the minor 300-kD cross-linked species remains to be determined. All of these findings establish that the c-*met* product is the cell surface receptor for HGF.

HGF is structurally related to the family of serine proteases that includes plasminogen, prothrombin, urokinase, and tissue plasminogen activator (*5, 9*). Several proteases, including members of this family, stimulate DNA synthesis presumably through a proteolytic mechanism similar to tryptic activation of the insulin receptor (*20*). Only urokinase has been found to associate with a specific cell-surface receptor, which itself bears no homology to the c-*met* protein or other tyrosine kinase receptors (*21*). HGF, however, lacks two amino acids in the catalytic triad required for proteolytic function (*22*). The direct interaction of HGF with the c-*met* receptor tyrosine kinase suggests a biochemical mechanism of mitogenic signal transduction similar to that of insulin, EGF, and others, and thus represents significant functional divergence from its serine protease homologs.

The *met* oncogene was originally identified in a chemical carcinogen-treated human osteogenic sarcoma cell line by transfection analysis in NIH 3T3 cells (*23*). Its cloning revealed that the oncogene encoded a truncated tyrosine kinase activated by chromosomal rearrangement (*24*). Although the oncogene product is predominantly a cytosolic kinase, the proto-oncogene product is a transmembrane receptor-like protein (*14, 15*), whose transcript is expressed in many tissues (*25, 26*). A high proportion of spontaneous NIH 3T3 transformants overexpress c-*met* (*27*). Moreover, NIH 3T3 transfection analysis has revealed that the murine c-*met* proto-oncogene exhibits transforming activity (*26*). Since this cell line produces HGF (*28*), an autocrine mechanism may provide the basis for transformation in each case. Tyrosine phosphorylation of apparently normal c-*met* protein has also been observed in certain human gastric carcinoma cell lines (*29*). Whether autocrine stimulation is responsible for the constitutive activation of c-*met* tyrosine kinase in such tumor cell lines remains to be determined. In any case, knowledge that the HGF receptor is the c-*met* tyrosine kinase provides the opportunity to explore the role of this ligand-receptor system in normal as well as disease states.

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Regulation of Polyphosphoinositide-Specific Phospholipase C Activity by Purified G_q

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The hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) by phospholipase C yields the second messengers inositol 1,4,5-trisphosphate ($InsP_3$) and 1,2-diacylglycerol. This activity is regulated by a variety of hormones through G protein pathways. However, the specific G protein or proteins involved has not been identified. The α subunit of a newly discovered pertussis toxin–insensitive G protein (G_q) has recently been isolated and is now shown to stimulate the activity of polyphosphoinositide-specific phospholipase C (PI-PLC) from bovine brain. Both the maximal activity and the affinity of PI-PLC for calcium ion were affected. These results identify G_q as a G protein that regulates PI-PLC.

REGULATION OF INTRACELLULAR activities by a wide variety of extracellular signals involves cell surface receptors that interact with GTP (guanosine triphosphate)-dependent regulatory proteins (G proteins) (*1*). The best understood pathways are the regulation of adenylyl cyclase by the G_s proteins and the cGMP (cyclic guanosine monophosphate)-specific phosphodiesterase by the G_t (transducin) proteins. The G_o and G_i subfamilies of G proteins are sensitive to modification by pertussis toxin (PTX) and have been implicated in the inhibition of adenylyl cyclase

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and in the regulation of various ion currents and phospholipase C in selected systems. The inability of PTX to inhibit hormonal regulation of other phospholipase pathways indicates that other G proteins exist. One such protein, G_z, was identified by molecular cloning (*2*). Another class of these proteins (G_q and related proteins) was identified simultaneously by purification with βγ-agarose (*3*) and molecular biological techniques (*4*).

Several enzymes have been identified that specifically hydrolyze the phosphoinositides (*5*). They are probably responsible for the increased hydrolysis of these lipids in response to hormones, neurotransmitters, and growth factors. One of these phospholipases, designated PI-PLC$_\gamma$, is phosphorylated by receptors for epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), and has been proposed to mediate this aspect of growth factor regulation (*6*). Many hormones appear to regulate PI-PLC activity through G protein pathways. The evidence for this exists in two forms (*7*). In some systems, the attenuation of hormonal stimulation of PI-PLC by PTX suggests the participation of the G_o or G_i proteins. In the majority of tissues and cells, however, regulation is insensitive to the bacterial toxins. In these cases, participation of a G protein is indicated by the influence of guanine nucleotides on the hormonal stimulation.

Reconstitution of G protein stimulation of PI-PLC has only recently been achieved. A unique G protein α subunit was purified from bovine liver membranes that were preactivated with GTP$_\gamma$S by following its ability to activate PI-PLC (*8*). The major polypeptide that correlated with this activity had an apparent molecular size of 42 kD. In addition, a PI-PLC has been isolated from turkey erythrocytes (*9*). The regulation of this enzyme by G proteins was demonstrated by reconstitution with membranes and subsequent stimulation by purinergic agonists and GTP$_\gamma$S.

We have purified a new G protein α subunit, α_q, from bovine brain (*3*). This 42-kD protein was identified as unique by its failure to be recognized by antibodies to known G proteins and by its amino acid sequence. The partial amino acid sequence was identical to that encoded by a newly isolated cDNA for a G protein designated α_q (*4*). The amino acid sequence information indicated that our G protein preparation also contained a second highly similar α subunit (α_{11}) as a minor component. This α subunit is not a substrate for PTX and binds guanine nucleotides poorly (*3, 10*). This is probably due to high-affinity association with GDP as observed for the G_t protein (*1*).

We first tested whether α_q can activate PI-PLC. We made use of the compound AlF_4^- in these studies, which stimulates the GDP (guanosine diphosphate) form of some G protein α subunits. Under the conditions used in our assays, AlF_4^- has no effect on PLC activity alone. Therefore, any stimulation achieved in the presence of AlF_4^- probably occurs through stimulation of the GDP form of α_q. Purified α_q stimulated the activity of partially purified PLC (*11*) from bovine brain, and this stimulation was markedly increased by AlF_4^- (Fig. 1). So far, we have not observed significant

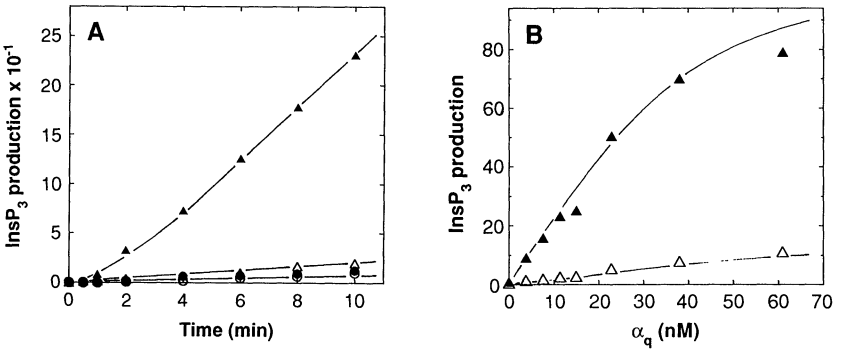

Fig. 1. Stimulation of phospholipase activity with α_q. (**A**) Time course of phospholipase activity. Units for $InsP_3$ production are micromoles per milligram of PLC. Assays contained α_q (18 ng) that had been preincubated either with (closed triangles) or without (open triangles) AlF_4^- (30 μM $AlCl_3$ and 10 mM NaF); assays contained no α_q but were done in either the presence (closed circles) or absence (open circles) of AlF_4^-. All reactions contained 3 ng of the PI-PLC preparation (*11*) and 300 nM free Ca^{2+}. (**B**) Titration of phospholipase activity with α_q. Units for $InsP_3$ production are micromoles per minute per milligram of PLC. Closed triangles, α_q as indicated plus AlF_4^-; open triangles, α_q only. Reactions contained 6 ng of PI-PLC preparation and 300 nM free Ca^{2+}. These results are representative of experiments performed with three different preparations of α_q. Stimulation of PI-PLC has been observed consistently with three independent preparations of lipase and at least five preparations of α_q. The PIP_2-specific phospholipase activity was assayed with [inositol-2-^{3}H{N}]-phosphatidylinositol 4,5-bisphosphate (50 μM) in mixed phospholipid vesicles with phosphatidylethanolamine (500 μM). All components were mixed on ice with Ca^{2+} added last. The final assay solution (60 μl) contained 20 mM Hepes, pH 7.2, 0.08% sodium cholate, 0.83 mM $MgCl_2$, 1 mM DTT, 3 mM EGTA, 0.2 mM EDTA, 30 mM KCl, 20 mM NaCl, and varying concentrations of $CaCl_2$ (*19*), α_q, and PI-PLC. Reactions were initiated by transfer from 0° to 30°C and terminated by transfer from 30° to 0°C and addition of methanol:$CHCl_3$:HCl (80:40:1) (375 μl). After mixing with an additional 150 μl of chloroform and 200 μl of 0.1 N HCl, the aqueous phase was removed and counted by liquid scintillation spectrometry. Assays were performed in duplicate; variance was less 10%.

Table 1. Attenuation of activation of PI-PLC by pretreatment of α_q. NEM treatments: α_q (140 nM) was incubated with NEM (4 mM) at 20°C for 30 min followed by addition of dithiothreitol (DTT) to 40 mM. In some experiments, DTT was added first followed by NEM. Trypsin treatments: α_q (140 nM) was incubated with TPCK-treated trypsin (0.02 mg/ml) for 30 min at 20°C followed by addition of soybean trypsin inhibitor (0.7 mg/ml). In some experiments, trypsin inhibitor was added first followed by trypsin. Immunoprecipitations: either preimmune serum (2 μl), antiserum to the COOH-terminus of α_q (X384) (*18*) (2 μl), or the antibody (X384) blocked with the peptide to which it was raised were incubated with α_q (183 ng) for 1 hour in a total volume of 22 μl. Pansorbin (8 μl of 10% solution) was added, and the mixture was incubated for 1 hour and centrifuged to remove precipitate. Aliquots of the supernatants were used to assay for their ability to activate PI-PLC. Assays were conducted as in Fig. 1 and contained 4 ng of the PI-PLC preparation. Values are the average of duplicate assays with a variance of less than 10%; these results are representative of at least two independent experiments for each condition.

Treatments	$InsP_3$ released (pmol)	
	$-AlF_4^-$	$+AlF_4^-$
− α_q	6	10
+ α_q	31	156
+ α_q + NEM (30 min), then DTT	15	19
+ α_q + DTT, then NEM (30 min)	35	194
+ α_q + trypsin (30 min), then trypsin inhibitor	11	5
+ α_q + trypsin inhibitor, then trypsin (30 min)	35	225
Removal of α_q with antibody	25	30
Blockage of removal by antibody with peptide	29	252
Preimmune serum	31	290

activation of the purified α_q with $GTP_\gamma S$, which was anticipated by the inability of these larger scale preparations to bind the nucleotide (*10*). When used in the same assay, neither purified βγ subunits or the purified α_o, α_{i1}, or α_{i2} subunits from bovine brain affected the activity of PI-PLC (*12*). The stimulation observed with α_q in the absence of AlF_4^- (Fig. 1, open triangles) is not understood at this point. While the GDP form of the α subunit could be causing this activation, it is more likely that the method of purification isolated an activated form of the α subunit. The α_o and α_i subunits are separated from α_q by activation with $GTP_\gamma S$. The $GTP_\gamma S$-liganded α_o and α_i subunits do not bind to the βγ-agarose, while the α_q is retained by the column and eluted with AlF_4^-. It is possible that some of this α_q has bound $GTP_\gamma S$ or that the activation of α_q with AlF_4^- during the elution is not entirely reversed.

The amount of α_q required for activation is substantial (Fig. 1B). Saturation has not yet been achieved with the available α_q. The data suggest that the affinity of AlF_4^--activated subunit for PI-PLC will be greater than 20 nM under these conditions. This observation and the poor exchange of guanine nucleotides may explain the difficulty in detecting the activity of these G proteins in crude solubilized systems.

The PI-PLC activation by α_q was sensitive to boiling, digestion with trypsin, and treatment with N-ethylmaleimide (NEM) (Table 1). The activity can also be eliminated by immunoprecipitation with an antibody to the COOH-termini of the α_q and α_{11} proteins (Table 1). Thus PI-PLC activation is attributable to a protein in the preparation that is a member of the α_q-α_{11} subfamily of G proteins.

The preparation of phospholipase used for these experiments was specific for polyphosphoinositides (Table 2). In this assay, only PIP_2 and phosphatidylinositol 4-phosphate (PIP) were effective substrates. Phosphatidyl inositol or dipalmitoylphosphatidylcholine were not hydrolyzed at either 0.1 or 20 μM Ca^{2+}. Activation of the lipase by α_q did not change this specificity. To determine which isozymes of PI-PLC were present, the preparations were examined by immunoblotting with monoclonal antibodies (*13*). The β but not γ or δ isoforms of PI-PLC were detected.

The phospholipase activity was dependent on Ca^{2+} (Fig. 2). Stimulation by α_q was observed over the full range of Ca^{2+} examined. Besides stimulation of maximal activity, the G protein changed the apparent affinity of the enzyme for Ca^{2+} from ~1 μM to 0.1 μM. Thus, activation was achieved by increasing both the intrinsic activity of the enzyme and its sensitivity to existing concentrations of free Ca^{2+}. Maximal fold activation was observed at lower concentrations of Ca^{2+}. This is consistent with effects that have been observed in diverse membrane systems (*14*).

Table 2. Substrate specificity of PI-PLC activated by α_q. Phosphatidylinositol, phosphatidylinositol-4-phosphate, and phosphatidylcholine, all labeled with tritium in the inositol or choline moieties, were substituted for PIP_2 at 50 μM each. Assays were conducted as in Fig. 1 for 10 min and contained PLC (7.5 ng) and α_q (53 ng). Free calcium concentrations were calculated as described (*19*). Results are representative of two independent experiments.

Substrate	Phospholipid hydrolyzed (pmol)			
	Free Ca^{2+} (0.1 μM)		Free Ca^{2+} (20 μM)	
	$-\alpha_q$	$+\alpha_q$	$-\alpha_q$	$+\alpha_q$
PI	<2	<2	<2	<2
PIP	13	430	240	1100
PIP_2	70	1050	390	1350
PC	<2	<2	<2	<2

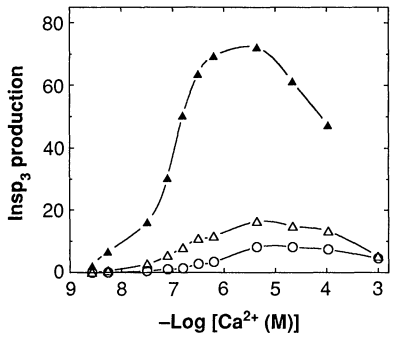

Fig. 2. Calcium dependence of phospholipase activity. Units for $InsP_3$ production are micromoles per minute per milligram of PLC. Closed triangles, 55 ng of α_q with AlF_4^-; open triangles, 55 ng α_q; open circles, no additions. Assays were conducted as in Fig. 1 and contained PI-PLC (7.5 ng). Free calcium was calculated according to (*19*). This is representative of two independent experiments.

The identification of a PTX-insensitive G protein that stimulates PI-PLC answers many of the questions about the regulation of this enzyme by GTP and hormones. The α_q and highly similar α subunits represent a subpopulation in the larger family of trimeric G proteins. Their purification by affinity chromatography on βγ-agarose indicates that their mechanism of activation is similar to that hypothesized for other G proteins. Specifically, a heterotrimer (αβγ) with bound GDP is the resting inactive state. Upon binding of GTP and Mg^{2+}, the protein is activated and dissociates into its respective α-GTP and βγ subunits. In the case of adenylyl cyclase and the retinal cGMP phosphodiesterase, the α subunits of G_s and G_t are sufficient to effect regulation. This has also been proposed for regulation of ion channels (*15*). Here, the α subunit of G_q appears to be sufficient to effect stimulation of the phospholipase. Other α subunits that are substrates for PTX have no effect on the lipase activity. Thus these data do not provide insight into the PTX-sensitive regulation of PIP_2 hydrolysis observed in several systems and may indicate the existence of a different G protein–regulated PI-PLC not represented in our preparation. While the βγ subunits do not stimulate PI-PLC, it is of interest that they can inhibit the stimulation observed with α_q in the presence of AlF_4^- (*12*); this agrees mechanistically with the inhibitory effects of βγ on α_s and the agonist stimulation of adenylyl cyclase (*1*), as well as effects of βγ on stimulation of PI-PLC by fluoride in turkey erythrocyte membranes (*16*).

The relation of the brain α_q to the stimulatory 42-kD α subunit from liver (*8*) remains to be determined. A similarity in apparent size suggests they may be the same gene product, and immunoreactivity with antisera to α_q indicates the presence of this protein (*17*). However, membranes from liver react only weakly with the anti-peptide antiserum (W082), which reacts strongly with α_q and brain membranes (*3*). In addition, other studies (*4*) indicate the existence of related α_q proteins with differential tissue distributions. These observations suggest that the actual compositions of the preparations will differ.

The nature of the interaction between α_q and PI-PLC remains to be explored. While the lipase preparation used is enriched and specific for the substrate, it is not pure. Therefore the participation of other protein factors cannot be ruled out. Furthermore, while PI-PLCβ was identified in these preparations, we cannot rule out the presence of another lipase that is responding to α_q. A broader question is whether α_q will activate other phospholipase activities, such as the production of diacylglycerol from other phospholipids or the generation of other potential second messengers (for example, phosphatidic acid or arachidonic acid and its metabolites). Finally, do G_q and related proteins actually represent the pathway for regulation of PI-PLC by hormones? Definitive evidence for this awaits reconstitution

of G_q with receptors and the development of reagents that specifically block the action of these proteins in membranes and whole cells.

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Translational Potentiation of Messenger RNA with Secondary Structure in *Xenopus*

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Differential translation of messenger RNA (mRNA) with stable secondary structure in the 5′ untranslated leader may contribute to the dramatic changes in protein synthetic patterns that occur during oogenesis and early development. Plasmids that contained the bacterial gene chloramphenicol acetyltransferase and which encoded mRNA with (hpCAT) or without (CAT) a stable hairpin secondary structure in the 5′ noncoding region were transcribed in vitro, and the resulting mRNAs were injected into *Xenopus* oocytes, eggs, and early embryos. During early oogenesis, hpCAT mRNA was translated at less than 3 percent of the efficiency of CAT mRNA. The relative translational potential of hpCAT reached 100 percent in the newly fertilized egg and returned to approximately 3 percent after the midblastula transition.

DURING OOGENESIS, THE EGGS OF many animal species accumulate large pools of maternal polyadenylated [poly(A)$^+$] RNA, of which only a variable fraction is recruited onto polysomes (*1*). Although β-actin mRNA is uniformly translated with up to 90% efficiency, the efficiency of translation of core histone, c-*mos*, and certain other mRNAs increases at the time of oocyte maturation (*2*). In addition, during the period of transcriptional quiescence that extends in many species from the time of oocyte maturation to the completion of the cleavage divisions of early embryogenesis, maternal mRNAs such as those encoding histone H1 and fibronectin become translationally activated (*3*). The mechanism by which differential translation of these various mRNAs is achieved remains obscure. It has been speculated that some maternal RNA molecules may be "masked" by binding to cytoplasmic proteins, which effectively removes them from the pool of translationally competent molecules (*4*)—although the nature of such "masking" proteins is not well understood. Alternatively, poly(A)$^+$ RNAs that contain repetitive sequences may hybridize with one another and form complex networks of intermolecular secondary structures that could interfere with oocytic and zygotic translation; the cytoplasmic polyadenylation of certain maternal mRNAs may also be required for their activated translation during oocyte maturation (*5*). We now report that intramolecular mRNA secondary structure can also be important in the regulation of translational activity during this critical phase of development.

A number of mRNA molecules from both eukaryotic viral and cellular sources contain stable palindromic sequences that may form hairpin or stem-loop secondary structures, usually in their 5′ or 3′ untranslated regions. These secondary structures may serve as binding sites for regulatory proteins, such as occurs in the control of ferritin, poliovirus, and human immunodeficiency virus–1 (HIV-1) mRNA translation (*6*), or they may enhance the stability of mRNAs, as is the case with mRNAs that encode histones (*7*), by reducing nuclease digestion. To determine whether such secondary structures may participate in posttranscriptional regulatory mechanisms during oogenesis and

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