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Review

Isozymes delta of phosphoinositide-specific phospholipase C and their role in signal transduction in the cell

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Phospholipase C (PLC, EC 3.1.4.11) is an enzyme crucial for the phosphoinositol pathway and whose activity is involved in eukaryotic signal transduction as it generates two second messengers: diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). There are four major types of phospholipase C named: β, γ, δ and the recently discovered ε, but this review will focus only on the recent advances for the δ isozymes of PLC. So far, four δ isozymes (named δ_{1-4}) have been discovered and examined. They differ with regard to cellular distribution, activities, biochemical features and involvement in human ailments.

Eukaryotic cells need to be capable of reacting to numerous extracellular and intracellular stimuli to function properly in an organism. One of the signal transduction pathways involves rapid hydrolysis of a membrane phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP_2), catalysed by phosphoinositide-specific phospholipase C (PLC)

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Abbreviations: ACN, acrylonitrile; α1-AR, α1-adrenergic receptor; ALT III of PLCδ4, alternatively spliced variant of PLC δ4; CRE, cAMP response element; CRM1, chromosome region maintenance 1 protein; CSA, coronary spastic angina; DAG, diacylglycerol; ERK, extracellular signal-regulated kinase; GAP, GTPase activating protein; GEF, guanine nucleotide exchange factor; GHIF, GTP hydrolysis inhibiting factor; IL-2R, interleukin-2 receptor; IP_3, inositol 1,4,5-trisphosphate; LPA, lysophosphatidic acid; MAPK, mitogen-activated protein kinase; NES, nuclear export sequence; PH domain, pleckstrin homology domain; PI, phosphoinositide; PI 3-kinase, phosphoinositide 3-kinase; PIP_2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PLC, phospholipase C; PLD, phospholipase D; PS, phosphatidylserine; SCI, spinal cord injury; TGII, tissue transglutaminase.

isozymes. In this reaction, two second intracellular messengers are generated: diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). These compounds mediate the release of Ca^{2+} from intracellular stores (ER) and activation of protein kinase C (PKC), respectively (Berridge, 1993; De Smedt & Parys, 1995; Nishizuka 1995). In addition, PIP_2 itself modulates the activities of several proteins, as well as being a cofactor for phospholipase D (PLD) and a substrate for phosphoinositide 3-kinase (PI 3-kinase) (Brown *et al.*, 1993; Rameh & Cantley, 1999). Furthermore, this phospholipid plays a role in actin polymerisation by interacting with many actin-binding proteins and serves as a membrane-attachment site for numerous signalling proteins containing the pleckstrin homology domain (PH domain) (Janmey, 1994). Thus, PLC activity is strictly regulated through several distinct mechanisms. Since the 1950s, eleven isozymes of PLC have been reported and characterised: PLC β1–4, PLC γ1–2, PLC δ1–4 and the recently discovered PLC ε (Cockroft & Thomas, 1992; Rhee & Bae, 1997; Kelley *et al.*, 2001). This review will only focus on the regulation of the δ isozymes, since they exist in lower and higher eukaryotes (yeast, slime molds, filamentous fungi, plants and mammals), suggesting that PLC β and γ evolved from the archetypal PLC δ (Flick & Thorner, 1993; Drayer *et al.*, 1994).

STRUCTURE AND CELL DISTRIBUTION OF PLC δ

Isozymes δ of PLC are multidomain proteins with molecular masses ranging from 83 to 87 kDa. The amino-acid sequence identity between the δ isozymes varies from 45% to 84% (Grosh *et al.*, 1997). The single polypeptide chain of PLCs comprises a pleckstrin homology domain, an EF-hand region, a catalytic centre with the X/Y linker region and a C2 domain. PLCs δ lack the *src* homology regions (present in PLCs γ) and are therefore unlikely to be substrates for tyrosine kinases. Recently, it has been discovered that PLCs δ1 and δ3, in contrast to PLC δ4, possess a putative nuclear export sequence (NES). Figure 1 shows a linear representation of PLC δ structure and Fig. 2 presents the parts of the EF-hand domains in which the NES is situated.

Figure 1. A linear representation of PLC δ structure exemplified by PLC δ1.

The grey box represents the nuclear export sequence (NES) located in the EF-hand domain.

The PH domain consists of about 120 amino-acid residues and is located in the NH_2-terminal part of PLCs. This domain is thought to tether the enzyme to the membrane surface during PIP_2 hydrolysis (Cifuentes *et al.*,1993; Harlan *et al.*, 1994; Yagisawa *et al.*, 1994; Ferguson *et al.*, 1995; Paterson *et al.*, 1995; Lomasney *et al.*, 1996; Lemmon & Ferguson, 2000). Although the EF-hand motif is believed to bind calcium or magnesium ions, the role of this domain is still not fully understood (Essen *et al.*, 1996; Pawelczyk & Matecki, 1997b). In the middle of all δ isozymes there is the catalytic centre formed by the X and Y regions and separated by a linker rich in acidic amino acids (Essen *et al.*, 1996; Matecki & Pawelczyk, 1997; Pawelczyk & Matecki, 1997b). At the COOH-terminus, the C2 motif of 120 residues is situated. This domain is believed to be involved in the calcium-dependent binding to the phospholipid membrane, although each isozyme requires a different number of calcium ions. It has also been postulated that the C2 motif orients and fixes the catalytic core to the membrane surface (Sutton *et al.*, 1995; Essen *et al.*, 1996; Essen *et al.*, 1997; Grobler & Hurley, 1998). The NES region, about 14 amino acid long

and rich in leucines, is likely to be responsible for the transport of PLC δ1 and δ3 from the cell nucleus. Blocking of the NES-dependent nuclear export results in nuclear accumulation of PLC δ1 that has been transported into the nucleus by an unknown mechanism(s). IP_3, generated in the nucleus, would then increase the nuclear calcium level causing a

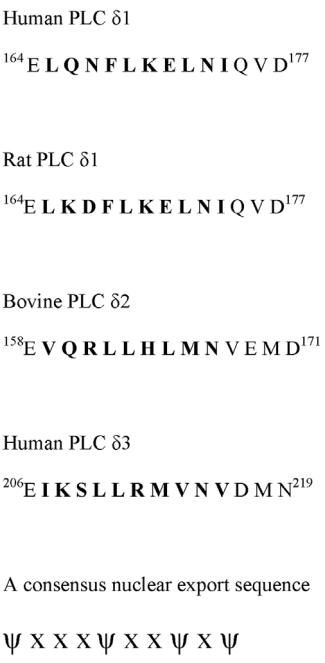

Figure 2. A comparison of the NES in the EF-hand domains of PLC δ isozymes.

Bold letters represent the NES present in the PLC δ_{1-3}. Ψ represents a hydrophobic residue (isoleucine, leucine, valine or methionine) and X represents any amino acid.

conformational change in the EF-hand domain of PLC δ1 leading to the exposure of the NES sequence to the highly conserved chromosome region maintenance 1 protein (CRM1). CRM1 is a nuclear export receptor for proteins containing leucine-rich NES that is situated at the nuclear pore. This would result in immediate export of PLC δ1 from the nucleus (Yamaga *et al.*, 1999).

The δ isozymes have been detected in various animal tissues and their expression levels vary significantly. Also, cultured cell lines, such as GH_4 pituitary cells, rat pheochromocytoma cells PC12, human W138 fibroblasts, simian kidney cells COS-7 and C6 glioma cells, express PLCs δ (Suh *et al.*, 1988; Kriz *et al.*, 1990; Meldrum *et al.*, 1991; Lee & Rhee, 1996; Martelli *et al.*, 1996; Bristol *et al.*, 1998; Lymn & Hughes, 2000; Fukami *et al.*, 2003). The mRNA for PLC δ1 has been found in rat skeletal muscles, spleen, testis, lung and brain (astroglial cells) (Suh *et al.*, 1988; Cheng *et al.*, 1995; Milting *et al.*, 1996). PLC δ2 has been purified only from bovine cerebral cortex but it is markedly expressed in type II intestinal metaplasia and in the adenocarcinoma (Meldrum *et al.*, 1989; Meldrum *et al.*, 1991; Marchisio *et al.*, 2001). It has been also determined that PLC δ2 is not expressed in rat muscles and digestive organs, nor in hematopoietic cells and lymphoid tissues (Lee *et al.*, 1994; Noh *et al.*, 1994; Shin *et al.*, 1994). PLC δ3 and δ4 are detectable in rat tissues, such as kidney, cardiac muscles, aorta, spleen, liver, testis and brain (Kriz *et al.*, 1990; Banno *et al.*, 1994; Lee & Rhee, 1995; Liu *et al.*, 1996; Bristol *et al.*, 1998; Pawelczyk & Matecki, 1998).

PLC δ1 is mostly a cytoplasmic protein, whereas PLC δ3 is detected in the membrane fraction (Mazzoni *et al.*, 1992; Divecha *et al.*, 1993; Banno *et al.*, 1994; Paterson *et al.*, 1995; Pawelczyk & Matecki, 1998; LaBelle *et al.*, 2002). PLC δ4 is predominantly located in the cell nucleus and its expression depends on the cell cycle (Liu *et al.*, 1996). The cellular distribution of PLC δ2 has not been determined yet.

REGULATION OF PLCs δ

Although four distinct PLC δ isoforms are known, only δ1 is relatively well characterised. There are two major stages of PLC action: binding to the membrane surface and interaction with the substrate (PIP_2). Therefore, its activity depends on factors modulating the association of the enzyme with the lipid membrane and factors changing the PLC interaction with the substrate. Nonethe-

less, the mechanism by which PLC δ is coupled to membrane receptors remains unclear.

It has been discovered that PLC δ1 binds to phospholipid vehicles containing PIP_2 and sphingomyelin with a high affinity (Rebecchi *et al.*, 1992; Pawelczyk & Lowenstein, 1993a), whereas phosphatidic acid has been reported to stimulate the binding of myocardial PLC to the plasma membrane (Henry *et al.*, 1995). For the interaction of PLC with the plasma membrane, the PH domain is required (Cifuentes *et al.*, 1993; Garcia *et al.*, 1995; Paterson *et al.*, 1995).

and kidney cell lines (Cos-7) but not in a liver line (Chang liver cells). It has also been found that the E-box and HFH binding sites are cell-type specific elements, whereas the major transcriptional activator in the majority of cell lines is Sp-1. The authors have suggested that a combination of several elements within the 5'-flanking elements of the *PLC δ1* gene is responsible for the limited expression of PLC δ1 in several cell lines (Kim *et al.*, 2002).

Under *in vitro* conditions, all eukaryotic PLC isozymes require Ca^{2+} for activity; the δ isozymes being the most sensitive to calcium

Table 1. Activators of PLCs δ

PLC δ isozymes	Activators	References
PLCδ1	Ca^{2+}	Cheng *et al.*, 1995; Allen *et al.*, 1997; Ghosh *et al.*, 1997; Lomasney *et al.*, 1999; Kim *et al.*, 1999.
	Spermine	Haber *et al.*, 1991.
	Phosphatidylethanolamine*	Pawelczyk & Lowenstein, 1992.
	Phosphatidylcholine*	Pawelczyk & Lowenstein,1992.
	4-hydroxysphingosine†	Pawelczyk & Lowenstein, 1992.
	Sphingosine†	Pawelczyk & Lowenstein, 1992; Matecki & Pawelczyk, 1997; Pawelczyk & Lowenstein, 1997.
	Thrombin	Banno *et al.*, 1994.
	p122GAP for RhoA	Homma & Emori, 1995.
	$G_h\alpha$	Feng *et al.*, 1996; Park *et al.*, 1998.
	Phosphatidic acid	Henry *et al.*, 1995.
PLC δ2	Ca^{2+}	Meldrum *et al.*, 1989.
PLC δ3	Ca^{2+}	Ghosh *et al.*, 1997; Pawelczyk & Matecki, 1997a; Pawelczyk & Matecki, 1998.
	Phosphatidic acid	Pawelczyk & Matecki, 1999.
PLC δ4	Ca^{2+}	Lee & Rhee, 1996
	Bradykinin	Fukami *et al.*, 2000.
	Lysophosphatidic acid	Fukami *et al.*, 2000.
	Serum	Fukami *et al.*, 2000.

(*), activation observed in the absence of 200 μM spermine in the detergent assay. (†), activation observed both in the detergent and liposome assays.

Recently, the PLC δ1 promoter region has been cloned and characterised in several cell lines. The potential transcriptional enhancement of reporter activities has been demonstrated in neuroblastoma cells (SK-N-BE(α)C)

ions. PLC δ1, as well as PLC δ3, is fully activated by calcium at a concentration of 1–10 μM (Cheng *et al.*, 1995; Allen *et al.*, 1997; Grosh *et al.*, 1997; Pawelczyk & Matecki, 1997a; Pawelczyk & Matecki, 1998). PLC δ4

exhibits a similar dependence on calcium ions as PLC δ1 (Lee & Rhee, 1996). Meldrum *et al.* (1989) have estimated that K_a for calcium ions amounts to 0.6 μM when PIP_2 is used as a substrate for PLC δ2. As PLCs δ are sensitive to calcium it is conceivable that intracellular calcium elevation alone may provoke PLCs δ to hydrolyse polyphosphoinositides *in vivo* or that calcium binding to the EF-hand motif may modulate the activation process of PLC δ1 such as the translocation to the plasma membrane or the putative interaction with a G-protein(s). Recently, it has been discovered that calcium ions regulate PLC δ1 activity by promoting the formation of an enzyme-PS-Ca^{2+} ternary complex (Lomasney *et al.*, 1999). This leads to PLC δ1 activation *via* a 20-fold reduction in the K_m for the substrate and an increase in the phospholipase affinity for PIP_2. The C2 domain is the structural motif responsible for mediating the PS-dependent Ca^{2+} binding. It is likely that the C2 domain can bind a minimum of two calcium ions (Essen *et al.*, 1997).

Table 2. Inhibitors of PLCs δ

PLC δ isozymes	Inhibitors	References
PLCδ1	Sphingomyelin	Pawelczyk & Lowenstein, 1992; Matecki *et al.*, 1997; Pawelczyk *et al.*, 1997a.
	Lysosphingomyelin	Pawelczyk & Lowenstein, 1992; Pawelczyk & Lowenstein, 1993b.
	Phosphatidylethanolamine*	Pawelczyk & Lowenstein, 1992;
	Phosphatidylcholine**	Pawelczyk & Lowenstein, 1992;
	Phosphatidylserine	Pawelczyk & Lowenstein, 1992;
	Hexadecylphosphorylcholine	Pawelczyk & Lowenstein, 1992; Pawelczyk & Lowenstein, 1993b.
	Ceramide	Matecki *et al.*, 1997.
	Ganglioside	Matecki *et al.*, 1997.
	4-hydroxysphingosine†	Matecki & Pawelczyk, 1997.
	RhoA	Hodson *et al.*, 1998.
	$G_h\alpha$	Murthy *et al.*, 1999.
	Sphingosine†	Pawelczyk & Lowenstein, 1992; Matecki *et al.*, 1997; Matecki & Pawelczyk, 1997.
	ALT III of PLC δ4	Nagano *et al.*, 1999.
PLC δ2	?	
PLC δ3	Spermine	Pawelczyk & Matecki, 1998.
	Sphingosine	Pawelczyk & Matecki, 1998.
	Phosphatidylethanolamine	Pawelczyk & Matecki, 1998.
	Phosphatidylcholine	Pawelczyk & Matecki, 1998.
	Phosphatidylserine	Pawelczyk &Matecki, 1998.
	Sphingosine	Pawelczyk & Matecki, 1998.
	cAMP	Liu *et al.*, 2001.
PLC δ4	ALT III of PLC δ4	Nagano *et al.*, 1999.

Abbreviations: ALT III of PLC δ4 – alternatively spliced variant of PLC δ4. (*), inhibition observed in the presence of 200 μM spermine in the detergent assay. (†), inhibition observed when the activity was measured with endogenous PIP_2 as a substrate in erythrocyte membranes ("ghosts").

Apart from calcium ions, under *in vitro* conditions PLC $\delta 1$ is also regulated by polyamines and phospholipids (Haber *et al.*, 1991; Pawelczyk & Lowenstein, 1992). Sphingomyelin is the most effective inhibitor of all the phospholipids (Pawelczyk & Lowenstein, 1992; Matecki *et al.*, 1997). The $\delta 1$ isozyme is also inhibited by hexadecylphosphorylcholine and lysophospholipids that show antitumour activity (Pawelczyk & Lowenstein, 1993b). The inhibition of PLC δ by sphingomyelin is promoted by calcium ions and spermine and is partially suppressed by sphingosine (Matecki & Pawelczyk, 1997; Pawelczyk & Lowenstein, 1997). In liposome and detergent assays, sphingosine and its homologue 4-hydroxysphingosine (phytosphingosine) activate PLC $\delta 1$ moderately. The PH domain has been reported to tether PLC $\delta 1$ to PIP_2-containing membranes in the absence of other signals (Paterson *et al.*, 1995).

Because glucose-stimulated insulin secretion appears to require an increase in intracellular calcium ions, it has been suggested that insulin secretion from pancreatic islets may be mediated in part by activation of phospholipase C and phosphoinositide hydrolysis. Nonetheless, overexpression of PLC δ-1 in INS-1 cells has had no effect on IP accumulation or insulin secretion in response to stimulatory glucose or glucose plus carbachol. Therefore, it has been concluded that overexpression of PLC either alone or with an important related G protein activator ($G_{11\alpha}$) is not sufficient to improve insulin secretion (Gasa *et al.*, 1999). When PLC $\delta 1$ has been overexpressed in CHO cells, it has been reported that thrombin-induced PLC $\delta 1$ activation is regulated *via* both a G protein and calcium (Banno *et al.*, 1994). Homma and Emori (1995) have reported that PLC $\delta 1$ binds to a rat GTPase activating protein (p122GAP) specific for RhoA protein and that the activation of $\delta 1$ occurs downstream of RhoA activation. Other researchers have suggested that RhoA exerts a negative modulatory influence on aortic PLC $\delta 1$ activity, on the basis of the fact that inhibition of RhoA by *Clostridium botulinum* toxin has resulted in a significant increase in aortic PLC $\delta 1$ activity (Hodson *et al.*, 1998). Other research groups working on the linking of PLC $\delta 1$ to the cell surface have discovered that the $G\alpha_h$ protein possessing tissue transglutaminase activity (TGII) binds and activates PLC $\delta 1$ (Feng *et al.*, 1996). The $G\alpha_h$ protein has been demonstrated to associate with agonist-stimulated $\alpha 1$-adrenergic receptor ($\alpha 1$-AR) (Nakaoka *et al.*, 1994). In human myometrium PLC $\delta 1$ has been proposed to be an effector of oxytocin receptor signalling *via* the activation of $G\alpha_h$. This interaction results in stimulation of PLC $\delta 1$ activity (Park *et al.*, 1998). It has been demonstrated that $\alpha 1$-AR couples to PLC $\delta 1$ *via* an interaction with $G\alpha_h$ which leads to a significant inhibition of PLC $\delta 1$ activity (Murthy *et al.*, 1999). This result is in contrast with the data obtained by Feng *et al.* (1996). Furthermore, TGII alone does not lower PLC $\delta 1$ expression. It is likely that the binding of $G\alpha_h$ with PLC δ is regulated by GTP. Recently, it has been suggested that PLC $\delta 1$ displays two regulatory functions for TGII. One is a guanine nucleotide exchange factor (GEF) function and the other is inhibition of GTP hydrolysis by TGII (in such a situation, PLC $\delta 1$ acts as a GTP hydrolysis inhibiting factor – GHIF). The interaction of TGII with PLC $\delta 1$ induces a conformational change in TGII converting it into a GTPase. The GEF/GHIF activity of PLC $\delta 1$ is displayed independently of $\alpha 1$-AR. The PLC $\delta 1$ activity is positively and negatively regulated by TGII depending on calcium level, TGII expression level, as well as binding of guanine nucleotides which promotes IP_3 and DAG generation (Baek *et al.*, 2001). It has been found that stimulation of G-protein-coupled bradykinin receptors significantly strengthens the responses of the PLC $\delta 1$-overexpressing PC12 cells. In these cells, PLC $\delta 1$ is mainly activated by capacitative calcium entry following PLC β activation in the BK receptor-mediated signalling pathway. This regulation may have an impor-

tant physiological role as presenting a mechanism of positive feedback thanks to which the signalling mediated by PLC β-linked receptors could be potentiated and prolonged (Kim *et al.*, 1999). Experiments carried out on PLC δ1-deficient mice have shown that two major downstream signals of PLC, calcium and PKC activation are impaired in the keratinocytes and skin of the PLC δ1-deficient mice. The researchers have also observed epidermal hyperplasia, numerous cysts similar to interfollicular epidermis, hyperplasia of sebaceous glands, as well as spontaneous skin tumours that have had characteristics of both interfollicular epidermis and sebaceous glands. These recently published results suggest that PLC δ1 is required for skin stem cell lineage commitment (Nakamura *et al.*, 2003). Recently, it has been reported that ischemia-reperfusion induces alterations in PLC isozymes. In ischemia, there have not been changes in the PLC δ1 mRNA level, nonetheless PLC δ1 activity and content have been decreased in the cardiac sarcolemma membrane. In contrast, in the cytosol PLC δ1 activity has been increased although the protein level has been decreased. An increase in phospholipase C δ1 activity has occurred upon reperfusion although the observed changes have not been accompanied by alterations in mRNA and protein levels (Asemu *et al.*, 2003). The effect of acrylonitrile (ACN) on the levels of phospholipase isozymes in rat heart and brain has been also tested. ACN is thought to cause astrocytomas *via* induction of oxidative stress. It has been discovered that ACN causes a significant increase in PLC δ1 level in the rat heart and the cytosol of cerebral cortex. On the other hand, under hyperoxia conditions PLC δ1 level has been decreased (Nagasawa *et al.*, 2003).

In cultured skin fibroblasts obtained from patients with coronary spastic angina (CSA), PLC δ1 activity has been demonstrated to be enhanced. In these patients an increased expression of an abnormal PLC δ1 isoform has been discovered, with arginine 257 replaced by histidine (R257H). Since the site of this amino acid replacement is situated in the fourth lobe of the EF hand domain, which is necessary for the interaction of the PH domain with PIP_2, it seems that the R257H variant contributes to the altered enzyme activity induced by calcium ions (Nakano *et al.*, 2002). Recently, enhanced PLC δ1 activity has been discovered in patients suffering from essential hypertension, whereas the activities of other phospholipase C isozymes (β2, β3 and γ) have not been altered. The researchers have suggested that the increased PLC activity might be involved in human hypertension pathogenesis (Kosugi *et al.*, 2003).

As it has been mentioned above, PLC δ2 has been found in type II intestinal metaplasia and in adenocarcinoma. During the neoplastic transformation the specific expression of the PLC β isoforms characterising healthy human gastric mucosa is decreased whereas expression of PLC δ2 is increased. This fact suggests that PLC δ2 plays a role in neoplastic evolution and could be a predictive marker of cancer transformation (Marchisio *et al.*, 2001).

PLC δ3 shows a high specificity in binding to lipid membranes containing either PIP_2 or phosphatidic acid (Pawelczyk & Matecki, 1999). In experiments in which PIP_2 has been located in detergent micelles, this isozyme has been activated fully by calcium ions at 1–10 μM (Grosh *et al.*, 1997; Pawelczyk & Matecki, 1997a; Pawelczyk & Matecki, 1998), whereas when PIP_2 was in the phospholipid membrane (in a liposome assay), the Ca^{2+} concentration that fully activates this isozyme has to be one order of magnitude higher than the calcium concentration needed to activate PLC δ1 (Pawelczyk & Matecki, 1998). Under *in vitro* conditions, the regulatory properties of PLC δ3 differ from those of PLC δ1 as the former is inhibited by polyamines and sphingosine (Pawelczyk & Matecki, 1997a; 1998). In W138 and U373, but not in H1299 cells, the PLC δ3 mRNA level is decreased two-fold in a cAMP dose- and time-de-

pendent manner (Lin *et al.*, 2001). In the same cells, the Ca^{2+} ionofor A23187 lowers the PLC δ3 mRNA level. It has been discovered that PKC modulators do not affect PLC δ3, meaning that the transcription of the *PLC δ3* gene is not associated with PKC. It is possible that cAMP response element (CRE) which is the consensus sequence for the *cis*-element directing cAMP-regulated gene expression modulates the regulation of the *PLC δ3* gene by Ca^{2+} and cAMP.

Several alternatively spliced variants of PLC δ4 mRNA have been identified. The promoter region for the *PLC δ4* gene is activated by numerous growth factors, such as bradykinin, lysophosphatidic acid (LPA) and serum, in response to an increase in cytoplasmic Ca^{2+} concentration (Fukami *et al.*, 2000). The PH domain from an alternatively spliced variant of PLC δ4, termed ALT III, alone can completely block the activity of PLC δ4 suggesting that this domain is sufficient for its inhibitory effect due to its strong binding affinity for PIP_2 and PIP_3, although this inhibition is not caused by simple competition for PIP_2 as the substrate for PLCs. ALT III also inhibits the activity of PLC δ1, but it only partially suppresses PLC γ1 and does not affect the activity of PLC β1. This indicates that ALT III acts as a negative regulator of PLC δ (Nagano *et al.*, 1999). PLC δ4 has been suggested to be an essential protein for events preceding, or leading to, sperm-ova fusion during mammalian fertilisation and it may play an important role in mediating the zona pellucida-induced acrosome reaction. The mechanisms of this PLC δ4 function could involve: IP_3 production leading to persistently elevated intracellular calcium concentrations in the sperm, PIP_2 level alterations that induce changes in membrane stability facilitating exocytosis, an anomalous phosphoinositide turnover affecting cholesterol metabolism and direct promotion of calcium influx by regulating calcium channels coupled to IP_3 receptor (Fukami *et al.*, 2001). Nonetheless, it has been proposed recently that PLC δ4 is an important enzyme for intracellular calcium mobilisation in the zona pellucida-induced acrosome reaction and for the prolonged calcium increases through store-operated channel induced by zona pellucida and progesterone in sperm (Fukami *et al.*, 2003). The mRNA for the ALT IV of PLC δ4 is highly expressed in intestines and in regenerating liver tissue and it is cell cycle dependent. In transformed cell lines its expression can be induced by serum. In fibroblast nuclei, the level of the ALT IV increases dramatically at the transition from the G1 to the S phase in response to mitogenic stimulation and is maintained throughout the metaphase (Liu *et al.*, 1996). A precise role for ALT IV of PLC δ4 in the nucleus has not been determined yet, although its action in several nuclear events has been suggested including the interaction between the nuclear matrix and nucleic acids, activation of PKC, as well as activation of DNA polymerase and topoisomerase resulting in cell proliferation. In contrast to PLC δ4, the action mechanisms of PLC β1 in the nucleus and the cell cycle are well known. It has been found that PLC β1 is a target protein of extracellular signal-regulated kinase — ERK and subsequent phosphorylation of PLC β1 plays a crucial role in the nuclear phosphoinositide (PI) cycle (Cocco *et al.*,1998; Xu *et al.*, 2001). Furthermore, PLC β1 is activated upon phosphorylation of the mitogen-activated protein kinase MAPK (Vitale *et al.*, 2001). It has been also demonstrated that rat *PLC δ4* gene has been down-regulated by more than 50% after spinal cord injury (SCI) along with six other genes for: lecithin:cholesterol acyltransferase, dipeptidyl aminopeptidase related protein, plasma membrane Ca^{2+}-ATPase isoform 2, G-protein G(O) α subunit, GABA transporter 3 and neuroendocrine protein 2, whereas three genes for heat shock 27-kDa protein, tissue inhibitor metalloproteinase-1 and epidermal fatty acid-binding protein have been up-regulated (Tachibana *et al.*, 2002). The observation of PLC δ4 down-regulation has lead the authors

to suggest selective impairment of the intracellular signalling system in SCI tissue.

CONCLUSIONS

Although PLCs δ are rather well characterised in regard to their structure and regulation mechanisms, there are still many questions and issues to be answered. Tables 1 and 2 summarise the known activators and inhibitors for all δ isozymes of phospholipase C. Scientists are interested in better defining the pathways that regulate PLCs δ and want to investigate the basis of responses to extracellular and intracellular stimuli. They are examining the roles of the various isozymes under physiological and pathological conditions and are investigating how the intracellular location relates to the function of these enzymes. In yeast and higher plants, PLCs δ are implicated in the response to different stresses, especially as regards cyclin-dependent growth control and nuclear mRNA export (Flick & Thorner, 1998; York *et al.*, 1999). It has been discovered that PLCs δ of primitive organisms function as stress response proteins, helping the organisms to adapt to a changing environment. It has yet to be determined whether some PLCs are stress related proteins (Hirayama *et al.*, 1995). As a key enzyme involved in the signalling at the plasma membrane and regulation of various cell functions, PLC δ has been investigated regarding its possible role in pathogenesis of numerous diseases. Abnormal expression of PLCs δ has been found for example in hypertension, coronary spastic angina, Alzheimer's disease, spinal cord injury, Pick's disease, progressive supranuclear palsy and diffuse Lewy body disease, as well as in the mentioned above carcinomas (Shimohama *et al.*, 1993; Marchisio *et al.*, 2001; Nakano *et al.*, 2002; Tachibana *et al.*, 2002; Kosugi *et al.*, 2003). The new approaches used in molecular biology and medicine should find answers to the various questions and ought to help to fully understand the nature of the δ isozymes of phospholipase C.

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