Chapter 11

Inositol Phosphates and Phosphoinositides in Health and Disease

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1. INTRODUCTION

Inositol is an essential molecule found ubiquitously in biological systems. Phosphorylation of the cyclic inositol ring produces two related families of molecules, inositol phosphates and phosphinositides. Research into the roles of inositol and its derivatives has been hampered by the complex and multitudinous interactions of these molecules in multiple cellular pathways. However, the potential rewards of such studies are immense, as inositol phosphates and phosphoinositides (PIs) play a role in numerous human diseases. This review addresses current knowledge of the role of inositol phosphates and PIs in human health and disease.

Part one of this review focuses on the role of inositol phosphates in cellular signaling pathways. Specifically, we focus on inositol 1,4,5-triphosphate ($InsP_3$) and inositol hexaphosphate ($InsP_6$) because these are the most highly studied inositol phosphates in relation to human disease. $InsP_3$ plays an essential role as a secondary messenger in the $InsP_3/Ca^{2+}$ signal transduction pathway, which is responsible for modulating the activity of numerous cellular processes. Perturbation of this pathway has been implicated in a variety of disorders including bipolar affective disorder, Alzheimer's disease (AD), Parkinson's disease, and malignant hyperthermia (MH). $InsP_6$ may be the most abundant inositol phosphate and is found ubiquitously in mammalian cells. Recently, $InsP_6$ has been identified as a potential antineoplastic therapy due to

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its antioxidant properties. As a result of the important functions of these molecules, increasing interest in examining the roles of other inositol phosphates has led to identification of novel functions. We briefly discuss the significance of recent findings regarding a variety of other inositol phosphates that provide promising avenues for future research.

In part two, we review the role of PIs in human disease. Although PIs are not abundant in biological systems, they have displayed numerous important functions in multiple signal transduction pathways. A number of human diseases are characterized by dysfunctional PI pathways, including cancer, type 2 diabetes, Lowe syndrome, myotubular myopathy, and Charcot-Marie-Tooth disease.

2. INOSITOL PHOSPHATES

The existence of inositol phosphates has been known for over 80 years (Posternak, 1919). Inositol, a six-carbon cyclitol found ubiquitously in all biological systems (Bachhawat and Mande, 1999; Chen *et al*., 2000; Majumder *et al*., 2003), exists in eight possible isomeric forms (*myo, chiro, scyllo, neo, cis, epi, allo, and muco*), of which *myo* is physiologically the most common and important stereoisomer. Phosphorylation of the inositol ring at one or more positions generates numerous PIs and inositol phosphates. The study of and interest in inositol phosphates is complicated by three major factors (Irvine and Schell, 2001): (1) there are many of them (63 possible isomers for inositol monophosphates alone), a potential that can be expanded further by attaching more than one phosphate on the same position, as in inositol pyrophosphates; (2) the multiplicity of metabolic pathways makes it hard to understand how their levels are regulated in cells; and (3) inositol phosphates are suspected to play a role in multiple signaling pathways (especially due to their involvement in Ca^{2+} metabolism), and, therefore, it is difficult to "bring them together" in one place.

Although the cellular roles of these molecules are not fully understood, inositol phosphates have been shown to convey signals for a variety of hormones, growth factors, and neurotransmitters (Berridge, 1993; Berridge and Irvine, 1989). As mentioned, cells contain a large array of inositol phosphates, some of which respond to receptor stimulation, providing the basis for multiple and complex responses. Among the inositol phosphates, we focus on $InsP_3$ and $InsP_6$, the most widely studied in relation to human health and disease.

2.1 Inositol 1,4,5-triphosphate ($InsP_3$) – A major role in neurological disorders

Streb and coworkers (1983) discovered that $InsP_3$ is a Ca^{2+}-mobilizing second messenger. Since then, a huge body of data has accumulated regarding its pivotal roles in the regulation of multiple cellular pathways, and its possible association with multiple illnesses, mainly neurological disorders.

The demonstration that $InsP_3$ causes release of Ca^{2+} from intracellular stores laid the foundation for the current understanding of the function of the phosphatidylinositol turnover pathway (Streb *et al*., 1983). Briefly, the pathway involves receptor-mediated activation of phospholipase C (PLC), which cleaves phosphatidylinositol(4,5)biphosphate[PtdIns(4,5)P_2]to produce 1,2-diacylglycerol (DAG) and Ins(1,4,5)P_3 (here referred to as $InsP_3$) (Gould *et al*., 2004a; Irvine and Schell, 2001; Irvine *et al*., 1984; Streb *et al*., 1983; Woodcock, 1997). DAG activates various isomers of protein kinase C (PKC), and $InsP_3$ initiates rises in Ca^{2+} (Berridge, 1987; Streb *et al*., 1983). $InsP_3$ amplifies its cellular effects (Ca^{2+}-mobilization) by activating $InsP_3$ receptors (IP_3Rs) (Streb *et al*., 1983). The IP_3R has a tetrameric structure similar to other Ca^{2+} channels (Patterson *et al*., 2004). The wide distribution of these receptors in cells likely reflects the multiplicity of functions of $InsP_3$. Initially, these receptors were identified in the endoplasmic reticulum, but they also reside in the Golgi apparatus, plasma membrane, nucleoplasmic reticulum, and other cellular organelles (Patterson *et al*., 2004).

The $InsP_3/Ca^{2+}$ signal transduction pathway modulates the activity of a multitude of intracellular events. Numerous receptors (mostly G-protein coupled receptors, particularly $G_{q/11}$) are associated with PLC-induced $InsP_3$ release. For example, in the central nervous system, the excitatory neurotransmitters glutamate and aspartate, and also M_1 and M_3 muscarinic, β_1-adrenergic, 5-HT_2 serotonergic, H_1 histaminic, and vasopressin V_1 receptors, among others, are all known to increase $InsP_3/Ca^{2+}$ release (Bloom, 2001).

Calcium is a very important signaling molecule within cells (Berridge, 1993). Many cellular functions are directly or indirectly regulated by free cytosolic Ca^{2+} ($[Ca^{2+}]_i$). The Ca^{2+} ions needed to control the activity of the cell can be supplied to the cytosol from the extracellular space or from intracellular stores (mainly from endoplasmic and sarcoplasmic reticulum, but also from mitochondria and Golgi apparatus). Interestingly, $InsP_3/Ca^{2+}$ release is known to take place in the nucleus of various cell types, and IP_3Rs have been found in the inner nuclear membrane (Martelli *et al*., 2004). $InsP_3$ regulation of nuclear Ca^{2+} levels is important for several processes that take place in the nucleus, such as protein transport across the nuclear envelope and regulation of gene expression, among others (Martelli *et al*., 2004).

The *time*, *space*, and *amplitude* of the fluctuating changes in ($[Ca^{2+}]_i$) concentration are very important and strictly regulated because cells extract specific information from these three parameters. Because Ca^{2+} is such an important signaling molecule, mutations causing drastic functional alteration in ($[Ca^{2+}]_i$) homeostasis are most likely not compatible with life (Lorenzon and Beam, 2000). Mutations or abnormalities in the proteins involved in ($[Ca^{2+}]_i$) regulation, which may cause only trivial alterations in the function of the protein *in vitro*, often lead to a diverse array of diseases (Missiaen *et al*., 2000). For example, alterations in the function of proteins related to ($[Ca^{2+}]_i$) regulation are associated with AD, skeletal muscle

pathology, heart disease, MH, visual disturbances, and skin diseases, among others (Missiaen *et al*., 2000).

The role of $InsP_3$ in the regulation of Ca^{2+} release is not universal for all cell types and signal transduction pathways. The effects of $InsP_3$ on Ca^{2+} mobilization from intracellular stores primarily occur in non-excitable cells, in which appropriate receptor stimulation causes rapid release of $InsP_3$. However, it should be kept in mind that this pattern is not observed in some excitable cells, *e.g.*, cardiomyocytes, smooth muscle, and skeletal muscle cells, in which voltage-regulated channels are major contributors to Ca^{2+} control mechanisms and release (Woodcock, 1997). Nevertheless, it is an oversimplification to say that $InsP_3$ does not play a role in these excitable cells (cardiomyocytes for instance). IP_3Rs are present ubiquitously, and their presence opens up the possibility that these receptors have other functions (Kijima *et al*., 1993). In addition, evidence suggests that $InsP_3$ is not always the primary inositol phosphate released, is not always formed from $PtdIns(4,5)P_2$, and is often present in unstimulated cells at concentrations sufficient to activate/saturate its receptors (Woodcock, 1997). This further complicates the understanding of the regulation and cellular functions of this pivotal second messenger.

Perturbation of the $InsP_3/Ca^{2+}$ signaling pathway leads to a variety of disorders. However, our focus in this section is on the role of $InsP_3/Ca^{2+}$ signaling in the pathophysiology of neurological disorders, mainly bipolar affective disorder and AD.

2.1.1 Bipolar disorder (BAD)

Bipolar disorder (BAD, manic-depressive illness) is a severe and chronic illness, which is a major public health problem, in any given year affecting approximately 1–3% of the US population (Narrow *et al*., 2002). In the World Health Organization Global Burden of Disease study, BAD ranked sixth among all medical disorders in years of life lost to death or disability worldwide, and is projected to have a greater impact in the future (Murray and Lopez, 1996).

Although a number of mood-stabilizing drugs are commonly used in the treatment of BAD, lithium and the anticonvulsants valproate (VPA) and carbamazepine are the only drugs for which long-term efficacy has been established, and are therefore used for maintenance treatment of BAD (Belmaker, 2004). However, these agents are far from the perfect medications, and they are ineffective and not well tolerated by a significant portion of patients.

The mechanism of action of mood-stabilizing drugs in the treatment of BAD is not fully understood. The inositol-depletion hypothesis has been suggested to explain the mechanism of action of lithium (Berridge and Irvine, 1989; Berridge *et al*., 1982). This hypothesis postulates that the therapeutic effects of lithium are due to uncompetitive inhibition of inositol monophosphatase (IMPase), which leads to depletion of *myo*-inositol in brain cells, and

consequently, to dampening of PI signaling. The action of IMPase is the final step in inositol synthesis. Inositol polyphosphate 1-phosphatase (IPPase) removes phosphate from Ins (1,4)-biphosphate. Both enzymes appear to be critical for the maintenance of *myo*-inositol levels and continuation of PI-mediated signaling. Therefore, direct inhibition of IMPase by lithium (Hallcher and Sherman, 1980; Naccarato *et al*., 1974) and IPPase (Inhorn and Majerus, 1988) could potentially lead to inositol depletion. Indeed, lithium has consistently been shown to decrease free inositol levels in human brain sections and in brains of rodents (Atack, 2000). Lithium treatment also decreases inositol levels in human subjects (Moore *et al*., 1999). Furthermore, lithium and VPA were found to normalize the altered PI cycle in BAD patients (Silverstone *et al*., 2002).

Controversy regarding the inositol depletion hypothesis has centered around a number of arguments. Lithium produces therapeutic effects only after chronic administration, whereas direct inhibition of IMPase by lithium is rapid (Atack, 2000; Pollack *et al*., 1994). As there are currently no specific IMPase inhibitors available for clinical use, it is difficult to directly test the inositol depletion hypothesis in BAD patients (Atack, 2000). Recent evidence points toward other signaling molecules and signal transduction pathways as targets for the mood-stabilizing effects of anti-bipolar drugs (Gould *et al*., 2004a; Jope, 2003). Furthermore, accumulating data suggest that severe mood disorders are associated with impairments of structural plasticity and cellular resilience, and that BAD patients may suffer from a reduction in CNS volume (Gould *et al*., 2004b). Gould and coworkers have suggested that the fact that currently used mood stabilizers take weeks to produce their therapeutic effects may implicate changes in gene expression, protein function, and more importantly, general neural plasticity (Gould *et al*., 2004a). The anti-bipolar effect of lithium is now attributed, at least in part, to its neuroprotective effect, achieved mainly by inhibiting the activity of glycogen synthase kinase-3 (GSK-3) (Gould *et al*., 2004a,b). GSK-3 is the only kinase known to be inhibited by lithium at nearly therapeutic concentrations (~1 mM) (Jope, 2003). Lithium inhibits GSK-3 activity in two ways: (i) by *directly* inhibiting catalytic activity, and (ii) by *indirectly* increasing phosphorylation that inhibits activity (Jope, 2003). Since GSK-3 plays a major role in multiple cellular pathways (Gould *et al*., 2004a,b), inhibition of its activity by lithium may have far-reaching effects. Consistent with these observations, GSK-3 inhibitors were found to possess mood-stabilizing effects in the forced swim test in mice and rats (Gould *et al*., 2004c; Kaidanovich-Beilin *et al*., 2004). The neuroprotective effects of lithium are also attributed to its ability to increase the levels of the neuroprotective protein bcl-2 (Manji *et al*., 1999). Similar to lithium, VPA was also found to elicit neuroprotective effects through the inhibition of GSK-3 and increase of bcl-2 levels (Chen *et al*., 1999a,b).

The inositol depletion hypothesis was revived by the observations that VPA, like lithium, also decreases intracellular inositol levels (Vaden *et al*., 2001),

and that anti-bipolar drugs increase the growth cone area of sensory neurons in an inositol-dependent manner (Williams *et al*., 2002). VPA decreases intracellular inositol in yeast, and this decrease is accompanied by a significant derepression of *INO1*, the gene encoding 1-D-*myo*-inositol-3-phosphate (MIP) synthase, which is derepressed in response to inositol limitation (Vaden *et al*., 2001). VPA decreases intracellular inositol levels by indirectly inhibiting MIP synthase activity *in vivo* (Ju *et al*., 2004). MIP synthase catalyzes the rate-limiting step in inositol synthesis, the conversion of D-glucose-6-phosphate (G-6-P) to MIP (Loewus *et al*., 1980). VPA was shown to decrease MIP synthase activity (by 50%) in crude homogenate of human postmortem prefrontal cortex (Shaltiel *et al*., in press). The inositol depletion hypothesis was further strengthened by the observation that three mood-stabilizing drugs – lithium, VPA, and carbamazepine – inhibit the contraction of sensory neuron growth cones and increase growth cone area in an inositol-dependent manner, providing evidence correlating inositol depletion and neuronal function (Williams *et al*., 2002).

As mentioned above, anti-bipolar drugs cause a rapid decrease in intracellular inositol levels, but their therapeutic effects are apparent only after chronic treatment (Pollack *et al*., 1994). While this may seem to argue against the inositol depletion hypothesis, it is important to view inositol depletion in light of the pivotal role of inositol as a major metabolic sensor for regulating major cellular pathways, including protein secretion, the unfolded protein response pathway, and the glucose response pathway (Carman and Henry, 1999; Ju, 2004). Therefore, the acute depletion of inositol by anti-bipolar drugs may have far-reaching cellular responses that contribute to their therapeutic effects.

Taken together, the data reviewed suggest that inositol may play a role in the pathophysiological mechanisms underlying BAD, and that the inositol depletion hypothesis may help to explain the mood-stabilizing effects of anti-bipolar drugs.

2.1.2 Alzheimer's disease (AD)

Alzheimer's disease (AD) is the leading cause of dementia in the elderly population; in the United States alone, almost 4 million patients suffer from the disease (Brookmeyer *et al*., 1998). Each year, approximately 360,000 new cases of AD are identified, and by the year 2050, more than 14 million persons in the United States alone may likely suffer from this neurodegenerative disease (Brookmeyer *et al.*, 1998). The pathophysiological mechanisms underlying AD are unclear. However, one of the most characteristic neuropathological lesions in the brains of patients with AD are amyloid plaques that are composed primarily of a peptide known as amyloid-β (Aβ) (Selkoe, 1991). The formation and aggregation of Aβ plaques are known to cause accumulation of microglia and reactive astrocytes, leading to acute phase/inflammatory response, which may damage neurons and exacerbate the pathological

processes of the disease (Aisen, 1997; McGeer and McGeer, 1995; Selkoe, 1991). It has also been suggested that cerebrovascular disease may contribute to the severity of the disease (Riekse *et al*., 2004).

Interestingly, disruption of the $InsP_3/Ca^{2+}$ signaling pathway is implicated in AD and other neurogenerative disorders (LaFerla, 2002; Mattson *et al*., 2000a). For example, it was shown that IP_3R levels were significantly decreased in the cerebellum, superior temporal, and superior frontal cortices of AD patients, as compared to matched control brains (Garlind *et al*., 1995). Similarly, in a postmortem analysis of eight AD patients, IP_3R protein levels were decreased significantly in the temporal and frontal cortices, compared to matched control cases (Haug *et al*., 1996). These degenerative changes may be responsible, at least in part, for the dysregulation of calcium homeostasis seen in AD. Moreover, abnormal function of IP_3R1 was observed in animal models of AD (Mattson *et al*., 2000b). $InsP_3$-induced Ca^{2+} release is known to serve numerous signaling functions in neurons, including modulation of membrane excitability (Yamamoto *et al*., 2002), synaptic plasticity (Fujii *et al*., 2000), and gene expression (Mellstrom and Naranjo, 2001). Recent attention has focused on presenilin 1 (PS1), an endoplasmic-reticulum-localized protein which modulates $InsP_3$-induced Ca^{2+} release, and is required for the proteolysis of amyloid precursor protein (Selkoe, 2001). Mutant forms of the *PS1* gene have been shown to contribute to the majority of early-onset AD cases (Selkoe, 2001). Recently, it was found that dysregulation of $InsP_3/Ca^{2+}$ signaling, which was induced by a *PS1* mutation, has enhanced neuronal Ca^{2+} liberation and signaling, and altered membrane excitability (Stutzmann *et al*., 2004). These data indicate that perturbation of the $InsP_3/Ca^{2+}$ signaling pathway may contribute to the pathology of AD.

2.1.3 Other neurological disorders

IP_3R1 is predominant in cerebellar Purkinje cells and is also present in other neural and peripheral tissues (Matsumoto *et al*., 1996). Importantly, the binding sites for $InsP_3$ correlated with IP_3R levels were significantly decreased in multiple regions of postmortem brain tissue from Parkinson's disease patients, compared with those of age-matched controls (Kitamura *et al*., 1989). Similar results were observed in postmortem brain regions of patients with Huntington's disease, in which the binding sites for $InsP_3$ were significantly reduced (Warsh *et al*., 1991). Consistent with these findings, it was found that IP_3R1 is related to the dysregulation of calcium signaling in a mouse model of Huntington's disease (Tang *et al*., 2003). Abnormal IP_3R1 function in humans is associated with neurological abnormalities, the most prominent of which is ataxia (Zecevic *et al*., 1999). Disruption of the IP_3R1 gene in mice resulted in a severe reduction of $InsP_3$-induced Ca^{2+} release in brain tissue (Matsumoto *et al*., 1996). Very few embryos carrying the disruption survive to birth, pointing to a significant role for IP_3R1 during embryonic development. Those

animals that did survive to birth exhibited severe neurological alteration, including ataxia and epilepsy. Accordingly, Street and coworkers have also observed that abnormal function of IP_3R1 is associated with epilepsy in mice (Street *et al*., 1997).

2.1.4 Other pathologies

Alteration in $InsP_3/Ca^{2+}$ signaling is one of the suggested mechanisms for MH in humans, a disorder characterized by uncontrolled, severely elevated body temperature and muscle contractions (Wappler *et al*., 1997). Interestingly, this study has shown that $InsP_3$ levels were significantly increased in the skeletal muscles but not in the plasma of MH patients (Wappler *et al*., 1997). The underlying mechanism of $InsP_3$-induced MH is not clear. However, in a swine model of MH (induced by halothane challenge), it was suggested that an increase in $InsP_3$ activity may cause a drastic increase in Ca^{2+} concentration, leading to metabolic changes resulting in MH (Tonner *et al*., 1995). Moreover, altered $InsP_3/Ca^{2+}$ signaling was observed during myocardial ischemia/reperfusion cycles in rats (Mouton *et al*., 1991). Adrenergic stimulation led to ischemia that was accompanied by a significant increase in $InsP_3$ levels in the myocardial tissue. Interestingly, an increase in $InsP_3$ levels was also observed during the reperfusion phase (Mouton *et al*., 1991).

Calcium overload appears to be involved in ischemia/reperfusion cycles. However, due to the differences in the ischemia-inducing protocols, the exact role of Ca^{2+} overload is unclear. Mouton and coworkers have concluded that calcium overload (due to the increased $InsP_3$ activity) is involved in the pathophysiology of myocardial ischemia/reperfusion cycles. In contrast to this study, Woodcock and coworkers have shown that during global myocardial ischemia, the levels of $InsP_3$ were significantly decreased in rat ventricles, whereas levels were increased during reperfusion (Woodcock *et al*., 1997). These authors have also shown that increased Ca^{2+} concentrations during ischemia (induced by coronary artery ligation) may further enhance $InsP_3$ release, and this may account for the Ca^{2+} overload seen in the ischemic myocardial tissue (Woodcock *et al*., 1996). This Ca^{2+} overload was suggested as a major contributor to the severe cardiac arrhythmias seen during the ischemia/reperfusion cycles.

2.2 Inositol hexaphosphate ($InsP_6$) – A potential antineoplastic therapy

$InsP_6$, also known as phytic acid, was the first inositol phosphate discovered (Posternak, 1919). Initially, $InsP_6$ was thought to exist only in plants (mostly legumes) and in the erythrocytes of a few animals. However, it is now known to be ubiquitous in mammalian (and probably all eukaryotic) cells (Heslop *et al*., 1985), and may actually be the most abundant inositol phosphate.

Chemically, $InsP_6$ is a highly charged molecule. It interacts with positively charged groups on proteins and with low molecular weight cations, and in doing so, can compete with other molecules that would bind these proteins and cations *in vivo*.

The earliest (and probably the most important) proposed function for $InsP_6$ in plants is that of a phosphate store for seeds (Posternak, 1919). It is now known that $InsP_6$ exhibits strong antioxidant properties (Hawkins *et al*., 1993) and can cause complete inhibition of Fe^{3+}-catalyzed hydroxyl-radical formation. Functions proposed more recently for $InsP_6$ include protein phosphatase inhibition (Larsson *et al*., 1997) and activation of PKC (Efanov *et al*., 1997). $InsP_6$ was also found in the nucleus, and York and coworkers observed that $InsP_6$ may modulate mRNA transport out of the nucleus (York *et al*., 1999).

One of the most striking findings regarding $InsP_6$ is that it may confer anticancer properties (Fox and Eberl, 2002; Vucenik and Shamsuddin, 2003). Numerous studies suggest that wheat bran is antineoplastic, especially for colon cancer (Fox and Eberl, 2002). Further research has indicated that the potential active ingredient for this effect is $InsP_6$. It has been extensively studied *in vitro* and in *in vivo* animal models of cancer, and in the majority of them it exhibited anticancer properties (Fox and Eberl, 2002; Vucenik and Shamsuddin, 2003). Interestingly, the most consistent and potent anticancer results were obtained from the combination of $InsP_6$ with inositol (which itself has a moderate anticancer effect) (Vucenik and Shamsuddin, 2003). For example, $InsP_6$ was found to inhibit neoplastic growth in many types of cancer, including breast, colon, liver, lung, prostate, rhabdomyosarcoma, skin, and others (Fox and Eberl, 2002; Vucenik and Shamsuddin, 2003). $InsP_6$ was effective (as an antineoplastic) in a dose-dependent manner given either before or after carcinogen (such as 1,2-dimethylhydrazine and azoxymethane) administration to rats and mice with colon cancer. Importantly, $InsP_6$ was found to act synergistically with standard chemotherapeutic agents, and therefore, significantly augmented the anticancer effect of the treatment (Tantivejkul *et al*., 2003). One of the most important studies to elucidate the anticancer properties of $InsP_6$ was the pilot clinical trial performed in patients with advanced colorectal cancer (Druzijanic *et al*., 2002). The results demonstrated enhanced antitumor activity of the standard treatment when $InsP_6$ (plus inositol) was added, without compromising the patients' quality of life.

The molecular mechanisms underlying the anticancer effect of $InsP_6$ are not understood. Proposed mechanisms of action for $InsP_6$ are (i) increase in natural killer cell activity and enhanced host immunity, (ii) alteration in signal transduction, (iii) stimulation of genes toward greater cell differentiation, (iv) reduction in cell proliferation, and (v) antioxidant activity (Fox and Eberl, 2002).

Exogenously administered $InsP_6$ is rapidly taken up by cells and dephosphorylated to other inositol phosphates, *e.g.*, $InsP_4$ and $InsP_5$, which interfere with signal transduction pathways and cell cycle arrest (Vucenik and

Shamsuddin, 2003). One of the advantages attributed to $InsP_6$ as a potential anticancer agent is that it has only a minimal effect on normal cells (Deliliers *et al*., 2002; Vucenik and Shamsuddin, 2003). Other beneficial health effects were attributed to $InsP_6$ treatment *in vivo*, including inhibition of kidney stone formation and reduction in the risk of developing cardiovascular diseases (via hypocholesterolemic and platelets antiaggregation effects) (Vucenik and Shamsuddin, 2003). The inhibitory effect on kidney stone formation may be attributed to potent inhibition of calcium salt crystallization (Grases *et al*., 2004). Because $InsP_6$ is abundant in the normal diet, is efficiently absorbed from the gastrointestinal system, and is considered safe, $InsP_6$ holds promise as a new strategy for the prevention and treatment of cancer. Not coincidently, $InsP_6$ is now being promoted extensively in health food stores as a "natural" anticancer compound. Importantly, the antineoplastic effects of $InsP_6$ were accompanied by serious side effects, such as chelation of multivalent cations and an increase in bladder and renal papillomas.

In summary, *in vivo* and *in vitro* studies indicate that $InsP_6$ may have significant potential as an effective agent for the prevention and treatment of cancer. To the best of our knowledge, no published controlled clinical trials have examined the anticancer effect of $InsP_6$.

2.3 Other inositol phosphates

The significance of $InsP_3$ as an important second messenger in multiple cellular pathways has led to the examination of the functions of other inositol phosphates.

2.3.1 Inositol 1,4-biphosphate ($InsP_2$)

The termination of $InsP_3$ intracellular signaling is achieved via the activity of a 5-phosphatase that converts $InsP_3$ to $InsP_2$. $InsP_2$ is a substrate for IPPase that removes a phosphate to form $Ins(1)P_1$. The latter is dephosphorylated by IMPase to give free *myo*-inositol. To the best of our knowledge, no established physiological function has been attributed to $Ins(1)P_1$ to date. On the other hand, $InsP_2$ was found to stimulate DNA polymerase-α (Sylvia *et al*., 1988) and may play a role in the control of DNA replication (York *et al*., 1994). Furthermore, in the heart, $InsP_2$ appears to be the primary inositol phosphate released (more than $InsP_3$) following "normal" adrenergic stimulation. $InsP_2$ levels were significantly reduced (70–90%) during global myocardial ischemia in rats (Woodcock *et al*., 1997). This study has demonstrated that $InsP_3$ content is also reduced significantly during the ischemic phase but predominates under conditions of postischemic reperfusion. It is possible that the release of $InsP_2$ substitutes for $InsP_3$ release because in some experimental models, enhanced $InsP_3$ signaling (and Ca^{2+} accumulation) is proarrhythmic in the heart (Jacobsen *et al*., 1996).

2.3.2 Inositol-tetrakisphosphates

Inositol-tetrakisphosphates are inositol phosphates containing four phosphates. Of these, inositol-1,3,4,5-tetrakisphosphate ($Ins(1,3,4,5)P_4$), along with its immediate catabolic product $Ins(1,3,4)P_3$, were among the first wave of inositol phosphates to be discovered (Irvine *et al*., 1984). One of the most important functions of $Ins(1,3,4,5)P_4$ is that it can protect $Ins(1,4,5)P_3$ against hydrolysis, probably by competing for the hydrolytic enzyme inositol 5-phosphatase (Connolly *et al*., 1987). $Ins(1,3,4,5)P_4$ is hydrolyzed by the same 5-phosphatase that hydrolyzes $Ins(1,4,5)P_3$, but the enzyme has a 10-fold higher affinity for $Ins(1,3,4,5)P_4$. In addition, $Ins(1,3,4,5)P_4$ can act in synergy with $Ins(1,4,5)P_3$ to mobilize Ca^{2+} and to activate its entry to cells (Morris *et al*., 1987; Smith *et al*., 2000). Furthermore, in endothelial cells (Luckhoff and Clapham, 1992) and in neurons (Tsubokawa *et al*., 1996), $Ins(1,3,4,5)P_4$ can *independently* activate Ca^{2+} channels in the plasma membrane.

Another member of the inositol-tetrakisphosphates is $Ins(3,4,5,6)P_4$. In epithelial cells, $Ins(3,4,5,6)P_4$ seems to be a physiologically important inhibitor of Ca^{2+}-regulated chloride channels (CLCA), and is now considered to be a regulator of chloride secretion (Vajanaphanich *et al*., 1994). Similar findings were observed by Ismailov and coworkers, in which the effect of $Ins(3,4,5,6)P_4$ on CLCA was found to be *bi-phasic*, namely, an initial phase of activation, followed by a prominent inhibition phase (Ismailov *et al*., 1996). These effects were not observed under treatment with other tetrakisphosphate isomers. The significance of this effect is particularly important for patients with cystic fibrosis (Ismailov *et al*., 1996) in whom the epithelial cyclic AMP-regulated chloride channel [or cystic fibrosis transmembrane conductance regulator, (CFTR)] is compromised, and CLCA is probably the most important remaining functional channel (Rudolf *et al*., 2003). It is worth noting that the majority of cystic fibrosis patients have a mutation in the gene encoding the CFTR (Modiano *et al*., 2004), resulting in defective hydration of mucosal membranes. Interestingly, the activity of CLCA was found to be enhanced in CF patients, a phenomenon that was suggested as a compensatory mechanism for the defect in CFTR activity (Leung *et al*., 1995). Therefore, restoring transepithelial chloride secretion by augmenting the activity of CLCA may have beneficial effects in the treatment of CF. This hypothesis was tested in a human colonic epithelial cell line by blocking the inhibitory effect of $Ins(3,4,5,6)P_4$ [using $Ins(3,4,5,6)P_4$ derivatives that can bind to but not inhibit the CLCA] on CLCA, which resulted in enhanced chloride secretion (Rudolf *et al*., 2003). These findings may serve as a basis for a new direction in the treatment of CF.

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

$Ins(1,3,4,5,6)P_5$ is the $InsP_5$ isomer that predominates in mammalian cells (Stephens *et al*., 1991). In most cells, receptor-stimulated release of

Ins(1,4,5)P_3 is accompanied by hydrolysis of Ins(1,3,4,5,6)P_5 and the generation of Ins(3,4,5,6)P_4 (Ye *et al*., 1995). Interestingly, InsP_5 acts as a potent antagonist of the IP_3R by competitive receptor binding (Lu *et al*., 1996). This inhibitory effect may have physiological significance because it dampens calcium mobilization. One of the most documented physiological functions for InsP_5 is regulation of the hemoglobin–O_2 interaction, as it was found that InsP_5 alters the affinity of hemoglobin for O_2 (Liang *et al*., 2001; Riera *et al*., 1991). A role for nuclear InsP_5 (and InsP_4) production in regulating gene expression was observed in yeast (York *et al*., 2001). The significance of these observations is still not well understood and now seems to be more complex than originally thought.

2.3.4 Inositol pyrophosphates

A fascinating discovery was made that an inositol ring containing six phosphates can accommodate additional phosphates (Menniti *et al*., 1993; Stephens *et al*., 1991). The simplest versions of inositol pyrophosphates are InsP_7 and InsP_8 [diphosphoinositol pentakisphosphate (InsP_5PP) and bis (diphospho) inositol tetrakisphosphate (Ins$P_4(PP)_2$), respectively] In InsP_7, the pyrophosphate occurs at the 1 position, whereas in InsP_8, the pyrophosphates occur at the 1 and 2, or 1 and 4 positions (Stephens *et al*., 1993). In mammalian cells, these metabolites turn over very rapidly and their most likely function is as an ATP-generating system (Voglmaier *et al*., 1996), as they were found to supply energy for vesicle formation and/or transport systems. These results suggest that pyrophosphates constitute an energy reservoir in mammalian cells, analogous to InsP_6 in plants. Additional pyrophosphates may yet be identified on different positions of the ring, further expanding the family of inositol phosphates. The physiological functions of these inositol phosphates remain to be ascertained.

3. PHOSPHOINOSITIDES

Phosphoinositides (PIs) are low in abundance but have been shown to play important roles in many signal transduction pathways. Various PIs have been found to interact with high-affinity PI-binding proteins and affect the activity and/or localization of these proteins (Toker, 2002). These specific PI-binding proteins further recruit and regulate specific signaling proteins that mediate many physiological processes, including cell growth, proliferation, apoptosis, insulin action, cytoskeletal assembly, and vesicle trafficking. The spatiotemporal control of signal transduction requires precise regulation of PI generation and turnover by PI-metabolizing enzymes, including specific kinases and phosphatases.

There are five free hydroxyl groups on the inositol ring of PIs. To date, three of these, D3, D4, and D5 are known to be phosphorylated *in vivo* (Toker,

2002). Eight PIs have been identified in the cell, including PI, PI(3)P, PI(4)P, PI(5)P, $PI(3,4)P_2$, $PI(4,5)P_2$, $PI(3,5)P_2$, and $PI(3,4,5)P_3$ (Figure 1). The intracellular levels of the PIs are strictly regulated by PI-metabolizing enzymes. In this part of the review, we focus on diseases caused by perturbation of PI metabolism.

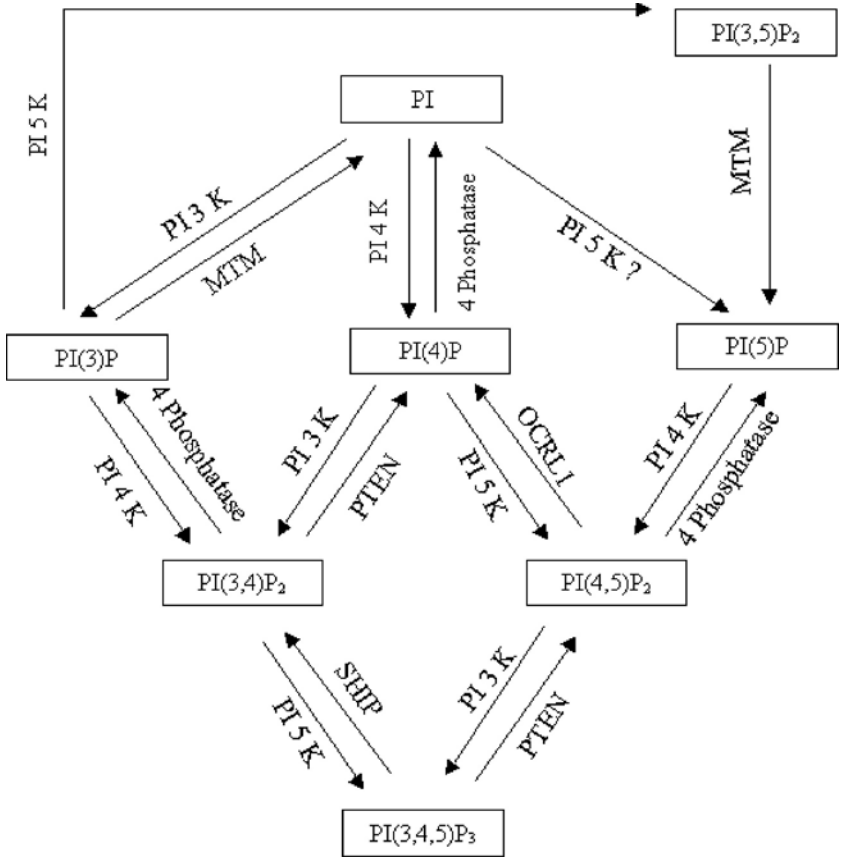

Figure 1. Synthesis of phosphoinositides (Martin, 1998; Pendaries *et al*., 2003).

3.1 PI3K/AKT pathway in cancer

PI3 kinase (PI3K) was first brought to the attention of cancer researchers in the mid-1980s, when it was discovered that the viral oncoprotein SRC and polyomavirus middle T-antigen could induce PI3K activity (Whitman *et al*., 1985). PI3Ks are classified into three groups according to their structures and specific substrates. Activated PI3Ks specifically phosphorylate PI, PI(4)P, and $PI(4,5)P_2$ to generate PI(3)P, $PI(3,4)P_2$, and $PI(3,4,5)P_3$. PI3Ks are heterodimeric

enzymes composed of a catalytic subunit, p110, and a regulatory subunit, p85 (Cantley, 2002). The class I PI3Ks are activated by *r*eceptor *t*yrosine *k*inases (RTK), and catalyze the conversion of PI(4,5)P_2 to PI(3,4,5)P_3. PI(3,4,5)P_3 is a quantitatively minor PI, normally undetectable and transiently increased when PI3K is activated by various agonist-mediated stimuli. Acting as a second messenger, PI(3,4,5)P_3 recruits pleckstrin homology (PH) domain-containing proteins, such as the cellular homolog of Akt retroviral oncogene protein serine–threonine kinase (AKT), also called protein kinase B (PKB), and PI-dependent kinase 1 (PDK1) to the plasma membrane. AKT is subsequently phosphorylated at Thr308 by PDK1 and at Ser473 by a putative PDK2. Activated AKT phosphorylates multiple downstream proteins on serine and threonine residues (Vivanco and Sawyers, 2002). Through phosphorylation of these targets, AKT carries out its important role in the regulation of many aspects of cellular physiology, including glucose metabolism, cell proliferation, cell growth, and survival (Figure 2). Constitutive activation of PI3K/AKT has been implicated in various human cancers. Increased expression of the gene encoding the catalytic subunit (p110) of PI3K was found in ovarian, breast, and colon cancer. In addition, a gain of function mutation in the regulatory subunit p85 has been identified in ovarian and colon cancer (Vivanco and Sawyers, 2002).

PTEN (*p*hosphatase and *ten*sin homolog deleted on chromosome *ten*)/*MMAC* (*m*utated in *m*ultiple *a*dvanced *c*ancers)/*TEP-1* (*T*GF-β-regulated and *e*pithelial cell-enriched *p*hosphatase) was originally identified in 1997 by two different groups as a tumor suppressor gene on chromosome 10q23, using traditional positional-cloning strategies (Li *et al*., 1997; Steck *et al*., 1997). Mutations in the *PTEN* gene were associated with breast cancer, glioblastomas, prostate, endometrial, renal and small cell lung carcinoma, melanoma, and meningioma. Subsequent studies confirmed that the *PTEN* gene is defective in a large number of human cancers (Cantley and Neel, 1999). In addition, germ-line mutations in *PTEN* result in three rare, dominant, inherited diseases: Cowden disease, Lhermitte-Duclos disease, and Bannayan-Zonana syndrome (Liaw *et al*., 1997; Marsh *et al*., 1998; Nelen *et al*., 1997). These disorders are characterized by multiple hamartomas and increased risk of developing cancers. These genetic data suggest that PTEN is important for normal cell growth and that *PTEN* dysfunction contributes to carcinogenesis.

Although analysis of the *PTEN* sequence suggested that it is a dual-specificity phosphatase, it is difficult to identify the biologically relevant targets of PTEN. In 1998, Maehama and Dixon showed that PTEN is actually a PI phosphatase, rather than a protein phosphatase (Maehama and Dixon, 1998). PTEN could dephosphorylate the D3 position of PI(3,4,5)P_3 both *in vitro* and *in vivo*, leading to decreased PI(3,4,5)P_3 levels. This finding led to a model in which PTEN is a negative regulator of the PI3K/AKT pathway. The deletion or inactivation of *PTEN* results in constitutive PI3K/AKT activation and aberrant cell growth.

Aside from PTEN, SH-2 domain-containing *i*nositol 5' *p*hosphatases SHIP1 and SHIP2 also regulate PI(3,4,5)P_3 levels by removing the D5 phosphate.

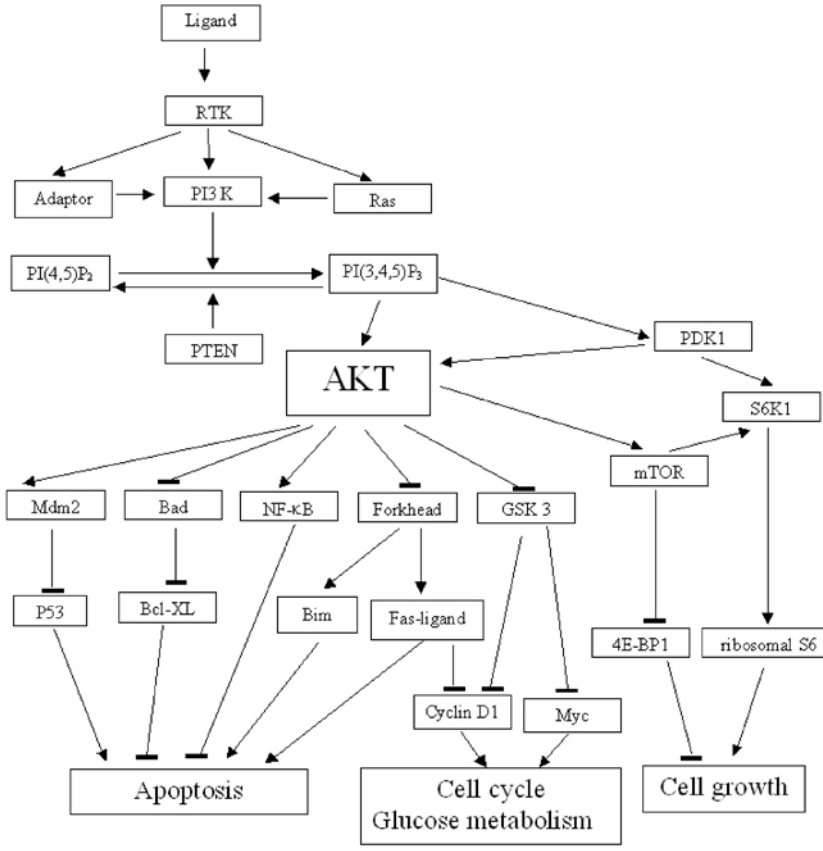

Figure 2. The PI3 Kinase (PI3K)/AKT pathway (Luo *et al*., 2003). Growth factor *r*eceptor *t*yrosine *k*inases (RTKs) receive ligand stimulation and recruit class I PI3K through direct interaction, adaptor molecules, or Ras. At the membrane, PI3K phosphorylates PI (4,5) P2 to PI(3,4,5)P_3. PTEN removes the D3 phosphate from PI (3,4,5) P3, thus regulating the intracellular PI(3,4,5)P_3 level. PI (3,4,5) P3 recruits the cellular homolog of Akt retroviral oncogene protein serine–threonine kinase (AKT) and *p*hosphoinositide-*d*ependent *k*inase 1 (PDK1) to the membrane. AKT is subsequently phosphorylated by PDK1 and a putative PDK2. Activated AKT regulates a number of downstream targets. AKT promotes cell survival by inhibition of apoptosis, regulates cell cycle, and stimulates glucose metabolism. By activating the *m*ammalian *t*arget *o*f *r*apamycin (mTOR) pathway, AKT also stimulates protein synthesis and boosts cell growth.

SHIP1 is expressed exclusively in hematopoietic cells (Geier *et al*., 1997; Liu *et al*., 1998). Homozygous disruption of *SHIP1* in mice leads to a myeloproliferative syndrome characterized by a dramatic increase in the number of granulocyte–macrophage progenitor cells in the marrow and spleen (Helgason *et al*., 1998). Based on the *SHIP1* gene knockout mouse phenotype and the hydrolysis of PI(3,4,5)P_3, SHIP1 protein is likely a negative regulator of the PI3K/AKT pathway. Consistent with this hypothesis, *SHIP1* gene expression

and protein half-life were decreased in primary neoplastic cells from patients with chronic myelogenous leukemia (Sattler *et al*., 1999). Expression of constitutively active SHIP1 protein in the leukemic Jurkat cell line decreases the $PI(3,4,5)P_3$ level and the activity of AKT (Freeburn *et al*., 2002). Recently, a mutation in the human *SHIP1* gene has been found in acute myeloid leukemia (Luo *et al*., 2003). The mutation reduced the catalytic activity of SHIP1 and led to enhanced PI3K/AKT activation following IL-3 stimulation. Overall, these data suggest that SHIP1 plays an important role in controlling the $PI(3,4,5)P_3$ level and downstream PI3K/AKT pathway activity.

3.2 Type 2 diabetes

3.2.1 PI3K/AKT pathway in type2 diabetes

One of the downstream targets of the PI3K/AKT pathway is glucose metabolism (Figure 2). AKT phosphorylates and regulates GSK-3, which is critical in insulin-mediated glucose metabolism (Shepherd *et al*., 1998). Overexpression of AKT or the PI3K catalytic subunit p110 stimulates insulin-mediated glucose metabolism (Katagiri *et al*., 1996; Ueki *et al*., 1998), which is blocked upon loss of function of the PI3K regulatory subunit p85 (Calera *et al*., 1998; Sharma *et al*., 1998). Insulin stimulates glucose uptake in adipose and muscle tissue by translocating the glucose transporter (GLUT4) from intracellular sites to the cell surface. Deficiency in GLUT4 translocation and glucose uptake in response to insulin stimulation is an important cause of type 2 diabetes (Shulman, 2000). GLUT4 translocation has been shown to be regulated by the PI3K/AKT pathway (Baumann *et al*., 2000; Kanzaki and Pessin, 2003; Saltiel and Kahn, 2001). Insulin-stimulated translocation of GLUT4 to the cell surface and glucose uptake into cells is blocked by the PI3K inhibitors wortmannin and LY294002 (Kanai *et al*., 1993; Okada *et al*., 1994).

As discussed above, the activity of the PI3K/AKT pathway is also regulated by $PI(3,4,5)P_3$ D3 phosphatase (PTEN) and D5 phosphatase (SHIP1, SHIP2). Overexpression of PTEN reduces insulin-induced PI3K/AKT pathway activity, GLUT4 translocation, and glucose uptake into cells (Nakashima *et al*., 2000; Ono *et al*., 2001). Microinjection of an anti-PTEN antibody increases insulin-stimulated translocation of GLUT4 to the cell surface and glucose uptake into cells (Nakashima *et al*., 2000). These results suggest that PTEN reduces insulin sensitivity, which is increased upon inhibition of PTEN.

SHIP1 is expressed only in hematopoietic cells, while SHIP2 is expressed ubiquitously. SHIP2 dephosphorylates $PI(3,4,5)P_3$ to $PI(3,4)P_2$ and attenuates insulin-stimulated PI3K/AKT activity. Overexpression of SHIP2 protein inhibits insulin-stimulated PI3K/AKT activity and leads to GSK-3 inactivation followed by reduced glycogen synthetase activity (Blero *et al*., 2001). Genetic deletion of *SHIP2* in mice resulted in increased PI3K/AKT activity and insulin sensitivity *in vivo* (Clement *et al*., 2001). Interestingly, mutations in SHIP2 that

result in increased SHIP2 activity have been associated with type 2 diabetes in both mice and humans (Marion *et al*., 2002). Altogether, these results suggest that SHIP2 also reduces insulin signaling, and inhibitors of SHIP2 may be candidates for the treatment of type 2 diabetes.

3.2.2 PI5P in insulin signaling

As mentioned above, alteration of GLUT4 translocation contributes to the development of insulin resistance and type 2 diabetes. Aside from the PI3K/AKT dependent pathway, GLUT4 translocation is also mediated by a PI3K/AKT independent pathway (Bachhawat and Mande, 1999). Recently, it has been suggested that PI5P is a novel intermediate for insulin signaling and GLUT4 translocation (Sbrissa *et al*., 2004). PI5P is the most recently identified member of the PI family and its physiological function is not clear. After insulin stimulation, a transient increase in PI5P was observed in both CHO-T and 3T3-L1 adipocytes. The PI3K inhibitor wortmannin could not block this PI5P increase. In 3T3-L1 adipocytes, microinjected PI5P, but not other PIs, mimicked the effect of insulin in translocating GLUT4 to the cell surface. Taken together, these results suggest that PI5P mediates insulin signaling and GLUT4 translocation. However, there is currently no direct evidence for altered levels of PI5P in type 2 diabetes patients.

3.3 Lowe syndrome

Lowe syndrome is a rare X-linked disorder characterized by severe congenital cataracts, mental retardation, and renal tubular dysfunction. It is also called OCRL syndrome (*o*culo-*c*erebro-*r*enal syndrome of *L*owe) because of the three major organ systems affected. The gene responsible for Lowe syndrome, *OCRL1*, has been cloned and different mutations have been identified (Addis *et al*., 2004; Attree *et al*., 1992; Lin *et al*., 1997; Monnier *et al*., 2000). *OCRL1* encodes a 105 kDa PI(4,5)P_2 5-phosphatase (Zhang *et al*., 1995). The OCRL1 protein is expressed in almost all tissues examined, except hematopoietic cells (Janne *et al*., 1998; Olivos-Glander *et al*., 1995). It was originally reported that OCRL1 protein was localized in the Golgi apparatus in fibroblasts (Olivos-Glander *et al*., 1995; Suchy *et al*., 1995). Cells deficient in the *OCRL1* gene accumulate PI(4,5)P_2, consistent with loss of function of the phosphatase activity (Zhang *et al*., 1998). In cells from Lowe syndrome patients, high levels of extracellular lysosomal enzymes were found, which may contribute to the pathogenesis of the disease (Olivos-Glander *et al*., 1995; Suchy *et al*., 1995; Ungewickell and Majerus, 1999; Zhang *et al*., 1998). A subsequent study showed that OCRL1 was located in the trans-Golgi network in fibroblasts and two kidney epithelial cell lines (Dressman *et al*., 2000; Suchy *et al*., 1995). Recent evidence also indicates that OCRL1 was also localized in endosomes (Ungewickell *et al*., 2004). The localization of OCRL1 in organelles involved

in trafficking suggests that defective OCRL1 may lead to abnormal vesicle trafficking.

Abnormalities in the actin cytoskeleton, including decreased actin stress fibers and altered response to depolymerizing agents were also observed in Lowe patients (Suchy and Nussbaum, 2002). Two actin-binding proteins, gelsolin and alpha-actinin, which are regulated by both PIP_2 and Ca^{2+} were found to be abnormally distributed. Actin polymerization plays a key role in cell – cell contacts, which may be defective in Lowe syndrome.

3.4 Diseases associated with the myotubularin family

The myotubularin (MTM) family was originally identified as a member of the *p*hospho*t*yrosine *p*hosphatases (PTP)/*d*ual-*s*pecificity *p*hosphatases (DSPs), which are able to dephosphorylate phosphotyrosine and phosphoserine/threonine residues (Laporte *et al*., 1996). MTMs contain the conserved sequence H*C*SDGWD*R*TXE, which fits with the CX_5R signature of the PTP/DSPs. Recent studies demonstrate that the preferred substrates of MTMs are PI 3-phosphatases (Blondeau *et al*., 2000; Taylor *et al*., 2000). The MTM family in humans consists of 14 members, including MTM1 and MTM-related (MTMR) proteins 1–13 (Laporte *et al*., 1998; Maehama *et al*., 2001; Wishart *et al*., 2001). Among these, *MTM1* was found to be mutated in X-linked myotubular myopathy (XLMTM). *MTMR*2 and *MTMR*13 are responsible for Charcot-Marie-Tooth disease type 4B1 (CMT4B1) and Charcot-Marie-Tooth disease type 4B2 (CMT4B2), respectively.

3.4.1 Myotubular myopathy (XLMTM)

Myotubular myopathy (XLMTM) is an X-linked severe recessive disorder characterized by muscle weakness and hypotonia that affects 1 out of 50,000 newborn males. The histological analysis of patients' muscles shows small rounded fibers with central nuclei. This is a characteristic of fetal myotubes, whereas mature muscle fibers have peripherally located nuclei (Sarnat, 1990). *MTM*1, the gene responsible for XLMTM and located on chromosome Xq28, was cloned in 1996 (Laporte *et al*., 1996). Mutations in *MTM*1 have been reported in more than 300 patients (de Gouyon *et al*., 1997; Herman *et al*., 2002; Laporte *et al*., 2000; Nishino *et al*., 1998; Tanner *et al*., 1999). *MTM*1 codes for myotubularin and is expressed ubiquitously. Although MTM1 shows a dual-specificity protein phosphatase activity *in vitro*, the preferred substrate of MTM1 *in vivo* is PI(3)P (Blondeau *et al*., 2000; Taylor *et al*., 2000). Thus, MTM1 may regulate PI(3)P levels by directly degrading PI(3)P to PI. A recent study found that MTM1 could dephosphorylate both $PI(3,5)P_2$ and PI(3)P. The GRAM (*G*lucosyltransferase, *R*ab-like GTPase *A*ctivator and *M*yotubularins) domain of MTM1 binds to $PI(3,5)P_2$ with high affinity *in vivo*. This GRAM-$PI(3,5)P_2$ interaction is essential for translocation of MTM1 to the late endosomal compartment in response to growth factor stimulation (Tsujita *et al*., 2004). These findings

suggest that MTM1 may also regulate PI(3,5)P_2 levels, and may function in late endosomal trafficking *in vivo*. *MTM1* knockout mice are viable and show normal muscle differentiation at birth, but a progressive myopathy appears a few weeks after birth (Buj-Bello *et al*., 2002). This indicates that MTM1 is essential for skeletal muscle maintenance but not for myogenesis in mice. Studies in knockout mice will help to elucidate the function and the regulatory mechanisms of MTM1 and increase understanding of the pathology of XLMTM.

3.4.2 Charcot-Marie-Tooth (CMT) disease

Charcot-Marie-Tooth (CMT) disease is a group of disorders characterized by chronic motor and sensory polyneuropathy. CMT4B1 is an autosomal recessive neuropathy with focally folded myelin sheaths and demyelination. Mutations in *MTMR*2 on chromosome 11q22 cause CMT4B1 (Bolino *et al*., 2000). In CMT4B1 patients, 3' phosphatase activity is dramatically reduced. PI(3)P is a substrate of both MTM1 and MTMR2. But the preferred substrate of MTMR2 is PI(3,5)P_2 (Berger *et al*., 2002). Misregulation of PI(3,5)P_2 in neurons may affect neural membrane recycling and membrane trafficking. The different substrate preferences of MTMR2 and MTM1 may account for the different pathologies of the diseases associated with mutations of these two genes.

Mutations in the *MTMR*13 gene cause CMT4B2 (Azzedine *et al*., 2003). *MTMR*13 is located on chromosome 11p15 and encodes an inactive phosphatase (Senderek *et al*., 2003). How this inactive phosphatase causes disease remains uncertain. However, it is possible that MTMR13 acts as an adaptor or directly regulates MTMR2 activity due to the similar phenotype of CMT4B2 and CMT4B1(Pendaries *et al*., 2003).

4. SUMMARY

In the past two decades, considerable progress has been made toward understanding inositol phosphates and PI metabolism. However, there is still much to learn. The present challenge is to understand how inositol phosphates and PIs are compartmentalized, identify new targets of inositol phosphates and PIs, and elucidate the mechanisms underlying spatial and temporal regulation of the enzymes that metabolize inositol phosphates and PIs. Answers to these questions will help clarify the mechanisms of the diseases associated with these molecules and identify new possibilities for drug design.

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