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*Review Letter*

# Receptor-phosphoinositidase C coupling

## Multiple G-proteins?

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Recent evidence has suggested that receptor-mediated phosphoinositide turnover, like that of the adenylate cyclase cAMP pathway, is regulated by guanine nucleotides. It is likely that one or more guanine nucleotide-binding proteins (G-proteins) couple calcium-mobilizing receptors to the activation of phosphoinositidase C. Recent studies utilizing various bacterial toxins have strongly suggested the presence of multiple G-proteins in the regulation of receptor-phosphoinositidase C coupling in a variety of cell types.

G-protein; Phosphoinositidase C; Phosphoinositide; Pertussis toxin; Cholera toxin; *ras* ocogene

In 1984, Berridge and Irvine [1] wrote that "...the well established role of GTP-binding proteins in controlling cAMP production may thus be extended to include the regulation of other transduction mechanisms such as inositol trisphosphate formation by calcium-mobilizing receptors...". Since then, a large number of reports have accumulated which strongly suggest a role for a GTP-binding protein (G-protein) in receptor-mediated phosphoinositidase C-activated polyphosphoinositide metabolism [2]. Although it is now clear that a G-protein is essential for phosphoinositidase C activation, unlike that responsible for adenylate cyclase activation the identity of this G-protein is still uncertain.

Bacterial toxins have proved to be useful tools to distinguish the G-proteins of the adenylate cyclase system. The toxins from *Vibrio cholerae* and *Bordetella pertussis* ADP-ribosylate G-proteins. While pertussis toxin ADP-ribosylates $G_i$ and thus impairs the receptor-mediated inhibition of cyclase, cholera toxin ADP-ribosylates $G_s$ which leads to persistent stimulation of cyclase [3]. Using these two toxins as probes to identify the G-protein that regulates phosphoinositide response, it seems that there are at least three (or even more) putative G-proteins involved in receptor-phosphoinositidase C coupling (table 1).

The first, $G_P$ is a substrate for neither pertussis toxin nor cholera toxin and is likely to be coupled to muscarinic receptors in astrocytoma cells [4] and cardiac myocytes [5]; angiotensin, vasopressin and $\alpha_1$-adrenergic receptors in rat liver membranes [6]; TRH receptors in $GH_3$ cells [7] and thrombin receptors in fibroblasts [8]. In addition, the GTP-binding protein encoded by *ras* (proto)oncogenes may also be involved in receptor-linked inositol phospholipid hydrolysis. When overexpressed, the p21 protein encoded by the N-*ras* protooncogene of normal, non-transformed cells greatly amplifies phosphoinositidase C activation to bombesin, vasopressin, bradykinin and platelet-derived growth factor (PDGF) [9]. (It is important to note that the analogy between *ras* gene products and other G-proteins only applies to the $\alpha$-subunits of the latter. A *ras*-specific equivalent of the $\beta/\gamma$-subunits of other G-proteins has not been identified nor does p21 interact with $\beta/\gamma$-subunits of

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Table 1

Possible G-proteins involved in receptor-phosphoinositidase C coupling in some cell types

| G-protein | Toxin substrate | Receptor | Cell type |
|---|---|---|---|
| $G_P$ | – | muscarinic [4] | astrocytoma cells |
| | | angiotensin II, vasopressin [6] | liver membranes |
| | | TRH [7] | $GH_3$ cells |
| $G_o$, $G_i$ or $G_o/G_i$-like | pertussis toxin | f-Met-Leu-Phe [14–16] | HL-60 cells |
| | | | PMN leukocytes neutrophils |
| | | compound 48/80 [17] | mast cells |
| | | thrombin [19] | CCL39 cells |
| | | bradykinin [20] | F-11 cells |
| $G_c$ | cholera toxin | T-cell antigen [22] | Jurkat cells |
| | | CCK-8, muscarinic, bradykinin [25,26] | Flow 9000 cells |
| | | secretin [24] | pancreatic acinar cells |
| | | vasopressin[a] | WRK-1 cells |

[a] Kirk, C.J. (personal communication)

other G-proteins [10].) Since p21 is a substrate of neither cholera toxin nor pertussis toxin [11], it is possible that p21 may represent the putative $G_P$ protein. However, it must be emphasized that opposite effects on receptor-phosphoinositidase C coupling have been observed in some other cell systems when overexpressed with the *ras* gene(s). Benjamin et al. [12] showed that in NIH-3T3 cells transfected with the *ras* oncogene isolated from EJ human carcinoma (EJ-ras) there was a reduction of PDGF-stimulated phosphoinositidase C activity. Similarly, a loss of PDGF receptor-activated phosphoinositidase C activity was observed in NIH-3T3 cells transfected with the v-Ha-gene [13]. Additional evidence must be provided to explain these discrepancies and to establish the role of the *ras* gene product in receptor-mediated phosphoinositide metabolism.

In contrast, activation of phosphoinositidase C by chemotactic peptide in HL-60 cells [14], human polymorphonuclear leukocytes [15] and rabbit neutrophil [16], by compound 48/80 in mast cells [17], by $\alpha_1$-adrenergic agonists in adipocytes [18], by thrombin in hamster fibroblastic CCL39 cells [19] and by bradykinin in clonal F-11 dorsal root ganglion hybrid cells [20] is inhibited by pertussis toxin pretreatment. These data suggest that the pertussis toxin substrate $G_i$, $G_o$ or a $G_i/G_o$-like G-protein may be involved in the phosphoinositide response in some cell types. Indirect evidence from Snyder's laboratory showed that immunohistochemical localizations of $G_o$ and protein kinase C correspond in many areas of the brain suggesting a potential role for $G_o$ in the regulation of the phosphoinositide response [21]. Reconstitution experiments using purified $G_o$ or $G_i$ will provide valuable information to the above observations.

A novel cholera toxin-sensitive G-protein ($G_c$) might also be involved in mediating receptor-phosphoinositidase C coupling. Using the T-cell line Jurkat, Imboden and his colleagues reported that cholera toxin inhibits T-cell antigen receptor-mediated increases in both inositol trisphosphate level and cytoplasmic calcium concentration [22]. In the mouse macrophage cell line RAW264, cholera toxin has been shown to inhibit chemotaxis via a cAMP-independent mechanism, possibly through impairment of the PI response [23]. Moreover, secretin receptor-stimulated inositol trisphosphate formation in rat pancreatic acinar tissues was also inhibited by cholera toxin treatment [24]. In our laboratory, we found that

pretreatment of Flow 9000 cells (a human embryonic pituitary cell line) with cholera toxin (but not pertussis toxin) produces a dose-dependent inhibition of cholecystokinin, acetylcholine and bradykinin stimulation of inositol phosphate formation [25,26]. Cholera toxin treatment did not affect GTP analogue-induced inositol phosphate accumulation in permeabilized Flow 9000 cells but inhibited the GTP[S] potentiation of hormonal responses, indicating that the impairment is not at the GTP- or phosphoinositidase C-binding sites on $G_c$ but influences the site responsible for receptor-$G_c$ coupling. In WRK-1 mammary tumour cells, vasopressin-activated $PIP_2$ hydrolysis is mediated via a G-protein [27]. Pretreatment of WRK-1 cells with cholera toxin, but not with pertussis toxin, produced a dose-dependent inhibition on vasopressin-induced inositol phosphate accumulation (Kirk, C.J., University of Birmingham, personal communication). These results thus provide further evidence for the involvement of a novel cholera toxin-sensitive G-protein in inositol phospholipid signalling. In all of the above observations, the cholera toxin effects cannot be mimicked by cAMP-generating agents indicating that these effects are not a consequence of ADP-ribosylation of $G_s$.

Taken together, these findings indicate that at least three different G-proteins are responsible for regulating receptor-phosphoinositidase C coupling. Structural evidence similar to that achieved for the G-proteins coupling to adenylate cyclase [11] will be required to confirm these observations.

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