COMMENTARY

The role of protein kinase C isoenzymes in the regulation of cell proliferation and differentiation

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Introduction

The protein kinase C (PKC) family of enzymes has been the subject of a great deal of research in the last few years because of the recognized importance of these enzymes in the control of cell growth and division on the one hand, and cell differentiation on the other. Originally identified as a calcium- and phospholipid-dependent protein kinase activity with specificity for serine and threonine residues, the PKC family is now understood to contain at least nine members, not all of which in fact require calcium for their activity (Nishizuka, 1988, 1989a; Parker et al. 1989).

The excitement which arose over the roles of these enzymes in growth regulation of mammalian cells initially stemmed from the finding that PKC constitutes the receptor for the mitogenic phorbol esters, which are tumour promoters (Leach et al. 1983; Blumberg et al. 1984). The naturally occurring activators of PKC in vivo are the diacylglycerols and arachidonic acid. These compounds are generated by hydrolysis of phosphoinositides and other phospholipids in the plasma membrane and function as second messenger molecules (Berridge and Irvine, 1989; Huang, 1989; Lester et al. 1991). Thus, the activation of PKC plays a major role in the signal transduction pathways by which many hormones, growth factors and other extracellular ligands that activate phospholipases mediate their effects on target cells.

The PKC family can be classified into two sub-groups, depending on whether the enzymes require calcium for activation (PKC-α, β1, β2 and γ or not (PKC-δ, -ε, -ζ, -η and -L). These different species of PKC have different tissue distribution patterns (Wetsel et al. 1992). The structure of these proteins and their genes, and the function of the PKC family in cellular signal transduction, have been reviewed extensively in this journal (Pears and Parker, 1991) and elsewhere (Kikkawa et al. 1989; Nishizuka, 1989a,b; Krauss et al. 1990; Kuo et al. 1990; Weinstein, 1990; Stabel and Parker, 1991). In this article we will address the question of how PKC activity can be involved in such a wide variety of cellular responses, some of which involve mitogenic activation while others show the opposite effect, viz. inhibition of cell proliferation and concomitant differentiation. Since the activation of PKC enzymes is usually associated with their translocation from the cytoplasm to the plasma membrane or other intracellular compartments, and is often followed by their down-regulation by proteolytic degradation, we will also examine the question of the relative roles of PKC activation versus turnover in cellular growth control. The article ends with a look to the future, with particular consideration of the possible roles that PKC species might play in the cell nucleus.

Involvement of PKC in the induction of mitogenesis versus cell differentiation

A number of cultured cell lines are responsive to treatment with phorbol esters such as 12-Ο-tetradecanoylphorbol-13-acetate (TPA, also designated PMA by some workers). The responses of different cell types fall into two categories, viz. stimulation of quiescent cells into growth and subsequent mitosis - as seen in the case of 3T3 mouse fibroblasts (Rozengurt, 1986) or resting T lymphocytes (Berry and Nishizuka, 1990), or inhibition of cell proliferation and stimulation of terminal differentiation. The most widely studied example of the latter response is the induction of differentiation of the HL-60 promyelocytic leukaemia cell line towards a macrophage-like phenotype (Harris and Ralph, 1985; Vandenbark et al. 1984), but other cell types also undergo differentiation following exposure to phorbol esters (Oberg et al. 1991; Exley et al. 1987; Yamauchi et al. 1989). As will be discussed later, these two types of response are not necessarily mutually exclusive in any one cell type. For example different concentrations of TPA can induce either a proliferative response or a differentiation response, at least in HL-60 cells (Trayner and Clemens, 1992).

There is good evidence that the responses to phorbol...
esters are mediated by the PKC family since these enzymes are the only known receptors for phorbol esters, the relative potency of different phorbol esters matches their relative affinities for PKC, and stimulation of cellular responses is usually accompanied by translocation and subsequent down-regulation of PKC. HL-60 cell variants in which expression of PKC is decreased are resistant to induction of myeloid differentiation by phorbol esters (Perella et al. 1986; Nishikawa et al. 1990). It has been suggested, however, that some cellular responses to phorbol esters occur independently of PKC (Murphy et al. 1991).

Another family of compounds that activate PKC in a similar manner to phorbol esters are the bryostatins (Kraft et al. 1986, 1987; Stone et al. 1988; Fields et al. 1990). Although some of the effects of these agents are very similar to those induced by the phorbol esters, the bryostatins can also antagonize the actions of TPA in some cases. It is possible that the bryostatins and phorbol esters activate and/or induce the translocation of different isoforms of PKC, leading to different cellular responses (Hocevar et al. 1992).

The weight of current evidence suggests that the activity of one or more PKC species is required both for cell proliferation and cell differentiation and that these enzymes play a positive role in both types of cell behaviour. For example, sustained activation of PKC is necessary both for the mitogenic activation of resting T lymphocytes (Berry et al. 1990) and for the differentiation of HL-60 cells to macrophages (Aihara et al. 1991), even though the consequence of such chronic activation is down-regulation of PKC. In other systems, PKC activation by phorbol esters can at least partially mimic the mitogenic effects of a growth factor such as interleukin-3 (Whetton et al. 1986) or insulin (Trayner and Clemens, 1992). PKC activity has also been implicated in mediating some of the effects of growth inhibitory cytokines such as the interferons (Pfeffer et al. 1990, 1991; Tiefenbrun and Kimchi, 1991; James et al. 1992), and in the mouse erythroleukaemia (MEL) cell line. Elevated expression of the β isozyme leads to accelerated erythroid differentiation and loss of proliferation when the cells are treated with hexamethylenebisacetamide (Melloni et al. 1989, 1990). Although there would thus appear to be a number of paradoxes in suggesting a role for PKC in both the mitogenic effects of some agents and the growth-inhibitory or differentiation-inducing effects of others, it is likely that these difficulties can be resolved by considering the relationships between PKC and other signal transduction pathways. Ultimately the problem becomes one of understanding the nature and mechanisms of regulation of the genes that must be expressed for cell proliferation to take place under one set of conditions and for terminal differentiation to take place under another. It is clearly important to understand the roles played by PKC species in controlling the expression of such genes in these different circumstances.

The relationship of PKC activity to other signal transduction pathways

The protein kinase C family is part of a complex network of signal transduction pathways involving not only other protein kinases (e.g. growth factor receptor tyrosine kinases and mitogen-activated serine/threonine kinases) but also GTP-binding proteins of the ras proto-oncogene family. The details of the many regulatory interrelationships between PKC and these other proteins are beyond the scope of this article (for reviews see Nishizuka, 1988; Rana and Hokin, 1990) but we will draw attention to certain significant observations. Expression of a mutationally activated p21ras gene results in elevated levels of diacylglycerols in cells (Wolfman and Macara, 1987; Lacal, 1990), which would be expected chronically to activate PKC. It also seems likely that PKC and ras genes can cooperate to transform cells to a tumourigenic phenotype since overexpression of PKC renders cells more susceptible to transformation by the H-ras oncogene (Hsiao et al. 1989). This phenomenon may also constitute the basis of the cooperation between the myc and ras oncogenes in cell transformation since expression of the c-myc gene can induce alterations in PKC expression (Barr et al. 1991). Other evidence suggests that ras may be activated downstream of PKC (Yu et al. 1988; Downward et al. 1990). Transfection of the PKC-B1 gene into rat embryo fibroblasts also revealed cooperativity of this PKC species with the adenovirus E1A oncogene in converting these cells to a more transformed phenotype (Su et al. 1992). These observations are consistent with the finding that some cell types that overproduce PKC proliferate more vigorously and are more susceptible to the mitogenic effects of growth factors (Krauss et al. 1989; Hoshina et al. 1990; Finkenzeller et al. 1992).

Activation of PKC by phorbol esters leads to a cascade of changes in cellular signalling, including stimulation of mitogen-activated protein (MAP) kinase activity (Adams and Parker, 1991; Nori et al. 1992) and the phosphorylation of components of the transcriptional and translational machinery (Boyle et al. 1991; Morley et al. 1991). These effects will cause an amplification of the initial signal and are likely to result in changes in gene expression at both the transcriptional and translational levels. Tyrosine kinase activity may also be required for PKC-mediated cellular responses (Munoz et al. 1991). Given the likelihood that different cell types will contain different species of ras, MAP kinases and tyrosine kinases, as well as the corresponding substrates for these proteins, it is not surprising that activation of PKC can result in such a diversity of cellular responses.

Activation versus down-regulation of PKC

As stated earlier, activation of PKC usually results in the translocation of the enzyme from cytoplasm to plasma membrane and this is often followed by the rapid cleavage of the protein (initially into its regulatory and catalytic domains - Pontremoli et al. 1990; Pears and Parker, 1991) and its further proteolytic degradation. It follows from this that chronic exposure to phorbol esters normally leads to overall down-regulation of PKC levels in the cell. In this respect the use of metabolically stable phorbol esters as substitutes for the natural PKC activators, the diacylglycerols, may be misleading, for the latter are turned over very rapidly in vivo by conversion to other products and rarely
lead to long-term activation of PKC in normal cells. In contrast to the effect of TPA, treatment of a mouse macrophage line with dioctanoylglycerol fails to inhibit proliferation or induce PKC down-regulation unless this diacylglycerol is added repeatedly to the cells, and another diacylglycerol (OAG) actually stimulates proliferation (Goode et al. 1990). Where long-term effects of phorbol ester treatment are studied the question therefore arises of whether the cellular responses are due to the initial PKC activation or to the subsequent loss of PKC by down-regulation (Brooks et al. 1991). In some cases short-term and long-term phorbol ester treatment have opposite effects on cell proliferation (Mond et al. 1991). Such considerations, together with the current absence of a reliable assay for PKC activity in intact cells, complicate the interpretation of experiments concerned with the role of PKC in the control of proliferation and differentiation.

The literature is in conflict over whether high level expression of PKC stimulates cell growth and contributes to the transformed phenotype. Borner et al. (1991) were unable to transform fibroblasts with either a wild-type or a mutant form of PKC-α. Moreover, although the evidence described in the previous section suggests that overexpression of PKC cooperates with activated oncogenes to transform cells, the growth and tumourigenic phenotype of HT29 colon cancer cells was suppressed by such overexpression (Choi et al. 1990). However, there are several reports suggesting a correlation between PKC activity and cell growth rate (Katayama et al. 1989, 1992; Couldwell et al. 1991; Minana et al. 1991; Aflalo et al. 1992; Benizli et al. 1992), or a correlation between inhibition of PKC and the induction of differentiation (Minana et al. 1989; Felipo et al. 1990; Taoka et al. 1990). Inhibition of PKC has been associated with a decrease in c-myc expression in myeloid leukaemia cells (Bernstein et al. 1991), a phenomenon that would be expected to result in growth inhibition and/or removal of a block to cell differentiation (Holt et al. 1988). A note of caution is needed at this point however. The effects of pharmacological inhibitors of PKC have been widely interpreted as evidence for a need for the activity of PKC in specific cellular processes. However such conclusions may be flawed because many PKC inhibitors [e.g. 1-(5-isouquinolinsulphonyl)-2-methylpiperazine (H7) or staurosporine] also affect the activity of other protein kinases. Even the newer generation of more specific staurosporine analogues (Davis et al. 1989; Dieter and Fitzke, 1991; Grove and Mastro, 1991) cannot be assumed not to inhibit other as yet uncharacterized protein kinases. More reliable approaches in the future may involve the use of specific antisense oligonucleotides or the delivery of PKC isoform-specific antibodies intracellularly; the latter procedure has been demonstrated to induce differentiation in neuroblastoma cells (Leli et al. 1992). Conversely, where studies have relied on the down-regulation of PKC by chronic phorbol ester treatment to show that PKC activity is not required for a particular cellular response it cannot safely be assumed that the cell has been equally depleted of every isoform of PKC, e.g. PKC-ζ is neither translocated nor down-regulated by TPA treatment (Ways et al. 1992; Liyanage et al. 1992).

As indicated previously, some cell types show a mitogenic response to treatment with phorbol esters (Rozengurt, 1986) whereas others exhibit inhibition of proliferation (Issandou et al. 1988; Duronio et al. 1990). In some cases the difference in these effects may be more apparent than real. For example, in the case of the Daudi line of Burkitt’s lymphoma cells TPA is a potent inhibitor of cell proliferation (Clemens et al. 1988; Menaya and Clemens, 1991) and induces the expression of surface antigens characteristic of differentiated plasma cells (Ekley et al. 1987). However, in these cells DNA synthesis is not blocked by TPA treatment and is even stimulated at low concentrations of the phorbol ester (Menaya and Clemens, 1991). TPA treatment of Daudi cells results in the appearance of large binucleate cells, suggesting that the inhibition of proliferation is due to a block in cytokinesis rather than DNA replication.

Another way in which the apparently opposite effects of phorbol ester treatment on cell proliferation in different systems may be reconciled is suggested by the dose-dependence of the response of HL-60 cells to TPA. We have observed that, whereas high concentrations of TPA (10 nM and above) cause an inhibition of cell growth and induce the characteristic differentiation to a macrophage-like phenotype, low concentrations of the phorbol ester (0.1-1 nM) are mitogenic and do not induce differentiation (Trayner and Clemens, 1992). The stimulation of cell growth can only be seen when cells are grown in serum-free, defined medium in the absence of insulin, and it appears that low TPA partially replaces the requirement for insulin for proliferation in this system. It is likely that these conditions mimic more accurately the situation in vivo during stimulation of PKC by diacylglycerols, where major down-regulation of PKC does not occur, whereas high TPA concentrations rapidly down-regulate at least some species of PKC (Zylber-Katz and Glazer, 1985; Kraft et al. 1987). The most simplistic interpretation of these results would be a model in which low level stimulation of PKC without down-regulation leads to a mitogenic response in cells, while maximal stimulation that results in down-regulation is associated with growth inhibition and/or differentiation (summarized in Table 1A). This model is also consistent with reports that leukaemic T cell lines, which are dependent on TPA for optimal growth, express lower than usual levels of PKC but may be deficient in other mitogenic signalling pathways (Tanaka et al. 1989; Tchou-Wong and Weinstein, 1992). Thus chronic low level PKC activity may replace other pathways normally sufficient to sustain proliferation. It must be recognized, however, that there are alternative explanations of these observations. For example, the effects of low and high concentrations of TPA might involve distinct signals such as the activation of different isoforms of PKC, with one pathway stimulating proliferation and another leading to differentiation (Table 1B). This would be supported by data from systems where differentiation takes place without long-term down-regulation of PKC (Aihara et al. 1991). Ultimately it may be that our current notions of “activation” and “down-regulation” are too simple and that what is actually important is the localization and activity of individual PKC isoforms. Even the concept of down-regulation could be misleading since a low level of fully active PKC may have more dramatic effects.
on cellular function than a high level of inactive enzyme protein.

Future directions

The size of the family of PKC isoenzymes continues to grow and it is likely that further members will be added in the future as additional protein kinases are cloned and sequenced. It is becoming clear that the different family members can have distinct properties, in terms of both their regulation and the substrates they can phosphorylate (Kikkawa et al. 1989; Ogita et al. 1990; Liyanage et al. 1992). Thus certain signal transduction pathways may involve only single PKC species and this will complicate the interpretation of experiments employing techniques such as the use of general PKC inhibitors or the overall down-regulation of PKCs by chronic phorbol ester treatment (Kelleher and Long, 1992). Examples of differential effects on PKC isoenzymes include the selective translocation or activation of PKC-β and PKC-ε in HeLa cells and Daudi cells, respectively, in response to interferon treatment (Pfeffer et al. 1990, 1991) and the translocation of only PKC-β following activation of B lymphocytes by lipopolysaccharide (Marquez et al. 1991). The differential expression or action of PKC isoforms may also play a role in the control of cell differentiation, for example in response to nerve growth factor (Wada et al. 1989; Wooten, 1992). The induction of granulocytic differentiation of HL-60 cells by retinoic acid is associated with differential increases and decreases in PKC species, as well as the appearance of a new PKC isoform (Hashimoto et al. 1990; Tanaka et al. 1992). It seems likely that the roles of individual PKC isoforms will only become clear when their genes are differentially (over)expressed in transfected cells and, conversely, when the synthesis of different isoforms is inhibited by the use of specific antisense oligonucleotides.

Although at present the full significance of the differential induction, activation or translocation of individual PKC species is not clear, there is growing evidence that different PKCs may act in very different ways to control cell function. An extreme view is the possibility that different isoforms may even exert opposite effects on proliferation versus differentiation, even in a single cell type, due to the phosphorylation of different substrates, perhaps in different subcellular locations. Furthermore, different cell types may respond differently to agents that activate the same species of PKC because they possess a distinct range of substrate proteins.

One of the most dramatic aspects of recent studies on PKC isoforms is the possibility of a nuclear function for some of them. Selective localization or translocation to the nucleus has been described for PKC-βII in HL-60 cells (Hocevar and Fields, 1991), PKC-βII in K562 erythroleukaemia cells (Hocevar et al. 1992) and PKC-L(τ) in a number of carcinoma and epidermal cell types (Greif et al. 1992). Nuclear PKC has been suggested to play a role in the mitogenic response to platelet-derived growth factor (Fields et al. 1990), transformation of cells by the ras oncogene (Chiarugi et al. 1990) and in the chemical induction of cell differentiation (Hocevar and Fields, 1991; Beckman, 1992). In general, increased nuclear levels of PKC are associated with cell proliferation and decreased levels are associated with differentiation. Nuclear targeting of PKC may be mediated by sequences in the hinge and catalytic domains (James and Olson, 1992). The mechanism of action of nuclear PKC is not yet known but it could be responsible for changes in the phosphorylation state (and hence activity) of proteins in transcriptional complexes such as AP-1 (Boyle et al. 1991). These factors in turn may cause the rapid (immediate-early) induction of further proteins with a regulatory role in transcription (Karim, 1990; Shimizu et al. 1992). Another potential nuclear effect of some PKC species may be the phosphorylation of DNA topoisomerase II, with consequent effects on DNA structure and function (DeVore et al. 1992).

There seems little doubt that a large body of information is still to be discovered concerning the roles of PKC isoforms in the regulation of gene expression and changes in cellular phenotype. Only when we understand events at the genetic level will the many mechanisms by which the PKC family controls cell proliferation and differentiation become apparent.

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### Table 1. Mitogenesis versus cell differentiation in response to stimulation of protein kinase C - alternative models

<table>
<thead>
<tr>
<th>Possible factors favouring mitogenesis</th>
<th>Possible factors favouring growth inhibition and cell differentiation</th>
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<tbody>
<tr>
<td>Constitutive low level PKC activity (e.g. due to moderate over-expression of one or more isozymes)</td>
<td>Constitutive high level PKC activity (e.g. due to strong over-expression of one or more isozymes)</td>
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<tr>
<td>Chronic stimulation by low levels of PKC activators (phorbol esters or diacylglycerols)</td>
<td>Acute stimulation by high levels of PKC activators</td>
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<td>Lack of down-regulation of PKC</td>
<td>Long-term down-regulation of PKC</td>
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<tr>
<td>Stimulation of PKC isoform(s) with specificity for phosphorylation of substrates in mitogenic pathways</td>
<td>Stimulation of PKC isoform(s) with specificity for phosphorylation of substrates in growth-inhibitory or differentiation-inducing pathways</td>
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<tr>
<td>Absence of PKC substrates that inhibit growth or induce terminal differentiation</td>
<td>Absence of PKC substrates that stimulate mitogenesis</td>
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<tr>
<td>Differential stimulation of PKC species with subcellular locations appropriate to a mitogenic effect</td>
<td>Differential stimulation of PKC species with subcellular locations appropriate to a growth inhibitory or differentiation-inducing effect</td>
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The induction of granulocytic differentiation of HL-60 cells by retinoic acid is associated with differential increases and decreases in PKC species, as well as the appearance of a new PKC isoform (Chiarugi et al. 1990) and in the chemical induction of cell differentiation (Hocevar and Fields, 1991; Beckman, 1992). Another potential nuclear effect of some PKC species may be the phosphorylation of DNA topoisomerase II, with consequent effects on DNA structure and function (DeVore et al. 1992).
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