 has a direct action on Ca^{2+} channels in the plasma membrane[12–14], and might have a complex action on the Ca^{2+} stores mobilized by Ins(1,4,5)P_3 (REF. 19). On the right, Ins(1,3,4)P_3 derived from Ins(1,3,4,5)P_4 inhibits Ins(3,4,5,6)P_4 1-kinase[34,102], which results in an increase in Ins(3,4,5,6)P_4 and thus a decrease in chloride efflux[29]. SOC, store-operated Ca^{2+} channel; VOC, voltage-operated Ca^{2+} channel; $InsP_3R$, Ins(1,4,5)P_3 receptor; I_{Cl}, chloride channel.

inhibitor of Ca^{2+}-regulated Cl^- channels (FIG. 3). The original discovery was made when Kachintorn and colleagues[27] found that if T-84 CELLS are stimulated for a prolonged period with an agonist that activates Ins(1,4,5)P_3 production, the activation of Cl^- secretion is only transient, even though the level of Ca^{2+} remains elevated. The time course of the inhibition of Cl^- secretion matched that of the production of various $InsP_4$s, of which Ins(3,4,5,6)P_4 correlated best[28]. Moreover, some elegant and pioneering use of cell-permeable inositol phosphate analogues supported the suggestion that Ins(3,4,5,6)P_4 is indeed the likely natural regulator of chloride secretion[28].

Since this discovery, the story has grown in strength and interest, although not without its controversies (see REF. 29 and the Shears' lab web site). The molecular mechanism of Ins(3,4,5,6)P_4 action was shown to be by inhibition of a Cl^- channel[30,31], but it is still not certain which channel is being inhibited. Recombinant bovine CLCA1 from bovine trachea is inhibited *in vitro* by Ins(3,4,5,6)P_4 (REF. 31), although several aspects of the inhibition differ from the action of Ins(3,4,5,6)P_4 in a PATCH-CLAMPED cell[29]. In human epithelial cells, the probable target of Ins(3,4,5,6)P_4 is a novel 1 pS Cl^- channel, which is activated by Ca^{2+} or by CaMKII, although only the kinase-dependent activation is counteracted by Ins(3,4,5,6)P_4 (REF. 32). Inhibitory effects of Ins(3,4,5,6)P_4 in patched cells are removed when protein phosphatases are inhibited by okadaic acid or microcystin[32,33], although the effect of Ins(3,4,5,6)P_4 on CLCA1 channels observed in membrane fragments fused with lipid bilayers[31] implies a direct (protein-kinase-independent) inhibition of those particular channels.

T-84 CELLS
A colonic epithelial cell line.

PATCH-CLAMP
The technique of attaching a pipette to the outside of a cell, and either pulling the small piece of membrane captured within it off the cell ('excised patch') or rupturing this piece, thus making the interior of the cell continuous with the inside of the pipette ('whole cell patch').

PI3K AND PTDINS(3,4,5)P_3
The phosphatidylinositol 3-kinases are a family of enzymes that phosphorylate the 3-position on inositol lipids. The type I varieties are the most relevant for signal transduction because they are receptor-regulated; they prefer phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) as a substrate and thus make the second messenger PtdIns(3,4,5)P_3.

The mechanism by which levels of Ins(3,4,5,6)P_4 increase when Ins(1,4,5)P_3-generating agonists are used has been clarified considerably by the recent work from Shears' laboratory. They have shown that the predominant route by which Ins(3,4,5,6)P_4 is removed is by a dual-specificity kinase, whose catalysis of an Ins(3,4,5,6)P_4 1-kinase reaction is inhibited by its co-substrate, Ins(1,3,4)P_3 (REF. 34) (see FIG. 2 and BOX 2). We are still not sure, however, how Ins(3,4,5,6)P_4 is synthesized. If a cell homogenate is presented with Ins(1,3,4,5,6)P_5 it will hydrolyse it to Ins(3,4,5,6)P_4, although this generation of Ins(3,4,5,6)P_4 depends on ATP[35]. As Ins(1,4,5,6)P_4 was the predominant product of Ins(1,3,4,5,6)P_5 dephosphorylation in these experiments, Oliver and colleagues[35] discussed the possibility of an isomerase interchanging the two enantiomers (BOX 1), but this has not been explored further.

The effects of Ins(3,4,5,6)P_4 on Cl^- secretion take on a particularly interesting practical slant when it is considered that, for patients with cystic fibrosis in whom the epithelial cyclic AMP-regulated Cl^- channel is compromised, the Cl^- channel regulated by Ca^{2+} and by Ins(3,4,5,6)P_4 is the only one that they have left. Stimulating this pathway by drugs that activate phospholipase C is a possible therapy, but if Ins(1,4,5)P_3 is generated by such a stimulation, this will of course ultimately raise the levels of inhibitory Ins(3,4,5,6)P_4 too. The alternative strategy[29] of trying to alleviate this inhibitory action of Ins(3,4,5,6)P_4 at the level of the Cl^- channel is an enticing possibility for the first clinical exploitation of the physiological action of an inositol phosphate.

Inositol-1,4,5,6-tetrakisphosphate

The evidence that this inositol phosphate has a physiological function is indirect and largely conjectural, but intriguing. It is the main $InsP_4$ formed when cell homogenates are incubated with Ins(1,3,4,5,6)P_5 *in vitro*[35], and the one that accumulates in cells transformed with *src*[36], although this might just be a long-term indirect consequence of the increase in Ins(1,4,5)P_3 3-kinase and other inositol phosphate kinases in these transformed cells.

The reason for devoting a small section of this review to Ins(1,4,5,6)P_4 is because its levels go through a marked increase when cells are infected with *Salmonella dublin*[37], and this leads to an inhibition of epidermal-growth-factor-receptor-stimulated phosphatidylinositol 3-kinase (PI3K) signalling (by an unknown mechanism, although possibly because the high levels of Ins(1,4,5,6)P_4 antagonize interactions between PTDINS(3,4,5)P_3 and its targets). This discovery is made more intriguing by the observation that a *Salmonella* virulence protein, SopB, has some sequence similarity to the inositol polyphosphate 4-phosphatases (reviewed by Majerus and colleagues[38]). The protein shows phosphatase activity against inositides and, among a number of reactions that it will catalyse, it generates Ins(1,4,5,6)P_4 from Ins(1,3,4,5,6)P_5 (REF. 39). Moreover, transfection of mammalian cells with SopB increases Cl^- transport[40], although recent studies on *Salmonella* virulence factors[41], which reveal SopB to have many

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GUARD CELLS
The cells found on the underside of plant leaves, which pair up to form stomata, or leaf pores. Guard cells control the size of the stomata, and thus in turn regulate gas exchange in the leaf.

ABSCISIC ACID
A plant hormone originally discovered (and named) for its ability to regulate leaf detachment; also a key regulator of guard cell shape (and thus gas exchange) in the leaf.

SYNAPTOTAGMINS
A group of Ca^{2+}-binding proteins that are generally understood to be involved with the secretion of granules and vesicles, especially in the nervous system.

AP-2 AND AP-180
Two members of a family of so-called 'clathrin adaptor proteins', which facilitate the early stages of endocytic vesicle formation through their ability to bind clathrin coats.

ARRESTIN
Protein that, when phosphorylated, associates with G-protein-coupled receptors, thereby inhibiting the receptors' actions.

complex effects on cell architecture and function (for example, profound actions on the cytoskeleton), urge some caution in interpreting its actions too simplistically. SopB will also hydrolyse inositol lipids *in vitro*[39], and if it does this *in vivo* then this is an alternative explanation for its actions.

Inositol-1,3,4,5,6-pentakisphosphate

This inositol phosphate was unambiguously identified in a classic study by Johnson and Tate[42], as a constituent of avian erythrocytes. It is also the $InsP_5$ isomer that predominates greatly in most mammalian cells[43], with a few exceptions (such as in some Jurkat T cells[44] and T5-1B cells[45], the latter having significant levels of $Ins(1,2,4,5,6)P_5$ and its enantiomer (BOX 1), $Ins(2,3,4,5,6)P_5$). It is evident from FIG. 1 that $Ins(1,3,4,5,6)P_5$ serves as a metabolic 'hub' in higher inositol phosphate metabolism, but whether it serves any other physiological function in mammalian cells remains unknown.

In several animal species with nucleated erythrocytes, $Ins(1,3,4,5,6)P_5$ is believed to decrease the affinity of haemoglobin for O_2, while increasing the cooperativity for O_2 binding, a function fulfilled by ATP or 2,3 bisphosphoglycerate (2,3-BPG) in most animals (see Coates[46] for review). In birds and (appropriately) turtles, a developmental switch from 2,3-BPG to $Ins(1,3,4,5,6)P_5$ occurs after the young animal hatches from the egg[47].

However, this generally accepted function of $Ins(1,3,4,5,6)P_5$, to modulate haemoglobin–O_2 interactions, might now require re-examination. For example, Isaacks *et al.*[48] stripped organic phosphates off the haemoglobin of two turtle species by column chromatography and dialysis, and showed that they had removed more than 90% of the original phosphates, but they found that with adult haemoglobin (in contrast to embryonic haemoglobin), adding back $Ins(1,3,4,5,6)P_5$ made no difference to the oxygen affinity[48]. In another study[49], they manipulated 2,3-BPG levels in either mature or embryonic avian erythrocytes, which respectively do or do not contain $Ins(1,3,4,5,6)P_5$, and found that the effects of 2,3-BPG on haemoglobin–O_2 interactions were indistinguishable between the two sets of erythrocyte. These observations do not necessarily mean that haemoglobin–$Ins(1,3,4,5,6)P_5$ interactions have no significance, but they do indicate that the physiology might be more complex than originally thought. Finally, we cannot avoid being intrigued by an Amazonian fish, the Pirarucu, which progresses from a juvenile, fully aquatic existence to later life in an anaerobic swamp where it becomes almost entirely air-breathing. During this time, the $Ins(1,3,4,5,6)P_5$ levels in its erythrocytes increase from about 1 mM to 7 mM[50]. The discovery of $Ins(1,3,4,5,6)P_5$ might be the oldest observation of inositol phosphates in animals, but perhaps it has not yet yielded all its secrets.

Inositol hexakisphosphate

$InsP_6$, also known as phytic acid, was the first inositol phosphate discovered, when Posternak produced conclusive evidence that the principal storage phosphate in the seeds of green plants was identical to synthetic $InsP_6$ that he prepared from inositol[1]. It is the most abundant inositol phosphate in the world — look at a field of wheat and you are gazing at tons of $InsP_6$. It was not until more than 60 years later that the new interest in inositol phosphates led to high performance liquid chromatography separation techniques that revealed[5] what has turned out to be the ubiquitous presence of $InsP_6$ in animal cells.

The original proposed function of $InsP_6$ in plants remains the most likely — that it serves as a phosphate store for the seed[1], although that might not be all that it does. For example, a tissue actively synthesizing $InsP_6$ (and therefore a very useful source of radiolabelled $InsP_6$) is the germinating mung bean seed[43], and superficially one might expect this tissue to be devoted entirely to mobilizing (hydrolysing) $InsP_6$ if the only function of $InsP_6$ were as a phosphate store. Recently, an exciting new possibility for $InsP_6$ in plants has been suggested, in the activation of K^+ channels in GUARD CELLS[51]. Consistent with its much faster rate of synthesis compared with in animal tissues (BOX 3), the levels of $InsP_6$ increased in response to hormonal (ABSCISIC ACID) stimulation within a few minutes. Application of submicromolar concentrations of $InsP_6$ specifically inhibited the same inward-rectifying K^+ channel that is an intrinsic part of the response of these cells to abscisic acid.

Mass assays based either on nuclear magnetic resonance spectroscopy, or on a technique for quantifying inositol phosphates that is based on their ability to compete for metal binding with a dye[52], have estimated the intracellular $InsP_6$ concentration to be 10 μM (REF. 53) or 60 μM (REF. 52) in animal cells, and up to 700 μM in slime moulds[54]. The available evidence indicates that it might not be compartmentalized, but that it might be in free equilibrium with the cytosolic compartment[55]. One limit to its free levels is probably the insolubility of its Mg^{2+} salt — simply loading anything higher than 100-μm $InsP_6$ into an 'intracellular buffer' at pH 7.5 will generate a precipitate. In cells, $InsP_6$ is probably extensively bound to proteins (see below).

In recent years, several more suggestions for $InsP_6$ functions (other than in plants) have been made, for example, protein phosphatase inhibition[56] and the activation of protein kinase C[57]. $InsP_6$ has also been shown to interact *in vitro* with several intracellular proteins, and these all seem to fall into the general area of secretion or vesicular recycling. They include SYNAPTOTAGMINS[58,59], the vesicle adaptor proteins AP-2 (REF. 60) AND AP-180 (REFS 61,62), and ARRESTIN[63]. Shears[64] has recently reviewed this area, and raised several concerns about possible artefacts that to some degree have troubled all those who have worked on this compound. $InsP_6$ is an unusual and highly charged molecule. It interacts strongly with positively charged groups on proteins or low molecular weight cations and, in doing so, can compete for other molecules that would bind these proteins and cations *in vivo*. So, it is particularly important to add $InsP_6$ to experimental systems in the presence of physiological Mg^{2+} concentrations (see above), and to use other inositol

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Box 3 | **Pathways of synthesis**

Mapping the pathways illustrated in FIG. 1 has been difficult, and is still largely incomplete, because the methods generally used to elucidate metabolic pathways present their own particular problems when dealing with inositol phosphates.

First, the most direct way of exploring a synthesis pathway is by pulse labelling and following the radiolabel through the components of the pathway. However, most cells do not take radiolabelled inositol up quickly enough for this approach to work effectively — the higher inositol phosphates incorporate label at a similar rate (very slowly). An alternative is to label with ^{32}P for a short time such that the ATP pool is not at the same specific activity as inorganic phosphate or other phosphate esters, and then analyse the specific activities of the individual phosphate moities in the inositide under consideration[103]. In simple terms, the 'hottest' phosphate is the one that was added last in the main synthesis pathway (see REF. 103 for the full discussion of this strategy — it is made more accurate if the cells are dual-labelled with ^{3}H-inositol). This approach has several interpretative complications, but it has been successfully used to sort out the main synthesis routes of the polyphosphoinositol lipids[104–106], and also on two tissues where inositol phosphate synthesis is fast enough to permit efficient labelling of the highly phosphorylated species[103,107].

Second, another approach to mapping pathways is to follow the labelling of intermediates *in vitro*, and explore their input into the pathway by adding them to a cell-free system. This allowed the mapping of the inositol-1,4,5-trisphosphate (Ins(1,4,5)P_3)/Ins(1,3,4,5)P_4 pathway[4,108], and also the InsP_6 synthesis pathway in the slime mould *Dictyostelium discoideum*[69], but these are exceptionally active pathways. *Dictyostelium* seems to be something of a freak in that it synthesizes InsP_6 from inositol much more quickly than any other known organism.

Third, as the preceding methods are unsuitable for mapping many of the inositol phosphate synthesis pathways, the only serious alternative is isolating, cloning and exploring the specificity of the enzymes responsible for a synthesis pathway. The genes for some inositol phosphate kinases have been cloned (see BOX 2), but this breeds its own problems. Most of these kinases use more than one substrate, and substrate testing is confined to the few tritiated inositol phosphates that are available at present. This is because assaying any such activities with ^{32}P-ATP is technically difficult, as the ATP contains so many decomposition products that have similar chromatographic properties to inositol phosphates. (Compare this with assaying lipid kinases, where these contaminants can be left behind on the origin of a thin-layer chromatography plate.)

Even when an inositol phosphate kinase is well characterized, its physiologically relevant action might still not be known. An example is the phosphorylation of Ins(1,3,4)P_3 to Ins(1,3,4,6)P_4. Ins(1,3,4)P_3 6-kinase has been cloned[101], but we do not know whether Ins(1,3,4,6)P_4 is actually synthesized by this route[103,109], or whether the physiological function of the enzyme is to act as an Ins(3,4,5,6)P_4 1-kinase regulated by Ins(1,3,4)P_3 (REF. 102 and FIG. 2).

A final point to make about mapping inositol phosphate synthesis pathways is, does it matter much to anyone other than afficionados? In the main text we discuss, for example, several possible functions of InsP_6 in mammalian cells, but, remarkably, we are still uncertain how this compound is synthesized *de novo* in animals (BOX 4), or where.

phosphates (or inositol hexakis-sulphate) as controls[64].

One puzzle about the function of InsP_6 in animal cells, is that it seems unlikely that its levels change extensively or rapidly (BOX 4), implying that it might do no more than act as an ever-present buffer of some kind of cation- or protein-dependent function. For example, it interacts strongly with some synaptotagmins[58] and inhibits their functions; but PtdIns(3,4,5)P_3 and PtdIns(4,5)P_2 interact more strongly with these proteins[59], at least *in vitro*, and it is more likely that the lipid interaction has physiological relevance[59]. Perhaps InsP_6 decreases resting levels of synaptotagmin activity, and thus increases the signal-to-noise ratio of a lipid signal. Another intriguing function for InsP_6 in plants and animals might lie in its antioxidant properties[65,66]. It is unique among naturally occurring compounds in that it completely inhibits Fe^{3+}-catalysed hydroxyl-radical formation. This is specifically due to the 1-,2- and 3-phosphates, in particular their equatorial–axial–equatorial orientation[66,67] (BOX 1).

Recently, three exciting new functional possibilities in animals have emerged for InsP_6, none of them being necessarily mutually exclusive. First, Hilton and colleagues[68] have purified an InsP_6-stimulated protein kinase (still unidentified) that specifically phosphorylates pacsin/syndapin I, a protein involved in synaptic vesicle recycling. The kinase is almost completely dependent on InsP_6, with high specificity for this inositol phosphate over many others (with the exception of InsP_7, which was equipotent with InsP_6). This again brings InsP_6 into 'familiar' territory — vesicle recycling.

The other two recent discoveries about InsP_6 focus on its potential functions in the nucleus. Some years ago, Van Haastart and co-workers showed that slime moulds apparently have two separate pathways of InsP_6 synthesis: a cytosolic route from inositol[69] (see FIG. 1 and BOXES 3, 4) and a route from Ins(1,4,5)P_3 in the nucleus[70]. New significance of this emerged with the observations of York and colleagues[71,72], who screened for yeast genes that are involved in mRNA transport out of the nucleus, and isolated four[71]. Three of these turned out to encode proteins that could constitute a previously suggested pathway [73] to synthesize InsP_6: yeast's only phosphoinositide-specific phospholipase C; an Ins(1,3,4,5,6)P_5 2-kinase; and a third gene that they[72] and others[74,75] subsequently identified as an enzyme that can phosphorylate Ins(1,4,5)P_3 to Ins(1,4,5,6)P_4 and then phosphorylate that to Ins(1,3,4,5,6)P_5 (here we will call it 'InsP multikinase'; BOX 2). The simple interpretation of this would be that

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Box 4 | How do animal cells make $InsP_6$?

How are inositol-1,3,4,5,6-pentakisphosphate ($Ins(1,3,4,5,6)P_5$) and $InsP_6$ synthesized *de novo* in animal cells? Recently, a mammalian enzyme (which we call InsP multikinase, see BOX 2) was cloned that can phosphorylate $Ins(1,4,5)P_3$ twice in the 3 and 6 positions to convert it to $Ins(1,3,4,5,6)P_5$ (REFS 74,90). Although the $Ins(1,3,4,5,6)P_5$ 2-kinase recently described in yeast[77], which finished this pathway at $InsP_6$ in that organism, has yet to emerge from the mammalian databases, the existence of InsP multikinase nevertheless seems to solve the problem of $InsP_6$ synthesis. The $Ins(1,4,5)P_3$ substrate for this enzyme is, at least in yeast, derived from phosphoinositide-specific phospholipase C (PI-PLC) activity on $PtdIns(4,5)P_2$ (FIG. 1), because mutants devoid of PI-PLC activity seem to make no $InsP_6$ (REF.71).

However, yeast has no $Ins(1,4,5)P_3$ receptors to mobilize Ca^{2+}, whereas in animal cells the role of $Ins(1,4,5)P_3$ as a Ca^{2+}-regulator would clash with its function as a precursor of $InsP_6$ synthesis unless there was a separate pool of $Ins(1,4,5)P_3$. There is evidence for such a pool in animal cells[110,111]. This pool of $Ins(1,4,5)P_3$ has a much slower turnover than receptor-generated $Ins(1,4,5)P_3$ (REF. 111), and labels to equilibrium with ^3H-inositol in 1–2 days. However, in the same cells $Ins(1,3,4,5,6)P_5$ takes three days, and $InsP_6$ at least four days, to reach equilibrium[35]. Simplistically, this would suggest that the route from $Ins(1,4,5)P_3$ to $InsP_6$ must be incredibly slow, which is not consistent with data suggesting that levels of $Ins(1,3,4,5,6)P_5$ and $InsP_6$ in animal cells can change (albeit only by a few per cent) over a timescale of minutes[52,56].

Slime moulds[69] and higher plants[107] synthesize $InsP_6$ rapidly from inositol through Ins3P (FIG. 1). Do mammals also have this pathway, albeit operating at a slower rate? The extremely slow incorporation of labelled inositol into $Ins(1,3,4,5,6)P_5$ and $InsP_6$ in mammalian cells would not be inconsistent with a moderate rate of synthesis of $InsP_6$ from Ins3P, if we assume that mammals lack only the inositol kinase known to exist in slime moulds[112] and plants[113]. The major source of Ins3P for the pathway to $InsP_6$ would then be glucose 6-phosphate rather than inositol (see REF. 95 and FIG. 1). The genetic evidence for the existence of the plant/slime mould pathway to $InsP_6$ in animals is at present ambiguous, given that inositol phosphate kinases are so heterogeneous in their primary sequence (BOX 2), so the principal route of $InsP_6$ synthesis in animals remains an open question.

the final product of this synthesis pathway from $PtdIns(4,5)P_2$ to $InsP_6$ (FIG. 1 and BOX 4) was required for mRNA transport out of the nucleus.

The possibility that $InsP_6$ is the active player, rather than $InsP_7$ or $InsP_8$ (see next section), is supported by the observation that deletion of $InsP_6$ kinase from yeast has no effect on mRNA transport[76]. However, the relationship between $InsP_6$ and mRNA transport is unlikely to be simple, because at least one mutant in the *ipk-1* gene (the $Ins(1,3,4,5,6)P_5$ 2-kinase), is defective in the mRNA transport assay, but still has significant $Ins(1,3,4,5,6)P_5$ 2-kinase activity[77]. Finally, some evidence that this might have relevance beyond yeast comes from recent data showing that transfection of mammalian cells with SopB causes the expected decline (indeed, almost complete removal) of $InsP_6$, and a coincident inhibition of mRNA transport[40] (although, as pointed out in REF. 41, this observation must be tempered with the caveat that SopB has several catalytic activities and several effects on cells).

The other suggested nuclear function for $InsP_6$ has come from the observations of Hanakahi and colleagues[78]. When assaying DNA end-joining *in vitro* with a nuclear extract that they expected to be fully active, they found that end-joining was dependent on the addition of a constituent of a cell fraction, which they purified and identified as $InsP_6$. The target for $InsP_6$ was shown to be the DNA-dependent protein kinase, and a possible binding domain for $InsP_6$ was identified[78]. The requirement for $InsP_6$ seems to be specific (for example, $Ins(1,3,4,5)P_4$ and $Ins(1,3,4,5,6)P_5$ are less active, and inositol hexakis-sulphate is inactive). It will be fascinating to see if $InsP_7$ or $InsP_8$ (see below) are more or less active, and how other inositol phosphates substituted in the 1-, 2- and 3-positions behave.

$InsP_7$ and $InsP_8$

Even for those of us attuned to the 'proliferation' of inositol phosphate in the 1980s, the discovery that you could actually cram more than six phosphates — seven or eight — onto an inositol ring was a surprise[79,80]. The simplest versions of these are a fully phosphorylated inositol ring, containing either one or two pyrophosphates. The correct names for these are $InsP_5PP$ and $InsP_4(PP)_2$, but here we shall call them by the simpler (if inaccurate) names $InsP_7$ and $InsP_8$, respectively. The possibility of placing pyrophosphates on inositol rings with vacant hydroxyls (BOX 2) leaves plenty of room for expansion of this family of inositol phosphates, but we shall ignore those until we know more about how widespread their existence is *in vivo*. In mammalian cells, the only $InsP_7$ identified has the pyrophosphate in the 5-position[81]. The only organisms in which $InsP_8$ isomers have been determined are slime moulds, which show some variety between species, but in *Dictyostelium discoideum* the principal $InsP_8$ has pyrophosphates in the 5- and 6-positions[82] (see FIG. 1; in this figure we have only shown one each of the known $InsP_7$, $InsP_8$ and $Ins(PP)P_4$ species for simplicity).

It is particularly interesting that, in *Dictyostelium*, the pyrophosphate groups on the principal $InsP_8$ are adjacent to each other, because this probably increases the energy released when they are hydrolysed. Voglmaier and colleagues[83] showed that the reverse reaction of $InsP_6$ kinase (the transfer of a phosphate from $InsP_7$ to ADP) was energetically favourable. Moreover, all the proteins (mentioned in the preceding section) that bind $InsP_6$ probably also bind $InsP_7$ and $InsP_8$, sometimes with a higher affinity (AP-180, for instance[61]). Many of these are involved in the assembly of multimolecular structures or membrane fusion events, so it becomes tempting to think that this might be some kind of local energy source that drives whatever processes these proteins control. In yeast cells with no $InsP_6$ kinase, the vacuoles are abnormally small and fragmented[76], indicating that $InsP_6$ and $InsP_7$ might interact with proteins involved in membrane dynamics (for example, AP-2 (REF. 60) and AP-180 (REFS 61,62) might be particularly relevant).

$InsP_7$ and $InsP_8$ apparently turn over much more quickly in animal cells than does $InsP_6$. For example, inhibiting their dephosphorylation with fluoride causes a very rapid rise in their levels, leading Menniti and colleagues[80] to calculate that as much as 20% of the cell's $InsP_6$ might be cycling through $InsP_7$ every hour (see the Shears' lab web site). Emptying Ca^{2+} stores with thapsigargin[84] or raising cAMP in some cells[85] can result in a rapid decrease in their levels, but we have no idea how or why.

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Box 5 | **Inositol phosphate phosphatases**

As with the kinases, our picture of the phosphatases is still incomplete.

5-phosphatases. **At present, there is only one definitively identified member of this group, designated 'type I', that is apparently exclusively an inositol phosphate phosphatase[114] as opposed to an inositol lipid phosphatase. The other members of the family can act on various inositol lipids and phosphates *in vitro*, but are now considered to be primarily inositol lipid phosphatases *in vivo* (see REFS 38,115 for reviews and discussion).**

Multiple inositol polyphosphate phosphatases and diphosphoinositol polyphosphate phosphatase. **Multiple inositol polyphosphate phosphatases (MIPP) was originally cloned from rat[116], and now from several animal species (see the Shears' lab web site for more details). Its name stems from the fact that it can hydrolyse several inositol phosphates *in vitro*, with its favoured substrates being those with four or more phosphates on them, but exactly which reactions shown in FIG. 1 that it catalyses *in vivo* is not known. This uncertainty is compounded by the fact that it is localized within the endoplasmic reticulum[116,117], and as far as we know there are no inositol phosphates there. A knockout of MIPP did not reveal any obvious physiological phenotype[118], although changes in levels of Ins(1,3,4,5,6)P_5 and $InsP_6$ indicated that MIPP might contribute to their hydrolysis. Chi and colleagues[118] also showed that it is unlikely to be the only enzyme hydrolysing Ins(1,3,4,5,6)P_5 and $InsP_6$, and, as is so often true for knockouts, the authors found that compensatory upregulation of another phosphatase had taken place.**

There are now three diphosphoinositol polyphosphate phosphatases (DIPPs), which are probably responsible for the removal of the β-phosphate from the pyrophosphates in $InsP_7$ and $InsP_8$ (REFS 119,120). DIPP2α and DIPP2β differ in only a single amino acid, and some recent evidence has indicated that these might be generated by intron-boundary skidding[121]. The DIPPs contain a NUDT (Nudix-type) DOMAIN, previously found in a group of enzymes that protect cells from various threats such as oxygen radicals[119]. These enzymes can also hydrolyse diadenosine phosphates.

Other phosphatases. **The inositol monophosphate phosphatases and the inositol polyphosphate 1- or 4-phosphatases finish off the dephosphorylation pathways to generate inositol (FIG. 1). They have been extensively reviewed elsewhere[38]. Plants need to hydrolyse phytic acid ($InsP_6$) rapidly, and several phytases have been purified and cloned and their activities characterized, but we have omitted them here for reasons of space.**

The enzymes that phosphorylate $InsP_6$ to $InsP_7$ have been cloned[74,76,86] and are related to some other inositol phosphate kinases (BOX 2). $InsP_7$ kinase has been purified[87], but has yet to be cloned. The principal dephosphorylation route probably involves the DIPP family of phosphatases discussed in BOX 5.

Inositol phosphates and transcription?

As mentioned in the $InsP_6$ section, the third gene identified in yeast that can complement the defect in mRNA export in *Saccharomyces cerevisiae* coded for the yeast InsP multikinase, Arg82 (BOX 2), and the complementation is probably due to its ability to provide precursors (specifically, Ins(1,3,4,5,6)P_5) for $InsP_6$ synthesis[71]. The multikinase activity has also been suggested to have a role in transcriptional regulation[72]. Arg82 regulates yeast growth on nitrogen-containing carbon sources, through its ability to modulate the expression of genes involved in arginine metabolism[88]. Arg82 is targeted to the nucleus, where it binds and sequesters at least two members of the MADS-BOX family of transcription factors, thereby protecting them from rapid degradation and localizing them near their DNA targets[88]. These transcription factors, Arg80 and Mcm1, control the transcription of arginine-metabolizing enzymes by forming a complex on DNA with Arg81, an arginine-sensing transcription factor[88]. Arg82 is not a necessary component of this complex, because overexpression of Mcm1 or Arg80 can reverse the inability of Arg82 mutants to grow on arginine.

How the Ins(1,4,5)P_3 kinase activity of Arg82 relates to growth on nutrients remains unclear. Point mutations that destroy kinase activity do not change the ability of yeast to respond to arginine, whereas point mutations that destroy the ability of Arg82 to bind to Arg80 do[89]. Other aspects of the *ARG82* null phenotype (a sporulation defect and an inability to grow at 37 °C) are not reverted by overexpressing its binding partners Mcm1 or Arg80. Moreover, kinase-dead mutants of Arg82, although not defective in growth on arginine, grow more slowly at 30 °C compared with wild type. Does this indicate that there are nuclear processes that require kinase activity (or can this slow growth be accounted for by effects on mRNA transport — see the section on $InsP_6$)? Alternatively, are there other transcription factors that can be modulated by Arg82, with this modulation being dependent on its kinase activity?

The recent cloning of a mammalian homologue of Arg82 (REF. 90) indicates that such processes could be conserved across species. But it is worth noting that multikinases in other species lack an acidic stretch of amino acids that are crucial for mediating Arg82's interactions with the MADS-box family. Further clarification might come from the unification of earlier observations that, on the one hand, osmotic stress in *Schizosaccaromyces pombe* greatly stimulates the multikinase pathway leading to $InsP_6$ (REF. 73) and, on the other hand, osmotic stress activates numerous changes in transcription, some of which involve MADS-box transcription factors[91].

Conclusion

Myo-inositol is a simple building block on which we now know that nature has wrought great variety. But less than 20 years ago the only suggested functions for inositol phosphates were phosphate storage in plants ($InsP_6$) and haemoglobin modulation in some erythrocytes (Ins(1,3,4,5,6)P_5), and we could not possibly have imagined then the complexity of metabolism, structures and functions that we have reviewed here. But it is nevertheless sobering to think that we have described the known or suspected functions of only seven inositol phosphates (eight if you include Ins(1,3,4)P_3 as a regulator of Ins(3,4,5,6)P_4 levels), and one question on which we would not want to wager money is, how many more will be functional? Scattered through the literature are suggestions for functions of several other inositol phosphates, and at present it seems that all seven polyphosphoinositol lipids have distinct cellular actions. Although that extreme surely cannot be true of the inositol phosphates, we would be disappointed if the functional list is now complete, and when we consider the great structural variety and scope for metabolic independence, it seems impossible to imagine that evolution has not exploited this potential further.

MADS BOX
A superfamily of transcription factors (including Mcm1, agamous, deficiens and serum response factor), which bind DNA and control a plethora of cellular functions.

NUDT DOMAIN
Nudix-type domain, previously known as a MutT domain. Discovered in a group of enzymes that protect cells from threats such as oxygen radicals.

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Understanding how cells regulate inositol phosphate metabolism will be a difficult task (BOX 3), but because the powerful techniques of modern molecular cell biology focus on the enzymes and regulatory proteins with which inositol phosphates interact, they will no doubt facilitate the clarification of those functions suggested above and will also lead to the identification of new functions. Along with this renewed interest and enthusiasm will surely come breakthroughs in even the most intransigent of problems, as has happened with the inositol lipids. In the lipids, the turtle (BOX 1) is anchored to the membrane by its front right flipper, and there is some irony in the fact that it was free-swimming (as Ins(1,4,5)P_3) when it spawned an explosion in cellular signalling that has since then been largely lipid-based. Perhaps it is now safe for it to go back in the water.

The turtle lives 'twixt plated decks
Which practically conceal its sex.
I think it clever of the turtle
In such a fix to be so fertile.

Ogden Nash (1902–1971)

Links

DATABASE LINKS **Src | Ins (1,4,5)P_3 3-kinase | CaM | CaMKII | phospholipase C | Ins(1,4,5)P_3 receptors | GAP1^m | CLCA1 | Ins(3,4,5,6)P_4 1-kinase | SopB | protein kinase C | synaptotagmins | pacsin | phosphoinositide-specific phospholipase C | DNA-dependent protein kinase | AP-180 | AP-2 | Arg82 | Arg80 | Mcm1 | Arg81 | MIPP**

FURTHER INFORMATION **Shears' lab | Pirarucu | Another view of inositol phosphate metabolism | Sources of inositol phosphates | IUBMB nomenclature of inositides**

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