1. Introduction

Myo-Inositol (Ins; see Fig. 1), one of nine distinct cyclohexanehexol isomers, is an essential dietary requirement for the growth of mice (Woolley, 1940) and is greatly concentrated in the milk of lactating mammals (Burton & Wells, 1974, 1976). Although not strictly a vitamin, as it can be synthesized from glucose by many eukaryotic cells (Eisenberg, 1967), yeast mutants which lack this synthetic capacity de novo do not survive in an Ins-free medium (Hanson & Lester, 1980).

Our understanding of the importance of this ubiquitous component of eukaryotic cells has accelerated dramatically during the last few years. It is a constituent of phosphatidylinositol (PtdIns) which accounts for approximately 5–10% of the phospholipids in mammalian cell membranes. In addition to this structural role, PtdIns also is the precursor of phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2]. This minor membrane phospholipid is the substrate for a receptor-dependent phosphoinositidase C (PIC) which cleaves it to give two divergent intracellular signals: inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3], which functions to release Ca^{2+} from microsomal membranes; and 1,2 diacylglycerol, which activates protein kinase C (PKC; Berridge & Irvine, 1984; Nishizuka, 1984; Downes & Michell, 1985). PtdIns also is a component of the PtdIns-glycans which serve as the membrane anchors for an increasing variety of membrane-attached proteins (Low, 1987). Given these functions of PtdIns, it is perhaps not surprising that disruption of the recently cloned yeast PtdIns synthase, which is responsible for the incorporation of Ins into PtdIns, is lethal (Nikawa et al., 1987).

In addition to the lipid metabolites of Ins noted above, cells contain many distinct species of water-soluble inositol phosphate. Many of these are the products of PIC activity or are specific metabolites of Ins(1,4,5)P_3. This situation is complicated by the occurrence in plant seeds, avian erythrocytes and a variety of mammalian tissues of inositol polyphosphates such as inositol pentakisphosphate (InsP_5) and inositol hexakisphosphate (InsP_6; Cosgrove, 1980; Szewczuk et al., 1987). Despite the fact that InsP_6 was isolated from plant seeds more than a century ago relatively little is known of the functions of inositol polyphosphates or of their biosynthesis.

In this article I shall focus upon the receptor-dependent hydrolysis of PtdIns(4,5)P_2; metabolism of the Ca^{2+}-mobilizing second messenger, Ins(1,4,5)P_3; and the identification of novel Ins metabolites including intermediates in the biosynthesis of InsP_6, and a fourth species of inositol phospholipid, phosphatidylinositol 3-phosphate (PtdIns3P). A recurring theme will be the versatility of the Ins structure which allows enormous diversity among metabolites such as the inositol phosphates. Cells may have exploited this potential for diversity by evolving segregated pathways of inositol phosphoester metabolism, perhaps with distinct functions.

Abbreviations used and nomenclature of inositol phosphates: InsP_1, InsP_2, ..., InsP_6; myo-inositol phosphate, myo-inositol bisphosphate, ..., myo-inositol hexakisphosphate (with isomeric numbering of inositol phosphates as appropriate; see Fig. 1). Note that, in line with the forthcoming NC-I.U.B. recommendation which allows relaxation of the lowest number rule of the J.C.B.N. if it clarifies the relationships in biochemical pathways, all numbering is D- unless otherwise stated; PtdIns, phosphatidylinositol; PtdIns3P, phosphatidylinositol 3-phosphate; PKC, protein kinase C; PIC, phosphoinositidase C; GTP[S], guanosine 5'-[γ-thiophosphosphate; GDP[S], guanosine 5'-β-thio-diphosphate; γNH_{2}ppG, guanosine 5'-β,γ-imidodiphosphate; 2SMeATP, 2-thiomethyl ATP.

2. Receptor-stimulated inositol phospholipid metabolism

In 1953 the Hokin (Hokin & Hokin, 1953) observed that stimulating the muscarinic cholinergetic receptors of excocrine pancreas specifically enhanced the metabolic turnover of PtdIns. This report triggered a trail of research that culminated in the discovery of two divergent intracellular signals whose derivation and roles are depicted in Fig. 2. A variety of cell surface receptors for hormones, neurotransmitters,
The physiological role of diacylglycerol came with the availability of myo-inositol in the preferred chair conformation; note that the 2-hydroxyl is axial to the 6-membered ring with the remaining hydroxyls all being equatorial. The $\alpha$-substitution sites are numbered in an anticlockwise direction. With the 2- and 5-positions remaining the same, the $\gamma$-substitution positions are arrived at by numbering in the clockwise direction. The $\alpha$-substitution positions are used throughout the text to denote the structures of all identified inositol phosphate isomers (except where otherwise specified). The total number of possible inositol phosphate isomers is indicated in the lower half of the Figure.

![Structure of myo-inositol](image)

Fig. 1. Structure of myo-inositol allows enormous diversity amongst its derivatives

The discovery of this signal transduction system was not made overnight and in fact took some 30 years to come to fruition. After the pioneering work of the Hokins, a large number of tissues and stimuli were added to the list documenting the occurrence of agonist-stimulated inositol phospholipid metabolism. However, it was not until 1975 that Michell combined knowledge of phospholipid metabolism, receptor pharmacology and cell physiology to propose the unifying hypothesis that hydrolysis of PtdIns by PIC could be an essential reaction responsible for coupling cell surface receptors to the generation of a Ca$^{2+}$ signal. That Ins(1,4,5)P$_3$ fulfilled this role, by acting as the second messenger for internal Ca$^{2+}$ release, was finally demonstrated by Berridge, Irvine and their colleagues (see Streb et al., 1983; Berridge & Irvine, 1984). Diacylglycerol remains in the membrane where it forms a complex with and activates PKC (Michell, 1984). The simultaneous generation of a Ca$^{2+}$ signal and activation of PKC regulates diverse cellular functions including smooth muscle contraction, secretion, activation of inflammatory cells and cell division.

PKC in intact cells (Kaiubuchi et al., 1982; Davies et al., 1985) and with the exciting discovery that PKC was the long-sought-after 'receptor' for tumour-promoting phorbol esters (Castagna et al., 1982). 2.1. Substrate specificity of receptor-dependent PIC. In addition to PtdIns, mammalian cells contain at least two other inositol phospholipids, namely phosphatidylinositol 4-phosphate (PtdIns4P) and PtdIns(4,5)P$_2$ (collectively termed the polyphosphoinositides) which each comprise between about 1 and 5% of the total PtdIns pool. Each of these lipids is degraded by PIC activity in vitro (Irvine et al., 1984; Wilson et al., 1984), but erythrocyte membranes contain a PIC that apparently is specific for the polyphosphoinositides (Davies & Michell, 1981). So an important question that arose in the early 1980s that had an important bearing on future work in this field was which of these lipids is hydrolysed during receptor stimulation (Davies & Michell, 1982)?

Early experiments which addressed this question analysed the phospholipid content of cells exposed to appropriate stimuli. PtdIns(4,5)P$_2$ breakdown was observed when iris smooth muscle was exposed to cholinergic and adrenergic stimuli, but this response appeared to be a consequence rather than a cause of the Ca$^{2+}$ signal in such cells (Akhtar & Abdel Latif, 1980). J. Creba and I, working in the laboratories of C.J. Kirk and R.H. Michell at the University of Birmingham, then observed that the PtdIns(4,5)P$_2$ content of rat hepatocytes fell rapidly in response to vasopressin and other Ca$^{2+}$-mobilizing hormones that were known to stimulate inositol phospholipid turnover. Since the initial rate of
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PtdIns(4,5)P₂ disappearance was similar to the overall rate of PtdIns breakdown, we proposed that PtdIns(4,5)P₂ might be the true substrate for a receptor-sensitive PIC with PtdIns being consumed as a substrate for PtdIns(4,5)P₂ resynthesis. Moreover, as PtdIns(4,5)P₂ breakdown in these cells was relatively insensitive to experimental manipulation of the intracellular Ca²⁺ signal, it was a plausible candidate for a reaction coupling vasopressin receptor activation to Ca²⁺ mobilization (Michell et al., 1981; Downes & Michell, 1982).

Similar results to those described above were obtained by a number of laboratories using a variety of tissues and stimuli, suggesting that receptor-sensitive PtdIns(4,5)P₂ breakdown was a widespread phenomenon (Weiss et al., 1982; Bilah & Lapetina, 1982). However, the results of such experiments are ambiguous because they do not define the mechanism of PtdIns(4,5)P₂ breakdown, nor do they distinguish between the proposed model and one in which PtdIns is the substrate for PIC with PtdIns(4,5)P₂, disappearing as it equilibrates with the reduced level of PtdIns. To establish the involvement of PIC, it was necessary to demonstrate accumulation of the expected products of such a reaction. In practice, this meant developing methods for the detection and analysis of cellular inositol phosphates, as their accumulation in cells would not only indicate participation of a PIC, but would also provide essential information on its substrate specificity.

2.2. Accumulation of inositol phosphates in stimulated cells. Durrell was the first to demonstrate the accumulation of inositol phosphates in stimulated cells (Durrell et al., 1969). His studies with cholinergically stimulated brain slices were largely ignored for many years yet they provided the most convincing evidence at the time for the participation of a PIC in cellular responses and, since an inositol bisphosphate was detected, suggested that a polyphosphoinositide must be at least one of its substrates.

With the need to determine which of the inositol phospholipids was the primary substrate for receptor-sensitive PIC as a major goal in the early 1980s, routine techniques were established for the extraction, detection and separation of cellular inositol phosphates (Downes & Michell, 1981; Berridge et al., 1982, 1983). These techniques, which involved labelling of cells with [³H]inositol and separation of water-soluble inositol phosphates on small anion-exchange columns, were subsequently adopted by many different groups and rapidly established the nature of the early biochemical events after exposure of cells to agonists. Inositol trisphosphate and inositol bisphosphate were both found to accumulate within seconds of stimulation, but inositol monophosphate accumulated only after a significant time lag (Berridge, 1983; Downes & Wusteman, 1983; Drummond et al., 1983; Rebecchi & Gershengorn, 1983; Martin, 1983; Aub & Putney, 1984; Thomas et al., 1984). These results suggested that agonists stimulate a polyphosphoinositide-specific (possibly PtdIns(4,5)P₂-specific) PIC and, with knowledge of the second messenger functions of Ins¹,4,5P₃ and diacylglycerol, helped to establish the dual-signal hypothesis of receptor-stimulated inositol phospholipid metabolism.

3. The molecular basis of PIC regulation

The experimental evidence described above pinpoints a polyphosphoinositide-specific PIC as the agonist-sensitive component of inositol phospholipid metabolism. But how do cell surface receptors communicate with the enzyme to greatly accelerate the rate of PtdIns(4,5)P₂ hydrolysis?

It has been known for some time that coupling of hormone receptors to adenylyl cyclase and light-activated rhodopsin to cyclic GMP phosphodiesterase is effected by a family of guanine-nucleotide-dependent regulatory proteins (G-proteins: Gilman, 1984, 1987). More recently, it has become clear that a distinct G-protein(s) serves to couple receptors to PIC. For example, non-hydrolysable analogues of GTP (such as guanosine 5'-y-thio)triphosphate (GTP[yS]) modulate the binding of agonists to receptors that are coupled to PtdIns(4,5)P₂; hydrolysis, evoke Ca²⁺-dependent secretion when introduced into permeabilized mast cells, and enhance the Ca²⁺-sensitivity of the secretory response of permeabilized platelets (see Taylor & Merritt, 1986 and references therein).

Cockroft & Gomperts (1985) and Litosch et al. (1985) were the first to demonstrate that guanine nucleotides could directly stimulate the hydrolysis of inositol phospholipids in membrane preparations obtained from ³P-labelled neutrophils and [³H]inositol-labelled blowfly salivary glands, respectively. These original observations were later confirmed by using [³H]inositol-labelled membranes from a wide variety of tissue sources (see Taylor & Merritt, 1986).

The results of experiments with guanine nucleotides thus provide compelling evidence that hydrolysis of endogenous membrane phosphoinositides by membrane-bound PIC is regulated by guanine nucleotides in a manner suggesting the involvement of a G-protein. However, whereas much is known of the molecular events involved in receptor and G-protein-dependent regulation of adenylyl cyclase (Levitzki, 1982, 1986; Gilman, 1984, 1987; Helmreich & Pfueffer, 1985), until recently, comparatively few details were understood of the mechanism of PIC activation. The currently accepted model describing the function of a G-protein (termed G₁) in the activation of adenylyl cyclase is shown in Fig. 3. The known G-proteins are heterotrimers with α-, β-, and γ-subunits. The guanine-nucleotide-binding site, which binds GDP in the resting state, is located on the α-subunit as is the GTPase which is a characteristic function of all G-proteins. Activation of a G-protein receptor with a suitable agonist, in the presence of GTP, leads to the formation of the hormone/receptor/G-protein ternary complex and greatly accelerates the rate of exchange of GTP for GDP. This is a first-order process the rate of which is dependent upon the proportion of activated receptors and, moreover, the function of the receptor appears to be catalytic in that a single agonist-occupied receptor is capable of activating many molecules of G-protein. In its GTP-bound form the α-subunit now dissociates from the βγ complex and is capable of activating adenylyl cyclase. Thus the G-protein functions as a shuttle between the receptor and effector proteins. The lifetime of the active G-protein species is limited by the GTPase function of G₁, which reverts to the GDP-γS form and consequently reassociates with the βγ complex. Inhibition of the GTPase activity of G₁ by either the use of non-hydrolysable analogues of GTP or after cholera-toxin-catalysed ADP-ribosylation, greatly enhances adenylyl cyclase activity as the G-protein is converted into a quasi-permanently active state.

A similar mechanism to that described above apparently is involved in light-induced inhibition of cyclic GMP phosphodiesterase in the retina, where rhodopsin and transducin are, respectively, the light receptor and G-protein. Hormone-controlled inhibition of adenylyl cyclase is mediated by a family of receptors distinct from those that stimulate the enzyme, and involves a distinct inhibitory G-protein (Gᵢ). Activation of this G-protein is analogous to the activation of G₁, but the mechanism of inhibition of the adenylyl cyclase catalytic moiety is still debated, as there appears to be both a direct inhibitory influence of GTP-bound Gᵢ as well as an indirect mechanism involving the association of free βγ subunits with Gᵢ. In this respect it is noteworthy that the βγ subunits of known G-proteins are all remarkably similar, if not identical.
the presence of purinergic agonists together with GTP or one of its non-hydrolysable analogues [Harden et al., 1988; Boyer et al., 1989].

Compared with G-protein-stimulated PIC activities reported for mammalian cells (see Taylor & Merritt, 1986), the activity of the turkey erythrocyte enzyme is notable in both the magnitude and stability of response to G-protein and receptor stimulants. Consequently it has been possible to determine several mechanistic features of this membrane transduction system.

3.1.1. Kinetics of PIC activation and inactivation. Addition of GTP[S] to [3H]inositol-labelled turkey erythrocyte membranes induced only a very slow release of labelled inositol phosphates with a pronounced lag phase. By contrast, a potent and selective P2 receptor agonist, 2-thiomethyl ATP (2SMeATP), which stimulated inositol phospholipid breakdown when applied to intact turkey erythrocytes, had no effect when added to membranes in the absence of GTP. However, the purinoceptor agonist greatly potentiated the response to GTP[S]. The construction of detailed time courses revealed that the activation process obeyed first-order kinetics. Although the eventual extent of PIC activation depended on guanine nucleotide concentration, the first-order rate constant for the activation process increased in proportion to agonist concentration. Thus the effect of receptor stimulation was to greatly decrease the lag phase for PIC activation by GTP[S], a finding that is remarkably similar to that made for β-receptor and G-protein-dependent stimulation of adenylate cyclase using the same membrane preparation (Levitzki, 1982, 1986).

Two additional findings on the activation of turkey erythrocyte PIC are worthy of note at this point. The first is that although a clear lag phase was still apparent when PIC was activated using optimal concentrations of GTP[S] and 2SMeATP, no lag phase was observed when GTP itself replaced GTP[S]. This suggests that agonist-dependent stimulation of PIC in intact cells will occur rapidly enough to account for the observed increase in Ins(1,4,5)P3 within seconds of stimulation. It also suggests that the lag phase cannot be accounted for solely by the relatively slow off-rate of bound GDP from the G-protein, as has been proposed for adenylate cyclase activation by Gs. The second is that the maximum activity of PIC achieved by adding a large excess of GTP[S] was only about half that attained when it was activated by GTP[S] plus the receptor agonist. At present we have no simple explanation for the latter observation.

The above experiments define factors that influence the on-rate for G-protein-dependent activation of PIC, but what is the evidence that the off-rate is determined by an endogenous GTPase? Most reports of guanine nucleotide stimulation of PIC activity have noted that non-hydrolysable analogues of GTP are much more effective than GTP itself (Litosh et al., 1985; Hepler & Harden, 1986; Martin et al., 1986; Uihling et al., 1986). We have found this also to be the case for turkey erythrocyte PIC with GTP[S] being the most effective guanine nucleotide tested, guanosine 5′-β,γ-imidodiphosphate (p[NH]ppG) having 50–70% of the efficacy of GTP[S] and GTP only about 20% (Harden et al., 1987; Boyer et al., 1988). Indeed, GTP itself was completely ineffective unless a receptor agonist was present.

Guanosine 5′-β-thiodiphosphate (GDP[S]) was a competitive inhibitor with respect to each of the GTP analogues and could, therefore, be used as a tool to study rates of PIC inactivation [Boyer et al., 1988]. In these experiments PIC was first stimulated by 2SMeATP and one of each of the GTP analogues. After the attainment of a maximum rate of inositol phosphate release, a large excess of GDP[S] was added and the subsequent decline in PIC activity monitored. When GTP was used in the activation phase of the experiment, activity returned rapidly to basal upon addition of

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Fig. 3 **G-protein mediated regulation of adenylate cyclase**

G-proteins involved in the regulation of adenylate cyclase (Gs, which is responsible for cyclase stimulation and Gi, which is responsible for cyclase inhibition) are heterotrimers which bind GDP to their α-subunits in the resting state. Agonist-occupied receptors catalyse a nucleotide exchange (GTP for GDP) reaction and the resulting GTP liganded α-subunit dissociates from the βγ complex. αGTP is responsible for stimulation of adenylate cyclase while αGDP and/or free βγ subunits account for inhibition of the enzyme (see text for details). The lifetime of the active species is limited by an endogenous GTPase located on G-protein α-subunits which converts bound GTP to GDP. Reassociation of αGTP with βγ completes the cycle of G-protein activation and inactivation. Note that this model predicts that the concentration of the active αGTP species will be proportional to the number of agonist-occupied receptors and that the use of non-hydrolysable analogues of GTP will interrupt the cycle by generating a quasi-permanently activated state.
GDP[S]. However, when pNHppG was used to activate, the off-rate was very much slower than was observed for GTP and when GTP[S] was used, inactivation after addition of GDP[S] was barely perceptible. These results are exactly as would be anticipated given the relative efficacies of the three GTP analogues and are at least in agreement with the notion that the rapid off-rate observed with GTP is probably due to its relatively rapid conversion to GDP by an endogenous GTPase.

3.2. Conclusions and future work. Studies of turkey erythrocyte membranes have provided much valuable information on the mechanistic principles underlying hormone-dependent stimulation of adenylate cyclase and PIC. However, whereas the G-proteins involved in regulation of adenylate cyclase have been unequivocally identified, the same cannot yet be said, either of the G-protein(s) responsible for PIC activation, or of PIC species capable of communicating with cell-surface receptors. As there are at least two PIC stimulatory G-proteins [one apparently sensitive to inhibition by pertussis toxin and the other not; see Taylor & Marine (1986) and references therein] and considerable heterogeneity among PICs (for example, several distinct PICs have recently been cloned and sequenced; Stahl et al., 1988; Suh et al., 1988; Bloomquist et al., 1988; Bennett et al., 1988) the successful reconstitution of PIC and G-protein species will be greatly facilitated if both proteins are isolated from a single cell type. In this respect turkey erythrocytes provide a good, albeit somewhat abundant supply of simply prepared plasma membranes from a predominantly homogeneous cellular source. The isolation and selective reconstitution of turkey erythrocyte PIC and G-protein species thus represent important landmarks in future work.

4. The metabolism of inositol phosphates

As noted in section 2.2 above, most early studies of cellular inositol phosphates used chromatography on small anion-exchange columns to separate distinct fractions of water-soluble inositol metabolites. These methods have since been augmented by h.p.l.c. techniques which have revealed an unexpected heterogeneity of cellular inositol metabolites, starting in 1984 and references therein) and considerable intracellular inositol trisphosphate that accumulated in carbachol-stimulated parotid glands was not Ins(1,4,5)P3, but Ins(1,3,4)P3 (Irvine al., 1987a). However, at neutral pH and at physiological ionic strength, Ins(1,4,5)P3 predominates (Harden et al., 1988). The cyclic derivative is metabolized rather slowly (Hawkins et al., 1987) and so can accumulate to levels that eventually approach those of Ins(1,4,5)P3 (Sekar et al., 1987). The relatively slow kinetics of Ins(1,2-cyclic,4,5)P3 metabolism suggest it is unlikely to function as a Ca2+-mobilizing signal and, as a quantitatively minor product of PIC activity, it contributes little to the inositol homeostatic mechanisms discussed in section 4.2. For a more detailed consideration of the metabolism and possible functions of inositol cyclophosphates see Majerus et al. (1986, 1988).

There are two independent pathways for the metabolism of Ins(1,4,5)P3, both of which yield compounds that do not release Ca2+ from intracellular stores. A 5-phosphomonoesterase converts Ins(1,4,5)P3 to Ins(3,4,5)P3 (Downes et al., 1982; Connolly et al., 1985), while a 3-kinase is responsible for the synthesis of Ins(1,3,4,5)P4 (Batty et al., 1985; Irvine et al., 1986). The latter pathway is especially interesting because the kinase in many tissues is regulated by Ca2+/calmodulin (Biden et al., 1987; Morris et al., 1987a; Ryu et al., 1987; Johansen et al., 1988; Takezawa et al., 1988; Daniel et al., 1988; Yamaguchi et al., 1988) and because it has been proposed that Ins(1,3,4,5)P4 functions as a signal in its own right, either by stimulating Ca2+ influx into cells, or by enhancing Ca2+ movements between cellular compartments (Irving & Moor, 1986; Morris et al., 1987b). Ins(1,3,4,5)P4 can itself be metabolized by 5-phosphomonoesterase activity, a reaction that appears to account for the accumulation of Ins(1,3,4)P3 in many cells (Batty et al., 1985; Connolly et al., 1987).

4.2. Conservation of intracellular inositol. Ins(1,4,5)P3 and Ins(1,3,4)P3 are dephosphorylated in a series of reactions whose primary and, very probably, only function is the recovery of Ins (see Downes, 1988). These pathways are catalysed by a minimum of four distinct enzymes, two of which are Mg2+-dependent and are involved in the manufacture of an anti-manic drug, Li+. The other two are metal-ion-independent enzymes (see Fig. 4; Majerus et al., 1988).

Ins(1,3,4)P3 and Ins(1,4)P3 can both be hydrolysed by a Li+-sensitive 1-phosphomonoesterase to give Ins(3,4)P2 and Ins4P, respectively (Inhorn & Majerus, 1987; Gee et al., 1988a). There is also an alternative pathway for Ins(1,3,4)P3 hydrolysis which involves two distinct Mg2+-independent, Li+-insensitive enzymes. One converts Ins(1,3,4)P3 to Ins(1,3)P2, and may also utilize Ins(3,4)P2 as a substrate converting it to Ins1P. The second enzyme converts Ins(1,3)P2 to Ins3P (Majerus et al., 1988; Bansal et al., 1987).

The dephosphorylation reactions described above converge upon a series of inositol monophosphate isomers each of which appears to be a substrate for a single inositol monophosphate phosphomonoesterase (Sherman, 1988). This enzyme has been purified to homogeneity from bovine brain, and is Mg2+-dependent and is potentially inhibited by Mg2+. Moreover, it occupies a pivotal position in inositol homoestasis because it resides at the confluence of two pathways for maintenance of intracellular inositol levels; the conservatory pathways were described above; and the other, which is Mg2+-independent, is via active and/or passive uptake from the medium as depicted in Fig. 5. Li+ inhibits both the inositol monophosphate phosphomonoesterase and the inositol polyphosphate 1-phosphate phosphomonoesterase by a non-competitive mechanism (Hallcher & Sherman, 1980; Majerus et al., 1988; Gee et al., 1988b). As this is a rare form of inhibition, it seems likely that these distinct enzymes are structurally and evolutionarily related.

A non-competitive inhibitor binds preferentially to the enzyme substrate complex so that the observed k is substrate-dependent (1.0 and 0.26 mm when Ins1P and Ins4P, respectively, are used as substrates of the monophosphate phosphomonoesterase; Gee et al., 1988b). This is an important aspect of the therapeutic utility of Li+ because with conventional, competitive inhibitors the block will eventually be overcome as the substrate concentration rises, but with a non-competitive mechanism the inhibitor exerts a stranglehold on the enzyme which tightens as the substrate level...
There are three possible sources of intracellular inositol hydrolysis involve Li⁺ by a Na⁺-dependent pump, appears to be insensitive to Li⁺. From the medium, either by a low-affinity, passive carrier, or the extent of activation of PIC-coupled receptors. Uptake of single tissue, may be influenced by external factors such as inositol from stimulus-dependent products of inositol lipid synthesis, which relative contributions to cellular inositol homoeostasis vary substantially between different tissues and, within a single tissue, may be influenced by external factors such as the extent of activation of PIC-coupled receptors. Uptake from the medium, either by a low-affinity, passive carrier, or by a Na⁺-dependent pump, appears to be insensitive to Li⁺. Both synthesis de novo from glucose and the recovery of inositol from stimulus-dependent products of inositol lipid hydrolysis involve Li⁺-sensitive steps.

Fig. 4. Recovery of myo-inositol from inositol phosphates generated during receptor activation

The Figure depicts the pathways for dephosphorylation of Ins(1,4)p; and Ins(1,3,4)p involving four distinct enzymes. Non-competitive inhibition by Li⁺ of two of these activities at the points indicated leads to a large accumulation of several inositol phosphate isomers at the expense of intracellular inositol, especially in agonist-stimulated cells.

Fig. 5. Cellular mechanisms of myo-inositol homoeostasis

There are three possible sources of intracellular inositol whose relative contributions to cellular inositol homoeostasis vary substantially between different tissues and, within a single tissue, may be influenced by external factors such as the extent of activation of PIC-coupled receptors. Uptake from the medium, either by a low-affinity, passive carrier, or by a Na⁺-dependent pump, appears to be insensitive to Li⁺. Both synthesis de novo from glucose and the recovery of inositol from stimulus-dependent products of inositol lipid hydrolysis involve Li⁺-sensitive steps.

Increases. This suggests that Li⁺ will selectively affect those cells in which PIC-coupled receptors are being actively stimulated because the inositol phosphate levels in such cells will be elevated (Berridge et al., 1982).

A major consequence of the inhibitory activity of Li⁺ is a stimulus-dependent fall in the cellular content of Ins which inhibits resynthesis of PtdIns (Allison & Stewart, 1971; Drummond & Raeburn, 1984; Downes & Stone, 1986). This in turn may reduce the supply of substrate for second messenger synthesis providing a hypothetical mechanism for the therapeutic utility of Li⁺. Moreover, Li⁺ may be used as a tool in situations, such as embryo development, where the conservation pathway is a dominant source of intracellular Ins, to probe the cellular mechanisms which depend upon it (Busa, 1988).

4.2. Synthesis of inositol polyphosphates. InsP₃ and InsP₅ are now known to be relatively ubiquitous cellular components, yet, despite the fact that they were among the earliest inositides to be identified, little is known of their cellular functions or of how they are made. InsP₃ is a major phosphoric ester in plant seeds, where it has been suggested to function as a phosphorus reserve, and InsP₅ is present at very high levels in avian erythrocytes, where it may function as an allosteric regulator of oxygen binding to haemoglobin, as does 2,3-bisphosphoglycerate in mammalian erythrocytes (Cosgrove, 1980). More recently, however, [³H]inositol-labelled compounds with the chromatographic properties of InsP₃ and InsP₅ have been found in an increasing variety of cultured cell lines (Heslop et al., 1985; Jackson et al., 1987), and they have been detected in animal tissues by [³P] n.m.r. at concentrations ranging between 5 and 15 μM (Szwergold et al., 1987).

Hanley and his colleagues recently have proposed that inositol polyphosphates may have extracellular functions and they have demonstrated potent excitatory effects following the microinjection of either InsP₃ or InsP₅ into the nucleus tractus solitarius, a brain stem nucleus involved in cardiovascular and respiratory control (Vallejo et al., 1987). However, the widespread tissue distribution of inositol polyphosphates suggests a more general role in addition to their proposed activity as excitatory neurotransmitters and it seems likely that there is still much to learn of their cellular functions.

As a first step towards defining the cellular significance of inositol polyphosphates, recent studies, devised and carried out in my laboratory mainly by Leonard Stephens and Phillip Hawkins, have focused upon the biosynthesis of InsP₅ in avian erythrocytes and mammalian tissues.

4.3.1. Identification of InsP₅ isomers. To determine whether different cells contain common precursors of InsP₅, a structural analysis of InsP₅ present in chick erythrocytes, (which contain large amounts of InsP₃) and murine macrophages was undertaken (Stephens et al., 1988a). InsP₅ fractions were isolated by anion-exchange h.p.l.c. of acid extracts prepared from [³H]inositol-labelled cells and analysed using the procedure outlined in Fig. 6. Periodate oxidation, which cleaves vicinal hydroxyl-bearing carbon-carbon bonds in the parent inositol phosphate, followed by reduction and dephosphorylation, yielded [³H]-labelled polyols that could be identified by co-chromatography with known standards. In the past this was achieved using tedious paper chromatography procedures, but we have developed a rapid and versatile h.p.l.c. system that greatly simplifies polyol separation.

[³H]inositol and [³H]dipicolinate were the only polyols present after subjecting the InsP₅ fractions from chick erythrocytes and murine macrophages to the above procedure. Inositol, which comprised between 20 and 30% of the polyols derived from both cellular sources, could arise from any one of the nine InsP₅ isomers that are not susceptible to periodate oxidi-
Periodate oxidation of this compound leaves the inositol ring between carbons-1 and -2 yielding, after reduction with NaBH₄ and dephosphorylation using alkaline phosphatase, a hexitol that could be identified as iditol by its mobility on a polyprop carbonate column. The enantiomeric configuration of the iditol, and hence of the original InsP₄, was established by monitoring its conversion to l-sorbose by a commercially available preparation of L-iditol dehydrogenase.

![Diagram](image)

**Fig. 6. Strategy for the identification of the predominant InsP₄ isomer present in avian erythrocytes and murine macrophages**

Ins(3,4,5,6)P₄ was the major InsP₄ present in unstimulated chick erythrocytes and murine macrophages. Periodate oxidation of this compound leaves the inositol ring between carbons-1 and -2 yielding, after reduction with NaBH₄ and dephosphorylation using alkaline phosphatase, a hexitol that could be identified as iditol by its mobility on a polyprop carbonate column. The enantiomeric configuration of the iditol, and hence of the original InsP₄, was established by monitoring its conversion to l-sorbose by a commercially available preparation of L-iditol dehydrogenase.

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5. Discovery of a novel inositol phospholipid: PtdIns3P

Since the elucidation of the headgroup structures of PtdIns4P and PtdIns(4,5)P$_2$, a number of unsuccessful attempts have been made to detect additional polyphosphoinositide species (Santiago-Calvo et al., 1964; Downes et al., 1986; Hawkins et al., 1986). However, studies in collaboration with L. C. Cantley and his colleagues at The Department of Physiology, Tufts University School of Medicine in Boston, have identified PtdIns3P as a product of a novel PtdIns kinase and as a minor constituent of several cell types (Whitman et al., 1988; Stephens et al., 1989). As with the occurrence of multiple inositol phosphate isomers, the synthesis of this novel lipid is no metabolic accident, but rather is the product of a specific enzyme species.

Cantley and his colleagues have identified two distinct PtdIns kinases in fibroblasts (Whitman et al., 1987). The major activity is a 'conventional', adenine-inhibitable PtdIns kinase that we have shown to phosphorylate the D-4 position on the inositol ring of PtdIns to give PtdIns4P. A relatively minor PtdIns kinase activity is inhibited by detergents such as Triton X-100, is insensitive to adenosine and associates specifically with activated tyrosine kinases. Examples of the latter include immunoprecipitates of the middle T/p$_{60}$- from middle T-transformed NIH-3T3 cells and anti-phosphorysine immunoprecipitates from platelet-derived growth factor stimulated Balb/c-3T3 cells. This PtdIns kinase, which the Cantley group have termed 'type I', specifically phosphorylates the D-3 position of PtdIns. Thus, a more useful terminology, which I propose here, uses the terms PtdIns 3-kinase and PtdIns 4-kinase for the 'type I'and conventional activities, respectively.

To confirm that PtdIns 3-kinase activity is expressed in intact cells it was necessary to demonstrate the occurrence of PtdIns3P in cell membranes. Preliminary experiments revealed the presence in 3T3 cells of a phospholipid which upon deacylation gave a glycerol derivative with the chromatographic properties of glycerophosphorylinositol 3-phosphate. The putative PtdIns3P comprised about 3% of the total PtdIns pool (Whitman et al., 1988). More recently, PtdIns3P has been definitively identified in 1321N1 astrocytoma cells, where it comprised between 5 and 15% of the PtdIns pool (Stephens et al., 1989).

The function of PtdIns3P is currently unknown, but the specific association of PtdIns 3-kinase with activated tyrosine kinases suggests that it may have a role in the transduction of mitogenic signals. One idea is that it may be further phosphorylated to give a series of novel phosphoinositides, a possibility that is strengthened by the observation of a putative PtdIns$_3$P$_1$ that appears transiently after stimulation of neutrophils with the chemotactic peptide, N-Met-leu-Phe (Traynor-Kaplan et al., 1988). Alternatively, PtdIns3P itself, rather than any putative metabolites, could act as a mediator of mitogenic signals.

Whatever is the function of PtdIns3P, the occurrence of this novel phospholipid is further testimony to the extraordinary versatility of Ins as a building block for regulatory molecules. Distinct pathways of inositol phospholipid and inositol phosphate metabolism can coexist within a single cell, apparently segregated by the positional specificity of the phosphotransferase activities that catalyse all of the interconversions I have discussed in this article. Most of the participants in these pathways act simply as metabolic stepping stones between molecules of likely functional significance. The complete elucidation and functional assignment of all of the pathways of Ins metabolism represent an important challenge for future work in this field.

Fig. 7. Inositol phosphate metabolism: inactivation of signal molecules, recovery of myo-inositol and synthesis of co-signals

The current knowledge of the complexities of inositol phosphate metabolism is summarized in this Figure. Ins(1,3,4,5,6)$P_5$ is metabolized by 5-phosphomonoesterase and 3-kinase activities and the products of these reactions are dephosphorylated to replenish the cell's reserves of myo-inositol (see Fig. 4). Ins(1,3,4,5,6)$P_5$ can be synthesized from Ins(3,4,5,6)$P_4$, whose metabolic origin is still unknown, by a 1-kinase. A second route for synthesis of the same Ins$P_4$ isomer potentially links inositol polyphosphate metabolism with the fast signalling events initiated by PtdIns4(5,4,5)$P_3$ hydrolysis. The route involves sequential phosphorylation of Ins(1,3,4)$P_3$ and Ins(1,3,4,6)$P_4$ by distinct 6- and 5-kinases. Note that although many tissues have been shown to contain Ins$P_3$, the Ins$P_4$ isomer that presumably acts as its precursor has not been defined.

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