Regulation of AMP Deaminase by Phosphoinositides*

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AMP deaminase (AMPD) converts AMP to IMP and is a diverse and highly regulated enzyme that is a key component of the adenylate catabolic pathway. In this report, we identify the high affinity interaction between AMPD and phosphoinositides as a mechanism for regulation of this enzyme. We demonstrate that endogenous rat brain AMPD and the human AMPD3 recombinant enzymes specifically bind inositol-based affinity probes and to mixed lipid micelles that contain phosphatidylinositol 4,5-bisphosphate. Moreover, we show that phosphoinositides specifically inhibit AMPD catalytic activity. Phosphatidylinositol 4,5-bisphosphate is the most potent inhibitor, effecting pure noncompetitive inhibition of the wild type human AMPD3 recombinant enzyme with a \( K_i \) of 110 nM. AMPD activity can be released from membrane fractions by *in vitro* treatment with neomycin, a phosphoinositide-binding drug. In addition, *in vivo* modulation of phosphoinositide levels leads to a change in the soluble and membrane-associated pools of AMPD activity. The predicted human AMPD3 sequence contains pleckstrin homology domains and (R/K)X(R/K)XXK sequences, both of which are characterized phosphoinositide-binding motifs. The interaction between AMPD and phosphoinositides may mediate membrane localization of the enzyme and function to modulate catalytic activity *in vivo*.

Phosphoinositides and inositol polyphosphates (referred to collectively as inositides) are components of many pathways in eukaryotic cells, functioning in second messenger cascades, acting as regulators of many proteins, and operating as membrane localization signals (1–3). Numerous protein and lipid kinases, adaptor proteins, ion channels, phospholipases, modulators of small GTPases, and actin-binding proteins are regulated by inositides (1–3). To identify novel targets for inositides, our laboratories and others have used purification schemes employing affinity resins that contain tethered inositides (3–9). These affinity purifications employing affinity resins could be the 80-kDa protein isolated using the inositide affinity resin.

AMPD is a diverse and highly regulated enzyme located at a branchpoint in the adenine nucleotide catabolic pathway and is important in regulating nucleotide pools. AMPD is also a component of the purine nucleotide cycle, an energy-generating pathway reportedly operative in many animal tissues (reviewed in Ref. 12). The AMPD1 gene encodes human isoform M and rat isoform A (13); the AMPD2 gene encodes the human isoform L and rat isoform B (14, 15); and the AMPD3 gene encodes the human isoform E and the rat isoform C (16, 17). A single AMPD gene has also been identified in yeast (18). All human AMPD isoforms contain similar C-terminal regions and substantially divergent N-terminal domains. The AMPD1 isoform is found almost exclusively in skeletal muscle, whereas the AMPD2 and AMPD3 isoforms are widely expressed in many tissues and cells (19, 20), including mammalian brain (21, 22).

AMPD activity is highly regulated through interactions with other proteins (23–25), phosphorylation (26, 27), and small molecules (10, 11, 28–32). Regarding the latter, polyphosphates (32) and inositol (1,3,4,5)-tetrakisphosphate (10) inhibit AMPD, whereas inositol (1,3,4,5)-tetrakisphosphate (Ins(1,3,4,5)P4) modestly stimulates AMPD activity (11). AMPD activity is also modulated by lipids. In particular, non-skeletal muscle AMPD activities are regulated by fatty acids, fatty acyl coenzyme As, phosphatidic acid, and phosphatidylcholine (PC) with \( K_i \) values ranging from 10 to 100 \( \mu \)M (33–39). The high concentrations required for these modulatory effects suggest that these lipid interactions may be low affinity or nonspecific. However, the interaction with lipids may prove to be physiologically relevant. Whereas hydropathy analysis suggests that AMPD isoforms do not contain putative transmem-

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bran spanning domains, the AMPD3 enzyme can associate with erythrocyte membrane fractions (40, 41). In this report, we provide several independent lines of evidence that the specific, high affinity interaction between the AMPD3 isoform and phosphoinositides constitutes an important mechanism for enzyme regulation and localization.

**EXPERIMENTAL PROCEDURES**

**Materials**

Aminopropyl-InsP4 affigel, (1),1-O-["32P]34,5,6-trisphosphate (ASA-InsP4), and 1-O-["32P]3,4,5-trisphosphate (ASA-InsP3) and myo-inositol 3,4,5-trisphosphate (BZDC-InsP4) were synthesized as described (3–5). PC, PS, and PE were from Avanti Polar Lipids (Alabaster, AL). PtdIns(4,5)P2, PtdIns(4)P, and PtdIns(3,4,5)P3 were from Matreya Inc. (Pleasant Gap, PA) or were gifts from Echelon Research Laboratories Inc. (Salt Lake City, UT). All other reagents were from Sigma. PtdIns(3,4,5)P3 and PtdIns(4,5)P2 were synthesized as described previously (42).

**Methods**

**Purification of Rat Brain AMP Deaminase**—The purification was performed as described (3, 5–7) with the following minor modifications. Rat brain superior cervical ganglia (CSG) extracts and mixed micelles were prepared in 1 M NaCl and washed with 0.25 M NaCl Triton X-100, and 0.1 M EDTA. Human AMPD3 recombinant enzymes were produced in Sf9 (Amicorp Corp., Beverly, MA) to a final volume of 1–2 ml/10–15 rat brains, diluted with 50 mM Tris, pH 7.4, 1 mM EDTA, and loaded on an aminopropyl-InsP4 column at a fast performance liquid chromatography (Amersham Pharmacia Biotech; column dimensions, 10 × 3 cm) at a rate of 0.2 ml/min. The column was washed with 10 ml of 0.2 M NaCl and eluted with a NaCl gradient of 0–2 M NaCl.

**Expression and Purification of Human AMPD3 Recombinant Enzymes**—Wild type and N-terminally truncated (ΔM90) human AMPD3 recombinant enzymes were produced in Sf9 (Spodoptera frugiperda) insect cells using a baculoviral expression system and partially purified by phosphocellulose chromatography as described previously (42).

**AMPD Assay**—AMPD activity was determined using a phenol/hypo- chlorite reaction (11). The reaction contained 25 mM sodium citrate, pH 6.0, 50 mM potassium chloride, 10 mM AMP, and samples were incubated at 37 °C for 10 min, followed by addition of 2.5 ml of 100 mM phenol, 200 mM sodium nitroprusside in H2O, 2.5 ml of 125 mM sodium hydroxide, 200 mM dibasic sodium phosphate, 0.1% sodium hypochlorite. Absorbance was measured at 625 nm. Absolute ammonia was determined with ammonium sulfate. For kinetic determinations, AMPD activity was assayed using a high pressure liquid chromatography (Amersham Pharmacia Biotech; column dimensions, 10 × 3 cm) at a rate of 0.2 ml/min. The column was washed with 10 ml of 0.2 M NaCl and eluted with a NaCl gradient of 0–2 M NaCl.

**Immunoblot Analysis**—Western blotting was performed using [125I]ASA-InsP4 photoaffinity label (Fig. 1B, odd fractions). Immunoblot analysis of batch eluted aminopropyl-InsP4 eluate with an anti-AMPD3 serum (Fig. 1C) indicated a single strongly immunoreactive band at approximately 80 kDa. Because both AMPD2 and AMPD3 are expressed in brain (21, 22) and all the AMPD activity was recovered in a single peak, the AMPD2 and AMPD3 isoforms appear to behave similarly in this purification. Thus, comparable with other high affinity inositol-binding proteins, endogenous rat brain AMPD is effectively purified using an inositol affinity resin (3–9, 45, 46).

**Unilamellar Mixed Micelle Assay**—.Binding of AMPD to unilamellar mixed micelles was performed as described in Ref. 43. 400 μg of PE was added to various concentrations of other phospholipids and dried under N2. The lipids were resuspended in 1 ml of 180 mM sucrose, pelleted at 10,000 × g for 15 min, and resuspended in 1 ml of 50 mM HEPES, 1 mM EDTA, and 1 mM EGTA. Human AMPD3 recombinant protein (2 μg) was added to the micelles for 30 min at 25 °C, and centrifuged at 400,000 × g for 40 min. The pellets were separated by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and probed with the anti-AMPD3 antibody.

**Results**

**Endogenous Brain AMPD Binds Inositol Affinity Probes**—Using an aminopropyl-InsP4 resin to purify inositol-binding proteins from rat brain, we previously identified a protein at approximately 80 kDa that eluted in fractions containing the cationin adaptor/assembly protein AP-2 (3, 5, 6, 45). Published reports have shown that AMPD, an enzyme family whose native purified subunit molecular masses are between 66–88 kDa, is regulated by inositol polyphosphates (10, 11). Therefore we tested whether AMPD activity was enriched in fractions containing the 80-kDa protein eluted from the aminopropyl-InsP4 resin (Fig. 1A). All of the AMPD activity bound to the resin, and a symmetrical peak of AMPD activity was eluted in the fractions containing AP-2 and the 80-kDa protein. Similar to AP-2, the 80-kDa protein in the peak activity fractions incorporated an [125I]ASA-InsP4 photoaffinity label (Fig. 1B, odd fractions). Photolabeling was specific because including 30 μM unlabeled Ins(1,3,4,5)P4 displaced the label (Fig. 1B, even fractions). By batch purification, the aminopropyl-InsP4 resin gave a 71-fold purification with a specific activity of 917.1 nmol/min/mg and an 86% yield. Immunoblot analysis of batch eluted aminopropyl-InsP4 eluate with an anti-AMPD3 serum (Fig. 1C) indicated a single strongly immunoreactive band at approximately 80 kDa. Because both AMPD2 and AMPD3 are expressed in brain (21, 22) and all the AMPD activity was recovered in a single peak, the AMPD2 and AMPD3 isoforms appear to behave similarly in this purification. Thus, comparable with other high affinity inositol-binding proteins, endogenous rat brain AMPD is effectively purified using an inositol affinity resin (3–9, 45, 46).

**Human AMPD3 Recombinant Enzyme Binds Phosphoinositides**—Recent advances in recombinant expression of human AMPD cDNAs have provided larger quantities of higher purity enzymes than can be obtained from endogenous sources (42). Therefore, the human AMPD3 recombinant enzyme was used to determine the specificity and affinity of inositol binding in AMPD. AMPD was specifically labeled using a high efficiency [3H]BZDC-Ins(1,3,4,5)P4 photoprobe (Fig. 2, A–C). A variety of inositides and phospholipids were tested for displacement of the photolabel. PtdIns(4,5)P2 and PtdIns(4)P were the most potent inhibitors of photolabeling, with 50% displacement of the label (IC50) effected by addition of 200 μM (Fig. 2, A and B). PtdIns(3,4,5)P3, washed PC, and C20 phosphatidic acid gave IC50 values of 300 μM (Fig. 2B). Ins(1,3,4,5)P4 and inositol (1,2,3,4,5,6)-hexakisphosphate were substantially less effective and displaced the photolabel only when added at 10 μM. Phosphatidylinositol (PI) and PS displaced the photolabel when added at concentrations greater than 10 μM (Fig. 2C), whereas PE or PC did not displace the label even at 30 μM. AMPD could also be...
photolabeled with a \(^{3}\text{H}\)BZDC-PtdIns(4,5)P\(_2\) probe (Fig. 2D).

Lower concentrations of PtdIns(4,5)P\(_2\) were required to effect displacement in this label (IC\(_{50}\) \( \approx 100 \) nM) presumably because lower concentrations of the \(^{3}\text{H}\)BZDC-PtdIns(4,5)P\(_2\) label were used. These data show that the human AMPD3 recombinant enzyme binds phosphoinositides with high affinity, with both the inositol head group and glycerolipid moieties essential for high affinity binding.

To ensure that differences in lipid accessibility or micellar size were not the basis for the apparent specificity differences observed, we examined the interaction of AMPD3 recombinant protein with unilamellar mixed lipid micelles. Binding of AMPD to mixed micelles that contained PE as the core lipid and increasing concentrations of either PtdIns(4,5)P\(_2\), PS, PI, or PC, is shown in Fig. 3. A fraction of the human AMPD3 recombinant enzyme consistently associated with the PE micelle pellet, presumably through a low affinity interaction with PE that is present in high concentrations in the micelles. However, addition of PtdIns(4,5)P\(_2\) to the PE micelles produced a substantial increase in the binding of AMPD to the micelles. The increase in AMPD associated with the lipid micelle was concentration-dependent and saturable with half-maximal association observed at approximately 200 nM. In contrast, addition of PS, PI, or PC to the PE micelles did not lead to any additional increase in association of AMPD compared with PE alone, indicating that the enhanced binding was selective for PtdIns(4,5)P\(_2\). These data demonstrate that the human AMPD3 recombinant enzyme recognizes PtdIns(4,5)P\(_2\) in a mixed micelle with an affinity and selectivity similar to that determined in the photoaffinity labeling.

**Phosphoinositides Inhibit AMPD Activity**—We next examined the effect of phosphoinositides on AMPD catalytic activity. Phosphoinositides potently inhibit human AMPD3 recombinant activity in a dose-dependent manner (Fig. 4). PtdIns(4,5)-
P2 was the most effective inhibitor of enzyme activity, with an IC50 of approximately 100 nM (Fig. 4A, open symbols). The only other phospholipids that inhibited activity in the submicromolar range were phosphoinositides (Fig. 4, B and C). PtdIns(3)P, PtdIns(4)P, PtdIns(3,4)P2, and PtdIns(3,4,5)P3 were between 1.5- and 3-fold less potent than PtdIns(4,5)P2 but did not effect complete inhibition even at 3 μM. Other phospholipids, such as PI, PC, and PS, produced either no or only slight (<20%) inhibition when the concentration was increased to 3 μM (Fig. 4D). Inositol-1,4,5-trisphosphate, Ins(1,3,4,5)P4, and inositol (1,2,3,4,5,6)-hexakisphosphate had no effect on activity, even at 30 μM (Fig. 4D). Endogenous rat brain AMPD is also potently inhibited by phosphoinositides (Fig. 4A, closed symbols). Phosphoinositides were approximately 2-fold less effective on endogenous brain AMPD than the recombinant enzyme, with an IC50 for PtdIns(4,5)P2 of 250 nM. This decreased affinity may be a result of interference by other inositide-binding proteins in the preparation or may be a consequence of N-terminal proteolytic cleavage of endogenous AMPD (see below). These data indicate that interaction with phosphoinositides leads to potent inhibition of human AMPD3 recombinant and endogenous rat brain activity, with phosphoinositides more than 500 times more effective than other phospholipids or inositol polyphosphates in inhibition of AMPD activity.

A kinetic analysis of inhibition by PtdIns(4,5)P2 on the human AMPD3 recombinant activity was performed by varying PtdIns(4,5)P2 and substrate concentrations. A Lineweaver-Burk plot of the inhibition data (Fig. 5A) shows decreasing Vmax values but no effect on the Km in the presence of increasing concentrations of PtdIns(4,5)P2, indicative of noncompetitive inhibition. Moreover, a linear replot of slope versus PtdIns(4,5)P2 concentration (Fig. 5B) demonstrates pure noncompetitive inhibition. The Kf for PtdIns(4,5)P2 inhibition of the human AMPD3 recombinant activity is 110 ± 41 nM (n = 5) (Fig. 5).

Mammalian AMPD isoforms have conserved C-terminal catalytic domains and divergent N-terminal regions. To determine whether the N terminus contributes to phosphoinositide recognition, we tested effects of phosphoinositides on a truncated human AMPD3 recombinant enzyme that is missing 89 N-terminal amino acids (ΔM90AMPD3). The activity of the N-terminally truncated protein is also inhibited noncompetitively

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**Fig. 4.** Phosphoinositide inhibition of AMPD activity. AMPD activity was determined in the presence of increasing concentrations of the compounds shown. A, effect of PtdIns(4,5)P2 on human AMPD3 recombinant (open squares) or endogenous brain (closed circles) AMPD activity. B—D, effect of addition of the compounds shown on AMPD3 recombinant activity. In A, data from four inhibition experiments were averaged, and the standard deviation is shown by the error bars. In B—D, representative inhibition curves are presented from experiments performed in triplicate.

**Fig. 5.** Kinetic analysis of PtdIns(4,5)P2 inhibition of the wild type human AMPD3 recombinant enzyme. A, Lineweaver-Burk double-reciprocal plot using the following concentrations of PtdIns(4,5)P2: 0 nM (open squares); 25 nM (closed triangles); 50 nM (closed squares); 100 nM (open triangles). B, replot of slope versus inhibitor concentration.
AMPD activity is inhibited or conformationally restricted while significant increase in the total AMPD activity, suggesting that the membrane fraction, without recentrifugation, led to a 2–3-fold increase in activity in the supernatant fraction (Fig. 6). Addition of neomycin to PC12 cells led to a significant increase in phosphoinositide-binding domains in the AMPD3 enzyme.

Membrane Association of AMPD May Involve Phosphoinositides—To investigate whether AMPD may interact with endogenous phosphoinositides within the membrane, we examined the effects of neomycin, a phosphoinositide-binding drug (47), on AMPD activity in membrane fractions. Addition of neomycin to the membrane fraction, followed by recentrifugation, released AMPD activity from the membranes, resulting in a 2–3-fold increase in activity in the supernatant fraction (Fig. 6A, black bars). Furthermore, addition of neomycin directly to the membrane fraction, without recentrifugation, led to a significant increase in the total AMPD activity, suggesting that AMPD activity is inhibited or conformationally restricted while associated with membranes (Fig. 6A, gray bars). These data are consistent with endogenous AMPD association with membranes involving phosphoinositide interactions.

To investigate further whether phosphoinositide interactions may mediate membrane association of AMPD, we incubated cells in the presence of taurine, which increases the intracellular levels of PtdIns(4,5)P_2 and PtdIns(4)P (44). Incubation with taurine led to an approximately 2-fold increase in the level of total phosphoinositides in PC12 cells (Fig. 6B). In the taurine-treated cells, AMPD activity in the supernatant fraction was 24% lower compared with the control (Fig. 6C), whereas activity associated with the membrane was 31% higher than in untreated cells (Fig. 6D). We also tested the effects of neomycin, which has been documented to block interac-
tions between PtdIns(4,5)P_2 and its target proteins (47). Addition of neomycin to PC12 cells led to a significant increase in the supernatant AMPD activity, consistent with release of AMPD from the membrane (data not shown). Together these data suggest that modulation of endogenous phosphoinositide levels can produce a change in the distribution between the soluble and membrane-associated pools of AMPD.

Phosphoinositide-binding Domains in AMPD—One characteristic motif that has been shown to mediate phosphoinositide binding in many proteins, including pleckstrin, spectrin, PLCβ, and Bruton’s tyrosine kinase, is a pleckstrin homology (PH) domain (1, 2, 48). Analysis of the predicted AMPD3 sequence indicates that it contains two tandem PH domains in the C-terminal half (Fig. 7A). Interestingly, one of the proposed catalytic regions of AMPD lies within the second PH domain (Fig. 7C). The second AMPD3 PH domain also contains conserved basic residues (denoted by the asterisks) in several loops, which have been shown to be essential for inositide binding in the PLCβ PH domain (48). The AMPD2 sequence contains similar PH domains (data not shown). In addition to the PH domain, another motif has been implicated in phosphoinositide binding: the highly basic domain (K/R)X(X/K/R/X/K/R), which is found in the actin-binding proteins profilin, gelsolin, and coflin, as well as the synaptic vesicle protein synaptogamin (49–51). In the AMPD3 isoform there are two (R/K)X(K/R/K/X)XKK sequences that fit this consensus (Fig. 7B). One is present within the first PH domain, and the other lies in the N terminus (Fig. 7C). These sequence data identify candidate phosphoinositide-binding domains in the AMPD3 enzyme.

**Discussion**

In this report, we demonstrate that phosphoinositides are specific regulators of AMPD. Six lines of evidence support this conclusion. First, we identified AMPD in a screen for high affinity inositide-binding proteins expressed in mammalian brain. Second, AMPD was specifically photolabeled by inositide-based photoprobe and bound to PtdIns(4,5)P_2 in mixed lipid micelles. Third, endogenous rat brain AMPD and human AMPD3 recombinant enzymes were potently inhibited by phosphoinositides. PtdIns(4,5)P_2 was the most effective inhibitor and displayed pure noncompetitive inhibition with a Ki of 110 nM. Fourth, AMPD activity was released from and disinhibited in membrane fractions by neomycin, a PtdIns(4,5)P_2-binding drug. Fifth, in vivo modulation of phosphoinositides led to a change in AMPD pools. Finally, several characterized phosphoinositide-binding domains are present in the predicted AMPD3 amino acid sequence.

The C-terminal region of AMPD2 and AMPD3 isoforms contains two PH domains, structural domains shown to mediate phosphoinositide binding in a variety of proteins (48). Interestingly, the second PH domain in AMPD contains several conserved basic residues shown to be critical for inositide binding in the PLCβ PH domain (48). In addition, the divergent N-terminal region of the AMPD3 isoform may also participate in
phosphoinositide binding because the ΔM90AMPD3 recombinant enzyme displayed a 5-fold lower affinity for PtdIns(4,5)P2 than the wild type activity. In AMPD3, both N-terminal and C-terminal domains contain an (R/K)Xn(R/K)XKK sequence, a motif that has been implicated in phosphoinositide binding in several actin-binding proteins and synaptotagmin (49–51). These sequence data, together with the fact that the AMPD3 isoform has a very high affinity for PtdIns(4,5)P2, support the likelihood that several domains in the enzyme contribute to this interaction.

Specificity determinations show that the human AMPD3 recombinant enzyme was most potently inhibited by PtdIns(4,5)P2 with a $K_i$ of 110 nM. Both the inositol phosphate head group and the glycerolipid are required for inhibition because neither PtdIns nor inositol polyphosphates inhibited activity effectively, even in the micromolar range. Given that other phosphoinositides, including PtdIns(3)P, PtdIns(4)P, PtdIns(3,4)P2, and PtdIns(3,4,5)P3, were only 1.5–3-fold less potent than PtdIns(4,5)P2, an important question is which phosphoinositides bind to AMPD in vivo. In the brain, the concentration of PtdIns, PtdIns(4)P, and PtdIns(3,4)P2 are 78, 4, and 14 nmol/mg wet weight, respectively (52). Cellular levels of PtdIns(3)P, PtdIns(3,4)P2, and PtdIns(3,4,5)P3 are estimated to be less than PtdIns(4,5)P2 (see references in Refs. 1 and 2). Therefore, based on relative phosphoinositide abundance and AMPD specificity, we anticipate that the in vivo ligand for the AMPD3 isoform is PtdIns(4,5)P2.

Binding to phosphoinositides may contribute to localizing AMPD to membranes, regulating catalytic activity, sequestering PtdIns(4,5)P2, and/or sequestering a mobilizable pool of AMPD. Phosphoinositide binding is essential for membrane association of several PH domain-containing proteins, including PLCδ and protein kinase B/Akt kinase (48). A function for phosphoinositide binding in membrane association of AMPD is supported by the demonstration that neomycin treatment can release the enzyme from membranes, and treatments that lead to increases in phosphoinositide levels also lead to increased AMPD in membrane fractions. Recent studies have shown that PtdIns(4,5)P2 is present in plasma membrane and internal membrane compartments, including endoplasmic reticulum, Golgi, and nuclear membranes (2). Subcellular fractionation studies in brain indicate that AMPD activity is associated with multiple membrane compartments (53). Whether phosphoinositide binding is sufficient for localizing AMPD to specific membrane compartments and whether additional domains or protein-protein binding is required are important issues for future studies.

The interaction between AMPD and phosphoinositides may contribute to sequestering the enzyme in an inactive pool. In this regard, interaction between phosphoinositides and AMPD would be analogous to phosphoinositide regulation of the actin-binding protein profilin. Following receptor-activated, phospholipase C-mediated PtdIns(4,5)P2 hydrolysis, profilin is released from the membrane and can interact with actin monomers to promote actin polymerization (49, 50). Similarly, AMPD may be released from membranes and activated following PtdIns(4,5)P2 hydrolysis or by altering the accessibility of PtdIns(4,5)P2, via translocation of other phosphoinositide-binding proteins or activation of PtdIns(4,5)P2-metabolizing enzymes (1, 2). In addition, interactions between AMPD and molecules that enhance or diminish the affinity or access to phosphoinositides could lead to changes in AMPD membrane association.
association and activity. For example, inositol polyphosphates that bind to but do not inhibit AMPD may act as competitive antagonists of phosphoinositides for AMPD. Competition between inositol polyphosphates and phosphoinositides has been proposed in regulation of PLCβ, AP-2, Brun’s tyrosine kinase, and synaptotagmin (1–3, 45, 46, 48, 50).

A mobilizable pool of AMPD may be required for a variety of purposes, for regulation of adenylate pools, to compete with cytosolic 5’ nucleotidases in modulation of adenosine levels, or for ammonia generation. Investigation of the cellular function of phosphoinositide binding to AMPD requires additional studies that appear warranted based on the results presented here.

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