Odor-Stimulated Phosphatidylinositol 3-Kinase in Lobster Olfactory Receptor Cells

ASYLBEK B. ZHAINAZAROV, 1 RICHARD DOOLIN, 1,3,4 JOHN-DAVID HERLIHY, 1,3,4 AND BARRY W. ACHE 1– 4

INTRODUCTION

Phosphoinositides are integral membrane constituents in eukaryotic cells that also function in transmembrane signaling (review: Zhang and Majerus 1998). The discovery of D-3 phosphorylated inositol lipids and their synthesis by a family of phosphoinositide 3-OH kinases (PI3Ks), LY294002 and Wortmannin, reduced the magnitude of the receptor potential in lobster olfactory receptor neurons (ORNs) recorded by patch clamping the cells in vivo. An antibody directed against the c-terminus of human PI3K-P110β detected a molecule of predicted size in the outer dendrites of the ORNs. Two 3-phosphoinositides, PI(3,4)P₂ (1–4 μM) and PI(3,4,5)P₃ (1–4 μM) applied to the cytoplasmic side of inside-out patches taken from cultured lobster ORNs, reversibly activated a Na⁺-gated channel previously implicated in the transduction cascade in these cells. 3-Phosphoinositides were the most effective phosphoinositide (1 μM) in enhancing the open probability of the channel. Collectively, these results implicate 3-phosphoinositides in lobster olfactory transduction and raise the need to consider the 3-phosphoinositide pathway in olfactory transduction.

Received 27 November 2000; accepted in final form 14 March 2001

Zhainazarov, Asylbek B., Richard Doolin, John-David Herlihy, and Barry W. Ache. Odor-stimulated phosphatidylinositol 3-kinase in lobster olfactory receptor cells. J Neurophysiol 85: 2537–2544, 2001. Two antagonists of phosphoinositide 3-OH kinases (PI3Ks), LY294002 and Wortmannin, reduced the magnitude of the receptor potential in lobster olfactory receptor neurons (ORNs) recorded by patch clamping the cells in vivo. An antibody directed against the c-terminus of human PI3K-P110β detected a molecule of predicted size in the outer dendrites of the ORNs. Two 3-phosphoinositides, PI(3,4)P₂ (1–4 μM) and PI(3,4,5)P₃ (1–4 μM) applied to the cytoplasmic side of inside-out patches taken from cultured lobster ORNs, reversibly activated a Na⁺-gated channel previously implicated in the transduction cascade in these cells. 3-Phosphoinositides were the most effective phosphoinositide (1 μM) in enhancing the open probability of the channel. Collectively, these results implicate 3-phosphoinositides in lobster olfactory transduction and raise the need to consider the 3-phosphoinositide pathway in olfactory transduction.

Address for reprint requests: B. W. Ache, Whitney Laboratory, University of Florida, 9505 Ocean Shore Blvd., St. Augustine, FL 32086.
activate a Na\(^{+}\)-gated channel previously implicated in the transduction cascade in these cells, and that 3-phosphoinositides are the most effective phosphoinositides (1 μM) in enhancing the open probability of the channel. These results collectively implicate 3-phosphoinositides in lobster olfactory transduction and raise the possibility of a general role for the 3-phosphoinositide pathway in olfactory transduction.

**METHODS**

**Animals and preparations**

Adult specimens of the Caribbean spiny lobster, *Panulirus argus*, were collected in the Florida Keys and maintained in the laboratory in running seawater on a diet of fish, squid, and shrimp. Data were obtained from two different preparations. Whole cell, current-clamp recordings were obtained from the ORNs in vivo as described in detail elsewhere (Michel et al. 1991), with several modifications. In the present study, the olfactory organ (lateral antennular filament) was hemisected in a dorsal/central axis, and the sections were mounted flat on the bottom of a recording chamber, allowing odorants and/or drugs to be “spritzed” directly on to the olfactory sensilla (aesthetascos) from a multibarrel pipette. To digest away the sheath covering the clusters of ORNs, the recording chamber was filled with L-cysteine–activated papain [Sigma Type IV, 0.17 mg/ml in *Panulirus* saline (PS); see Solutions] for 1 min. The cells were then rinsed with Ca-free PS (see Solutions), and the bath was replaced with trypsin (1 mg/ml in Ca-free PS) for 1 min, before returning to PS for recording. Unitary currents were obtained from the ORNs in vitro. Primary cultures of the ORNs were prepared as described earlier (Fadool et al. 1991). Cells were given fresh medium every third day and used within 1–7 days of plating.

**Whole cell recording**

The receptor potential was recorded from the soma of the cells using conventional whole cell patch-clamp recording. Patch pipettes were fabricated from borosilicate filament glass (1.50 mm OD, 0.86 mm ID; Sutter Instrument) and fire-polished to a tip diameter about 1 μm. The pipette resistance was 5–9 MΩ when filled with normal patch pipette solution and formed seals with resistances of 4–10 GΩ. Signals were recorded with a Dagan 3900 amplifier, low-pass filtered at 2 kHz (4-pole low-pass Bessel filter), digitally digitized at 2–5 kHz, and analyzed using PClamp 8 software (Axon Instruments). A reference electrode was connected to the bath solution through a 3 M KCl/agar bridge. The series resistance was <10 MΩ. All potentials were corrected for the junction potentials at the pipette tip and at the indifferent electrode as described by Neher (1995). The magnitude of the receptor potential was determined as the maximum amplitude of the “plateau” since membrane voltage was not clamped and a few cells crossed the threshold for discharging, giving the appearance of a phaso-tonic receptor potential. Experiments were carried out at room temperature (20–22°C).

**Single-channel recording**

Membrane patches were pulled from the soma of the cultured ORNs, and voltage-clamp recordings were performed using the inside-out configuration of the patch-clamp technique, as described earlier (Zhainazarov and Ache 1995). The pipettes were filled with PS. Single-channel currents were measured with an Axopatch 200A patch-clamp amplifier (Axon Instruments, Foster City, CA), low-pass filtered at 1 kHz (3 dB; 4-pole Bessel filter), digitized at 10 kHz (A/D, D/A interface, DigiData-1200A; software, pClamp 7.0; Axon Instruments), and stored on a computer hard disk for later analysis. A rotary perfusion system (RSC-100, Bio-Logic, Clax, France) was used to apply different solutions to the isolated membrane patches from one of up to nine different reservoirs (Zhainazarov and Ache 1999). Unless stated otherwise, recordings were performed at a holding potential of −60 mV. The recordings were referenced to a Ag-AgCl wire electrode connected to the bath solution through a 3 M KCl agar bridge. All recordings were made at room temperature (20–22°C).

Single-channel current was analyzed using pClamp 7.0 software as described earlier (Zhainazarov and Ache 1999). Briefly, membrane patches typically contained more than one channel, so the open probability of a channel was calculated using the equation 

\[ P_o = \frac{N}{i} \]  

\( P_o \) is the mean current over the interval of interest, \( N \) is the number of channels in the patch, and \( i \) is the single-channel current amplitude. Current amplitude histograms were used to measure single-channel current amplitudes. \( N \) is given in this context refer to the number of single-channel current traces, each of 1-min minimum duration, analyzed for a given membrane potential. When the number of channels in a patch was difficult to determine reliably, \( N \) was used as a measure of the channel activity. In estimating the relative efficacy of a phosphoinoside in activating a Na\(^{+}\)- activated channel, the first minute of channel activity after application of phosphoinoside was excluded from calculation of the channel open probability. The baseline of the single-channel current traces are depicted by dashed lines. The data points are presented as means ± SE of \( n \) observations.

**Western blotting and immunocytochemistry**

Protein preparation was carried out as described by Xu et al. (1999). Briefly, olfactory sensilla were shaved from 20–30 frozen lobster olfactory organs and the sensilla transferred to a 1.5-ml microcentrifuge tube containing 300 μl of homogenization buffer consisting of 120 mM NaCl, 5 mM KCl, 1.6 mM KH\(_2\)PO\(_4\), 1.2 mM MgSO\(_4\), 25 mM NaHCO\(_3\), 7.5 mM glucose, 2 mM ethyleneglycoltetraacetic acid, 3 μg/ml phenylmethylsulfonyl fluoride, 1 μg/ml pepstatin, 1 μg/ml leupeptin, and 10 μg/ml benzamidine, pH 7.4. Hairs were homogenized with a plastic Teflon pestle, sonicated for 10 s, and centrifuged (1,000 × g) to remove the cuticle. ORN somata were dissected from 10 shaved olfactory organs and homogenized and prepared as above. Protein concentrations were determined by Bio-Rad Protein Assay. Protein samples were boiled for 5 min in 1 × sodium dodecyl sulfate (SDS) loading buffer then run on 4–12% NuPAGE Bis-Tris Gels with a MDES SDS Running Buffer. Ten micrograms of sample were loaded per well. Electrophoresis was carried out with a Hoefer MiniVE Vertical Electrophoresis System. Separated proteins were transferred onto nitrocellulose using semidry electrophoresis apparatus. Once transferred, the blots were blocked overnight in 5% dried milk powder in Tris-buffered saline plus Tween 20 (TBST). Blots were probed for 2 h with a commercially available goat anti-PI3K antibody raised against the carboxy terminus of PI3-kinase p110β, but cross reactive with all isoforms of p110 PI 3-kinase (Santa Cruz Biotechnology). As a control, the primary antibody was preabsorbed for 2 h in a 10-fold excess of the blocking peptide (Santa Cruz Biotechnology) prior to probing the blot. Both the primary and the preabsorbed control were used at a final dilution of 1:1,000 in blocking solution. The blots were washed three times in TBST for 5 min and then probed with a donkey anti-goat horseradish peroxidase–conjugated secondary antibody at 1:10,000 (Jackson Immunoresearch Laboratories) in blocking solution and washed three times in TBST for 5 min. The blots were developed using enhanced chemoluminescence (Amersham Pharmacia Biotech) exposed to Hyperfilm (Amersham Pharmacia Biotech).
**Solutions**

PS contained (in mM) 458 NaCl, 13.4 KCl, 13.4 Na₂SO₄, 13.6 CaCl₂, 9.8 MgCl₂, 2 glucose, and 10 N-2-hydroxethylpiperazine-N'-2-ethanesulfonic acid (HEPES), at a pH of 7.4 adjusted with 1 M NaOH. Patch pipettes were filled with either PS (cell-free recording) or normal patch pipette solution consisting of (in mM) 180 K-acetate, 30 NaCl, 11 ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 1 CaCl₂, 10 HEPES, and 522 glucose, pH 7.2 adjusted with Tris base (whole cell recording). Sodium-free solution consisted of (in mM) 210 KCl, 11 EGTA, 1 CaCl₂, 696 glucose, and 10 HEPES, at a pH of 7.4 adjusted with 1 M Tris[hydroxymethyl]aminomethane (Tris base). The calculated free calcium concentration in the sodium-free solution was 10 nM (Chelator) (Schoenmakers et al. 1992). In some experiments, part of the KCl in the sodium-free solution was substituted by an equivalent concentration of NaCl as described in the text and figure legends. PI(3)P, PI(3,4)P₂, and PI(3,4,5)P₃ stock solutions (1 mM) were prepared by dispersing the inositol phosphates in distilled water with 30 min sonication on ice, aliquoted, and stored at −20°C for use within 3 days. Stock solutions were diluted to working solutions of the stated concentration and sonicated for an additional 30 min on ice before use. PI(3)P, PI(3,4)P₂, and PI(3,4,5)P₃ were purchased from Matreya (Pleasant Gap, PA).

The odorant consisted of an extract of a commercial marine aquarium fish food, TetraMarin (TET; Tetra Werke, Melle, Germany), made by mixing 2 g of dry flakes in 60 ml PS, centrifuging the resulting suspension at 1,400g, and filtering through no. 3 Whatmann paper. The resulting stock solution was adjusted to pH 7.4 with 1 M NaOH and stored frozen in 5-ml aliquots until needed, at which point aliquots were thawed and diluted the stated number of times with PS.

**RESULTS**

**Blockers of PI3K activity block the receptor potential in lobster ORNs in vivo**

LY294002 (50 μM), a synthetic membrane-permeant inhibitor of PI3K (Vlahos et al. 1994), markedly and reversibly reduced the amplitude of the receptor potential (Fig. 1A). It was not necessary to preincubate the cell with the drug to see this effect. There was no obvious effect of the drug on the membrane potential in the absence of the odor. The drug was effective on all cells tested, and reduced the receptor potential on average to 24 ± 10% of its initial magnitude (means ± SE in this and all subsequent whole cell data, n = 12, Fig. 1B). The effect of the drug was fully reversible on wash out. The effect of the drug was concentration dependent; lowering the concentration to 10 μM reduced the receptor potential to 64 ± 11% of its initial amplitude (n = 5, data not shown).

Wortmannin (100 nM), a naturally occurring membrane-permeant inhibitor of PI3K (e.g., Okada et al. 1994), also reduced the amplitude of the receptor potential in a manner similar to that seen with the synthetic inhibitor in 8 of 14 cells tested (Fig. 2A). In these cells, the drug reduced the receptor potential on average to 64 ± 10% of its initial amplitude, with no obvious effect on the membrane potential in the absence of the odor (Fig. 2B). The effect of the drug was fully reversible on wash out, with the receptor potential recovering on average 96 ± 9% of its initial amplitude. Ten micromolar LY294002 and 0.1 μM Wortmannin reduced the amplitude of the receptor potential to essentially the same level, suggesting that Wortmannin was the more potent of the two inhibitors in this system. The effect of Wortmannin on the receptor potential in the remaining six cells was confounded by the fact that the drug alone depolarized the cells and increased the amplitude of the receptor potential to 138 ± 2% of its pretreatment magnitude (data not shown). Testing 200 nM LY294002 on two of these cells, however, inhibited the receptor potential in the same manner as it did in all other cells (data not shown), suggesting that Wortmannin acted nonspecifically on these six cells, perhaps as an odorant. Unlike the synthetic drug LY294002, Wortmannin is naturally derived and more likely to contain water-soluble compounds capable of activating these chemosensitive cells.

**PI3K is enriched in the transduction compartment lobster ORNs in vivo**

Scraping the olfactory sensilla from the lobster olfactory organ produces a membrane preparation that is highly enriched...
in the outer dendrites of the ORNs. An antibody raised against the c-terminus of human PI3K-P110β recognized a protein of predicted size, approximately 110 kDa, in a Western blot of membranes obtained from the olfactory sensilla (Fig. 3). The staining in this tissue was enriched compared with that in the remainder of the organ, which consists primarily of the soma and inner dendrites of the ORNs. The immunostaining could be eliminated by preabsorbing the antibody with the antigenic peptide (Fig. 3).

Exogenous PI(3,4,5)P$_3$ and PI(3,4)P$_2$ target a component of the transduction cascade, the Na$_{\text{v}}$-activated nonselective cation channel, in cultured lobster ORNs

Applying 1 μM Pl(3,4,5)P$_3$ to inside-out patches containing Na$_{\text{v}}$-activated channel activity evoked single-channel openings, even in the absence of Na$_{\text{v}}$, one instance of which is shown in Fig. 4. The effect of Pl(3,4,5)P$_3$ was immediate, reversed within 10–30 s of wash out, and was seen in all of 12 patches. In none of 10 attempts did 1 μM Pl(3,4,5)P$_3$ elicit single-channel openings in patches that failed to display Na$_{\text{v}}$-activated channel activity (data not shown). The effect was concentration dependent. In a typical experiment, increasing Pl(3,4,5)P$_3$ from 1 to 4 μM in the absence of Na$_{\text{v}}$ increased channel activity (Fig. 5A). The $N_{\text{p}}$, in this instance increased from 0.24 to 0.52. Similar concentration-dependent effects of Pl(3,4,5)P$_3$ were observed in all of five patches tested. Co-application of 30 mM Na$_{\text{v}}$ with 1 μM Pl(3,4,5)P$_3$ induced a greater level of channel activity in the same patch than did application of 30 mM Na$_{\text{v}}$ alone (Fig. 4; $n = 5$). In a typical experiment, the channel activity ($N_{\text{p}}$) increased from 0.02 at 30 mM Na$_{\text{v}}$ to 0.52 at 30 mM Na$_{\text{v}}$ + 1 μM Pl(3,4,5)P$_3$. At a membrane potential of −60 mV, the channel opened to a single level with an amplitude of −1.5 ± 0.1 pA ($n = 3$) for 30 mM Na$_{\text{v}}$, −1.6 ± 0.1 pA ($n = 3$) for 1 μM Pl(3,4,5)P$_3$, and −1.5 ± 0.1 pA ($n = 3$) for 30 mM Na$_{\text{v}}$ + 1 μM Pl(3,4,5)P$_3$. The slope conductance was not affected by Pl(3,4,5)P$_3$ (Fig. 5B). Between −100 and −50 mV, the slope conductance was 42.2 ± 2.6 pS for 30 mM Na$_{\text{v}}$, 43.7 ± 2.7 pS for 1 μM Pl(3,4,5)P$_3$, and 48.2 ± 5.7 pS for 30 mM Na$_{\text{v}}$ + 1 μM Pl(3,4,5)P$_3$.

Applying 1–4 μM Pl(3,4)P$_2$ also activated the channel in the absence of Na$_{\text{v}}$ and increased the channel open probability in the presence of 30 mM Na$_{\text{v}}$, as shown for one patch exposed to 1 μM Pl(3,4)P$_2$ in Fig. 6, A and B. The same effect was seen in 13 other patches, each exposed to a single concentration Pl(3,4)P$_2$ ranging from 1 to 4 μM. As with Pl(3,4,5)P$_3$, the effect of Pl(3,4)P$_2$ was also rapid and concentration dependent. In contrast to Pl(3,4,5)P$_3$, however, Pl(3,4)P$_2$ typically required about 1 min for channel activity to reach a stable level. On wash out, the effect of Pl(3,4)P$_2$ reversed slowly over 20–30 min. Applying 1–4 μM Pl(3)P failed to activate the channel in the absence of Na$_{\text{v}}$, or increase the open probability of the channel when activated by 30 mM Na$_{\text{v}}$, as shown for one patch exposed to 1 μM Pl(3)P.
PI(3)P in Fig. 6, C and D. The same effect was seen in four other patches, each exposed to a single concentration PI(3)P ranging from 1 to 4 mM. PI(3,4,5)P_3 is the most effective phosphoinositide on the Na\textsuperscript{+}-activated nonselective cation channel

We estimated relative efficacy of phosphoinositide activation (\(P_R\)) using the following equation

\[
P_R = \frac{NP_o}{NP_o + c} \tag{1}
\]

where \(NP_o\) is the channel open probability in the presence of 30 mM Na\textsuperscript{+} and 1 \(\mu\)M of one of the following phosphoinositides: PI, PI(3)P, PI(4)P, PI(3,4)P\(_2\), PI(4,5)P\(_2\), and PI(3,4,5)P\(_3\), and \(NP_c\) is the channel open probability in the control (30 mM Na\textsuperscript{+}) condition. The relative efficacy of the phosphoinositides in enhancing the channel open probability in the presence of 30 mM Na\textsuperscript{+} was as follows: PI(3,4,5)P\(_3\) (36.0 ± 3.5; \(n = 4\)) > PI(3,4)P\(_2\) (25.0 ± 0.5; \(n = 4\)) > PI(4,5)P\(_2\) (2.5 ± 0.5; \(n = 4\)) (Fig. 7). PI and PI(3)P had no appreciable effect on the channel when applied in either the presence or absence of Na\textsuperscript{+} (Fig. 7).

**DISCUSSION**

We pharmacologically implicated PI3K in the odor activation of lobster ORNs, immunocytochemically localized a PI3K-like molecule to the transduction zone, identified an ion channel in the transduction cascade as a potential target for the action of 3-phosphoinositides, and showed that 3-phosphoinositides are the most effective phosphoinositide activating the channel. These results collectively suggest that 3-phosphoinositides play an important role in lobster olfactory transduction.

Four lines of evidence support the involvement of PI3K in the transduction sequence. 1) LY294002, a synthetic antagonist of PI3K, reduced the magnitude of the receptor potential in the cells. The drug has a very selective structure-activity relationship for PI3K since analogues of LY294002 with only slight changes in structure cause marked decreases in inhibition in binding assays (Vlahos et al. 1994). 2) Wortmannin, a naturally occurring antagonist of PI3K, mimicked the action of LY294002 in those cells in which it could be tested effectively. 3) An antibody directed against the c-terminus of human PI3K-
P110β stained a molecule of appropriate molecular weight in the olfactory sensilla, suggesting that an appropriate molecular target for the two pharmacological probes occurs in the transduction zone of the cells. Finally, 4) the substrate for PI3K, phosphatidylinositol 4,5-bisphosphate (PIP2), is constitutively present in the ORNs. Depleting membrane patches of cultured lobster ORNs of endogenous PIP2 by applying recombinant PLCβ alters ion channel activity in the patch, which can be restored by subsequent application of exogenous PIP2 (Zhainazarov and Ache 1999).

We assume that the antibody directed against the c-terminus of human PI3K-p110β stained a lobster homologue, as it recognized an antigen of the correct molecular weight, and this immunoreactivity was lost when the antibody is preabsorbed with antigenic peptide. Although raised against human p110β, the anti-PI3K-p110β, antibody recognizes all the mammalian p110 isoforms (α, β, δ, and γ), allowing that the protein recognized in the lobster ORN could be any of these four isoforms. The most likely candidate, however, is likely to be a lobster p110 gamma, because p110 gamma can be activated by Gb/g, as well as Ga (Stoyanov et al. 1995), and olfactory signaling in lobsters utilizes G-protein–coupled receptors (Fadool et al. 1995).

The action of the 3-phosphoinositides on the lobster olfactory Na⁺-gated cation channel reveals a potential target for the products of PI3K activity in the transduction cascade. This channel has been proposed to secondarily amplify the primary transduction current in lobster ORNs (Zhainazarov et al. 1998). Here, we show that PIP(3,4,5)P3 and PIP(3,4)P2 directly activate the Na⁺-gated channel and enhance the Na⁺ sensitivity of the channel when co-applied with Na⁺. We assume that this action is selective since 1) neither phosphoinositide induced channel openings in patches that fail to exhibit Na⁺-gated channel activity, 2) the channel openings they evoked had a single-channel conductance similar to that of the Na⁺-gated channel (Zhainazarov and Ache 1999), and 3) PIP(3)P had no effect on the channel. Membrane phosphoinositides also have such dual trigger/regulatory action on ATP-sensitive K⁺ channels (Baukrowitz et al. 1998; Shyng and Nichols 1998), suggesting that this may be a common mechanism by which...
phosphoinositides control ion channel function, even if the specific effects (e.g., increased or decreased open probability) vary for different channel types.

The action of PI(3,4,5)P$_3$ and PI(3,4)P$_2$ on the channel in isolated membrane patches is consistent with the pharmacological effect of LY294002 and Wortmannin on the cells in vivo, suggesting that the olfactory Na$^+$-gated cation channel is indeed a potential target for one or more products of PI3K metabolism. Blocking the production of 3-phosphoinositides would be expected to reduce the amplifying function of the Na$^+$-gated cation channel on the receptor current. This in turn would reduce the magnitude of a depolarizing receptor potential, which was the pharmacological effect we obtained from treating the cells in vivo with LY294002 or Wortmannin.

PI(3,4,5)P$_3$ was the most potent phosphoinositide, as measured by the ability of the lipid to activate and regulate the channel, although PI(3,4)P$_2$, PI(4)P, and PI(4,5)P$_2$ also had measurable activity. The sensitivity to multiple phosphoinositides does not reflect a generalized effect of charge per se, with more highly charged molecules being more active, since InsP$_3$, for example, with six negative charges per molecule, had no significant effect on the Na$^+$-gated channel either in the presence or absence of Na$^+$ (Zhainazarov and Ache 1995). Presumably, both the hydrophobic tail and the negatively charged hydrophilic head are required for phosphoinositides to regulate the channel. It is not clear, however, that the channel would be activated/modulated by multiple phosphoinositides in vivo. PI(4,5)P$_2$ is the substrate for PI3K, so it is possible that application of exogenous PI(4,5)P$_2$, and perhaps PI(4)P as a precursor for PI3K, so it is possible that application of exogenous PI3K turnover pathway shown earlier to be involved in synaptic potentiation (Yang et al. 2001), the 3-phosphoinositide pathway works in concert with the canonical phosphoinositide turnover pathway (23: 719–732, 1991).

This study provides the first evidence that 3-phosphoinositides have signaling function in an olfactory receptor cell. Still to be resolved is the exact role of these lipids in the activation sequence and whether, as in NT3-induced odor-stimulated activation of odor-stimulated PI3K in the lobster cells.

We thank A. Hastings for preparation of the cultured cells, D. Migliaro for assistance with Western blotting, and M. Milstead for assistance with the illustrations.

This work was supported by National Institute on Deafness and Other Communication Disorders Grant DC-01655.

Present address of A. B. Zhainazarov: Dept. of Cardiology, Children’s Hospital and Harvard Medical School, John F. Enders Building, Rm. 1316, 320 Longwood Ave., Boston, MA 02115.

REFERENCES


Maier U, Babich A, and Nurnberg B. Roles of non-catalytic subunits in G$_{o1}$-induced activation of class 1 phosphoinositide 3-kinase isoforms $\beta$ and $\gamma$. J Biol Chem 274: 29311–29317, 1999.


