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COMMENTARY

METABOLISM AND BIOLOGICAL ACTIVITIES OF INOSITOL PENTAKISPHOSPHATE AND INOSITOL HEXAKISPHOSPHATE

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Phosphatidylinositol 4,5-bisphosphate, a phosphoinositide, is now recognized to be the precursor for several informational molecules in signal transduction, including Ins1,4,5-P_3§, 1,2-diacylglycerol, and phosphatidylinositol 3,4,5-trisphosphate [1, 2]. However, it has been appreciated for some time that Ins1,4,5-P_3 is but one member of an extraordinarily large family of naturally occurring inositol phosphate isomers that show complex patterns of interconversion. One of the first indications of this complexity was the discovery of a second "inositol trisphosphate" using high resolution ion exchange methods. This was shown to be Ins1,3,4-P_3, which derived from another unsuspected inositol phosphate, Ins1,3,4,5-P_4 [3]. Ins1,4,5-P_3 is phosphorylated rapidly by Ins1,4,5-P_3 3-hydroxykinase to give Ins1,3,4,5-P_4, which has been proposed to have regulatory functions on Ca^{2+} sequestration [4], Ca^{2+} influx [5, 6] and membrane ion currents [7]. These two metabolites are the only inositol phosphates for which biological activities have been demonstrated unambiguously, and, of the two, only Ins1,4,5-P_3 has an undisputed physiological role.

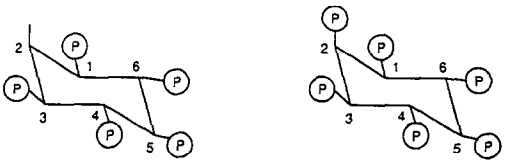

Fig. 1. Structures of inositol1,3,4,5,6-pentakisphosphate and inositol hexakisphosphate. Circled P = phosphate.

However, the most abundant inositol phosphates in many, if not all, cells are the higher inositol polyphosphates, $InsP_5$ and $InsP_6$ (see Fig. 1). These inositol phosphates were thought previously to be restricted to plants and the nucleated erythrocytes of lower vertebrates. In the erythrocytes of avian, reptilian and amphibian species, $InsP_5$ was suggested to modulate the oxygen binding affinity of hemoglobin [8], whereas in plant seeds and tissues $InsP_6$ (or "phytic acid") was believed to be a phosphate store, or a metal ion chelator (forming a specific ion complex, "phytin") [9]. However, metabolic labeling of mammalian cells [10, 11] and tissues [12] with [^{3}H]inositol and ion exchange fractionation of radioactively labeled inositol phosphates revealed that these higher inositol polyphosphates were widespread, perhaps ubiquitous, metabolic products of inositol. These compounds exhibited sluggish metabolic turnover, suggesting that they did not act like Ins1,4,5-P_3 in acute regulation of cellular responses. Similarly, the levels inferred from steady-state radiolabeling and measured directly by several techniques have, in all cases, suggested that they are maintained at relatively high levels, generally from 10 to 100 μM [10, 11, 13]. Detailed studies of the metabolism of the higher inositol polyphosphates have highlighted a remarkable investment of both energy and genetic complexity in their biosynthesis and catabolism. In this review, we summarize the metabolic pathways regulating levels of $InsP_5$ and $InsP_6$, and indicate possibilities for cellular functions.

Metabolism

Turnover of $InsP_5$ and $InsP_6$. Figure 2 summarizes

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§ Abbreviations: Ins1,4,5-P_3, inositol 1,4,5-trisphosphate; Ins1,3,4-P_3, inositol 1,3,4-trisphosphate; Ins1,3,4,5-P_4, inositol 1,3,4,5-tetrakisphosphate; Ins1,3,4,6-P_4, inositol 1,3,4,6-tetrakisphosphate; Ins3,4,5,6-P_4, inositol 3,4,5,6-tetrakisphosphate; $InsP_5$, inositol pentakisphosphate; Ins1,3,4,5,6-P_5, inositol 1,3,4,5,6-pentakisphosphate; Ins1,2,3,4,6-P_5, inositol 1,2,3,4,6-pentakisphosphate; Ins1,2,4,5,6-P_5, inositol 1,2,4,5,6-pentakisphosphate; $InsP_6$, inositol hexakisphosphate; $InsP_5P$, inositol pentakisphosphate pyrophosphate; $InsP_6P$, inositol hexakisphosphate pyrophosphate; Ins, inositol; InsP, inositol monophosphate; and $InsP_2$, inositol bisphosphate.

Note on nomenclature: Ins is an accepted IUPAR abbreviation for D-*myo*-inositol. Unless otherwise indicated, $InsP_4$ will refer to the specific isomer Ins1,3,4,5-P_4 and $InsP_5$ will refer to Ins1,3,4,5,6-P_5.

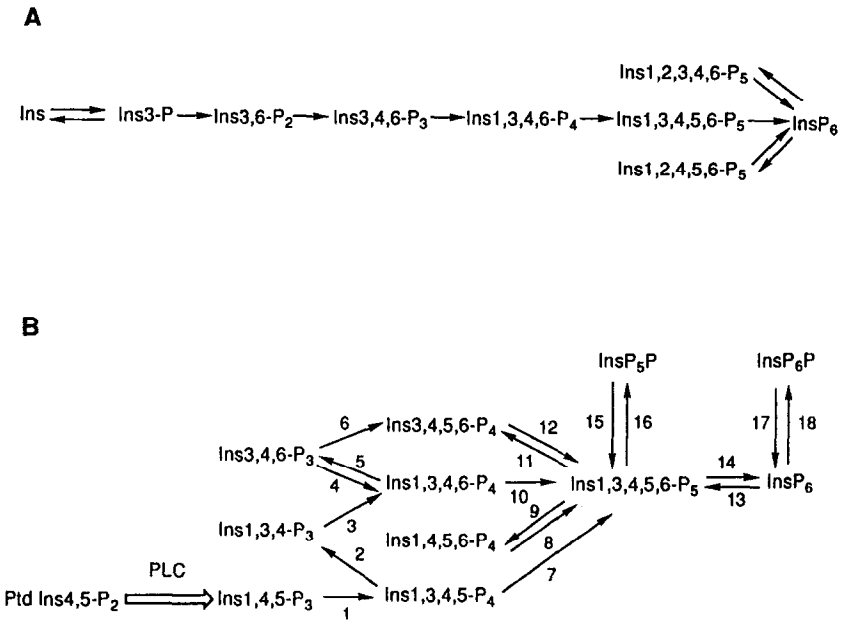

Fig. 2. Metabolism of inositol polyphosphates. (A) *De novo* biosynthesis of $InsP_5$ and $InsP_6$ in *Dictyostelium discoidium* (derived from [15]). (B) Identified inositol phosphate isomers contributing to the synthesis and breakdown of $InsP_5$ and $InsP_6$, including isomers produced from receptor activation. Each metabolic conversion (from **1** to **18**) has been reported (in the following papers), but it is unclear how many of these conversions may be cell-specific. For completeness we have illustrated all of these conversions in a single comprehensive figure. **1** and **2**: Ref. 3; **3**: Ref. 20; **4** Ref. 15; **5** and **6**: Ref. 78; **7**: Ref. 24; **8**: Ref. 22; **9**: Ref. 23; **10**: Refs. 19, 20 and 23; **11**: Ref. 21; **12** Refs. 21 and 79; **13**: Ref. 27; **14**: Refs. 16 and 80; **15–18**: Refs. 17 and 18.

the reported metabolic pathways for $InsP_5$ and $InsP_6$. The main route by which $InsP_5$ and $InsP_6$ are made *de novo* in animal cells is not established but is almost certain to be independent of Ins1,4,5-P_3 and inositol lipid metabolism [1, 14]. The most comprehensive analysis of the biosynthesis of $InsP_6$ was undertaken by chemical characterization of the radioactive products of [^{3}H]inositol incorporation in the slime mold *Dictyostelium* [15] (Fig. 2A). The key feature of the pathway, likely to be the major one for the *de novo* synthesis of $InsP_6$ in many cell types [16], is a series of positionally selective, sequential kinase reactions, beginning with the direct phosphorylation of *myo*-inositol. Thus, the production of $InsP_6$ does not proceed through a lipid intermediate, and is characterized by a distinctive set of inositol phosphate isomers that are distinct from those identified with inositol lipid metabolism. In addition, the 3- and 5-phosphates of $InsP_6$ are rapidly removed and replaced in metabolic "futile cycles," implying position-specific phosphatases engaged in continuous turnover. There is not, at this point, a comparable analysis of the pathway for formation of $InsP_5$, although it is likely to be formed by an independent series of positional isomers, and not simply as an intermediate in the synthesis of $InsP_6$.

An additional level of complexity has been the discovery of inositol pyrophosphates produced from both $InsP_5$ and $InsP_6$ [17, 18]. These compounds attract special interest because the conversion rates of $InsP_5$ and $InsP_6$ to the inositol pyrophosphates ($InsP_5P$ and $InsP_6P$) are remarkably high [17]. The positional specificity of the pyrophosphates has not been determined, but it is likely that there may be multiple forms of these novel inositol pyrophosphates. Moreover, the pyrophosphate bond in inositol pyrophosphates has been hypothesized as a new form of high energy phosphate [18] that could, in principle, participate in phosphotransferase or energetic reactions. Thus, these new findings may provide an additional impetus for resolving the complexity of the metabolism of $InsP_5$ and $InsP_6$.

Considerable attention has also been directed to cross-talk between the intermediates of inositol lipid signaling and the levels of $InsP_5$ and $InsP_6$. These are summarized in Fig. 2B. One pathway outlined by different reports which could generate Ins1,3,4,5,6-P_5 by a phosphorylation of Ins1,3,4-P_3 with the sequential actions of a novel $InsP_3$ 6-kinase and $InsP_4$ 5-kinase [19, 20], would be the following: Ins1,4,5-P_3 → Ins1,3,4,5-P_4 → Ins1,3,4-P_3 → Ins1,3,4,6-P_4 → Ins1,3,4,5,6-P_5. Furthermore, it has been shown that other inositol phosphates, such as Ins3,4,5,6-P_4 [19, 21] and Ins1,4,5,6-P_4 [22], can also be phosphorylated to Ins1,3,4,5,6-P_5. At present, the specific enzymes mediating these conversions are poorly, if at all, characterized. However, Ins1,3,4,6-P_4 5-kinase [19, 20, 23], Ins3,4,5,6-P_4 1-kinase [19, 21], Ins1,3,4,5-P_4 6-kinase [24], Ins1,4,5,6-P_4 3-kinase [22], and Ins1,3,4,5,6-P_5 1-phosphatase [21] have been reported and may contribute to the

homeostasis of Ins1,3,4,5,6-P_5. A novel cell surface enzyme capable of hydrolyzing $InsP_5$ or $InsP_4$, but not any inositol mono-, bis- or trisphosphates, or other phosphomonoester compounds, has been reported [25]. This enzyme is a candidate for an inactivation mechanism for the higher inositol polyphosphates in the extracellular environment.

In the case of $InsP_6$, a number of $InsP_5$ kinases [16, 26] and an $InsP_6$ phosphatase ("phytase") [27] have been inferred by the use of purified isomers of $InsP_5$.

Dynamics of changes during stimulation. In general, the effects of specific types of cell stimulation or perturbation upon $InsP_5$ or $InsP_6$ levels have been studied using [^{3}H]inositol metabolic labeling. There is no general pattern observed for the resulting changes, which may, in some cases, indicate problems of interpretation from steady-state radioactive labeling. $InsP_5$ and $InsP_6$ levels may respond to different forms of stimulation, either coordinately or independently, and may (a) decrease [21], (b) increase [13, 28–31], (c) show biphasic changes [23, 32], or (d) remain substantially unaffected [25]. Therefore, there may be a number of cell-specific or stimulus-specific factors contributing to the short- and long-term regulation of $InsP_5$ and $InsP_6$ levels. At the least, there is no simple generalization as to how the levels of $InsP_5$ and $InsP_6$ will be altered by any form of cell manipulation.

A new technique, using metal-dye detection analysis of chromatographic fractions, has permitted direct chemical measurement of the concentrations of unlabeled $InsP_5$ and $InsP_6$ [33]. When this technique was applied to resting and activated HL-60 cells, concentrations were reported to increase following stimulation, to reach estimated intracellular levels as high as 60 μM for $InsP_6$ and 52 μM for $InsP_5$ [13]. Subsequently, antibody activation of the U937 cell line also showed elevation in the concentrations of $InsP_5$ and $InsP_6$ [34].

The recognized role of Ins1,4,5-P_3 in calcium discharge calls attention to the possibility of calcium-dependent modulation of $InsP_5$ and $InsP_6$ metabolism. In stimulated Jurkat T-lymphocytes, the levels of $InsP_5$ isomers have been suggested to be regulated by a Ca^{2+}-dependent $InsP_5$ kinase [35]. The levels of $InsP_5P$ and $InsP_6P$ are reduced dramatically in hepatocytes by treatment with the calcium-mobilizing reagent thapsigargin [36]. Accordingly, the rate of $InsP_5$ and $InsP_6$ conversion to their corresponding pyrophosphates may be sensitive to sustained calcium elevation or persistent loss of calcium from stores. However, other than these observations, there is little to suggest a special importance of $[Ca^{2+}]$ in regulating $InsP_5$ and $InsP_6$ metabolism.

Overall, $InsP_5$ and $InsP_6$ are multi-step products, but possibly not endpoints, of complex metabolic pathways, which are distinct from those associated with the production and breakdown of phosphoinositides.

Biological actions of $InsP_5$ and $InsP_6$

Intracellular regulation of surface receptors and ion channels. The concentration- and time-dependent loss of responsiveness of cell-surface receptors during persistent stimulation with agonist has been well demonstrated in many hormone– and neurotransmitter–receptor systems [37–39]. The acute, reversible loss of receptor responsiveness has been classically called "desensitization," whereas the chronic, irreversible loss of receptors from the surface is "down-regulation." Together, these two adaptive processes control the overall sensitivity of a receptor system to short- or long-term patterns of stimulation. For the G-protein coupled receptors, the process of desensitization is caused by phosphorylation of the receptor by a specific class of kinases [40] that exclusively recognize the agonist-occupied receptor. The phosphorylation of the receptor uncouples it from G-proteins through the binding of arrestins, a specialized class of regulatory proteins [40]. Recently, in a screen of charged small molecules, it was discovered that $InsP_6$ was uniquely capable of blocking the arrestin interaction with phosphorylated rhodopsin [41], presumably through a direct binding to arrestin [42]. Subsequently, it was shown that $InsP_6$ could be used to eliminate light-induced inactivation of rhodopsin [41]. These results suggested that $InsP_5$ and $InsP_6$ may modify desensitization of other types of G-protein coupled receptors. In agreement with this prediction, $InsP_5$ and $InsP_6$, when injected into *Xenopus* oocytes, have been shown to protect against agonist-induced, but not phorbol diester-induced, desensitization of a substance P receptor [43]. The IC_{50} values (30–50 μM) for this action suggested a low-affinity interaction, but there was structural specificity for $InsP_6$ or $InsP_5$ isomers, in that inositol tris- or tetrakisphosphate isomers did not have this novel activity. Significantly, the potency of this desensitization-blocking action was very similar to the micromolar affinity estimates of $InsP_6$ binding to arrestins [42]. This arrestin affinity is in close correspondence with the steady-state concentrations of $InsP_5$ and $InsP_6$, so that changes in the physiological levels of these compounds would be in the most sensitive concentration range for altering arrestin interactions. However, whether such binding to arrestins can or does occur *in vivo* is unknown.

Down-regulation is initiated by internalization of receptor, leading to loss of receptors in plasma membrane. One of the components of plasma membrane internalization is the clathrin assembly protein AP-2, which is required to form coated pits and coated vesicles [44, 45]. A high-affinity $InsP_6$ binding protein, when characterized, was demonstrated to be AP-2 [46, 47]. It has a nanomolar affinity for $InsP_6$, but also recognizes $InsP_5$ and Ins1,3,4,5-P_4 with high affinity. The binding of $InsP_6$ to AP-2 blocks clathrin assembly [48]. Other work has shown that AP-2 protein, when reconstituted into planar bilayers, gives a K^+ conductance that is blocked by $InsP_6$ [49]. Therefore, the high-affinity binding of $InsP_6$ blocks two identified activities of AP-2: its ability to assemble clathrin complexes and its potassium channel conductance. More recently, the intracellular trafficking protein, coatomer, which is essential to forming coated vesicles for Golgi intracisternal transport, was also shown to bind $InsP_6$, but unlike AP-2, coatomer had a dramatically lower affinity for $InsP_5$ [50]. Unlike the interaction

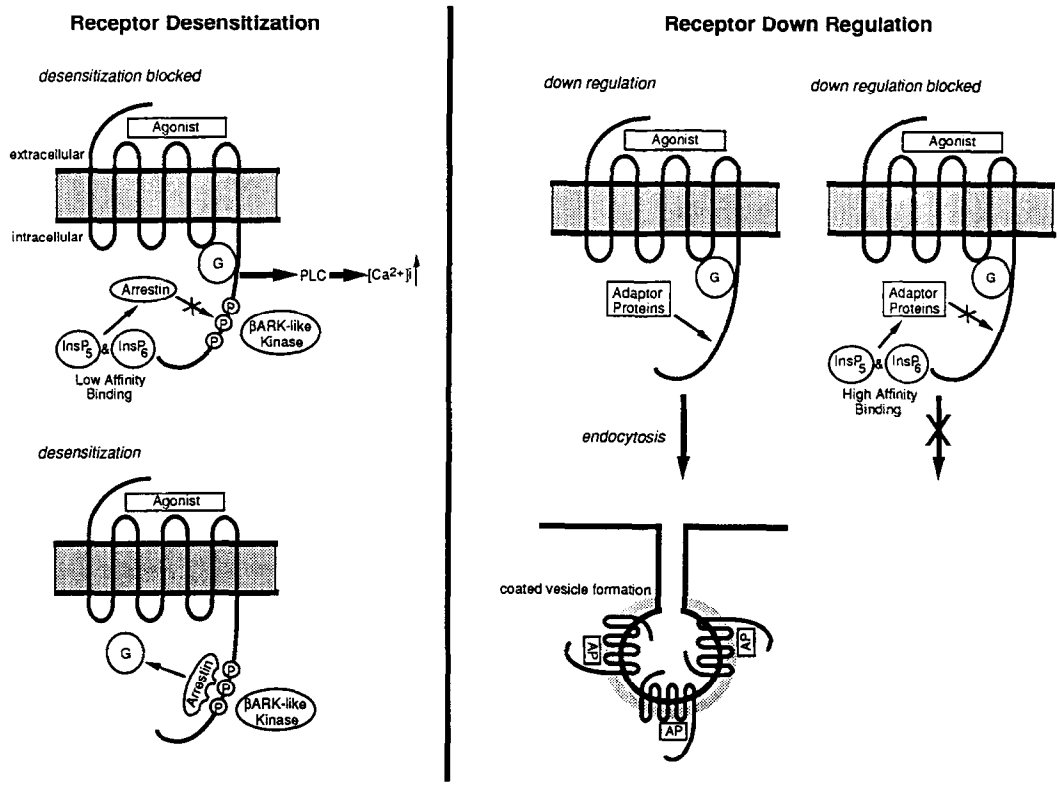

Fig. 3. Possible roles of $InsP_5$ and $InsP_6$ in the mechanisms of G-protein coupled receptor desensitization and down-regulation. Abbreviations: G, GTP binding protein; PLC, phospholipase C; βARK-like kinase, β-adrenergic receptor kinase-like kinase; P, phosphorylated amino acid residue; and AP, adaptor protein.

of $InsP_6$ with arrestin, however, it is difficult to envision how the high-affinity binding of higher inositol polyphosphates by AP-2 would be modulated. Indeed, the micromolar intracellular concentrations of these compounds would saturate such high-affinity binding sites, if the inositol polyphosphates are not compartmentalized. If this interaction is of physiological importance, it would predict that either AP-2 or an auxiliary protein is able to modulate inositol polyphosphate binding and release, by analogy to the guanyl nucleotide exchange factors regulating G-proteins.

Taken together, these intriguing observations suggest that $InsP_6$ interaction with regulatory proteins may contribute to setting the level of both rapid adaptation of the G-protein coupled class of receptors through desensitization and longer term adaptation of multiple classes of receptors through down-regulation. A hypothetical scheme is shown in Fig. 3.

Regulatory effects of $InsP_5$ and $InsP_6$ have also been observed on ion channels. It has been shown that intracellular injection of $InsP_5$ and $InsP_6$ induced current responses in neurones of *Aplysia kurodai*. $InsP_5$ induced a slow inward current associated with a nonspecific cation conductance [51]. On the other hand, $InsP_6$ induced a bisphasic current response, consisting of an initial inward current carried mainly by Na^+ and Ca^{2+}, followed by an outward K^+ current [52]. In these experiments, $InsP_5$ and $InsP_6$ had no effect when applied extracellularly. Thus, the intracellular regulation of membrane permeability by inositol polyphosphates, as indicated by the inhibition of AP-2-mediated K^+ conductances and the effects in neurones, is preliminary and should be examined further.

Extracellular actions. Many small molecules with intracellular functions, such as ATP, also have specialized properties as extracellular mediators. Early observations indicating that $InsP_5$ and $InsP_6$ levels may decline after neuronal activation [11] suggested that $InsP_5$ and $InsP_6$ may undergo secretion from storage compartments in specific cells. Thus, in a manner analogous to ATP, $InsP_5$ and $InsP_6$ may have widespread intracellular housekeeping functions, but also one or more extracellular luxury functions regulating excitable or non-excitable cells. Initially, this hypothesis was tested by microinjection of $InsP_5$ or $InsP_6$, but not $InsP_4$, into the nucleus tractus solitarious of the brainstem, a brain region known to receive a diversity of modulatory inputs. Both $InsP_5$ and $InsP_6$, but not any isomer of $InsP_4$, produced a potent and reversible reduction in heart rate and systemic blood pressure [12]. These results were confirmed and extended to show that local brainstem microinfusion of $InsP_6$ could rapidly

decrease both mean arterial blood pressure and respiratory rates [53].

To investigate the cellular mechanisms underlying these nervous system excitant effects of $InsP_5$ and $InsP_6$, defined inositol polyphosphate isomers were locally iontophoresed onto dorsal horn neurones, and single-unit responses were recorded [54]. Neurones were selectively excited by $InsP_5$ or $InsP_6$, but not by any $InsP_4$ isomer or other acidic compounds, such as inositol hexakissulfate. When compared with the recognized neuronal excitant, glutamic acid, both $InsP_5$ and $InsP_6$ exhibited longer latencies to responses, but reversed rapidly up on cessation of iontophoresis. Sustained administration of $InsP_6$ desensitized its own responses without altering sensitivity to glutamic acid. Collectively, these results indicated that $InsP_5$ and $InsP_6$ were acting directly on neurones, and were unlikely to be eliciting their actions by local precipitation or toxicity. Subsequently, $InsP_6$ microinjected onto medullary sympatoexcitatory neurones was shown to enhance the firing rate [55]. However, this effect was attenuated by co-administration of Ca^{2+}, and mimicked by the calcium chelator EGTA. These results were interpreted to indicate that $InsP_5$ and $InsP_6$ might, in part, elicit extracellular excitation of neurones by their recognized potential for calcium chelation [55]. This has been a consistent concern for the interpretation of the mechanisms of extracellular pharmacological actions, since $InsP_6$ can bind up to three calcium ions with micromolar affinity [56]. Indeed, $InsP_6$ has been shown to be a potent convulsant, whose activity is ablated in high extracellular calcium, when administered intracerebroventrically in rodents [57]. $InsP_5$ and $InsP_6$ are unlikely to function physiologically in neuronal communication, but may contribute to neuronal pathology, such as the propagation of hyperexcitability and the augmentation of seizures following neuronal damage. However, it is important to emphasize that the neuronal excitant effects of $InsP_5$ and $InsP_6$ are not found with all neurones, suggesting some cell-specific property that confers sensitivity. Whether an authentic surface receptor, analogous to the intracellular calcium channel gated by Ins1,4,5-P_3, is involved in these effects remains unknown, but is an attractive possibility. Circumstantial evidence providing support for this notion is the identification of a highly selective cell surface inositol polyphosphate phosphatase [25], which would be a putative inactivation mechanism for extracellular actions.

Several populations of isolated cells can be activated by exogenous $InsP_5$ or $InsP_6$, but not $InsP_4$. For example, $InsP_6$ enhanced $^{45}Ca^{2+}$ uptake in primary cultures or cerebellar granule cells [58], adrenal chromaffin cells [59] and anterior pituitary cells [60]. The enhanced Ca^{2+} uptake was also correlated with stimulation of hormone secretion in cerebellar granule cells [58] and adrenal chromaffin cells [59]. In non-excitable cell populations, $InsP_6$ has been suggested to act as a priming stimulus for the activation of neutrophils [61]. These extracellular actions were, in all cases, rapid, structurally specific, and reversible, reinforcing the possibility of specific recognition sites.

Binding studies using [^{3}H]$InsP_6$ have been used as an assumption-free route to identify high-affinity interactions relevant to possible extracellular and intracellular targets. Initially, high-affinity binding of [^{3}H]$InsP_6$ was reported in membranes derived from cerebellum [62] and cultured granule cells [63], and was also found in membranes from anterior pituitary cells [63] and adrenal chromaffin cells [59]. In the case of cerebellar membranes, the saturable, high-affinity membrane binding required a high ionic strength medium lacking divalent cations and containing EDTA. However, the addition of divalent or trivalent cations induced a large and unsaturable binding component, which could be ablated by treatment with metal ion chelators. These results suggest that $InsP_6$ may have complex interactions with membranes through specific protein recognition sites as well as non-protein interactions, possibly via phospholipids, in a manner dependent upon trace metals. It is worthwhile to consider that at least one biologically active species of $InsP_5$ or $InsP_6$ may be a metal-ion complex, rather than a free acid. Again, this would be directly analogous to the active species being Mg^{2+}-ATP in phosphotransferase enzymes.

Autoradiographic analysis has shown the distribution of metal-ion independent, high-affinity [^{3}H]-$InsP_6$ binding sites, which is very similar to the density of neuronal cell bodies marked by the Nissl stain [62], and which also resembles the pattern of ^{125}I-IGF II binding [64]. These results reinforce the notion that [^{3}H]$InsP_6$ binding sites are associated with neurones, and not non-neuronal cell types, in the brain, and that they may be involved with extrasynaptic functions, in view of their enrichment in somatic, and not dendritic or axonal regions.

The high-affinity recognition of $InsP_6$ has been used to purify both membrane-bound and cytosolic binding proteins, in one case, using an immobilized Ins1,3,4,5-P_4 as an affinity purification matrix [46]. Two groups [46, 47, 49] have identified one such protein as AP-2 (see above), a protein required for assembly of clathrin coats and endocytosis at the plasma membrane. As noted earlier, the intracellular counterpart of AP-2, coatomer, also has this property of binding $InsP_6$ with high affinity and selectivity (Table 1). It remains unresolved whether more than one subunit in these protein complexes can bind [^{3}H]$InsP_6$, but a single $InsP_6$ recognition site has been identified on the α-subunits of AP-2, whereas the site of $InsP_6$ interaction in the coatomer complex has not been identified. More recently, the specific binding of [^{3}H]$InsP_6$ has been used to purify cytosolic proteins from brain and liver of apparent molecular weights of 266,000 and 512,000, respectively [65]. These novel proteins have been shown to be distinct from the previously identified binding proteins, AP-2 and coatomer, and to have no $InsP_5$ or $InsP_6$ kinase activity or $InsP_6$ phosphatase activity.

The results for [^{3}H]$InsP_6$ binding to membrane and purified protein preparations are summarized in Table 1.

Although extracellular activities of $InsP_5$ and $InsP_6$ have focused on cell activation, there is emerging evidence that these inositol metabolites can act as antagonists of several types of cell surface recognition phenomena. For example, in the examination of the

Table 1. Summary of [^{3}H]$InsP_6$ binding

Preparation	K_d	Density	Specificity	Comment	Reference(s)
Crude membrane					
Rat cerebral hemisphere	33 nM	152 pmol/mg protein	$InsP_6 > InsP_4 > InsP_5$	Divalent cations enhance density	[63]
Rat cerebellum	60 nM	80 fmol/mg protein	$InsP_6 > InsP_5 \geq InsP_4$	Neuronal cell body localization	[62]
Bovine adrenal chromaffin cell	90 nM	700 fmol/mg protein	$InsP_6 > InsP_5 > InsP_4$	Catecholamine release enhanced	[59]
Purified protein					
Golgi coatomer (400 k, bovine liver)	0.2 nM	1.3 nmol/mg protein	$InsP_6 = InsP_4 >> InsP_5$	Stoichiometry ~1 site/molecule	[50]
$InsP_6BP$* (400 k, bovine cerebellum)	0.12 μM	1.3 nmol/mg protein	$InsP_6 > InsP_3 > InsP_4$	Stoichiometry ~1 site/molecule	[81]
$InsP_6BP$ (266 k, bovine brain cytosol)	5.1 nM	1.81 nmol/mg protein	$InsP_6 >> InsP_5 \geq InsP_4$	Stoichiometry 0.48 site/molecule	[65]
$InsP_6BP$ (512 k, rat liver cytosol)	18.5 nM	0.72 nmol/mg protein		Stoichiometry 0.37 site/molecule	[65]
Arrestin (48 k, bovine retine)	5.5 μM		$InsP_6 > InsP_5 > InsP_4$	Stoichiometry ~1 site/molecule	[42]
AP-2 (~115 k, rat cerebellar membrane)	14 nM		$InsP_6 > InsP_5 \geq InsP_4$	Stoichiometry ~1 site/molecule	[46, 47]
Hemoglobin (avian erythrocyte)	1 μM		$InsP_6 > InsP_5$	Oxygen affinity decreased	[82]

* $InsP_6BP$: $InsP_6$ binding protein.

apparent co-distribution of [^{3}H]$InsP_6$ and ^{125}I-insulin-like growth factor II (^{125}I-IGF II) binding [64], it was also shown that micromolar concentrations of $InsP_6$ could compete for ^{125}I-IGF II binding. Similarly, an *in vitro* action of basic fibroblast growth factor, the transdifferentiation of a Schwann cell precursor into a melanocyte, can be selectively blocked by the addition of $InsP_6$ (25 μM) to the extracellular medium [66]. Lastly, $InsP_5$ (IC_{50} = 1.4 μM) and $InsP_6$ (IC_{50} = 2.1 μM) were shown to inhibit the binding of carbohydrate ligands to the adhesion molecules L- and P-, but not E-, selectin [67]. This inhibitory action of selectin binding was shared, although reduced in potency, with the distinct inositol polyanion inositol hexakissulfate (IC_{50} = 0.2 mM). Strikingly, inositol polyanions appeared to show *in vivo* efficacy consistent with this biochemical action in that intravenously administered $InsP_6$ inhibited accumulation of immune effector cells in experimentally induced inflammation.

Although it would be premature to conclude that inositol polyphosphates act physiologically on extracellular sites, the extracellular actions nonetheless indicate promising routes to the design of novel reagents that may have greater potency and selectivity in perturbing these processes. In this regard, inositol polyanions appear to have promise for interference with both polypeptide growth factor and specific adhesion molecule interactions. Such reagents could have immense practical and therapeutic value.

Metal-ion chelation and anti-oxidant properties. It has been appreciated for decades that inositol phosphate isomers, particularly $InsP_5$ and $InsP_6$, have the ability to chelate with remarkable potency and specificity divalent and trivalent metal cations such as Ca^{2+}, Mg^{2+}, Fe^{3+}, and Zn^{3+}, to form soluble or insoluble complexes [68]. As a result, it is now appreciated that some of the biological actions of $InsP_5$ and $InsP_6$ may result from selective metal ion chelation [55, 69]. In membrane binding studies, enhancement of [^{3}H]$InsP_6$ binding by metal ions was shown to arise from the formation of ternary $InsP_6$–membrane–ion complexes, and $InsP_6$ was indirectly shown to bind Fe^{3+} with extraordinary affinity and selectivity [70]. Because there is evidence that $InsP_6$ may be normally complexed with a trace metal, Fe^{3+} is the best candidate for this metal-bound species of $InsP_6$. Indeed, this complex formation is comparable to that of the known iron chelator desferrioxamine, suggesting that such complexation would be highly favored with free Fe^{3+} within the cell. In this regard, it is intriguing to speculate whether $InsP_6$ may act as a physiological "iron chaperone" to facilitate movement of ionized iron from carrier proteins, such as transferrin, across membrane barriers to targeted sites of utilization, such as mitochondria [69]. Unlike many of the activities described thus far, this biological activity appears to be selective for $InsP_6$, since it requires the 1,2,3 (equatorial-axial-equatorial) phosphate grouping [69]. Thus, one of the housekeeping functions that may be uniquely correlated with $InsP_6$ could be regulation of iron homeostasis in animal cells.

The ability of $InsP_6$ to chelate iron efficiently has been reported to have other practical implications. Notably, iron-mediated oxidative damage to cells, arising from the catalysis of hydroxyl radical formation, has been well documented. $InsP_6$ effectively blocks iron-catalyzed hydroxyl radical formation and greatly diminishes lipid peroxidation [69, 71]. This suggests that extracellular $InsP_6$ may be an endogenous anti-oxidant and, therefore, anti-inflammatory modulator.

Antineoplastic action. One of the most mysterious, but also potentially important activities of $InsP_6$ is its reported antineoplastic action. Oral administration of $InsP_6$ with drinking water exerted chemopreventive and chemotherapeutic effects on rodent colon and mammary carcinogenesis models as well as in a transplanted fibrosarcoma model [72–74]. The antineoplastic effects may be related to the ability of $InsP_6$ to chelate a variety of metals, and to inhibit iron-catalyzed production of hydroxyl radicals and lipid peroxidation [71]. $InsP_6$ also inhibited colonic and mammary gland epithelial cell proliferation [75] and reduced multiple carcinogen-induced rat hepatocellular carcinomas [76]. This is an intriguing area for further exploration in that $InsP_6$ may inhibit both stimulated and malignant cell proliferation and may be an endogenous, as well as dietary-derived, reagent for attenuation of cancer initiation.

Concluding comments

$InsP_5$ and $InsP_6$ are now recognized as the most abundant inositol phosphates in many, perhaps all, animal cell populations. However, the significance of their ubiquitous biosynthesis remains obscure. In terms of their metabolism, a variety of phosphorylation/dephosphorylation interconversions and a possible biosynthetic pathway have been identified, but there is a paucity of information on the purification and properties of the relevant kinases and phosphatases mediating these conversions. One unexpected finding has been the realization that $InsP_5$ and $InsP_6$ may not be metabolic end-products, but rather could serve as precursors for rapidly metabolized inositol pyrophosphates. Overall, the metabolism of $InsP_5$ and $InsP_6$ is much more dynamic and sophisticated than was appreciated previously.

In terms of possible functions, there is a substantial gap between a variety of demonstrated pharmacological actions of $InsP_5$ and $InsP_6$, and their possible physiology. Moreover, in many instances, $InsP_5$ and $InsP_6$ appear to be functionally redundant in that they appear to be indistinguishable in their activities. However, an important exception is the iron-chelating activity of $InsP_6$, which makes it an attractive candidate for a long-hypothesized low molecular weight iron transporter ("iron chaperone") (see Ref. 77). Thus, a unique housekeeping function for $InsP_6$ may be in iron metabolism, whereas both $InsP_5$ and $InsP_6$ may play complex and overlapping housekeeping roles, including modulating receptor sensitivity and internalization. The possibility of "luxury" functions, such as neuronal activation, has also been suggested. Significantly, the documented activity of higher inositol polyphosphates to act as potent neuronal excitants, in fact endogenous convulsants, suggests that they should be considered

as possible contributory mediators to seizure propagation or excitotoxic damage. However, in all cases, the suggested functions for higher inositol polyphosphates are speculative and remain unproven.

An important missing clue to possible functions is the unambiguous determination of the cellular compartmentation of $InsP_5$ and $InsP_6$. Are they cytoplasmic? Are they normally bound to proteins? Are they normally bound to membranes? Are they stored in vesicles or other membrane-bound organelles? Are they capable of translocation to and from a number of intracellular sites? Are $InsP_5$ and $InsP_6$ co-localized within cells, or do they have different and independent distribution patterns?

The resolution of these issues will be an exciting challenge and will undoubtedly reveal multiple roles of inositol polyphosphates in regulating cell function.

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