ity, can result in the FCHL phenotype. Although decreased LDL receptor activity is not thought to be the primary defect in human FCHL (1–2), LDL cholesterol and APOB amounts appear to be influenced by LDL receptor activity (21). In APOC3 transgenic mice, hypertriglyceridemia arises from a delay in clearance of VLDL (7), reflecting an in vivo lipolysis defect (7, 22), which may be caused by decreased binding of VLDL to endothelial cell proteoglycans, impairing access to LPL (22–24). It is possible that low LDL receptor activity results in increased conversion of accumulating VLDL into IDL and LDL. Other hypertriglyceridemia genes also might produce the FCHL phenotype. For example, deficiency of LPL activity has been found in some FCHL families (6, 25). Although this is not usually because of alterations in the LPL gene itself (25), other genes that diminish LPL activity might underlie FCHL in some instances.

REFERENCES AND NOTES

11. Analysis of the plasma lipoprotein triglyceride distribution by gel filtration chromatography showed an increase in IDL–LDL triglyceride (858 mg/dl) and HDL triglyceride (57 mg/dl) in LDLR–/–/–/– mice compared with IDL–LDL triglyceride (550 mg/dl) and HDL triglyceride (10 mg/dl) in LDLR+/+ mice.
21. S. M. Grundy, A. Chait, J. D. Brunzell, Arteriosclerosis... 17 JANUARY 1997

The muscarinic receptor m2 was expressed in COS-7 cells together with an epitope-tagged MAPK (HA-ERK2) (1). Treatment of cells with the agonist carbachol induced activation of MAPK, and wortmannin, an inhibitor of phosphoinositide 3-kinase (PI3Ks), nearly abolished this effect (Fig. 1A). Furthermore, MAPK activation induced by transient expression of Gβγ, by coexpression of Gβγ, or by coexpression of Gβγ and the guanine nucleotide exchange factor Soxs was also inhibited by wortmannin (Fig. 1B). In contrast, no effect of wortmannin was observed when MAPK was stimulated by epidermal growth factor (EGF); by a mutually activated form of MEK, MEK E; or by a membrane-targeted form of Soxs, myrSoxs (Fig. 1, A and B). These results support an essential role for wortmannin-sensitive PI3K in signal transduction from G protein–coupled receptors to MAPK (2), separately from the EGF signaling pathway and upstream of Soxs and MEK.

Several pairs of PI3K have been cloned and characterized. Heterodimer PI3Kα and PI3KB, consisting of p110 catalytic subunits and different p85 regulatory molecules, are regulated by receptors with intrinsic or associated tyrosine kinase activity (3). Another PI3K isotype, termed PI3Kα, can be activated in vitro by both α and ββ subunits of heterotrimERIC G proteins but does not interact with p85 (4). We expressed the α and γ forms of PI3K in COS-7 cells and investigated their ability to induce MAPK activity (Fig. 2, A and B). PI3Kγ induced a concentration-dependent stimulation of MAPK. In contrast, expression of PI3Kα or a mutant of PI3Kγ lacking lipid kinase activity, PI3Kγ K799R (5), did not affect MAPK activity (Fig. 2, B and C). Stimulation of MAPK by overexpression of PI3Kγ was abolished by wortmannin (Fig. 2C). These observations indicate that PI3Kγ mediates Gβγ-dependent regulation of the MAPK signaling pathway.

**Linkage of G Protein–Coupled Receptors to the MAPK Signaling Pathway Through PI 3-Kinase γ**

Marco Lopez-Ilasaca,* Piero Crespo,* P. Giuseppe Pellicci, J. Silvio Gutkind,† Reinhard Wetzker

The tyrosine kinase class of receptors induces mitogen–activated protein kinase (MAPK) activation through the sequential interaction of the signaling proteins Grb2, Sos, Ras, Raf, and MEK. Receptors coupled to heterotrimeric guanine triphosphate–binding protein (G protein) stimulate MAPK through Gβγ subunits, but the subsequent intervening molecules are still poorly defined. Overexpression of phosphoinositide 3-kinase (PI3Kγ) in COS-7 cells activated MAPK in a Gβγ–dependent fashion, and expression of a catalytically inactive mutant of PI3Kγ abolished the stimulation of MAPK by Gαs, or in response to stimulation of muscarinic (m2) G protein–coupled receptors. Signaling from PI3Kγ to MAPK appears to require a tyrosine kinase, Shc, Grb2, Sos, Ras, and Raf. These findings indicate that PI3Kγ mediates Gβγ–dependent regulation of the MAPK signaling pathway.
nearly abolished MAPK activation induced by expression of m2 receptors and by stimulation of Gβγ (Fig. 2D). However, no inhibitory effect by this mutant was observed when MAPK was stimulated with EGF or in Gαs-induced activation of phospholipase C–βγ (PLC–βγ) (Fig. 2E). Thus, the mutated PI3Kγ appears to specifically inhibit the MAPK response to Gβγ. We also expressed a chimeric molecule combining the extracellular and transmembrane domain of CD8 fused to the COOH-terminal domain of βARK, which includes the βy-binding region (6). This chimeric molecule expressing the CD8 antigen at the cell surface and the βARK COOH-terminal domain at the inner face of the plasma membrane is expected to bind to and sequester free βy, thus blocking βy-dependent pathways (6). As expected, CD8-βARK inhibited activation of PLC–βγ by Gβγ (Fig. 2E). Coexpression of CD8-βARK with PI3Kγ nearly abolished MAPK activation by PI3Kγ, whereas CD8 alone had no demonstrable effect (Fig. 2F). CD8-βARK was ineffective in inhibiting MAPK activation by PI3Kγ when this kinase was expressed as a myristoylated form, upon fusing its coding region to that of the NH2-terminal myristoylation, membrane localization signal from c-Src (7). These results indicate that one function of Gβγ is to localize PI3Kγ to the plasma membrane, thereby allowing access to lipid substrates. We can conclude that PI3Kγ has a critical role linking G protein–coupled receptors and Gβγ to the MAPK signaling pathway.

We tested whether the small guanosine triphosphate (GTP)–binding protein Ras participates in signal transduction from PI3Kγ to the MAPK cascade. The dominant negative mutant N17-Ras suppressed the increase of MAPK activity induced by PI3Kγ, but the negative mutants of the small guanosine triphosphatases (GTPases) RhoA, Rac, and Cdc42 did not (Fig. 3, A and B). Expression of a dominant negative mutant of Raf or a mutant Sos protein lacking the domain involved in Ras-specific guanine nucleotide...
exchange activity, SosΔcdc25, also inhibited MAPK stimulation by PI3Kγ without affecting MAPK elevation by the activated form of MEK, MEK E (Fig. 3, C and D). These results support a crucial role for Ras, Raf, and Sos in signaling from G protein–dependent receptors, G<sub>pβ</sub>, and PI3Kγ to the MAPK pathway.

Finally, we investigated the possible involvement of Shc and Grb2 in this signaling route. These elements of the receptor tyrosine kinase–stimulated signaling cascade participate in G<sub>pβ</sub>–dependent signal transduction (8). Wortmannin inhibited binding of Shc and Grb2 induced by carbachol (Fig. 4A). Association of Shc with Grb2 was induced by expression of PI3Kγ in COS-7 cells (9). Expression of PI3Kγ also stimulated tyrosine-phosphorylation of Shc (Fig. 4B). Furthermore, a mutant of Shc lacking the tyrosine-phosphorylation site, Y317F (10), suppressed the stimulation of MAPK induced by lysophosphatic acid (LPA), expression of G<sub>pβ</sub>, carbachol in m2-transfected cells, expression of PI3Kγ, or expression of the Src-related tyrosine kinase Fyn (11) (Fig. 4C). In contrast, the Shc mutant did not affect MAPK activation induced by coexpression of v-Ras. Thus, stimulation of MAPK by PI3Kγ apparently requires a tyrosine kinase that, in turn, phosphorylates Shc and induces its association with Grb2 and leads to a Ras-dependent activation of MAPK. Consistent with this conclusion, the nonspecific tyrosine kinase inhibitor genestein or the Src-like specific inhibitor PP1 (12) potentely blocked MAPK activation by PI3Kγ (9).

The emerging picture is that agonist-activated G protein–coupled receptors first cause the exchange of guanosine diphosphate bound to Gα, thereby causing the dissociation of Gβγ from GTP-bound Gα. Free Gβγ would then recruit PI3Kγ to the plasma membrane, enhancing the activity of a Src-like kinase (13), which in turn leads to the activation of the Shc-Grb2-Sos-Ras pathway, resulting in increased MAPK activity.

**Fig. 3.** Ras and Sos mediate MAPK activation by PI3Kγ. (A) Inhibition of PI3Kγ induced MAPK activation by dominant negative N17ras. Expression plasmids of PI3Kγ WT, PI3Kγ K799R (DK), or MEK E were transfected together with N17ras (18) and MAPK was assayed (19). (B) Effects of dominant negative mutants of Rac (N17rac-1), Rho (N19rho-A), Cdc42 (N17cdc42), and Ras (N17ras) on MAPK stimulation induced by PI3Kγ. Cells were cotransfected with expression plasmids for PI3Kγ and the indicated small GTPases. Data are from a representative experiment that was repeated three times with nearly identical results. (C) Inhibition of PI3Kγ induced MAPK activation by the dominant negative SosΔcdc25. COS-7 cells were cotransfected with PI3Kγ WT, PI3Kγ K799R (DK), or MEK E and an expression plasmid of SosΔcdc25. MAPK activity was assayed. (D) Inhibition of PI3Kγ induced MAPK activation by the dominant negative Raf. COS-7 cells were cotransfected with PI3Kγ WT, PI3Kγ K799R (DK), or MEK E and an expression plasmid for a dominant negative mutant of Raf (DN raf) (1). MAPK activity was assayed.

**Fig. 4.** Involvement of Shc and Grb2 in MAPK activation by PI3Kγ. (A) Inhibition by wortmannin of association of Shc and Grb2 induced by carbachol and m2 receptors. The m2 muscarinic receptors or PI3Kγ were expressed together with an epitope-tagged (HA) Shc in COS-7 cells. The cells were treated as indicated with wortmannin for 30 min and lysed. Proteins were immunoprecipitated with antiserum to Grb2, and activation with Shc was assayed by protein immunoblotting with antiserum to Shc (20). (B) PI3Kγ induced increase of phosphotyrosine in Shc; inhibition by wortmannin. COS-7 cells transfected with PI3Kγ or m2 receptors were treated with the indicated effectors and lysed. Proteins immunoprecipitated with antibodies to Shc were probed with antibodies to phosphotyrosine (15). (C) Dominant negative Shc Y317F inhibits MAPK activation induced by LPA, m2 + carbachol, PI3Kγ, and activated Fyn (Fyn-c). COS-7 cells were transfected with the Shc Y317F mutant and constructs for PI3Kγ, the m2 receptor, v-Ras, and Fyn-c and treated with the indicated effectors; MAPK was then assayed (19).

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5. The mutant of PI3Kγ that lacks lipid kinase activity, PI3Kγ K799R (designated DK) exhibits less than 0.1% of the specific activity of the wild-type enzyme when expressed in vitro. It was provided by Dr. W. Wymann, Fribourg (14), Lysine 799 is the wortmannin-binding site of PI3Kγ (S. Stoyanova et al., in preparation).
For the MAPK assay, after centrifugation, proteins from lysates of total cellular protein or anti-HA immunoprecipitation with the respective antibodies. The cDNAs for human c-Src (designated MEK1) and myrSos were cloned into a modified version of the pEF-BOS vector (M. Lopez-Ilasaca, P. Crespo et al., unpublished data). The construct expressed in COS-7 cells as an increase in phosphatidylinositol 3,4,5-trisphosphate (PIP3) as assayed by lipid extraction, deacylation, and separation by thin-layer chromatography. The myr-PDKy was generated by subcloning of the wild-type construct into pcDNAs3-myr, a modified pcDNAs3 expression plasmid encoding the 21 NH2-terminal amino acids of chicken c-Src (16). The cDNAs for human RhoA, Rac1 and Cdc42Hs GTP-binding proteins, activated MEK1 (designated MEK1) and myrSos were expressed as described (16). CD8 and CD8-ARK1 were generated as described (17).


COS-7 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with fetal bovine serum (10%). Subconfluent cells were transfected with pcDNAs3-HA-MAPK and additional cDNAs (15) by the DEAE-dextran technique. The total amount of plasmid DNA was adjusted to 3 to 4 µg per plate with vector DNA (pcDNAs3; Invitrogen) when necessary; 2 days later, transfected and COS-7 cells were cultured overnight in serum-free medium. Cells were then left untreated or were stimulated with various agents, washed in cold phosphate-buffered saline (PBS), and lysed on ice in a buffer containing 20 mM Hepes (pH 7.5), 10 mM EGTA, 40 mM β-glycerophosphate, 1% NP-40, 2.5 mM MgCl2, 1 mM dithiothreitol, 2 mM sodium vanadate, 1 mM phenylmethylsulfonylfluoride, aprotinin (20 µg/ml) and leupeptin (20 µg/ml). The lysate was centrifuged at 14,000 g for 20 min at 4°C, and proteins were immunoprecipitated and assayed for kinase activity. Equivalent expression of cDNA constructs was verified with the respective antibodies.

For the MAPK assay, after centrifugation, proteins from clarified supernatants were immunoprecipitated with monoclonal antibody (mAb) to hemagglutinin 12CA5 (Babco, Berkeley, CA) for 1 hour at 4°C, and immunocomplexes were recovered with Gamma-bind G (Pharmacia). Bound proteins were washed three times with PBS supplemented with 1% NP-40 and 2 mM sodium vanadate, once with 0.5 M LiCl in 100 mM tris (pH 7.5), and once with kinase reaction buffer [10 mM Mops (pH 7.5), 12.5 mM β-glycerophosphate, 7.5 mM MgCl2, 0.5 mM EGTA, 0.5 mM sodium fluoride, and 0.5 mM vanadate in 30-µl volumes of kinase reaction buffer containing 1 µCi of [γ-32P]adenosine triphosphate (ATP) per reaction, 20 µM unlabeled ATP, and myelin basic protein (MBP) (1.5 mg/ml) (Sigma) at 30°C for 30 min. Reaction complexes were terminated by addition of 5× Laemmli buffer. Samples were boiled and proteins were separated by SDS–polyacrylamide gel electrophoresis (PAGE) (12%) gel. Phosphorylated MBP was visualized by autoradiography and quantified with a phosphorimager or a liquid scintillation detector. Parallel samples were immunoprecipitated with antibody to HA and processed for protein immunoblot analysis with a MAPK-specific antiserum.

Lysates of total cellular protein or anti-HA immunoprecipitates were analyzed by protein immunoblotting after SDS-PAGE (12) with the corresponding rabbit antiserum (inhouse mAb. Immunocomplexes were visualized by enhanced chemiluminescence detection (Amersham) with the use of goat antiserum to rabbit IgG coupled to horseradish peroxidase (Cappel, Westport, CT). Mouse mAbs to the HA epitope 12CAS were purchased from Babco. Rabbit polyclonal antisera to c-Src, Src-C, and TrkA were purchased from Santa Cruz Laboratories (Santa Cruz, CA) or Upstate Biotechnology (Lake Placid, NY).


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Unifying Two General Patterns in the Distribution of Species
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Two patterns in the distribution of species have become firmly but independently established in ecology: the species-area curve, which describes how rapidly the number of species increases with area, and the positive relation between species’ geographical distribution and average local abundance. There is no generally agreed explanation of either pattern, but for both the main hypotheses are essentially the same: divergence of species along the ecological specialist-generalist continuum and colonization-extinction dynamics. A model is described that merges the two mechanisms, predicts both patterns, and thereby shows how the two general, but formerly disconnected, patterns are interrelated.

The species-area (SA) curve is one of the few universally accepted generalizations in community ecology (1–3), but ecologists have failed to agree on the mechanisms that produce this pattern (3). According to the habitat heterogeneity hypothesis, large areas have more species than small ones because of their greater range of distinct resources, which facilitates the occurrence of ecological specialists (3). As an alternative, MacArthur and Wilson (2) advanced the dynamic theory of island biogeography, which predicts that species richness increases with area owing to decreasing extinction rate with increasing area.

Another general pattern in the distribution of species has been well documented only during the past 15 years (4, 5): species with wide distributions tend to be locally more abundant than species with narrow distributions. We call this relation the distribution-abundance (DA) curve. The most widely recognized explanations of the DA curve are Brown’s niche breadth hypothesis and metapopulation dynamics. According to Brown’s hypothesis (5), generalist species, or species using ubiquitous resources (6), are both locally common and widely distributed, whereas specialists are constrained to have narrow distribution and tend to be locally uncommon. Metapopulation dynamic models predict that locally common species become widely distributed because of their low extinction rates and high colonization rates (7, 8). High migration rates from existing large populations may additionally “rescue” small populations from extinction, in which case a wide distribution with many large populations tends to enhance average local abundance (7).

Surprisingly, although the two main hypotheses about the SA and DA curves are strikingly similar, the two patterns themselves have been studied without any reference to each other (9). To bring conceptual unity to this area of ecology, we demonstrate that the SA and DA curves are both predicted by the same model, which furthermore merges the two “competing” hypotheses, namely, ecological specialization (habitat heterogeneity) and extinction-colonization dynamics.

To construct the model, consider a set of R islands populated by a “pool” of Q species. The islands differ in area; we denote by mA and σA the mean and the variance of the logarithm of island areas (base e is used throughout this report). Likewise, the species differ in their abundances per unit area (density), with mA and σA denoting the mean and the variance of the logarithm of species densities (11). By definition, the “carrying capacity” (equilibrium population size) of species i on island j is given by Kj = wi Aj, where wi is the density of species i and Aj is the area of island j.

Following the standard approach to modeling metapopulation dynamics (7), we model changes in the probability pij(t) of species i being present on island j at time t, in the absence of interspecific interactions, as

$$\frac{dp_{ij}(t)}{dt} = C(t)(1 - p_{ij}(t)) - p_{ij}(t)$$

where C(t) is the colonization rate of empty islands and µj is the extinction rate of extant populations. Empirical studies suggest that µj is roughly proportional to 1/Kj (12, 13), and we use this approximation below. The appropriate expression for C(t) is different for two fundamentally different scenarios. In a mainland-island situation, the presence of species on islands is dependent on colonization from a permanent mainland community, where the density of species i is wi. In this case, C(t) is given by...
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