Distinct Phosphatidylinositol 3-Kinase Lipid Products Accumulate upon Oxidative and Osmotic Stress and Lead to Different Cellular Responses*

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Signaling by phosphatidylinositol (PI) 3-kinases is mediated by 3-phosphoinositides, which bind to Pleckstrin homology (PH) domains that are present in a wide spectrum of proteins. PH domains can be classified into three groups based on their different lipid binding specificities. Distinct 3-phosphoinositides can accumulate upon PI 3-kinase activation in cells in response to different stimuli and mediate specific cellular responses. In Swiss 3T3 mouse fibroblasts, oxidative stress induced by 1 mM H₂O₂ caused almost exclusive accumulation of phosphatidylinositol 3,4-bisphosphate (PtdIns(3,4)P₂), whereas osmotic stress increased both phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P₃) and PtdIns(3,4)P₂ levels. The increase in PtdIns(3,4)P₂ levels, caused by oxidative stress, correlated with the activation of protein kinase B, which has a promiscuous PH domain that binds both PtdIns(3,4,5)P₃ and PtdIns(3,4)P₂. P70 S6 kinase, another signaling component downstream of PI 3-kinase, however, was not activated by this oxidative stress-induced increase in PtdIns(3,4)P₂ levels. Increased PtdIns(3,4,5)P₃ and PtdIns(3,4)P₂ levels in response to osmotic stress did not correlate with protein kinase B activation, because of concomitant activation of an inhibitory pathway, but p70 S6 kinase was activated by osmotic stress. These results demonstrate that PtdIns(3,4)P₂ can accumulate independently of PtdIns(3,4,5)P₃ and exerts a pattern of cellular responses that is distinct from that induced by accumulation of PtdIns(3,4,5)P₃.

The significance of PI³ 3-kinases in the regulation of a wide spectrum of cellular signaling events has been well established (1, 2). Based on structural features and substrate specificity, three classes of catalytic subunits of PI 3-kinases have so far been recognized (2, 3). The Type I class, members of which phosphorylate PtdIns, PtdIns4P, and PtdIns(4,5)P₂ in vitro but utilize PtdIns(4,5)P₂ as the likely substrate in vivo, is subdivided into Type Iα and Type Iγ. Type Iα PI 3-kinases interact with adaptor proteins containing SH2 domains that bind phosphotyrosine residues, linking this class to tyrosine kinase signaling cascades. Type Iγ PI 3-kinases are stimulated by G-protein βγ subunits and do not interact with SH2 domain-containing adaptor proteins but with p101, a novel adaptor protein that has no homology with known proteins. Type II PI 3-kinases phosphorylate PtdIns and PtdIns4P in vitro (and not PtdIns(4,5)P₂) and contain a C2 domain, implicated in lipid binding. Type III PI 3-kinases only phosphorylate PtdIns and are thought to be constitutively active. They can be recruited and regulated by their adaptor, a dual specificity protein kinase, with which they form heterodimers.

The principle enzymatic activity of PI 3-kinases involves the phosphorylation of phosphoinositides on the 3-position of the inositol ring, resulting in the formation of 3-phosphoinositides, which can either directly interact with appropriate modules of specific target proteins or first undergo further phosphorylation. Examples of the latter mechanism include the observation that in platelets, PtdIns3P can be phosphorylated by a PtdIns3P 4-kinase activity to give PtdIns(3,4)P₂, which activates PKB (4), and in yeast, a PtdIns3P 5-kinase activity encoded by the FAB1p gene has been shown to be essential for vacuolar functioning (5). FYVE domains are the structural modules that specifically bind PtdIns3P (6, 7), whereas PH domains confer specificity for polyphosphoinositides (PtdIns(4,5)P₂, PtdIns(3,4)P₂, and PtdIns(3,4,5)P₃) (8, 9). More than 100 different proteins containing PH domains have been identified, and the majority require membrane association for their function, which is mediated via interaction with phosphoinositides. PH domains can be assigned to one of three groups based on their affinities for PtdIns(4,5)P₂, PtdIns(3,4)P₂, and PtdIns(3,4,5)P₃ (or their polar headgroup), although many details involved in binding specificity still have to be elucidated (8). Modelling of PH domains, based on sequence alignments, resolved crystal structures, and binding studies, are beginning to allow predictions of binding specificities and hence likely physiological ligands.

Cells respond to different extracellular inputs from their environment by activation of protein kinase cascades. These complex networks allow amplification of the signal and can mediate the required diversity of cellular responses. The classical MAP kinase pathway is activated by agonists that include growth factors and tumor-promoting agents and the SAPK/p38 pathway is activated by cellular stresses and inflammatory cytokines. PI 3-kinases can mediate cellular responses to stress, but their direct role and the mechanism of activation is not well defined.

There are relatively few reports on direct activation of PI
3-kinase upon exposure to cellular stresses. Heat shock and oxidative stress have both been reported to activate PI 3-kinase activity directly in NIH 3T3 cells (10, 11) or indirectly by manipulation of downstream signaling components (12). Both heat shock and oxidative stress effects were suramin-sensitive (10, 12), suggesting the involvement of growth factor receptors. Konishi et al. (13) showed that osmotic shock activated wild-type but not mutated PKB in transfected COS-7 cells, whereas others failed to detect this in Swiss 3T3 cells (14, 15). The involvement of PI 3-kinase in stress responses inferred by activation of PKB or other downstream signaling components, however, can be misleading as demonstrated by the following two observations in this report. Firstly, mannose-sensitive PKB activation was strongly enhanced upon H2O2 treatment in activation of PKB or other downstream signaling components, whereas the PKB activity in 4 ml of scintillant (FloScint IV).

**p70 S6 Kinase Activity Assay**—Measurement of p70 S6 kinase was done exactly as described for the PKB assay except that the antibody was raised against p70 S6 kinase, and the substrate peptide used was PKKRTKTV at 100 μM.

**3-kinase Activity Assay**—PI 3-kinase was immunoprecipitated from Swiss 3T3 mouse fibroblast cell lysates, and in vitro activity was measured as follows. After treatment (described above) the cell medium was aspirated, ice-cold lysis-buffer was added, cells were scraped, and the lysates were spun for 2 min at 20,800 g at 4 °C. The supernatants were snap frozen in liquid nitrogen and stored at −80 °C.

Anti-phosphotyrosine antibody (1 μg/sample) was precoupled to protein G-agarose beads (5 μl/sample) and added to each sample. After 90 min at a shaking platform at 4 °C, the beads were washed down, the supernatant was aspirated, and the beads were washed twice with 1 ml of ice-cold lysis-buffer containing 0.5 M NaCl and twice with 0.1 M Tris/HCl, pH 7.5, 0.1 mM EDTA, 0.1% β-mercaptoethanol, 20 μM eIF4EBP1 peptide, 50 μM protein G-agarose beads, and 1 μCi of [γ-32P]ATP. After 30 min at 30 °C on a shaking platform, the reactions were terminated by pipetting the suspension onto 2.5 × 2.5-cm phosphocellulose papers that were washed four times for 15 min in 0.5% ortho-phosphoric acid and then washed once with acetone. The dried filters were counted for radioactivity.

**Materials**

- Insulin-like growth factor I (IGF-1) and protein G-agarose were from Sigma.
- Anti-phosphotyrosine monoclonal antibody G410 was from Upstate Biotechnology, Inc. The horseradish peroxidase-conjugated anti-antibody was from Scottish Antibody Production Unit in Dundee.
- The anti-PKB Ser(P) 473 antibody, both raised in sheep, were a gift from the Medical Research Council protein phosphorylation unit in Dundee.

**Methods**

**Cell Culture and PKB Activity Assay—Swiss 3T3 mouse fibroblasts** were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum in a 5% CO2 atmosphere at 37 °C. Cells were grown to confluence in 6-well plates (sometimes in insitol-free Dulbecco’s modified Eagle’s medium with the omission of fetal bovine serum for parallel comparison with cell labeling experiments; see below). Prior to treatment, the medium was aspirated, and the cells were washed twice with 2 ml of modified Krebs Henseleit (KRH) buffer (0.1 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 1.3 mM CaCl2, 1 mM KH2PO4, 25 mM HEPES/NaOH, pH 7.4, and 2 mM d-glucose) and then conditioned for 60 min in 3 ml of KRH buffer in a 37 °C waterbath. Cells were treated as described above but quenched with 1 ml of ice-cold 10% trichloroacetic acid (w/v), scraped on ice and transferred to Eppendorf tubes. The wells were rinsed once with 0.5 ml of ice-cold 10% trichloroacetic acid, and the particulate material was pelleted by centrifugation (5 min at 20,800 × g). The supernatant was removed, and the pellet was washed once with 1 ml of ice-cold 5% trichloroacetic acid/1 ml EDTA. The lipids were extracted in ice for 20 min in 0.75 ml of CHCl3:MeOH:concentrated HCl (40:80:1), and two phases were obtained by adding 0.25 ml of CHCl3 and 0.25 ml of 0.1 M HCl. After centrifugation the upper phase was aspirated, and the organic phase was washed twice with 0.5 ml of synthetic upper phase and dried down. The lipids were analyzed by TLC on silica 60 plates using CHCl3:MeOH:ammonia:H2O (75:100:15:25) as the mobile phase. The bands were visualized by autoradiography and scraped, and the radioactivity was measured in a scintillation counter.

**Cell Labeling and Phosphoinositide Analysis—Swiss 3T3 mouse fibroblasts** were seeded in 6-well plates at 106 cells/cm2 in Dulbecco’s modified Eagle’s medium without insitol and supplemented with 10% dialyzed fetal bovine serum. After 48 h, the medium was replaced with 4 ml of fresh medium containing 10 μM [3H]inositol [10 Ci/mmol] and incubated for 20 min on a platform shaker at 37 °C. The reactions were terminated with 0.75 ml of CHCl3:MeOH:concentrated HCl (40:80:1), and two phases were obtained by adding 0.25 ml of CHCl3, and 0.25 ml of 0.1 M HCl. After centrifugation the upper phase was aspirated, and the organic phase was washed twice with 0.5 ml of synthetic upper phase and dried down. The lipids were analyzed by TLC on silica 60 plates using CHCl3:MeOH:ammonia:H2O (75:100:15:25) as the mobile phase. The bands were visualized by autoradiography and scraped, and the radioactivity was measured in a scintillation counter.

- The PKB C-terminal antibody and the anti-PKB Ser(P)473 antibody, both raised in sheep, were a gift from the Medical Research Council protein phosphorylation unit in Dundee.

**Experimental Procedures**

- [γ-32P]ATP (3000 Ci/mmol) and Enhanced Chemoluminescence kit was from Amersham Pharmacia Biotech.
- [3H]Inositol was from NEN Life Science Products. Partisphere SAX column was from Whatman.
- Insulin-like growth factor I (IGF-1) and protein G-agarose were from Sigma.
- Anti-phosphotyrosine monoclonal antibody G410 was from Upstate Biotechnology, Inc. The horseradish peroxidase-conjugated anti-sheep/goat antibody was from Scottish Antibody Production Unit in Dundee.

**Oxidative and Osmotic Stress Activates Different Pathways**

Oxidative and osmotic stress activates different pathways. Whereas oxidative stress and osmotic shock activates many different components of several kinase cascades (16–18), the role of PI 3-kinase in these events has been analyzed in more detail. This report shows the accumulation of distinct PI 3-kinase lipid products, in response to oxidative stress and osmotic shock treatment of Swiss 3T3 mouse fibroblasts, and the activation of different metabolic pathways and lead to distinct patterns of cellular response. Whereas oxidative stress caused a sustained accumulation of PtdIns(3,4,5)P3 and to subsequent activation of PKB but not p70 S6 kinase, osmotic shock increased PtdIns(3,4,5)P3 concentration and subsequent activation of p70 S6 kinase but did not activate PKB.
that had essentially the same characteristics. Recombinant GST-GAP154-488 (expressed in Escherichia coli and purified on glutathione-
agarose beads) replaced crude cerebellar membrane from sheep. Total cellular lipids were isolated from confluent 6-well plates as described above.

Western Blot—Cell lysates were obtained exactly as described for the PKB activity assay. Approximately 20 μg of cytosolic protein from each sample was subjected to SDS-polyacrylamide gel electrophoresis on 2 gels and transferred to polyvinylidene difluoride membranes. One membrane was incubated with the anti-PKB C-terminal antibody, and the other was incubated with anti-PKB Ser(473) antibody each at 1 μg/ml in Tris-buffered saline, 0.1% Tween-20, 3% dried milk. The secondary horseradish peroxidase-conjugated anti-sheep/goat antibody allowed visualization by enhanced chemiluminescence according to the manufacturer’s protocol.

RESULTS AND DISCUSSION

The Effects of Cellular Stresses on PKB Activity Do Not Correlate with Changes in PtdIns(3,4,5)P3 Levels—The potential of cellular stresses to activate the PI 3-kinase pathway was investigated using a radioligand displacement assay that specifically measures PtdIns(3,4,5)P3. Exposure of Swiss3T3 mouse fibroblasts to osmotic stress and heat shock increased PtdIns(3,4,5)P3 levels, but oxidative stress or treatment with anisomycin or UV irradiation did not (Fig. 1; data for heat shock, anisomycin, and UV radiation not shown).

These cellular stresses were subsequently tested for their ability to activate PKB, which, both in vitro and in vivo, requires binding of either PtdIns(3,4,5)P3 or PtdIns(3,4)P2 to its PH domain (22, 23). Consistent with the measured increase in PtdIns(3,4,5)P3 concentration, heat shock-activated PKB (14), but surprisingly, osmotic stress did not activate PKB, despite causing a wortmannin-sensitive increase in PtdIns(3,4,5)P3 production. UV radiation and anisomycin, which did not increase the PtdIns(3,4,5)P3 concentration, as expected showed no effect on PKB activity (14). Exposure to oxidative stress, however, strongly activated PKB, without significantly increasing PtdIns(3,4,5)P3 levels (Figs. 1 and 2).

Oxidative stress activates stress-activated MAP kinase cascades, including that culminating in MAPKAP-K2 activation (16), which has been shown to activate PKBα 5-fold in vitro by phosphorylating Ser473 (24) but was not responsible for PKB activation in vivo for the following reasons. The activation of PKB by oxidative stress was completely insensitive to the specific inhibitor SB203580, which prevents activation of MAPKAP-K2 through inhibition of p38 MAP kinase, its upstream activator. Moreover, other cellular stresses that activated MAPKAP-K2 to the same extent as oxidative stress failed to activate PKB (14). MAPKAP-K2, therefore, is unlikely to account for the oxidative stress-induced activation of PKB that occurs in the absence of a detectable increase in PtdIns(3,4,5)P3.

Wortmannin-sensitive Stimulation of PKB Activity by Oxidative Stress Is Mediated by Increased PtdIns(3,4)P2 Levels—The observation that PKB activation stimulated by oxidative stress in Swiss 3T3 mouse fibroblasts did not coincide with increased PtdIns(3,4,5)P3 levels when measured with a radioligand displacement assay called for a more detailed investigation. The increase in PKB activity in these cells upon treatment with 1 mM H2O2 has a slow onset and is transient, reaching a maximum at 20 min and declining to basal levels by 60 min. By contrast, IGF-1 stimulation of Swiss 3T3 mouse fibroblasts did cause an increase in PtdIns(3,4,5)P3 levels, which closely paralleled increased PKB activity (Figs. 1 and 2). Two possible explanations are that oxidative stress activated PI 3-kinase but a subsequent, significant PtdIns(3,4,5)P3 increase was too small to be detected or that the concentration of an alternative activator, such as PtdIns(3,4)P2, increased in the absence of a detectable rise in PtdIns(3,4,5)P3 levels.

To address these possibilities, the activity state of PI 3-kinase upon treatment with H2O2 was measured in vitro in anti-phosphotyrosine immunoprecipitates. Fig. 4 shows that IGF-1 strongly stimulated anti-phosphotyrosine associated PI 3-kinase activity, whereas H2O2 treatment resulted in a very small but significant increase at 10 min that was not detected at 30 min.

Studies on the mechanism of PKB activation have revealed that full activation of the enzyme requires phosphorylation on both Thr308 and Ser473, probably mediated by PDK1 (25). These phosphorylation events are preceded by binding of PtdIns(3,4,5)P3 or PtdIns(3,4)P2 to the PH domain of PKB. This not only serves to localize PKB to the plasma membrane but also exposes the phosphorylation sites to PDK1, which itself is constitutively localized at this site via its own PH domain (26). The question of whether PKB was phosphorylated upon oxidative stress was addressed by Western blot analysis using an antibody that specifically recognizes PKBα which is phosphorylated at the Ser473 residue. Fig. 3A, shows that IGF-1 and H2O2 specifically stimulated the phosphory-
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3-Phosphoinositide levels from in vivo cell labeling with [3H]inositol of Swiss 3T3 mouse fibroblasts were represented as the means ± S.D. from one representative experiment repeated three times and measured in triplicate. Cells were grown to confluence in 6-well plates and were labeled with 40 μCi/4 ml of [3H]inositol. After treatment at the indicated times, the cellular lipids were extracted and deacylated, and the resulting glycerophosphoinositolphosphates were analysed by HPLC. ND, not detected.

|                     | PtdIns3P | PtdIns3,4P2 | PtdIns3,4,5P3 |
|---------------------|----------|-------------|---------------|
| Control             | 15902 ± 377 | ND          | 185 ± 94      |
| IGF-1               | 17641 ± 659 | 516 ± 163  | 2157 ± 304    |
| H2O2 10’            | 10506 ± 647 | 3610 ± 516 | 562 ± 209     |
| H2O2 30’            | 11596 ± 209 | 3782 ± 241 | 187 ± 80      |
| Sucrose 10’         | 54381 ± 2152 | 402 ± 8   | 768 ± 137     |
| Sucrose 30’         | 46355 ± 3696 | 841 ± 327 | 528 ± 108     |

\[\text{3-Phosphoinositide levels in response to IGF-1, oxidative, and osmotic stress in Swiss 3T3 cells.}\]

The effect of oxidative stress on p70 S6 kinase activity, a signaling molecule whose activation usually requires PI 3-kinase activity, was also investigated. Although the regulation of p70 S6 kinase activity is not yet fully understood, several reports have suggested roles for the PI 3-kinase signaling molecules, PDK1, Rac, and PKB in its activation (28–31). The interaction of these molecules, and/or their activators, with 3-phosphoinositides is thought to be crucial for their activation. Because oxidative stress leads to increased PtdIns(3,4)P2 levels, its potential to activate p70 S6 kinase was examined. Fig. 5 shows that stimulation for 30 min with IGF-1 resulted in a ~20-fold activation of p70 S6 kinase, which was completely rapamycin- and wortmannin-sensitive. Treatment with H2O2 for 30 min showed no significant stimulation, suggesting that unlike for the stimulation of PKB, PtdIns(3,4)P2 was not sufficient for p70 S6 kinase activation (treatment for 20 min with...
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H₂O₂, at which time the transient activation of PKB is maximal, did not activate p70 S6 kinase (not shown).

Although an inhibitory pathway (other than SAPK/p38, because SB203580 did not rescue p70 S6 kinase activation) triggered by H₂O₂ cannot be ruled out, the results suggest that different patterns of 3-phosphoinositides can elicit distinct downstream responses. PtdIns(3,4,5)P³ alone appears sufficient for PKB activation, whereas p70 S6 kinase and other responses, perhaps mediated via more selective PH domains like those found in GRP1 and BTK (8), require significant production of PtdIns(3,4,5)P³.

Although H₂O₂ has been shown to mimic many of the metabolic effects of insulin (32), its working mechanism is unknown. Because many growth factor and cytokine receptors contain cysteine-rich motifs (33), oxidative stress can exert its effects via sulfhydryl reactivity of these cysteine residues (Cys-SH). Enhancement of the anti-oxidant potential of cells by pretreatment with N-acetyl cysteine and mannitol has indeed been shown to be effective in the inhibition of H₂O₂-induced PI 3-kinase signaling (12).

**Osmotic Stress-activated PI 3-Kinase Increases PtdIns(3,4,5)-P³ Levels without Apparent Activation of PKB—Osmotic stress increased PtdIns(3,4,5)P³ levels in a wortmannin-sensitive manner in Swiss 3T3 mouse fibroblasts as measured by radioligand displacement (Fig. 1) and cell labeling with [³H]inositol (Table I). This increase in PtdIns(3,4,5)P³ concentration was somewhat variable but typically had a magnitude comparable with that of IGF-1 stimulation. The relatively slow onset of PtdIns(3,4,5)P³ accumulation reached a maximum at 10 min and was sustained at 30 min, which was consistent with the increase of in vitro PI 3-kinase activity, measured in anti-phosphotyrosine immunoprecipitates (Fig. 4).

The strong increase in PI 3-kinase activity at 10 and 30 min and the increased PtdIns(3,4,5)P³ levels were not reflected by the expected large increase in PKB activity measured in parallel samples (Fig. 2). Compartmentalization of PtdIns(3,4,5)P³ production or activation of an inhibitory pathway might explain this observation. The fact that the IGF-1-induced PtdIns(3,4,5)-P³ increase was not additive with that induced by osmotic stress argues against the first possibility. The latter possibility might result from one of the many signaling pathways triggered by osmotic stress, for example, activation of SAPK1/JNK and SAPK2/p38/RK/HOG1 (17, 18). Western blot analysis, using an antibody specifically recognizing Ser⁴⁷³ phosphorylated PKBα revealed that upon exposure of cells to an osmotic stress, PKB was not phosphorylated on Ser⁴⁷³ (in contrast to IGF-1 or H₂O₂ treatment; Fig. 3).

To address whether osmotic stress negatively regulates PKB activation, its effect on IGF-1-stimulated PKB activity was tested. The activation of PKB by IGF-1 was strongly inhibited in osmotically stressed cells to 20% of full activation by IGF-1, and when cells stimulated with IGF-1 were subsequently subjected to osmotic stress, the PKB activity was diminished to 60% of the full activity within 1 min (Fig. 6). Because the activity state of PKB will be determined by the balance of its phosphorylation and dephosphorylation, the effect of osmotic stress could either be via inhibition of phosphorylation or stimulation of dephosphorylation of PKB. Meier et al. (15) proposed that the profound inhibitory effect on PKB activity exerted by osmotic stress could be mediated by PP2A, which dephosphorylates both Thr³⁰⁸ and Ser⁴⁷³ of PKB. Although no up-regulation of PP2A nor association of PP2A with PKB was detected upon osmotic stress, pretreatment with Calyculin A, a potent inhibitor of PP2A, blocked the inhibitory effect on PKB activation by osmotic stress. We confirmed these data by Meier et al., showing that the inhibition of osmotic stress on the activation of PKB can be blocked by pretreatment with Calyculin A. However, pretreatment for 20 min with 100 μM okadaic acid, another equally potent PP2A inhibitor, failed to do this.³ Two possible explanations are that Calyculin A stimulates the phosphorylation of PKB rather than inhibiting its dephosphorylation and that a phosphatase rather than PP2A is activated by osmotic stress, which is inhibited by Calyculin A and not by okadaic acid.

Because stimulation of PI 3-kinase by osmotic stress did not increase PKB activity, apparently because of the concomitant activation of an inhibitory pathway, the question remained as to whether other PI 3-kinase-driven signaling pathways could be triggered. p70 S6 kinase activation, which is PI 3-kinase dependent, was activated ~20-fold when Swiss 3T3 mouse fibroblasts were stimulated with IGF-1, whereas osmotic shock caused a ~6-fold stimulation of p70 S6 kinase. Both responses were completely rapamycin- and wortmannin-sensitive.

³ J. Van der Kaay, unpublished observation.
whereas the p38/SAPK2 inhibitor SB20350 had no effect.

Several possible mechanisms can account for the less pronounced p70 S6 kinase activation by osmotic stress compared with IGF-1. Because the activation of PKB is prevented under hyperosmotic conditions, this may result in impaired p70 S6 kinase activation. Although no direct phosphorylation of p70 S6 kinase by PKB has been demonstrated, overexpression of constitutively active forms of PKB have been shown to activate p70S6 kinase (31, 34). Alternatively a direct, simultaneous deactivation of p70 S6 kinase by PP2A may occur. For example, Peterson et al. (35) showed that PP2A interacts with p70 S6 kinase and deactivates the enzyme by dephosphorylation. N- and C-terminally truncated p70 S6 kinase could still be phosphorylated and activated but showed reduced interaction with PP2A and was rapamycin-resistant.

Another striking effect of osmotic stress was to induce a large increase in the level of PtdIns3P (Table I) in a wortmannin-sensitive manner (not shown). In many studies, the level of PtdIns(3)P in cells has shown to be unresponsive to growth factors. In Chinese hamster ovary cells treated with calcium ionophore (A23187), PtdIns3P showed a very slight increase (36), and in platelets, activation of αIIbβ3 integrin leads to a small transient increase in PtdIns3P via a type II PI 3-kinase (4). In platelets this increase in PtdIns3P precedes and appears to be required for subsequent synthesis of PtdIns(3,4)P2. The recently described FYVE domain of early endosomal protein 1A, which specifically binds PtdIns3P, suggests that this lipid also has a direct signaling potential. Osmotic stress provides a means to manipulate PtdIns3P levels and thus to study the cellular functions of this lipid signal.

Conclusions—This report shows that different cellular stresses activate distinct lipid signaling pathways that result in different cellular outputs. Oxidative stress resulted in a sustained increase in PtdIns(3,4)P2 levels that was responsible for the activation of PKB but not of p70 S6 kinase. Osmotic stress caused substantial accumulation of PtdIns(3,4,5)P3 because of the activation of a type I PI 3-kinase, resulting in p70 S6 kinase activation. Full activation of PKB was prevented, however, via the simultaneous activation of an inhibitory pathway that either prevents phosphorylation or promotes the dephosphorylation of PKB.

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