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REVIEW ARTICLE

Inositol-lipid-specific phospholipase C isoenzymes and their differential regulation by receptors

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INTRODUCTION

Activation of cell-surface receptors by appropriate ligands causes the generation of second messengers which are released in the cell interior. Such transmembrane signalling mechanisms pervade the whole of biology and the classic example is that of cyclic AMP produced as a result of an increase in the activity of an integral membrane enzyme, adenylate cyclase. In this case, receptor stimulation results in the association of the activated receptor with a GTP-binding protein, G_s, and this, in turn, regulates the activity of adenylate cyclase. The minimum requirement for this signalling cascade is three proteins residing in a single compartment, the plasma membrane with the cytosolic compartment providing the substrate, MgATP for adenylate cyclase.

A more recently recognized transmembrane signalling system is the cascade that regulates intracellular Ca^{2+} and protein phosphorylation. This remarkable system where hydrolysis of a phospholipid results in the generation of two second messengers has captured the imagination of many people working in disparate areas such as cell growth and differentiation, plant physiology, phototransduction in invertebrates, and a host of functional responses including contraction, secretion, metabolic responses. In many cases, the inositide signalling pathway is the major controller of subsequent events.

The enzyme(s) responsible for hydrolysing the inositol lipid, phosphatidylinositol bisphosphate (PtdInsP_2) is a phosphodiesterase of the phospholipase C type. By this we mean that this enzyme is responsible for rupturing the bond between the oxygen and the phosphate such that the products of the reaction are diacylglycerol and the water-soluble headgroup, Ins(1,4,5)P_3. Diacylglycerol is responsible for activating a large family of protein kinase C isoenzymes, which catalyse protein phosphorylation. InsP_3 on the other hand interacts with a receptor on the endoplasmic recticulum to release Ca^{2+} from internal stores. This simultaneous generation of two second messengers sets in motion a train of events that results in the culmination of the cellular response. The number of receptors that use this signalling pathway is extensive and what has now emerged is that phospholipase C is regulated by receptors by at least two distinct pathways. The presence of multiple isoforms of PLC which bear minimal relationship was already a clear indication that differential regulation may occur. Two pathways that have been clearly and unambiguously identified are G-protein-mediated and tyrosine kinase-mediated regulation of PLCs.

In this Review we focus on the different isoforms that constitute the phospholipase C family. Several reviews have appeared recently on the enzymology of phospholipase C (see [1–7]) and therefore this review focuses on the recent explosion in our knowledge regarding the regulation of the phospholipases by receptors. For this reason, a number of reconstitution systems have been developed to address this problem. The advantages of different reconstitution systems and their drawbacks will be discussed.

LOCALIZATION OF PLC ISOENZYMES: CYTOSOLIC VERSUS MEMBRANE-ASSOCIATED

Early studies on phospholipase C had clearly established that this enzyme was predominantly resident in the cytosol [8] although a case for a membrane location had also been made [9–12]. Membrane localization of PLC had been a source of controversy in the past [13]. The concept that the inositol lipids may be a source of second messengers goes back to the 1970s [14], and it was hypothesized that for phospholipase C to play a role in cellular signalling, the enzyme had to be located at the plasma membrane, by analogy with adenylate cyclase.

More recently, the majority of the phospholipases have been purified from the cytosolic fraction [15–21]. Although the activity is present mostly in the cytosolic fraction when the cells are disrupted, a membrane-associated localization of PLCs in the living cell cannot be excluded. Moreover, activity associated with the membranes has also been characterized [22–25]. Membrane-associated activity can generally be removed by high salt, indicating that it is ionic interactions that attach the PLC to the membranes. Indeed, one of the membrane-bound activities (PLC-β1) is distributed equally between the cytosolic compartment and the membrane compartment [25]. From the hydropathy analysis and amino acid sequence of PLC-β1, there is no indication of the presence of transmembrane spanning domains. In contrast, PLC-γ1 is found predominantly in the cytosol [25].

The existence of multiple isoforms of PLC was described very early on in studies of many different tissues including heart, brain, platelets, liver and kidney [26–29]. Purification of phospholipases C began in earnest when it was recognized that InsP_3 and diacylglycerol both function as second messengers (reviewed in [30]). This finally led to the purification, sequencing and cloning of the multiple isoforms of the phospholipases. The sequence homology between the isoenzymes was found to be limited.

Another approach that has provided a useful means to tackle the problem of localization of PLCs in living cells is cell permeabilization. There are many techniques available that allow selective permeabilization of the plasma membrane of cells while maintaining the intracellular architecture and their ability to respond to appropriate effectors. For the study of large protein molecules, permeabilization techniques that make pores allowing entry of macromolecules > 10 nm are necessary. Streptolysin O, a bacterial cytolysin, is a very effective tool for generating such pores. Loss of cytosolic proteins such as lactate dehydrogenase (142 kDa), actin (43 kDa) and phosphoglycerate kinase (40 kDa)

Abbreviations used: PLC, phosphoinositide-specific phospholipase C; PtdEtn, phosphatidylethanolamine; GTPγS, guanosine 5'-[γ-thio]triphosphate; PMA, phorbol myristate acetate; PDGF, platelet-derived growth factor; NGF, nerve growth factor; EGF, epidermal growth factor; TSH, thyrotropin; LH, lutrotropin; PTH, parathyroid hormone; PAF, platelet activating factor; FGF, fibroblast growth factor; IGF, insulin-like growth factor.

have been monitored from various cell types, e.g. mast cells and HL60 cells [31–34]. Following addition of streptolysin O to intact cells, the release of cytosol marker enzymes commences within 30 s, and by 5 min the release of cytosolic components is complete [33,34]. From this it can be inferred that it takes 5 min for truly cytosolic components to diffuse out of the cell. Since diffusion is governed by the size of the molecule, smaller molecules such as ATP or GTPγS equilibrate faster than larger components such as lactate dehydrogenase [35]. PLC isoenzymes have differing molecular sizes, ranging from 70 to 88 kDa and 145 to 155 kDa (see Table 1). What happens to PLC when streptolysin O is added to cells? PLC also diffuses out of the cells but 20 min is required for maximal loss, which is slower in comparison to the loss of lactate dehydrogenase [36]. This would imply that the distribution of some PLC isoforms is not limited to the cytoplasmic compartment and is possibly in dynamic equilibrium with cell membranes. Permeabilization disturbs this equilibrium such that the majority of the enzyme(s) finally leaves the cell. Stated differently, in living cells a proportion of the phospholipases are likely to be membrane-associated.

There is now strong evidence that phospholipase C may be associated with the nucleus [37,38]. In Swiss 3T3 cells, it has been shown that PLC-β1 localized at the nucleus was regulated by IGF-1 and is presumably responsible for the known metabolism of inositol lipids at this site [39].

CLASSIFICATION OF ISOFORMS OF PHOSPHOLIPASES C

Table 1 provides a classification of the currently identified phospholipases C. In the top section, (*a*), the classification is based on sequence homology and it only includes those enzymes that can be classified based on the deduced amino acid sequence from their cloned cDNA. Three families of phospholipase C have been identified, β, γ and δ. Within each family, there are several subtypes; subtypes are designated by adding Arabic numerals after the Greek letters as in PLC-β1 and PLC-β2, for example [7]. In the lower section, (*b*), of Table 1, the remainder of phospholipases that have been identified by protein purification and biochemical characterization are tabulated. These enzymes have not yet been sequenced or cloned. Many of the enzymes can be classified into the known PLC-β, γ and δ families. In addition, one can predict the existence of at least two more families, PLC-α and ε.

Fig. 1 compares the linear sequence of the PLC β, γ and δ families. It emerges that there are only two regions of homology shared by the three families, designated X and Y. The X and Y regions comprise 150 and 240 amino acids, respectively [6]. The two domains are about 60% and 40% identical respectively. These regions might constitute, separately or jointly, the catalytic domain. The PLC-γ isoform has been found to contain the *src* homology domains, SH2 and SH3. These domains govern protein–protein interactions; the SH2 domain targets the molecule to tyrosine phosphorylated sequences present in other proteins (e.g. the PDGF receptor) and the SH3 domain targets it to cytoskeletal components. These domains are found in a large number of unrelated proteins such as GTPase activating protein (GAP), PtdIns 3-kinase, tyrosine phosphatases and kinases (see [40] for review).

The PLC-β family

The PLC-β family contains three members, PLC-β1, -β2 and -β3, all present in mammalian cells. Tissue distribution of PLC-β1 indicates that it is expressed at different levels in various cell types [6]. For example, in brain it is present in significant amounts but is present in low abundance in other cells, e.g. HL60, a promyelocytic cell line that can be induced to differentiate towards a granulocyte [6]. Presumably other members of the β family are present at significantly higher levels in some cells. Our knowledge of the extent of the β family comes indirectly from cDNA analysis rather than from studies of the enzymes themselves.

The PLC-γ family

PLC-γ comprises γ1 and γ2, and both forms have been purified (see Table 1). A detailed comparison has yet to be undertaken with regard to their biochemical characterization. They are differentially expressed in cells. PLC-γ2 isoforms predominate in spleen, B-cells, HL60 cells and lung, whereas PLC-γ1 is widely distributed in many tissues.

The PLC-δ family and its biochemical comparison to the PLC-ε family

The PLC-δ family contains three members, δ1, δ2 and δ3.

Table 1. Classification of PLC isoenzymes characterized from different tissues

Proposed name	Size (kDa)	Source [reference]
(*a*) Classification based on sequence data		
β family		
β1	150–154	Bovine and rat brain [187,188]
β2	134	Human HL60 cDNA [2]
β3	–	Human fibroblast cDNA [2]
norp A	125	*Drosophila* genome [151]
plc-21	–	*Drosophila* genome [152]
γ family		
γ1	145	Bovine and rat brain [93]
γ2	146	HL60, spleen, lung [113–115,189]
δ family		
δ1	85	Rat and bovine brain [20,41]
δ2	85	Bovine brain [21,42]
δ3	84	Human fibroblast cDNA [2]
(*b*) Classification based on enzyme purification and biochemical characterization		
γ family		
γ	157	Melanoma [176]
δ family*		
δ	85	Seminal vesicular gland [190]
ε family*		
ε	85	Rat brain [20,36]
ε	88	Bovine brain [18]
ε	87	Rat liver [19]
ε	?	Human platelets [191]
α family		
α	70	Rat liver cytosol [192,193]
α	65	Seminal vesicular gland [190]
α	70	Murine thymocyte cytosol [194,195]
α	70	Murine thymocyte membrane [195]
α	62	Guinea pig uterus cytosol [17]
α	61	Human platelet membranes [22]
α	57	Bovine brain cytosol [196]
α	58	Vascular smooth muscle [197]
α	57	Human spleen [44]
Miscellaneous		
Fragment of β?	98	Human platelet cytosol [198]
Fragment of β?	110	Human platelet membranes [24]
??	18	Human spleen [44]

* The basis for classifying the 85 kDa phospholipase C enzymes into δ and ε families is based on the biochemical properties of the phospholipases. PLC-ε can only hydrolyse polyphosphoinositides. PtdIns is a poor substrate for this enzyme at any pH and in the presence of millimolar levels of Ca^{2+}, conditions which are optimal for PtdIns hydrolysis. PLC-δ on the other hand can hydrolyse all three phosphoinositides, PtdIns, PtdIns*P* and PtdInsP_2, in an assay *in vitro*.

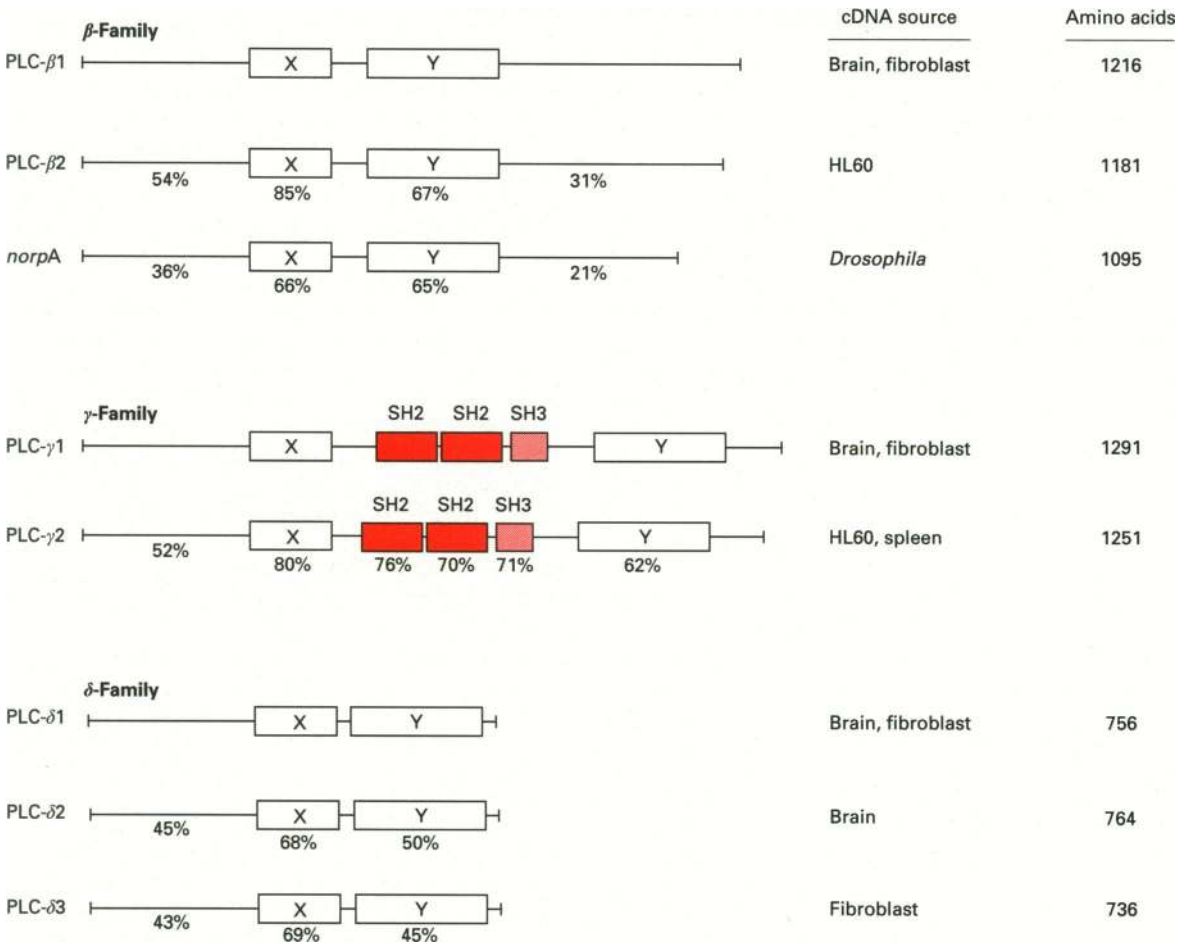

Fig. 1. Linear representation of the members of the PLC-β, γ and δ families

Percentages reflect the degree of sequence identity within each PLC family. Diagram adapted from [5].

Phospholipase δ1 and δ2 have been purified from brain, cloned and sequenced [16,21,41,42] whilst the presence of PLC-δ3 is inferred from cDNA cloning from a fibroblast (WI-38 cells) library [2]. The molecular mass of enzymes that constitute the PLC-δ family is 85 kDa. Many phospholipases having a molecular mass of 85–88 kDa have been purified by different groups of workers from many cell-types. What is the relationship between the PLC-δ family and the other 85 kDa proteins?

In section (*b*) of Table 1 we have classified these phospholipases into two distinct families. The PLC-δ family has been characterized biochemically and one property of this family (also shared by β and γ forms of PLC) is that, in an assay *in vitro* with pure substrate, the enzyme is able to hydrolyse both PtdIns and PtdInsP_2. Whilst PtdInsP_2 hydrolysis occurs at micromolar levels of Ca^{2+}, PtdIns hydrolysis is optimal at millimolar levels of Ca^{2+}. This feature has been used to tentatively assign some of 85–88 kDa PLCs identified from other sources to the δ family. Other 85–88 kDa PLC enzymes have also been described where the enzyme hydrolyses PtdInsP_2 but hydrolyses PtdIns minimally [18–20]. None of these enzymes have been sequenced as yet, but because of this difference in its biochemical property, they have been classified tentatively to another group, PLC-ε ([1,36] and see Table 1).

A second reason for classifying this enzyme into another category is the observation that an 86 kDa phospholipase C that is not δ1 or δ2 has been found to be G-protein-regulated [36]. This 86 kDa protein is also unable to hydrolyse PtdIns *in vitro*. The classification in Table 1 is thus tentative until the sequence of the enzymes designated in the PLC-ε is known. It is likely that some of the 85 kDa proteins characterized in other tissues may also be similarly regulated by G-proteins (Table 1, Section *b*).

Phospholipase C α family

Phospholipases C having a molecular mass of 57–70 kDa have been isolated from many tissues (Table 1). A sequence of PLC-α has been published [17] but since the cloned DNA was not expressed to show that it encodes a phospholipase C, it remains a possibility that the published sequence does not represent PLC-α. PLC-α does not contain any sequence homology to PLC-β1, γ1 or δ, including the absence of the X and Y domains (see Fig. 1). Indeed, it has been suggested that the cDNA clone of PLC-α may encode a previously uncharacterized lumenal endoplasmic reticulum protein [43]. A 58 kDa protein that showed near sequence identity with PLC-α sequence was purified from liver microsomes [43]. The 58 kDa protein, however, did not have PLC activity.

The strategy used to clone PLC-α was based on the use of an antibody against a purified PLC from uterus to screen the RBL-1 library [17]. It is always possible in this technique that the antibody was directed against a highly immunogenic contaminating protein rather than the PLC, for the clone of PLC-α shows very little homology with the proteins encoded by the other cloned mammalian cDNAs of PLC and in fact shows more similarity to thioredoxin, protein disulphide isomerase and ERp72 [43]. Although there is some doubt about the deduced sequence there is plenty of evidence at the protein level that there

is a family of PLCs with a molecular mass of 57–70 kDa which have been purified from a variety of cells (see Table 1). It remains possible that they may be proteolytic fragments derived from PLC-β, -γ, and -δ types [7].

Miscellaneous phospholipases

An 18 kDa phospholipase C has been purified from human spleen membranes which seems to be associated with a regulatory component by ionic interactions. When purified as a complex of 60–70 kDa, it has very little PLC activity but on dissociation, PLC activity is enhanced [44]. This provides a novel form of regulation, i.e. dissociation of an inhibitory component to obtain catalytic activity. Clearly, further work is required to characterize this enzyme and its distribution in other tissues.

REGULATION OF PHOSPHOLIPASE C ISOFORMS

The regulation of phospholipase C is distinct for two of the known phospholipase families, PLC-β and PLC-γ (Figs. 2 and 3). PLC-β is regulated by G-proteins whilst PLC-γ1 and γ2 are regulated by tyrosine kinases. In addition to PLC-β1, a second PLC designated PLC-ϵ has also been found to be under control by G-proteins. Another important regulator of PLC that has been recognized for a long time is Ca^{2+} and yet there is very little information regarding which isoforms are Ca^{2+}-regulated.

Various techniques have been used to analyse PLC regulation and, in particular, to address the problems inherent in studying an enzyme whose substrate normally resides in a membrane. Two approaches have been used to study the regulation of PLCs with their potential activators in cell-free preparations.

In the first approach, purified PLCs and their activators are incubated with lipid substrates presented either as mixed micelles or liposomes or as pure substrate, often in the presence of detergents. The main advantage of this system is its simplicity and the individual proteins that are added are clearly identified. Irvine *et al.* (1984) demonstrated that presentation of PtdInsP_2 in a non-bilayer configuration in the presence of excess PtdEtn enabled its hydrolysis by soluble brain PLC activity at physiological Ca^{2+} and Mg^{2+} concentrations [45,46]. In other words, the enzymes are constitutively active when assayed under conditions where the substrate is presented in a non-bilayer configuration. Therefore assay conditions have to be selected to suppress the catalytic activity so that potential activators can then be tested. The major limitation of this procedure is that the environment in which the enzyme is functioning is totally artificial. In such an assay system, phosphorylated versus non-phosphorylated PLCs do not always show a difference in activity and yet in cells the state of phosphorylation may control PLC activity, e.g. of PLC-γ1 and γ2.

The second approach is to use the native cell membranes as opposed to mixed micelles or pure substrate as a source of substrate and purified PLCs are then added back. Under these conditions, the PLCs cannot hydrolyse their substrate. The situation is more analogous to that of an intact cell. Since in cells the PLC will be presented to its lipid substrate, PtdInsP_2, in a membrane, this method mimics closely the events occurring in a cell. This approach has the added advantage that receptors will also be present. Also, because the normal complement of G-proteins will be present in the membrane preparation, one is examining interactions between G-proteins and PLC that are physiologically relevant. One drawback with this approach is that the membrane preparation can be the source of the activator, e.g. the G-protein and therefore its identity may not be unambiguously assigned. However, identification of the relevant G-proteins that interact with the PLC can be assessed using antibodies against G-proteins and has already been successfully used [47,48]. A further extension of this method is the use of membranes from cells which have been transfected with cDNA encoding different G-proteins [49]. In the case of the PLC-γ1 and γ2, the use of native membranes would also permit the question of whether tyrosine phosphorylation actually activates the enzyme *in vivo* to be asked. This has yet to be established.

G-protein regulation in PLC-β1 and PLC-ϵ isoforms

The involvement of a regulatory G protein termed G_p, in the signal transduction mechanism of agonists stimulating the hydrolysis of PtdInsP_2 has been strongly implicated for some time. The main evidence for a G-protein involvement in PLC activation by receptors can be summarized as follows: (*a*) non-hydrolysable analogues of guanine nucleotides and aluminium fluoride cause increased hydrolysis of endogenous PtdInsP_2, (*b*) GTP is essential for agonist-stimulated PLC activation, and (*c*) in some cell types, pertussis toxin inhibits PLC activation (reviewed in [50]). Progress in the identification of the G-proteins became possible when exogenously added PtdInsP_2 could be used as a substrate.

Studies with exogenous substrate—the deductive approach. The initial studies utilized exogenous substrates and cytosolic PLC to study G-protein-mediated regulation. The result of these studies were contradictory. For, example, early reports had demonstrated that a cytosolic PLC from platelets, brain, thymocytes and HL60 cells could be activated by guanine nucleotides [51–54]. Baldassare *et al.* identified a 29 kDa GTP-binding protein [53]. On the other hand, Rock & Jackowski demonstrated that, in a different assay system, platelet soluble PLC activity is non-specifically stimulated by several nucleotides [55,56]. In a recent study, the GTPγS-binding activity associated with cytosolic PLC from the platelet cytosol was purified and was identified as an actin–gelsolin (1:1) complex [57]. One of the obvious lessons emerging is that all effects of GTPγS may not always be mediated by G-proteins.

Other studies in which labelled PtdInsP_2 was added to membrane preparations showed that it could be hydrolysed as a consequence of activation of a membrane-bound PLC by either GTPγS or receptor-directed agonists. Such agonist- and guanine nucleotide-dependent stimulation of PLC activity has been observed in membranes from blowfly salivary glands [58,59], smooth muscle [60–62], fibroblasts [63], brain [64–69], squid photoreceptor [70], kidney cells (MCDK) [71] and liver [72]. This approach is not universally applicable. In many of the studies addition of detergent was necessary [68,69]. The incubation conditions used in these diverse studies varied; e.g. substrate presentation, inclusion of detergent and the buffer used in the assay. Conditions of the assay played a crucial role in obtaining activation of the PLC via a G-protein since other studies showed non-specific effects of nucleotides [73].

Using the liver system, Exton and his colleagues successfully exploited the above assay procedure to purify the G-protein present in the membrane responsible for activating the PLC [72]. The liver membranes provided both the G-protein and the phospholipase C. The assay could be used even when both the PLC and the G-proteins were released from the membranes by sodium cholate. The critical step here was to activate the G-proteins with GTPγS prior to cholate extraction. A 42 kDa PLC activator, immunologically identified as a G-protein α subunit and shown to belong to the G_q class of G-proteins, was found to be the activator [74]. In this *in vitro* reconstitution assay, the molar ratio of G-protein to PLC is 20:1 for maximal activation [75]. The efficiency is extremely low since it would be expected that a single G-protein may be able to activate many more PLC

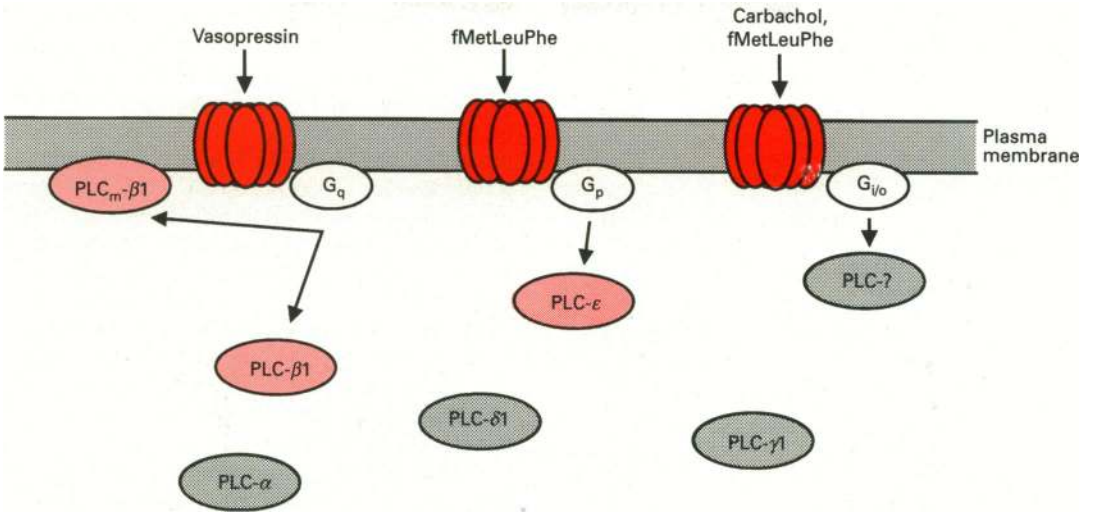

Fig. 2. G-protein-coupled receptors interact with specific G-proteins to stimulate different isoenzymes of phospholipase C

G_p denotes any G-protein that activates a PLC (a functional definition). G_q and $G_{i/o}$ are defined G-proteins which have been shown to interact with either specific receptors or PLC isoforms.

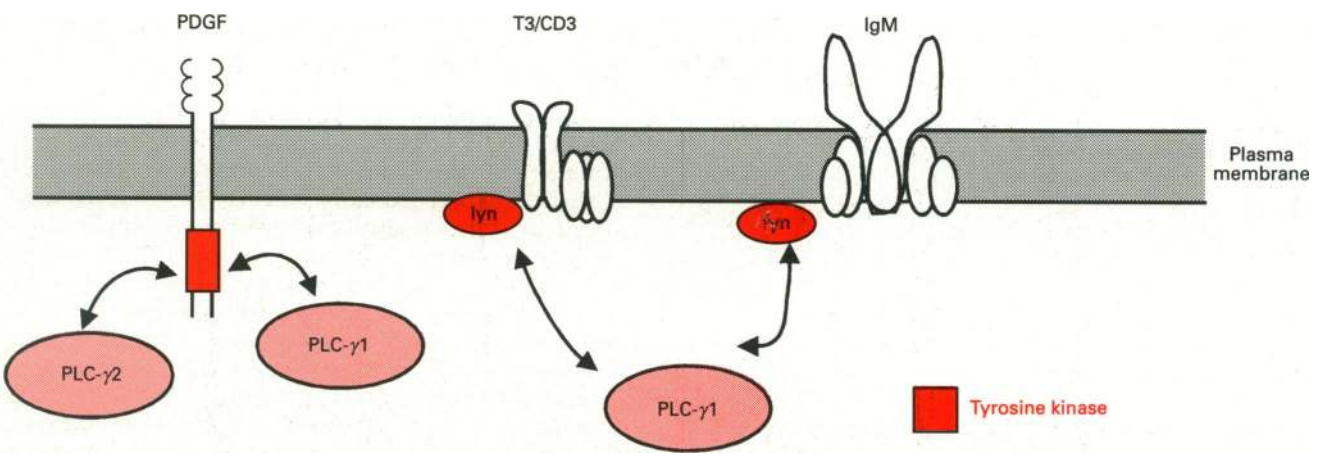

Fig. 3. Receptors for growth factors such as PDGF, the T cell receptor, T3/CD3 complex and the B cell antigen receptor (IgM) all stimulate the PLC-γ1 and -γ2 isoforms of phospholipase C

PDGF activates an intrinsic tyrosine kinase which autophosphorylates and the phosphorylated tyrosine residues on the receptor signals the attachment of PLC-γ1 via its SH2 domains. Phosphorylation of tyrosine residues on PLC-γ then activate the enzyme. The T3/CD3 complex and the IgM receptor interact with a soluble tyrosine kinase of the *src* family instead and these tyrosine kinases, lyn and fyn, phosphorylate PLC-γ1.

molecules. Instead, up to 20 G-protein molecules appear to be required to activate a single molecule of PLC-β1.

Identity of G-proteins that regulate phospholipases C. Clearly the use of exogenous substrate has opened the way to identify the G-proteins that regulate PLC. The G_q family of G-proteins were also purified by Sternweis and his colleagues independently [76] and shown to reconstitute with an enriched preparation of phospholipase C-β1 [77] using an *in vitro* assay method. The partial amino acid sequence was identical to that encoded by a cDNA designated α_q [78–80]. The G_q family comprises at least five G-proteins, G_q, G_{11}, G_{14}, G_{15} and G_{16} [80]. G_q and G_{11}, which are 88 % identical, are capable of activating PLC [49,81,82]. These two G-proteins are widely expressed [78,83,84] and can selectively activate PLC-β1 but not PLC-γ1 or PLC-δ1 [75]. G_{14}, G_{15} and G_{16} are expressed in a tissue-restricted fashion. G_{15} and G_{16} are specifically expressed in cells of the haematopoietic lineage [83,85] together with PLC-β2 [2]. $G\alpha_q$ purified from a βγ-affinity gel was found to stimulate PLC-β1 but not PLC-β2 [7,273] and indeed it was found that $G\alpha_{16}$ (and less effectively, $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$) when expressed in Cos-7 cells was most effective in reconstituting with PLC-β2 [7,274]. That different members of the G_q family activate PLC-β isoforms selectively provides ample room for subtle differences in activation characteristics. The mix and match approach of G-proteins and PLCs provides a system which is extremely versatile and can be easily tailored to the requirement of any particular cell.

Table 2 lists the receptors and cell types where the effect of pertussis toxin pretreatment on PLC activation has been examined. Pertussis toxin ADP-ribosylates a cysteine residue four amino acids from the *C*-terminus of G-proteins. This modification of the G-protein impairs its ability to communicate with the appropriate receptor. G-proteins, G_{i-1}, G_{i-2}, G_{i-3}, G_o and transducin all possess the cysteine residue and are therefore substrates for pertussis toxin. Thus pertussis toxin-sensitivity provides an important clue as to the nature of the G-protein.

Cell types fall into three different categories (Table 2). In the

Table 2. Pertussis toxin sensitivity of the PLC signalling pathway in different cell types stimulated with their specific agonists

Cell type	Agonist	Reference
(*a*) Inhibition by pertussis toxin pretreatment		
Astrocytoma cells	Mastoparan	[199]
Basophils	C5a, fMetLeuPhe	[200]
Endothelial cells	ATP	[201]
Fat cells	Adrenaline	[202]
Fibroblasts (CCL39)	Thrombin	[203–205]
HeLa cells	5-Hydroxytryptamine	[170]
HL60 cells (differentiated)	fMetLeuPhe, ATP, UTP	[86,206]
Liver	EGF	[207,208]
Macrophage	Leukotriene B_4, fMetLeuPhe, PAF	[209,210]
Mast cells	Compound 48/80,	[211]
	Mastoparan	[212]
Neutrophils	fMetLeuPhe	[213–215]
	ATP, C5a, PAF	[216]
	Leukotriene B_4, IgG	[217]
Neuroblastoma-glioma Hybrid cells (NG108-15)	Bradykinin	[218]
Platelets	Thrombin	[86,219]
Renal mesangial cells	Angiotensin II, PAF	[220]
	Prostaglandin E_2	[221]
Renal (MDCK) cells	Bradykinin	[71,222]
Renal tubular cells	Bradykinin	[223,224]
Sensory neurons (DRG)	Neuropeptide Y	[168]
U937 cells	IgG (Fc RII)	[185, 186]
Vascular smooth muscle	Noradrenaline, histamine	[225]
	Angiotensin II, 5-hydroxy-tryptamine	[226,227]
(*b*) Partial inhibition by pertussis toxin pretreatment		
AT10 smooth muscle cells	Vasopressin (V_1)	[224]
Fibroblasts	ATP, UTP	[228]
Fibroblasts (NIH)	Bombesin, bradykinin	[229,229a]
FRTL5 cells (thyroid)	ATP	[230,231]
Gastric cells	Gastrin	[232]
HL60 cells	ATP	[233,234]
NG108-15 cells	Bradykinin	[218]
Neutrophils	IgG (FcR_{42})	[217]
Vascular smooth muscle cells (renal artery)	Endothelin	[235]
(*c*) No effect of pertussis toxin pretreatment demonstrated		
A431 cells	Bradylinin	[236]
Adipocytes (brown)	Adrenaline (α_1)	[237]
Adrenal glomerulosa	Angiotensin II	[238]
	Vasopressin	[239]
Astrocytoma 1321N1 cells	Carbachol, bradykinin	[240,241]
Astrocytoma	Histamine, thromboxane A_2	[242]
Basophils	Anti-IgE	[200,243]
Cerebral striata	Carbachol	[244]
Coronary artery smooth muscle	Carbachol	[60]
Fibroblasts	Bombesin	[245,246]
Fibroblasts (rat-1)	Lysophosphatidate	[247]
Glomerulosa cells	Vasopressin, angiotensin	[239,248]
Heart cells (chick)	Carbachol	[249]
HeLa cells	Histamine	[170]
L cells	LH	[163]
Liver	Angiotensin II	[207,208]
	Vasopressin, adrenaline	[250]
Mast cells	Anti-IgE	[243]
Myometrium	Carbachol, oxytocin	[251]
NG108-15 cells	Bradykinin	[252,253]
Osteosarcoma cells	Thrombin	[254]
Pancreatic acinar cell	Carbachol, caerulein	[255,256]
PC12 cells	Carbachol	[257]
Platelets	Thromboxane A_2	[258]
Pituitary GH_3 cells	TRH	[259,260]
Pituitary cells (anterior)	Angiotensin II	[261]
Pituitary Flow9000 cells	Cholecystokinin, carbachol	[262]
Pituitary gonadotrophs	Gonadotropin releasing hormone	[263]
Sensory neurons (DRG)	Bradykinin	[168]
Thyroid FRTL-5 cells	Adrenaline (α)	[264,265]

first and third category are the cell types which are either sensitive or insensitive to pertussis toxin pretreatment. There is also the intermediate category where the responses are only partially inhibited. Whilst members of the G_q family are likely to be involved in coupling the systems that are pertussis toxin-insensitive, the G_o and G_i family of G-proteins are the potential candidates for the category of receptors that are sensitive to pertussis toxin. Although direct evidence that a member of the G_i or G_o family can activate a purified phospholipase C *in vitro* is not available, there are reports that purified G_i and G_o can activate PLC in membranes after inactivation of the endogenous G-proteins with pertussis toxin [86] or after purification of the PLC from platelet membranes [87]. Two forms of G_o, G_{oA} and G_{oB}, when injected into *Xenopus* oocytes are also able to specifically increase muscarinic receptor stimulation of phospholipase C [88].

Several important messages are implicit in Table 2. Firstly, in a single cell type, different agonists can stimulate PLC via pertussis toxin-sensitive and -insensitive pathways. A good example is the dorsal root ganglion sensory neurons. Here, neuropeptide Y uses a pertussis toxin-sensitive G-protein and bradykinin uses a pertussis toxin-insensitive G-protein. Secondly, a particular receptor does not always couple with a particular subset of G-proteins. Bradykinin is a good example as stimulation can be either pertussis toxin-sensitive (e.g. in renal cells) or pertussis toxin-insensitive (e.g. in endothelial cells). However, the receptor subtype has yet to be characterized for these two cell-types.

Receptor coupling will be a function of the co-expression of the receptors, G-proteins and phospholipases. The relative concentration of the proteins will also dictate the available interactions. Many receptors, when purified, are obtained as complexes with G-proteins, indicating that constraints may be imposed on receptors in native membranes. These constraints can clearly be violated in transfected cells. For example, the V_1 vasopressin receptor from liver co-purifies with the G-proteins of the G_q family, G_q and G_{11} [89]. On the other hand, transfection of the same vasopressin receptor into *Xenopus* oocytes now stimulates PLC via a pertussis-toxin sensitive G-protein. Clearly there is a lack of coupling fidelity and this must be influenced by both the native composition of G-proteins in these cells and the membrane environment. Since the cholecystokinin receptor is also able to couple to pertussis toxin-insensitive G-proteins in *Xenopus*, it is not due to absence of the pertussis toxin-insensitive family of G-proteins.

The use of exogenous substrate bypasses many of the possible control points present in native membranes. Many other factors could also play a role. For example, alterations of receptor–G protein–PLC coupling by protein kinases or provision of substrate for the PLC. The main substrate for the PLC is PtdInsP_2. However, the concentration of PtdInsP_2 is maintained by kinases that can phosphorylate PtdIns sequentially. As the initial steady state concentration of PtdInsP_2 is diminished the kinases catalyse a flux of substrate from the PtdIns substrate pool to that of PtdInsP_2. The initial amount of PtdInsP_2 present in the membranes is much smaller that the total amount of InsP_3, formed indicating the importance of substrate provision. Therefore, *in vitro* reconstitution studies with purified phospholipases and G-proteins need to be complemented with studies in native membranes where these reactions remain intact.

Since the majority of PLC is present in the cytosol, it is possible to remove cytosolic components from cells by permeabilization, leaving a leaky 'ghost' preparation where the membrane compartments remain intact. This 'ghost' preparation has been used for reconstitution [36]. Ghost preparations of red cells (avian) can also be made by hypotonic lysis and have also

been used for reconstitution successfully [90]. G-proteins, receptors and the machinery for synthesizing the polyphosphoinositides all remain intact and the 'ghost' preparation is therefore ideal for the study of G-protein-mediated coupling to specific phospholipases [36,90].

A 150 kDa PLC purified from turkey eythrocytes [91] has been added back to turkey red cell 'ghosts' and the purified enzyme was able to hydrolyse the endogenous substrate when the purinergic receptor was stimulated [90]. This reconstitution method does not have to be restricted to using PLC and G-protein from the same source, i.e. homologous reconstitution. A heterologous system has also been used where the membranes were from one source and the enzyme from another. HL60 'ghost' cells have been reconstituted with PLC-β1 and with PLC-ϵ, both prepared from rat brain cytosol [36]. Although, the G-proteins that are responsible for reconstitution have not been identified, it is known that HL60 cells contain G-proteins of the G_q family as well as $G_{i2/3}$. Identification of the G-proteins that reconstitute with these two enzymes in this experimental situation should provide a good indicator about selectivity of interactions. Clearly, this approach of reconstitution where the normal complement of G-proteins is already present in the membranes will make an important contribution towards understanding which phospholipases associate with which G-proteins under near physiological conditions. This approach essentially complements the *in vitro* reconstitution system.

Regulation of PLC-γ by tyrosine phosphorylation

The regulation of PLC-γ family by tyrosine phosphorylation has been reviewed [5]. The first indication that tyrosine phosphorylation may regulate phospholipase C activity came from the published sequence of phospholipase C-γ1 [92]. The predicted amino acid sequence for the phospholipase C derived from complementary DNA cloning revealed that it possessed regions homologous to the products of various tyrosine kinase-related oncogenes (*yes*, *src*, *fgr*, *abl*, *fps*, *fes*, *tck* and *crk*) [92–94]. The next significant finding in this story was the demonstration that PLC-γ1 was a substrate for receptor protein-tyrosine kinases. Both EGF and PDGF receptors when activated could phosphorylate PLC-γ1 on tyrosine and serine residues both *in vivo* and *in vitro* [95–98]. By virtue of being phosphorylated on tyrosine residues, phosphotyrosine antibodies immunoprecipitated PLC-γ1 from EGF-activated A-431 cells [99]. A physical association of PLC-γ1 with the PDGF and EGF receptor was also demonstrated [100,101].

Other receptors with tyrosine kinase activity (e.g. insulin, CSF-1) fail to phosphorylate PLC-γ1, indicating that all receptors that are tyrosine kinases are not necessarily able to target PLC-γ1 as a substrate [101,102]. The stimulation of PLC appears to require the intrinsic activity of the receptors because mutant PDGF and EGF receptors that lack tyrosine kinase activity bind the growth factors but fail to stimulate the hydrolysis of inositol phospholipids [103,104]. Phosphorylation at tyrosine residues leads to the activation of PLC-γ1 [105]. Dephosphorylation by tyrosine phosphatases (48 kDA T cell protein tyrosine phosphatase but not CD45), on the other hand, diminishes the activity of PLC-γ1 immunoprecipitated from EGF-treated cells [105].

In the early studies on PLC-γ1, it was not possible to demonstrate a change in PLC-γ1 activity after tyrosine phosphorylation when assayed *in vitro* using pure exogenous substrate. The PLC enzymes present in the cytosol have a high activity when assessed with pure substrate but are essentially inactive when the substrate is presented in a native membrane. In the recent study by Nishibe *et al.* [105], Triton-X100 (0.05 %) was shown to selectively inhibit the *in vitro* PtdInsP_2-hydrolysing activity of unphosphorylated PLC-γ1, mimicking the native membrane preparation. Enhancement of PLC activity could now be observed with phosphorylated PLC-γ1. However, another detergent, deoxycholate, could not differentiate phosphorylated versus non-phosphorylated PLC-γ1 at various concentrations. Under physiological conditions, other elements such as profilin, a regulator of actin polymerization, may take on this role [106,107].

Four sites of tyrosine phosphorylation in PLC-γ1 have been identified. Purified EGF receptor phosphorylates tyrosine residues 771, 783, 1254 and to a lesser extent, Tyr-472 [108,109]. The major sites of phosphorylation by EGF and PDGF receptors *in vivo* and *in vitro* appear to be identical [95]. Tyr-771 is adjacent to regions of PLC-γ1 that contain high homology to the non-catalytic, terminal region of the *src* tyrosine kinase. Tyr-1254 lies near the *C*-terminus of the PLC molecule. By site-directed mutagenesis, it was established that Tyr-783 and Tyr-1254 were essential for PLC-γ activation [110]. However, mutation at residues 771, 783 and 1254 does not change the catalytic activity of PLC-γ measured *in vitro* [110].

In unstimulated cells, PLC-γ is normally found in the cytosolic fraction after cell disruption. On activation by PDGF and EGF, a translocation from the cytosol to the membrane is observed [111,112]. The mechanism of translocation relies on the affinity of the phosphorylated tyrosine residues on the receptor for the SH2 domains of the PLC-γ1. This association permits the phosphorylation of PLC-γ1 tyrosine residues. Clearly, the association of PLC-γ1 with the membrane is a key step to enzyme activation.

Regulation of PLC-γ2. PLC-γ2 appears to be present in abundance in haematopoietic cells [113–115]. It also contains SH2 and SH3 domains as well as tyrosine residues analogous to 771 and 783 of PLC-γ1 but not tyrosine residue 1254. Nonetheless, PLC-γ2 appears to be regulated in a similar manner to PLC-γ1. *In vivo*, PDGF stimulates tyrosine phosphorylation of PLC-γ2 when overexpressed in rat-2 cells [116] or in NIH 3T3 fibroblasts [117]. Although the PDGF receptor can phosphorylate PLC-γ2, it is unlikely to be the natural regulator of this enzyme. PDGF receptors are normally not found in haematopoietic cells where PLC-γ2 is specifically expressed. However, there are several candidate receptors that are either intrinsic tyrosine kinases or can activate *src*-related soluble tyrosine kinases expressed in haematopoietic cells whose activation is linked to PLC activation. Examples include the IgE receptor, T-cell receptor and the Fc receptor.

Regulation of phospholipase C by Ca^{2+}: the Ca^{2+} paradox

It has been clear for some time that Ca^{2+} has profound effects on PLC activation either directly or by modulating the receptor-mediated responses. The direct effects of Ca^{2+} occur both in the physiological range of micromolar levels as well as at high millimolar levels which are of less relevance for the intact cell. Different methods have been used to raise cytosol levels of Ca^{2+}, e.g. depolarization by high potassium, Ca^{2+} ionophores or buffered Ca^{2+} in permeabilized cell preparations. In many cases, the increase in cytosol Ca^{2+} appears to stimulate PLC activity. For example, high K^+ induces PLC activation in adrenal chromaffin cells [118], neuronal cells in the guinea pig ileum longitudinal smooth muscle [119], and pancreatic islets [120]. Studies in permeabilized cells or membrane preparations also support the general view that Ca^{2+} can stimulate PLC in some situations [121]. Ca^{2+} has also been shown to induce the hydrolysis of PtdInsP_2 in human sperm [122]. The question that needs to be addressed now is whether there is a specific isoform of PLC that is only sensitive to Ca^{2+}.

REGULATION OF PLC ISOENZYMES BY PROTEIN KINASE C- AND A-MEDIATED PHOSPHORYLATION

Protein phosphorylation is a common mechanism for regulation of enzyme activity in cells. Treatment of a variety of cells with PMA, an activator of protein kinase C, or with cyclic AMP-elevating agents leading to protein kinase A activation result in the inhibition of receptor-coupled PLC activation. Both G-protein-coupled receptors as well as receptors which use tyrosine kinase as their transducing system appear to be similarly affected. A potential target for phosphorylation are the PLCs and evidence for phosphorylation on serine residues on PLC-γ1 by either protein kinase C or A has been presented.

PLC-γ1 (but not PLC-β1 or PLC-δ1) can be phosphorylated on a single serine residue (Ser-1248) by cyclic AMP-dependent protein kinase in rat glioma C6Bu1 cells [123], 3T3 cells [124] as well as a human leukaemic T cell line, Jurkat [125,126]. Intact cells when treated with forskolin, dibutryl cyclic AMP or cholera toxin all exhibit enhanced phosphorylation of PLC-γ1. Moreover, the enzyme can also be phosphorylated by cyclic AMP-dependent protein kinase *in vitro*. Prior treatment with agents that increased cyclic AMP levels inhibited the subsequent stimulation of PLC by noradrenaline in rat glioma [123] and by CD3 in Jurkat cells [125] respectively. However, phosphorylation of the enzyme did not lead to any changes in enzyme activity when assessed *in vitro* [124,125] indicating that phosphorylation alters interaction of PLC-γ1 with protein tyrosine kinase or the protein tyrosine phosphatase. This altered interaction may account for the decreased tyrosine phosphorylation of PLC-γ1 seen in cells where cyclic AMP levels were elevated [126].

PLC-γ1 can also by negatively modulated by protein kinase C. Treatment of Jurkat cells with PMA leads to phosphorylation of PLC-γ1 at Ser-1248, the same residue also phosphorylated by cyclic AMP-dependent kinase. This prior phosphorylation does not permit the tyrosine phosphorylation of PLC-γ1 to occur, thereby leading to inhibition of CD3-stimulated PLC-γ1 activation. Clearly, a prior protein phosphorylation by either protein kinase A or protein kinase C diminishes the ability for PLC-γ1 to be phosphorylated on tyrosine residues, hence leading to inhibition [126]. Protein kinase C-dependent inhibition of PLC-γ1 is not always observed [127,128].

PLC-β1 is phosphorylated by protein kinase C but not by protein kinase A. Treatment of PC12, C6Bul and NIH 3T3 cells with PMA stimulated the phosphorylation of serine residues (Ser-887) in PLC-β1 but not PLC-δ1 or PLC-γ1 [127]. (PLC-γ1 phosphorylated by protein kinase C in Jurkat cells may be an exception [126].) Phosphorylation of pure PLC-β1 by protein kinase C *in vitro* also results in a stochiometric incorporation of phosphate at a single serine residue (Ser-887) [127]. The catalytic activity in an assay *in vitro* is not affected by phosphorylation of PLC-β1 by protein kinase C [127]. The experiment that has yet to be done is to test the ability of the phosphorylated versus the unphosphorylated PLC-β1 enzyme both in the *in vitro* reconstitution system with purified G_q as well as in the permeabilized HL60 system. This may reveal whether the phosphorylation of PLC β1 has any effect on its function.

RECEPTORS THAT ACTIVATE PHOSPHOLIPASES C CAN BE CATEGORIZED BY THE MODE OF PLC ACTIVATION: G-PROTEINS VERSUS TYROSINE KINASE-DEPENDENT PHOSPHORYLATION

G-protein-coupled receptors

The G-protein-coupled receptors so far identified show differential pertussis toxin sensitivity (Table 2). Two phospholipases (PLC-β1 and PLC-ϵ) have been identified that can be G-protein-regulated. In Table 3, we have summarized some of the receptor systems that are now known to be coupled to either specific G-proteins and/or PLC. What emerges very clearly is that the G_q family of G-proteins regulate the PLC-β1 family and this accounts for the large proportion of the pertussis toxin-insensitive G-protein regulated PLC activity. This relationship also holds for the invertebrate system (see below).

Receptors that are intrinsic tyrosine kinases or regulate cytosolic tyrosine kinases

Many receptors are intrinsic tyrosine kinases, such as PDGF and EGF. These receptors appear to recruit the PLC-γ1 isoform of PLC. This is summarized in Table 4. Many other receptors have been identified that regulate cytosolic tyrosine kinases and increasing evidence suggests that many of these receptors also active PLC-γ1 by tyrosine phosphorylation. These receptors are summarized in Table 4 and are discussed individually below.

The ability to recognize and respond to foreign antigen is fundamental to the function of the cells of the immune system. These include T and B lymphocytes, mast cells, neutrophils and macrophages. The molecules involved in the actual recognition and binding of antigen are well-defined. In the case of T and B lymphocytes, the T cell receptor–CD3 complex and the surface immunoglobulin M and D are involved respectively. Early studies on the signalling pathway used by lymphocytes suggested that a G-protein was involved in coupling to PLC [129–132]. Antibody-mediated crosslinking of the antigen receptors of both B and T cells induces at least three rapid biochemical changes; an increase in the level of $[Ca^{2+}]_i$, the activation of protein kinase C and an increase in tyrosine protein phosphorylation [133]. Since both the increase in $[Ca^{2+}]_i$ and the activation of protein kinase C probably do result in part by activation of PLC, it has been suggested that tyrosine phosphorylation may be responsible for PLC activation, an alternative to G-protein-mediated activation of PLC [133,134].

Direct evidence for tyrosine phosphorylation of PLC-γ1 via the T cell receptor has now been presented [125,135–137]. PLC-γ1 phosphorylation on both serine and tyrosine residues is induced as a result of activation of CD3. The tyrosine phosphorylation is achieved via soluble tyrosine kinases belonging to the *src* family, $p56^{lck}$ and $p59^{fyn}$. $p59^{fyn}$ appears to be tightly associated with the T cell receptor [138].

For B lymphocytes, a number of *src*-related kinases have been identified. These include $p56^{lck}$ and $p53^{lyn}$. The *lyn* kinase has been reported to be physically associated with membrane IgG. PLC-γ1 has been found to be a substrate for tyrosine kinases in anti-IgG-activated B lymphocytes [139]. Since many B-cell lines contain a high level of PLC-γ2 with lower levels of PLC-γ1, this might suggest that, in B cells, PLC-γ2 is the main substrate for tyrosine phosphorylation [140].

Mast cells and basophils have a high-affinity receptor for IgE on their surface. Occupation of these receptors by IgE primes the cells to respond to antigen which triggers the cells by crosslinking the IgE receptors. Engagement of the high affinity IgE receptor activates *src* protein-related tyrosine kinases such as $p62^{yes}$ and $p56^{lyn}$ [141]. Since the tyrosine kinase inhibitor, genistein, can inhibit antigen-mediated PLC activation, it has been speculated that PLC-γ is activated by a soluble tyrosine kinase [142]. A direct demonstration that oligomeric IgE induces tyrosine phosphorylation of PLC-γ1 in RBL cells has been presented [143].

Another potential regulator of tyrosine-phosphorylated PLC-γ1 that is present in essentially all haematopoietic cells including T and B lymphocytes is CD45, a large (180–220 kDa) transmembrane tyrosine protein phosphatase [144]. Although no extracellular ligand has yet been identified for CD45, antibodies

Table 3. G-protein-coupled receptors known to regulate specific G-proteins and/or specific phospholipase C isoenzymes

Agonist	Cell type	Identity of G_p	PLC isoenzyme	Reference
Thromboxane A_2	Platelets	G_q/G_{11}	??	[47]
Histamine	1321N1 cells	G_q/G_{11}	??	[48]
Bradykinin	NG108-15 cells	G_q/G_{11}	??	[48]
Angiotensin II	Liver	G_q/G_{11}	??	[48]
GTPγS	Cos-7 cells	G_q/G_{11}	PLC-β1	[266]
AlF^{4-}	*In vitro*	G_q/G_{11}	PLC-β1	[81]
GTPγS	*In vitro*	G_q/G_{11}	PLC-β1	[81]
Not used	*In vitro*	α_q	PLC-β1	[75,77]
Carbachol	*Xenopus* oocytes	α_o	PLC-??	[88]
Carbachol m1	*In vitro*	$\alpha_{q/11}$	PLC-β1	[267]
GTPγS	HL60 cells	??	PLC-ε/β1	[36]
fMetLeuPhe	HL60 cells (differentiated)	??	PLC-ε	
Vasopressin V_1	Liver	G_q/G_{11}	PLC-β1?	[89]

Table 4. Receptors which are (*a*) intrinsic tyrosine kinases or (*b*) able to activate soluble *src*-related tyrosine kinases and their ability to stimulate the PLC-γ family by tyrosine phosphorylation

Agonist	Cell-type	Ability to stimulate PLC-γ	Reference
(*a*) Intrinsic tyrosine kinases			
PDGF	NIH 3T3 cells	Yes	[102]
EGF	Liver	Yes	[48]
	A431 cells	Yes	[96]
	HSC-1 cells	Yes	[108]
FGF(*FLG*)	*In vitro*	Yes	[268]
NGF	PC-12	Yes	[269]
	NIH 3T3	Yes	[270]
Steel factor (c-*kit* ligand)	Mast cells	Yes	[271]
CSF-1	NIH 3T3 cells	No	[102]
Insulin	NIH 3T3 cells	No	[101]
IGF	NIH 3T3 cells	No	[272]
(*b*) Able to activate soluble tyrosine kinases			
TD3/CD3	Jurkat T-cell-line	Yes	[125,135–137]
mIgM	B-cells	Yes	[140]
FcεR1	RBL-2H3	Yes	[142,143]
FcRI	U937 cells	Yes	[184]
FcRII	U937	Yes	[184]

to CD45 are inhibitory to activation of PLC via CD3 [145,146]. Nonetheless, the presence of CD45 is essential for the T cell receptor to couple efficiently to the PLC [147].

PHOTOTRANSDUCTION IN INVERTEBRATES

On the basis of both biochemical, physiological and genetic evidence, it has been proposed that the phototransduction cascade in invertebrate photoreceptors involves G-protein-coupled activation of PLC by light-activated rhodopsin [70,148]. A *Drosophila* visual mutant, *norp*A (no receptor potential A), has been described which is defective in the phototransduction process. Although the retinal cells show almost normal levels of rhodopsin, they fail to respond to light stimulation. These *norp*A mutants show virtual absence of PLC in the head region [149,150].

The *norp*A gene has now been shown to encode a 1095-amino-acid phospholipase C with extensive sequence similarity to PLC-β1 [151]. The gene is expressed in great abundance in the retina. A second gene encoding another phospholipase (*plc-21*) has also been identified. The *plc-21* gene encodes two transcripts, the protein products having 1305 and 1312 amino acids which are similar in sequence to the *norp*A protein and to the mammalian PLC-β family [152]. From biochemical studies the phospholipase C encoded by the *norp*A gene is the major PLC in these cells whilst the PLCs encoded by the *plc-21* gene are in the minority [153].

An antibody to the *norp*A protein recognizes an eye-specific protein of 130 kDa that is present in wild type head extracts but not in *norp*A mutants. The protein is associated with membranes and can be extracted with high salt, a property shared by PLC-β family.

Since PLC-β1 is regulated by the G-proteins of the G_q family, it would be predicted that a homologue of the G_q family may also be responsible for stimulating the photoreceptor-specific PLC encoded by the *norp*A gene of *Drososphila*. The principal G-protein in squid photoreceptor cells has been identified and is closely related to the G_q family of G-proteins. It is present at about 10% the concentration of rhodopsin, a ratio similar to that found in the vertebrate photoreceptor [154]. This strongly suggests that in the invertebrate phototransduction system, a G-protein which is homologous to the mammalian G_q regulates an invertebrate PLC which shares sequence similarity to the PLC-β family.

AVIAN ERYTHROCYTES

Historically, the avian erythrocyte has contributed enormously to our understanding of β-adrenergic regulation of adenylate cyclase via the G-protein, G_s. The turkey erythrocyte has more recently been used to analyse the activation of PLC by a G-protein. A purinergic receptor of the P_{2Y} class has been shown to be present on these cells and to be linked to phospholipase C via a G-protein [155–157]. The phospholipase C has been purified from the cytosol and reconstituted with turkey erythrocyte ghosts [90,91]. The enzyme is a 150 kDa protein which has two stretches common to mammalian PLC isoenzymes, but it diverges in sequence elsewhere. Homology with sequence in PLC-β outside the conserved X and Y domains suggests some similarity between the turkey 150 kDa PLC and the PLC-β family of isoenzymes [158].

In addition to the purinergic receptor present on turkey eythrocytes, these cells also have a β-adrenergic receptor coupled to adenylate cyclase. Surprisingly, the β-adrenergic receptor is also able to couple to phospholipase C directly via a G-protein that is not sensitive to cholera toxin (this excludes G_s as the transducer) [159,160]. This activation by a receptor which is

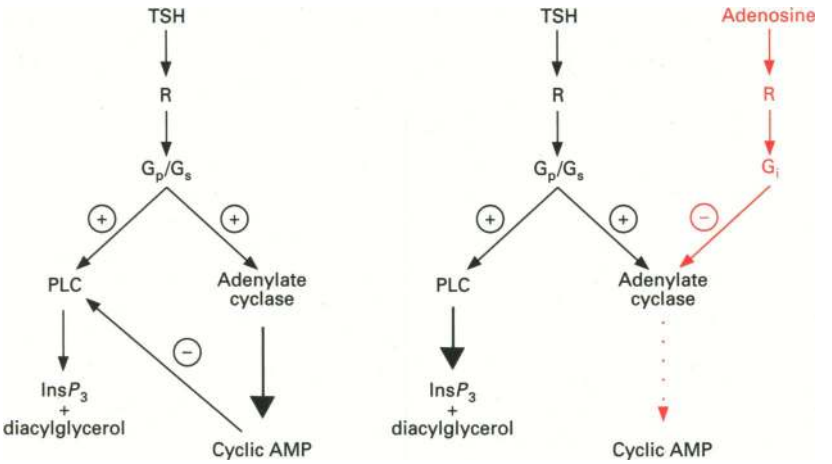

Fig. 4. Signalling networks

In (*a*), it is shown that the activated TSH receptor can couple to both adenylate cyclase and PLC. The PLC response is held under check by the presence of cyclic AMP-mediated phosphorylation. In (*b*) the response to TSH is modified if adenosine receptors are also activated. Adenosine inhibits the activity of adenylate cyclase via G_i, causing a decreased output of cyclic AMP. Cyclic AMP no longer keeps the PLC signal in check and now a good stimulation of PLC is observed with TSH.

classically regarded as coupled to adenylate cyclase via G_s is totally unexpected.

PARALLEL NETWORKS OF SIGNALLING

It is almost axiomatic to say that most receptors couple to multiple signalling pathways. For example, fMetLeuPhe stimulates three phospholipases, C, D and A_2 [161]; TSH, LH and PTH stimulate PLC and adenylate cyclase [162–164]. Since many of the studies have been done with transfected cells overexpressing the relevant receptors, it is clear that it is due to a single receptor coupling to multiple effector systems rather than to the presence of receptor subtypes. The products of one pathway can then have feedback effects on the other signalling pathway. This is best illustrated by the effect of Ca^{2+} on phospholipase A_2 activation by fMetLeuPhe. The rise in Ca^{2+} produced by the PLC pathway is essential for the G-protein-coupled phospholipase A_2 pathway to operate [165].

A further feature that is emerging is that activation of one receptor can modulate the response of another receptor. In FRTL-5 (thyroid) cells, TSH has been shown to stimulate both adenylate cyclase as well as phospholipase C [162,166]. The concentration-dependencies for these two responses are quite different. Cyclic AMP formation occurs at a lower concentration of TSH compared to PLC activation. These cells also possess a receptor for P_1-purinergic agonist, adenosine, coupled to a pertussis toxin-sensitive G-protein. Adenosine inhibits TSH-induced cyclic AMP formation but sensitizes the PLC pathway such that TSH activates the PLC at much lower concentrations [167]. The simplest and economical explanation is that cAMP-dependent phosphorylation attenuates the PLC activity. Adenosine, by blocking the rise in cyclic AMP, allows the PLC to respond normally. Cyclic AMP dampens the PLC response, presumably by phosphorylation of PLC, and therefore reduction in the cyclic AMP signal by adenosine removes this constraint. These interactions are summarized in Fig. 4.

In the physiological environment, cells will be exposed to a multitude of agonists, and therefore, cellular signalling by a single agonist will be dictated not only by its interaction with its own receptor but by the presence of other bioactive molecules. Parallel networks allow the cells to change output responses in a more sophisticated manner than is possible were cells to respond to agonists in a non-interactive manner.

A further twist to PLC signalling is the observation that signalling via different receptors all coupled to PLC can utilize different sets of G-proteins systems in the same cell type. Given that within a single cell there are likely to be a number of G-protein-coupled receptors all signalling via phospholipase C, do they access a common pool of G-proteins and/or phospholipases? The current evidence is suggestive of different G-protein-coupled receptors interacting with only a subset of G-proteins. This is based on the observation that in many cell types, different receptors appear to stimulate phospholipase C in an additive fashion [168–171].

PATHOPHYSIOLOGY OF PHOSPHOLIPASES

Since the phospholipases C play such a prominent role in the activation of cellular function, it is not surprising that phospholipases may be involved in causing some of the pathophysiology. This is just beginning to be explored as our knowledge of the phospholipases and their regulation is expanding [172–174]. Microinjection of PLC-γ1 or PLC-β1 has been shown to induce DNA synthesis in growth-arrested fibroblast cells [172] but inhibited when antibodies to PLC-γ1 are microinjected in serum-stimulated cells [173]. Since PLC-γ1 is a direct substrate for many growth factor receptors such as PDGF, EGF, NGF, it may provide signals for cell proliferation. It has been reported recently that the relative content of PLC-γ1 in primary mammary carcinomas is considerably higher in comparison to normal breast tissues [175]. Since over-expression of PLC-γ1 *per se* in 3T3 cells does not influence mitogenesis [174], other factors such as receptor upregulation must also be important [175]. A member of the PLC-γ family has also been purified from human melanomas [176], implying that cell proliferation in cancer cells may be influenced by the higher levels of PLC-γ.

Although the mechanism of regulation of the PLC-δ family by receptors is unknown at the present time, it is likely that their activity is tightly regulated. In aorta cytosol, analysis of the phospholipases revealed the presence of only two phospholipases (PLC-γ1 and -δ1). In spontaneously hypertensive rats, the activity

of PLC-δ1 is increased in the aorta compared with normal rats [177]. This increase in activity was not due to increased expression of the enzyme but was suggested to be due to a mutation. The abnormality may have resulted from point mutations in the X and Y regions of the enzyme [178]. Clearly, a further analysis of PLC-δ at the genetic level from normal and hypertensive rats is called for to locate the lesion. Abnormal distribution of PLC-δ1 (but not PLC-β1, -γ1 or γ2) has also been shown to be associated with the formation of paired helical filaments in human brains of patients suffering from Alzheimer disease [179]. Knowledge of the regulation of PLC-δ family by receptors and its mechanism of regulation has to be the next major goal.

CONCLUSIONS AND FUTURE PERSPECTIVES

What has clearly emerged from studies to date is that regulation of PLC is far more complex in comparison to adenylate cyclase regulation. Two independent mechanisms of regulation have been identified, a G-protein-regulated pathway (Fig. 2) and a tyrosine-kinase-mediated pathway (Fig. 3). The G-proteins involved in coupling the receptor to the phospholipases are just beginning to be defined and it is now fairly established that the newly identified G_q family of G-proteins regulate the PLC-β family. A second isoform, PLC-ϵ, is also regulated by an unidentified G-protein. PLC-γ1 and PLC-γ2, on the other hand, are regulated by tyrosine kinase-mediated phosphorylation. Some receptors (e.g. the T-cell receptor) use soluble tyrosine kinases of the *src* family whilst other receptors (e.g. PDGF receptor) contain tyrosine kinase activity. This differential form of regulation is summarized in Figs. 2 and 3.

What defines receptor–G-protein and G-protein–effector interactions? Answers to these questions will unravel some of the mysteries behind cellular signalling. Although more than 100 G-protein receptors have been cloned [180] and as many as 17 heterotrimeric G-protein α-subunits [80], we are still a long way from understanding what drives these interactions. A possibility that has emerged recently is that $\beta\gamma$-subunits of G-proteins may also be regulators of PLCs [181]. Studies of these questions will require detailed investigation using not just purified components but also studies done in native membrane preparations. The molecular architecture of the environment may play an important role in mediating some of the specificity that is observed in cells. It is worth noting that fidelity of coupling is also lost when receptors are transfected into an unnatural environment [182].

Just to provide a bit of spice to life, there appears to be some interaction between these two modes of PLC regulation. A prime example is the EGF receptor on hepatocytes. The EGF receptor is a tyrosine kinase and yet its activation of PLC can be blocked by pertussis toxin [48,183]. The EGF receptor co-immunoprecipitates with a G_i protein α subunit. Activation of the Fc receptor for IgG (FcγRII) has recently been shown to activate tyrosine phosphorylation of PLC-γ1 [184] and yet activation of this receptor can also be inhibited by pertussis toxin pretreatment [185,186]. Obviously, there is ample room for innovative interactions in cellular signalling between G-proteins and tyrosine kinases.

Of the three known families of PLCs, β, γ and δ, regulation of PLC-β and γ is now beginning to be understood. However, there is no information regarding the regulation of the entire PLC-δ family although it is subject to intense scrutiny. This is clearly an important area since it may be involved in such diseases as hypertension.

Note added in proof

Berstein *et al.* [275] have recently reported that PLC-β1 accelerates the receptor-stimulated GTPase activity of G_q. This result implies that PLC-β1 contains both GAP (GTPase-Activating Protein) activity as well as enzymic activity for hydrolysing PtdInsP_2. The increased turn-off rate is more ideally suited to that required for regulating physiological processes *in vivo*.

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