The intracellularly acting *Pasteurella multocida* toxin (PMT) is a potent mitogen that stimulates Gq-dependent formation of inositol trisphosphate. We show that PMT, a nontoxic mutant of PMT (PMT*C116S*), and bombesin each stimulate time-dependent phosphorylation of Goq at tyrosine 349. Although PMT and PMT*C116S* each cause phosphorylation of Goq, only the wild-type toxin activates Goq. Pretreatment of cells with wild-type or mutant PMT potentiated the formation of inositol phosphates stimulated by bombesin equally. These data show that PMT potentiates bombesin receptor signaling through tyrosine phosphorylation of Goq and distinguishes between the two proposed models of Goq activation, showing that tyrosine phosphorylation is not linked to receptor uncoupling.

The *Pasteurella multocida* toxin (PMT)*1* is a highly potent mitogen for mesenchymal cells, including Swiss 3T3 fibroblasts (1). Although the primary molecular targets of this intracellularly acting toxin have not been identified, a prominent role for heterotrimeric G proteins has been elucidated (2–4). The toxin affects several signal transduction pathways, resulting in increased inositol phosphate production, stimulation of protein kinase C activity, Ca2+ mobilization, actin rearrangements, and increased protein tyrosine phosphorylation (5).

Heterotrimeric G proteins are guanine nucleotide-binding proteins that function as molecular switches that transduce signals from G protein-coupled receptors (GPCR) to effector proteins such as enzymes or ion channels (6–8). The Go proteins are divided into four families: Goα, Goβγ, Goαq, and Goα12 (8, 9). The Goαq class are widely expressed and regulate various effector proteins including phospholipase Cβ and Bruton's tyrosine kinase (10). Activation of GPCRs results in a conformational change in the Go subunit, favoring the exchange of bound GDP for GTP. GTP binding results in the dissociation of Go-GTP and βγ complexes, each of which can modulate effector proteins. The regulation of these processes in vitro has yet to be fully elucidated.

Recently it has been demonstrated that the α subunit of Goq is a target for tyrosine phosphorylation. Interestingly, phosphorylation of Goq increased its ability to activate phospholipase Cβ in an in vitro model, suggesting that phosphorylation may modulate the activity of the G protein in vitro (11). Modulation of Goq phosphorylation using chemical inhibitors of tyrosine kinases or tyrosine phosphatases (12) had no effect on the production of inositol phosphates (IP3) in vitro (12, 13). Furthermore, transient expression of a dominant active mutant of the Fyn tyrosine kinase elevated the phosphorylation of Goq but blocked receptor-stimulated IP3 production (12). Taken together, these results implied that cellular kinases and phosphatases coordinately regulate the activity of Goq.

The experiments presented here were designed to determine whether PMT stimulated activation and tyrosine phosphorylation of Goq. We show that PMT is a potent stimulator of Goq tyrosine phosphorylation but that this phosphorylation step is not a prerequisite for Goq activation. Furthermore, using wild-type and mutant forms of PMT, we show that tyrosine phosphorylation of Goq can potentiate signaling through the Goq-coupled bombesin receptor.

**EXPERIMENTAL PROCEDURES**

Cell culture reagents were obtained from Invitrogen. All of the primary antisera were obtained from Santa Cruz Biotechnology, Inc. Horseradish peroxidase-conjugated donkey anti-rabbit and anti-mouse IgG were from Sigma-Aldrich. Recombinant rat and mouse mKY-GPCR (1200 ng/ml) was obtained from Amersham Biosciences. m KY-2 [3H]inositol (1 mCi/ml) was obtained from New England Nuclear, Ltd. All other reagents were of the highest available grade from standard commercial sources. SYF−/− (CRL-2459) and YF−/− (CRL-2498) cells were purchased from the American Type Culture Collection. Goq−/−/12−/− double deficient fibroblasts were a generous gift from Professor Stefan Offermanns (Pharmakologisches Institut, Universitaet Heidelberg, Germany).

**Cell Culture**—Cell culture procedures (14, 15), assays of mitogenicity by [3H](thyminidine incorporation (16) or by cell number (15), and measurements of total inositol triphosphates (17) were performed as described previously.

**Immunoprecipitation of the Goq Subunit**—Quiescent cultures of confluent Swiss 3T3, Goq−/−, or SYF−/−/YP−/− cells were incubated in DMEM containing PMT (70 μM), mutant PMT*C116S* (70 μM), or bombesin (10 nM) as indicated and extracted at 4 °C with RIPA buffer (50 mM Hepes, pH 7.4, 150 mM NaCl, 5 mM MgCl2, 1 mM EDTA, 1 mM Na3VO4, 1% (v/v) Nonidet P-40, 0.5% (w/v) deoxycholate, 0.1% (w/v) SDS, COMPLETE protease inhibitors; Roche Applied Science). The lysates were incubated for 18 h at 4 °C with rabbit anti-Goq (2 μg) antibody coupled to 50 μl of protein G-Sepharose. The immunoprecipitates were resolved by SDS-PAGE, transferred to nitrocellulose, and subjected to Western blotting.

**Preparation of Swiss 3T3 Cell Membranes**—Quiescent cultures of Swiss 3T3 cells were rinsed twice with cold phosphate-buffered saline and scraped into phosphate-buffered saline containing 1 mM sodium orthovanadate and protease inhibitors. Following centrifugation (200 × g, 10 min, 4 °C), washed cell pastes were frozen at −70 °C until required. The frozen cell pastes were thawed on ice and suspended in 5 ml of buffer A (10 mM Tris-HCl, 10 mM MgCl2, 0.1 mM EDTA, pH 7.5) containing 1 mM sodium orthovanadate and protease inhibitors. The cells were ruptured by 25 passes through a 23-gauge needle, and the resulting homogenate was centrifuged at 800 × g for 10 min to remove...
unbroken cells and nuclei. The supernatants were transferred to fresh tubes and centrifuged at 50,000 × g for 10 min. The pellet was washed and suspended in 10 ml of buffer A containing inhibitors. After a second centrifugation step the membrane pellet was suspended in buffer A to a protein concentration of 3 mg/ml and stored at −70 °C.

Determination of GDP-GTP Exchange on G – Determination of GTP·S binding was essentially as described previously (18). Briefly, membranes (30 µg) were suspended in 100 µl of assay buffer (50 mM Hepes, pH 7.4, 120 mM NaCl, 20 mM MgCl2, 2 mM KCl, 1 mM deoxycholate, 20 µM GDP, 0.2% bovine serum albumin) and incubated for 10 min at 37 °C. Following incubation, an equal volume of assay buffer containing either toxins (140 pM) or bombesin (40 nM) and 2 mM [35S]GTP·S (~100,000 cpm/tube) was added, and incubation at 37 °C was continued. The reactions were quenched by the addition of 3 ml of ice-cold assay buffer followed by centrifugation. The membrane pellets were solubilized in 50 µl of solution containing 1.5% (v/v) Triton X-100, 0.2% (w/v) SDS and then diluted to 1 ml with immunoprecipitation buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 20 mM MgCl2, 2 mM KCl, 1 mM EDTA, 1% (v/v) Triton X-100, 0.1% (w/v) SDS, protease inhibitors). The solubilized lysate was incubated for 18 h at 4 °C with rabbit anti-Gq11 (2 µg) antibody coupled to 40 µl of protein G-Sepharose. Immunoprecipitates were recovered by centrifugation and washed six times with immunoprecipitation buffer. The complexes were solubilized by boiling in 5% SDS, and the bound GTP·S was quantitated using a Wallac BetaRack scintillation counter.

Purification of Wild-type and Mutant PMT – Recombinant PMT and inactive, mutant PMT1165S were expressed and purified as described previously (19).

Expression of the Bombesin/GRP Receptor in SYF – Cells – Membranes were prepared from confluent cultures of cells as outlined above. The membranes (50 µg) were fractionated by SHP-GE, transferred to nitrocellulose, and Western blotted with a rabbit polyclonal antiserum to GRP receptor as described previously (20).

G Protein Mutation – Tyrosine residue 349 of Gq was converted to phenylalanine using a QuikChange kit (Stratagene) in accordance with the manufacturer’s instructions. The constructs were confirmed by restriction digestion and nucleotide sequencing. The constructs were directed against the C terminus of the G protein, failed to detect the relevant Gq11 phosphorylation in response to stimulation with PMT. Murine Gq (1–353) was cloned into the eukaryotic expression vector pcDNA3 and the C-terminal tyrosine residue (349), analogous to Gq11 tyrosine 352, was changed to phenylalanine by site-directed mutagenesis. The constructs were confirmed by restriction digestion and nucleotide sequencing. Embryonic fibroblasts deficient in both Gq and Gq11 (Gqq11−/− cells) were transfected with either wild-type or mutant (Y349F) Gq, and the expression of the Gα subunit was determined. In agreement with previous studies (28), an anti-Gq11 anti-serum directed against the C terminus of the G protein failed to detect the mutant Y349F Gq.

However, probing of the membranes with an anti-serum directed against an internal sequence of Gq (115–133) revealed equivalent expression of both constructs (data not shown). Because the C-terminal anti-serum fails to recognize mutant Y349F Gqα, it was not possible to use this anti-serum for immunoprecipitation. It was therefore decided to carry out Gqα immunoprecipitation using an anti-phosphotyrosine antibody and probe blots with the anti-serum directed against the internal sequence of Gqα.

Wild-type or mutant (Y349F) Gqα was expressed in Gqq11−/− cells, and cells were stimulated either with a mixture of bradykinin and thrombin or with wild-type or mutant PMT. In cells expressing wild-type Gqα treatment with GPCR agonists or toxins resulted in increased tyrosine phosphorylation of Gqα. In contrast, mutant Y349F Gqα was not immunoprecipitated using an antibody directed against phosphotyrosine in either untreated or treated cells (Fig. 2a). Parallel experiments revealed that the observed changes in phosphorylation were not due to major differences in the expression of either wild-type or mutant Gqα (Fig. 2b). This indicated that Gqα Tyr349 represents the major site of tyrosine phosphorylation in response to either GPCR or toxin stimulation.

PMT, but Not PMT−1165S, Stimulates Activation of Gq – It had been assumed that mutant PMT (PMT−1165S) failed to activate pathways associated with wild-type toxin, although this had never been demonstrated. We decided to determine whether PMT and PMT−1165S each stimulated activation of Gqα. The initial steps of G protein activation in response to either bombesin or toxins were determined by analyzing binding of the GTP analog GTP·S. Because PMT is predicted to act enzymatically, standard GTP·S binding assays were carried out at 37 °C for 1 h. The use of an in vitro assay system means prolonged incubation steps are not required because the toxin does not require cellular binding and internalization. PMT and bombesin each stimulated concentration-dependent binding of GTP·S to Swiss 3T3 membrane fractions (Fig. 3a). PMT potently stimulated GTP·S binding at concentrations (picomolar range) 100-1000-fold lower than the GPCR agonist bombesin. In contrast, PMT−1165S did not stimulate a significant increase in GTP·S binding in parallel experiments (Fig. 3a) under a
**FIG. 1.** **PMT stimulates tyrosine phosphorylation of G\(_{\alpha_q}\).** 

**a**, quiescent Swiss 3T3 cells were either left unstimulated or stimulated with increasing concentrations of PMT for 4 h and then lysed. The lysates were immunoprecipitated with anti-G\(_{\alpha_q}\) antibody (C-terminal sequence) and Western blotted with either anti-Tyr(P) (upper panel) or anti-G\(_{\alpha_q}\) antibody (lower panel). **b** and **c**, quiescent Swiss 3T3 cells were stimulated with 70 pM PMT (**●**), PMT\(^{C1165S} (**□**), or 10 nM bombesin (**) for various times and then lysed. The lysates were treated as for **a**. The results presented in **a–c** are representative of at least three independent experiments. The results shown in the right panels are expressed as fold stimulation over untreated controls (means ± S.E., n = 3).

**d**, Swiss 3T3 cells preincubated for 1 h with (+) or without (−) 10 mM methylamine (CH\(_3\)N) were treated with 70 pM toxins (4 h) or 20 nM bombesin (1 min) in the presence (+) or absence (−) of a rabbit polyclonal anti-PMT antiserum (pAb). The results presented in **d** and **e** are representative of at least two independent experiments.
To determine whether \( \Gamma_{\alpha_\gamma} \) was directly affected by both bombesin and PMT, we performed GTP\( \gamma \)S binding assays followed by immunoprecipitation with antisera against \( \Gamma_{\alpha_\gamma} \). Membranes from Swiss 3T3 cells were stimulated with 70 pM PMT or PMT\( \gamma_{\alpha_\gamma} \) or 20 nM bombesin as indicated. Nonspecific binding was determined in the presence of excess GTP\( \gamma \)S and by using normal rabbit serum. Bombesin and PMT each stimulated direct GTP\( \gamma \)S binding to \( \Gamma_{\alpha_\gamma} \) (Fig. 3b). The kinetics of GTP\( \gamma \)S binding differed between bombesin and PMT. As previously reported, bombesin stimulated a rapid increase in the levels of bound GTP\( \gamma \)S, which peaked 5–10 min after addition. By comparison, increases in GTP\( \gamma \)S binding stimulated by PMT occurred gradually, peaking 40–50 min after addition. This result would support the concept that PMT has an enzymatic action, with GTP binding occurring downstream of a toxin-catalyzed event. PMT\( \gamma_{\alpha_\gamma} \) had no effect on GTP\( \gamma \)S binding to \( \Gamma_{\alpha_\gamma} \) (Fig. 3b). Thus despite stimulation of \( \Gamma_{\alpha_\gamma} \) by tyrosine phosphorylation by PMT\( \gamma_{\alpha_\gamma} \), this mutant failed to stimulate this key indicator of \( \Gamma_{\alpha_\gamma} \) activation.

To further investigate the functional role of tyrosine phosphorylation in G protein activation, the production of inositol phosphates was determined. Activation of G protein-coupled receptors linked to members of the \( \Gamma_{\alpha_\gamma} \) subfamily stimulates phospholipase C\( \beta \)-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate. As previously reported (1), bombesin and PMT each stimulated activation of phospholipase C in a concentration-dependent manner (Fig. 4a). The increased phosphorylation of \( \Gamma_{\alpha_\gamma} \) in response to either PMT or bombesin was completely blocked by prior exposure to 50 \( \mu \)g/ml genistein (Fig. 4b). Daidzein, an analog of genistein, which lacks tyrosine kinase inhibitory activity, had no effect on the phosphorylation of \( \Gamma_{\alpha_\gamma} \) (data not shown). Application of 20 nM bombesin or 70 pM PMT to Swiss 3T3 cells pretreated with the solvent Me\( _2 \)SO or daidzein resulted in the production of inositol phosphates.

The increased phosphorylation of \( \Gamma_{\alpha_\gamma} \) in response to either PMT or bombesin was completely blocked by prior exposure to 50 \( \mu \)g/ml genistein (Fig. 4b). Daidzein, an analog of genistein, which lacks tyrosine kinase inhibitory activity, had no effect on the phosphorylation of \( \Gamma_{\alpha_\gamma} \) (data not shown). Application of 20 nM bombesin or 70 pM PMT to Swiss 3T3 cells pretreated with the solvent Me\( _2 \)SO or daidzein resulted in the production of inositol phosphates.
However, at concentrations that blocked tyrosine phosphorylation, genistein only inhibited inositol phosphate production stimulated by bombesin and did not inhibit the stimulation of inositol phosphate production by PMT (Fig. 4c). This result demonstrated that phosphorylation of G\textsubscript{q} is not an absolute requirement for phospholipase C activation in vivo. Furthermore, these data strongly argue against PMT acting directly as a tyrosine kinase.

**PMT Enhances Inositol Phosphate Production in Response to Bombesin**—It was previously reported that pretreatment of Swiss 3T3 cells with subsaturating concentrations of PMT enhanced the production of IP\textsubscript{3} in response to neuropeptides but not platelet-derived growth factor (2). To further investigate this effect, we asked whether PMT\textsuperscript{C1165S} could also enhance the production of IP\textsubscript{3}. As previously reported, PMT was able to facilitate the production of IP\textsubscript{3} in response to bombesin treatment (Fig. 5). Although treatment of Swiss 3T3 cells with PMT\textsuperscript{C1165S} alone did not stimulate production of IP\textsubscript{3}, pretreatment of Swiss 3T3 cells with PMT\textsuperscript{C1165S} significantly enhanced the production of IP\textsubscript{3} in response to bombesin (p < 0.01) (Fig. 5). The degree of potentiation stimulated by either wild-type or mutant PMT (approximately 140 and 142%, respectively, of additive values) was comparable (Table I) and thus argues that PMT facilitates the production of IP\textsubscript{3} in response to bombesin through phosphorylation of G\textsubscript{q} tyrosine 349.

**Role of Src Family Kinases in G\textsubscript{q/11} Phosphorylation**—The differential effects of wild-type and mutant PMT on the stimulation of phosphorylation and inositol phosphate production suggested that a cellular kinase phosphorylated G\textsubscript{q}. Src family kinases (subsequently referred to as Src kinases) have been postulated to be possible regulators of this process (11–13). To clarify the role of Src kinases, we utilized the recently described Src/Yes/Fyn-deficient (SYF\textsuperscript{−/−}) cell line (23). We compared activity in these cells with SYF\textsuperscript{−/−} cells rescued with a retroviral vector expressing murine c-Src (YF\textsuperscript{−/−} cells). PMT potently stimulated DNA synthesis in both cell lines, indicating that the cells were responsive to toxin (Fig. 6, a and b). PMT\textsuperscript{C1165S} did not stimulate DNA synthesis in either cell line under the same

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**TABLE I**

| Treatment                        | Inositol phosphates Potentiation |
|----------------------------------|----------------------------------|
| Control                          | 186 ± 14                         | NA                                |
| Bombesin                         | 519 ± 28                         | NA                                |
| PMT\textsuperscript{C1165S}      | 187 ± 15                         | NA                                |
| PMT                              | 1294 ± 20                        | NA                                |
| Bombesin with PMT\textsuperscript{C1165S} pretreatment | 659 ± 55                         | 141.6                             |
| Bombesin with PMT pretreatment   | 2206 ± 150                       | 140.2                             |

The data presented in Fig. 5 were analyzed to determine the level of potentiation in response to both wild-type and mutant PMT\textsuperscript{C1165S}, NA, not applicable. The degree of potentiation is calculated as follows:

\[
\text{Potentiation} = \frac{(\text{Bombesin with PMT/PMT}\textsuperscript{C1165S} pretreatment − Control)}{(\text{Bombesin − Control}) + (\text{PMT/PMTC1165S − Control})} \times 100\%
\]

(Eq. 1)
**Fig. 6. Effect of agonists on tyrosine phosphorylation of Gq in Src/Yes/Fyn-deficient (SYF−/−) cells.** a, quiescent SYF−/− cells were treated for 40 h with various concentrations of PMT ( ), PMT C1165S ( ) or bombesin ( ) in the presence of [3H]thymidine to determine induction of DNA synthesis. The results are expressed relative to incorporation stimulated by 10% fetal calf serum. The results for PMT C1165S and bombesin lie on the same line. b, quiescent YF−/− cells were treated for 40 h with various concentrations of PMT ( ), PMT C1165S ( ), or bombesin ( ) in the presence of [3H]thymidine to determine induction of DNA synthesis. The results are expressed relative to incorporation stimulated by 10% fetal calf serum. c, expression of bombesin/GRP receptor in SYF−/− and YF−/− cells. d, the focal adhesion kinase (p125FAK) in RIPA extracts of control or PMT-treated SYF−/− or YF−/− cells was immunoprecipitated with anti-FAK antibody and Western blotted with either anti-Tyr(P) (upper panel) or FAK antibody (lower panel). The Gq subunit in RIPA extracts of treated YF−/− cells (e) or SYF−/− cells (f) was immunoprecipitated with anti-Gq11 antibody and Western blotted with either anti-Tyr(P) (upper panel) or anti-Gq11 antibody (lower panel). The results presented in all panels are representative of at least two independent experiments.

**DISCUSSION**

Tyrosine phosphorylation of proteins can modulate their activity and/or promote interaction with other molecules (24). Several neuropeptides that regulate cell growth and differentiation induce tyrosine phosphorylation of Gq. Recent studies have indicated that Gq phosphorylation forms part of a novel cycle in which tyrosine kinases and phosphatases regulate Gq activation (11–13). The aim of the present study was to further investigate the role of phosphorylation using PMT.

The results presented here show for the first time that PMT induces both dose- and time-dependent increases in tyrosine phosphorylation of Gq. A nontoxic mutant of PMT (PMT C1165S) also stimulated phosphorylation, with kinetics matching those of wild-type PMT. Phosphorylation of Gq11 in response to wild-type or mutant PMT occurs at Gq tyrosine residue 349. Previous studies indicated that this tyrosine residue is also phosphorylated in response to GPCR agonists, an event confirmed in these studies. Our data demonstrate that the induction of tyrosine phosphorylation by either wild-type or mutant PMT is a specific event following toxin internalization. Methylamine, an agent that increases endosomal and lysosomal pH (25) and therefore inhibits the entry and processing of many toxins, selectively blocked the induction of tyrosine phosphorylation by PMT. Similarly, the early addition of neutralizing antisera to PMT selectively blocked phosphorylation of Gq.

The addition of PMT to cell membranes induced an increase in binding of GTPγS to Gq. This is the first description of an *in vitro* assay for PMT activity. PMT C1165S neither affected GDP/GTP exchange nor stimulated an increase in levels of inositol phosphates. Thus the stimulation of tyrosine phosphorylation by PMT C1165S does not lead to activation of Gq. These data demonstrate that stimulation of Gq tyrosine phosphorylation can occur in the absence of G protein activation and confirm that phosphorylated Gq is not constitutively active in the basal GDP bound state (11).

Lui *et al.* (11) reported that in Rat-1 fibroblasts transformed with the v-src oncogene, inositol phosphate production stimulated by endothelin-1 was increased 6-fold, without changes in the number of receptors. This increased response was mediated...
through $G_q$, which was phosphorylated on tyrosine residue(s). Moreover, when extracted G protein was reconstituted with exogenous phospholipase C, stimulation of $G_q$ activity was significantly increased in extracts from v-src transformed cells. These data implied that phosphorylation of $G_q$ may have a regulatory role in vivo.

Subsequent studies by Umemori et al. (12, 13) have further investigated the role of $G_q$ tyrosine phosphorylation in regulating G protein activity. These authors demonstrated that $G_q$ was phosphorylated upon ligand activation. This phosphorylation was suggested to prevent interaction of the GPCR with $G_q$ and was essential for the activation of $G_q$ by receptor stimulation (12). However, the data conflict with the previous report of Lui et al. (11), who clearly demonstrated that phosphorylated $G_q$ in v-src transformed cells could still interact with and be activated by the endothelin-1 receptor. Indeed, in such cells the production of IP$_3$ was potentiating by the phosphorylation of $G_q$.

Similarly, overexpression of c-Src in mouse fibroblasts potentiates both agonist-induced signaling through β-adrenergic receptors and cAMP accumulation in response to cholera toxin (26, 27). Analysis of the in vitro sites of phosphorylation catalyzed by c-Src identified residues Tyr$^{37}$ and Tyr$^{377}$ (27). Tyr$^{37}$ lies near the site of $G_{q}$ binding in the N terminus, whereas Tyr$^{377}$ is located in the extreme C terminus, within a region of $G_q$ important for receptor interaction. Furthermore, phosphorylation of $G_q$ by immune-complexed c-Src resulted in enhanced rates of receptor-mediated GTP$^\gamma$S binding and GTP hydrolysis (29). These data support the findings of Lui et al. (11) and suggest that tyrosine phosphorylation of $G_q$ subsists not, in itself, prevent interaction of the G protein with the GPCR.

Most recently, Liu et al. (28) reported that an aromatic group is required for efficient information transfer from an agonist occupied receptor to $G_{q1}$. However, their data established that tyrosine 352 could be substituted with phenylalanine or tryptophan without abolishing G protein activity. These findings, together with the data presented here, clearly demonstrate that tyrosine phosphorylation of $G_{q1}$ is not a requirement for G protein activation in vivo.

To further address this problem we investigated the effects of wild-type and mutant PMT on the production of IP$_3$ in response to bombesin. As reported previously (2), wild-type PMT facilitated the production of IP$_3$ in response to bombesin. Interestingly, we have now shown that mutant PMT can also potentiate signaling via the bombesin receptor. Wild-type and mutant PMT each stimulated phosphorylation of $G_q$, but importantly, only the wild-type toxin activated the G protein. These findings imply that phosphorylation of $G_q$ alone is not sufficient to uncouple the receptor from the G protein.

The mechanism through which tyrosine phosphorylation of $G_q$ is induced remains unclear. Our data support the suggestion that a cellular kinase is activated early in the G protein cycle to phosphorylate $G_q$. Umemori et al. (13) proposed that a kinase is activated in response to GTP-GDP exchange on $G_q$. However, the findings that mutant PMT can stimulate phosphorylation of $G_q$ in the absence of GTP-GDP exchange suggest that this model may be an oversimplification. To further understand the mechanisms through which phosphorylation of $G_q$ is regulated, the putative kinase must be identified. Previous work has indicated Src family kinases are critical mediators of this pathway. Our data using Src-deficient cell lines suggest that Src mediates phosphorylation of $G_q$ in response to PMT in vivo. However, because basal phosphorylation of $G_q$ is high in these cells, we cannot exclude the possibility that other kinases may also be involved.

In summary, these studies have conclusively demonstrated that PMT activates members of the $G_q$ family of heterotrimeric G proteins. Utilizing GPCR agonists and a mutant form of PMT, we have confirmed that tyrosine phosphorylation of $G_q$ can be dissociated from G protein activation. Moreover, the finding that wild-type and mutant PMT can potentiate GPCR signaling highlights as yet undefined roles for tyrosine phosphorylation of $G_q$.

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