Journal of Cellular Biochemistry 74:499–521 (1999)

PROSPECT

Multiple Biological Responses Activated by Nuclear Protein Kinase C

Alberto M. Martelli,[1]* Nianli Sang,[2] Paola Borgatti,[3] Silvano Capitani,[3] and Luca M. Neri[3,4]

[1]Dipartimento di Morfologia Umana Normale, Università di Trieste, 34138 Trieste, Italy
[2]Institute for Human Gene Therapy, Department of Molecular and Cellular Engineering, University of Pennsylvania, Philadelphia, PA 19104
[3]Dipartimento di Morfologia ed Embriologia, Sezione di Anatomia Umana, Università di Ferrara, 44100 Ferrara, Italy
[4]Istituto di Citomorfologia Normale e Patologica del C.N.R., 40137 Bologna, Italy

Abstract Protein kinase C is a family of serine-threonine kinases that are physiologically activated by a number of lipid cofactors and are important transducers in many agonist-induced signaling cascades. To date, 12 different isozymes of this kinase have been identified and are believed to play distinct regulatory roles. Protein kinase C was thought to reside in the cytosol in an inactive conformation and translocate to the plasma membrane upon cell activation by different stimuli. Nevertheless, a growing body of evidence has illustrated that this family of isozymes is capable of translocating to other cellular sites, including the nucleus. Moreover, it seems that some protein kinase C isoforms are resident within the nucleus. A wealth of data is being accumulated, demonstrating that nuclear protein kinase C isoforms are involved in the regulation of several critical biological functions such as cell proliferation and differentiation, neoplastic transformation, and apoptosis. In this review, we will discuss the most significant findings concerning nuclear protein kinase C which have been published during the past 5 years. J. Cell. Biochem. 74:499–521, 1999.

Key words: nucleus; protein kinase C; signal transduction; lipid second messengers; proliferation; differentiation; neoplastic transformation; apoptosis

Abbreviations used: ANG 2, angiotensin 2; ATRA, all-*trans*-retinoic acid; bFGF, basic fibroblast growth factor; CDDP, cis-diamminedichloroplatinum; CLSM, confocal laser scanning microscope; DAG, diacylglycerol; DNIG, dexniguldipine hydrochloride; EGF, epidermal growth factor; EPO, erythropoietin; FELC, Friend erythroleukemia cells; GFP, green fluorescent protein; GM-CFC, granulocyte/macrophage colony forming cells; 13-HODE: 13-(S)-hydroxyoctadecadienoic acid; HMBA, hexamethylene-bis-acetamide; IGF-I, insulin-like growth factor I; IFN, interferon; IL, interleukin; MAP kinase, mitogen-activated protein kinase; M-CSF, macrophage colony stimulating factor; NGF, nerve growth factor; NMAF, nuclear membrane activator factor; NLS, nuclear localization signal; NuMA, nucleus-mitotic apparatus; PKC, protein kinase C; PC, phosphatidylcholine; PDGF, platelet-derived growth factor; PG, phosphatidylglycerol; PI, phosphatidylinositol; PI 3-kinase, phosphatidylinositol 3-kinase; PIP_2, phosphatidylinositol 4,5-bisphosphate; PIP_3, phosphatidylinositol 3,4,5-trisphosphate; PLA_2, phospholipase A_2; PLC, phospholipase C; PLD, phospholipase D; PMA, phorbol 12-myristate 13-acetate; PS, phosphatidylserine; RA, retinoic acid; SCF, stem cell factor; TdT, terminal nucleotidyl transferase; VIP, vasointestinal active peptide.

*Correspondence to: Dr. Alberto M. Martelli, Dipartimento di Morfologia Umana Normale, Università di Trieste, via Manzoni 16, 34138 Trieste, Italy. E-mail: martelli@univ.trieste.it

Received 25 March 1999; Accepted 29 March 1999

PKC is family of serine/threonine kinases that transduce a bewildering number of signals in the regulation of cell growth and differentiation and a plethora of other cell functions [Clemens et al., 1992; Nishizuka, 1992, 1995; Livneh and Fishman, 1997]. At present, 12 PKC isoforms have been identified and cloned and are thought to have distinctly different regulatory roles. They are classified into three main groups that share a common requirement for phospholipid for activity, but differ in their structure and dependencies on other activators. Conventional PKCs (cPKCs), -α, -β_I, -β_{II}, -γ require PS, Ca^{++}, and DAG (or phorbol esters); novel PKCs (nPKC), -δ, -ϵ, -η,- θ, -μ require only DAG and PS; atypical PKCs (aPKCs), -ζ, -ι, -λ are dependent on PS [Jaken, 1996; Mellor and Parker, 1998]. The -λ isotype appears to be a mouse homologous of the human -ι isozyme [Nishizuka, 1995]. The structure of PKC isozymes includes conserved regions (C_1–C_4) that are interrupted by variable regions (V_1–V_5). Each isoform contains a catalytic and a regulatory do-

main [Hug and Sarre, 1993]. The catalytic domain can be active in the absence of cofactors after proteolytic removal of the regulatory domain by cleavage in the V_3 region. Typical protein kinase sequences (e.g., the glycine-rich ATP-binding motif present in the cAMP-dependent protein kinase and many other kinases) are present in the catalytic domain. On the other hand, the regulatory domain, which is responsible for dependence on cofactors, contains an autoinhibitory pseudosubstrate region as well as sequences that mediate the interactions of PKC with phospholipids and DAG or phorbol esters. The Ca^{++} dependency is mediated by the C_2 region (which is indeed absent in nPKCs), while phorbol ester binding requires the presence of two cysteine-rich zinc-finger regions within the C_1 domain. aPKCs lack one of the two cysteine-rich zinc-finger regions and therefore do not bind (and cannot activated by) phorbol esters [Hug and Sarre, 1993].

The biological functions of PKC have been mostly linked to events occurring at the plasma membrane level and/or in the cytoplasm, for PKC isotypes are thought to reside in the cytosol in an inactive state and, after stimulation, translocate to the plasma membrane where they become activated in the presence of specific co-factors [Hugh and Sarre, 1993; Newton, 1997]. However, increasing evidence has implicated a role for PKC in nuclear functions, suggesting that this may be a pathway to communicate signals generated at the plasma membrane to the nucleus [Olson et al., 1993]. The very first report showing the presence of PKC within the nucleus was published in 1987 by Capitani et al. These authors demonstrated that in demembranated rat liver nuclei PKC was associated with the nuclear matrix, a mainly proteinaceous, dynamic structure which acts as a nucleoskeleton [Berezney et al., 1995; Martelli et al., 1996]. Since then, papers dealing with nuclear PKC have been published at an ever increasing rate, underlining the pivotal importance of this research field for cell biologists.

Here, we will not examine in detail the regulation of nuclear PKC signaling systems. Rather, we will review the molecular basis which allow localization of PKCs within the nucleus, as well as emerging details on the fine subnuclear distribution of different isoforms, including their interactions with other nuclear components. Moreover, we will tackle the problem of lipid second messengers which are involved in the regulation of PKC activity within the nuclear compartment. Then, we will concentrate on the biological responses which appear to be mediated by nuclear PKCs, such as cell proliferation, differentiation, neoplastic transformation, and apoptosis. We have reviewed a period of time mainly concerning the past five years, which have seen the publication of many reports dealing with these issues. This is because the papers published prior to 1994 have already been summarized in a comprehensive review by Buchner [1995].

Molecular Basis for Nuclear Import of PKC

Targeting of proteins to the nucleus requires the NLS contained in its primary structure, that marks which protein goes to this compartment. Albeit ions and small molecules can passively diffuse through the 9-nm pores of the nuclear pore complex, macromolecules require a NLS in their sequence or in an interacting partner, which allows for an active transport through the 26-nm central channel [Silver, 1991; Rout and Wente, 1994]. NLSs are believed to be responsible for targeting and binding the imported protein to the receptor protein at the nuclear pores known as a NLS-binding protein. Most NLSs may be categorized into one of three classes: the first is composed of a single peptide region containing basic residues (RKRRxK), such as that found within the SV40 large T-antigen; a second class is typified by the NLS within the Xenopus protein nucleoplasmin, which is composed of two peptide regions containing basic residues that are separated by a spacer of 10 residues. By permitting a spacer of variable length many NLSs can be classified as bipartite. In fact, it has been suggested that this class of NLSs is the most common [Dingwall and Laskey, 1991]. The third and most unusual class is defined by the amino-terminal signal of the yeast protein Mat α2 (RKxxxKR) [Hicks and Raikhel, 1995]. Almost all the isoforms of PKC, except for the -μ isozyme, have been identified at the nuclear level: -α [e.g., Neri et al., 1994a], -β_I, and -β_{II} [e.g., Mason-Garcia et al., 1995], -γ [e.g., Zini et al., 1997a; Oehrlein et al., 1998), -δ [e.g., Borgatti et al., 1996], -ϵ [e.g., Gordon et al., 1997], -η [e.g., Borgatti et al., 1996], -θ [e.g., Jones et al., 1997], -ζ [e.g., Zauli et al., 1996a], -ι/ λ [e.g., Wooten et al., 1997]. These findings have prompted the question as to whether or not PKC isoforms possess NLS motifs. Initially, sequences resembling the bi-

partite nuclear targeting motif were found to be present in the regulatory domain of the -β and -γ isoforms, but not in PKC-α [Malviya and Block, 1992]. However, it was suggested that PKC-α might possess a cryptic NLS because deletion of its regulatory domain resulted in the constitutive localization of the enzyme to the nuclear envelope of COS cells [James and Olson, 1992]. The domain involved in nuclear translocation of PKC-α was mapped to a 75-amino acid segment near the hinge region that is not conserved in other PKC isozymes (the hinge region corresponds to V3 and separates the regulatory from the catalytic domain) [see Hug and Sarre, 1993]. In a classical model of nuclear translocation of PKC-α, phorbol ester induced the accumulation of the enzyme in nuclei of NIH 3T3 cells. When the cytoskeleton was disrupted by cytochalasin B or colchicine, nuclear translocation was inhibited. In contrast, the nuclear accumulation of a NLS-containing reporter protein in an in vitro nuclear transport assay was not affected by these drugs. Therefore, it was suggested that the cytoskeleton plays an important role in the nuclear translocation of PKC isoforms which do not possess a NLS [Schmalz et al., 1996]. Subsequently, it has been shown that in contrast to the NLS-dependent transport, the phorbol ester-induced transport of PKC-α is not affected by microinjection of antibodies against the nuclear import factor p97/importin/karyopherin β or microinjection of non-hydrolyzable GTP analogues. Therefore, the mechanism of PKC-α nuclear import is independent of both p97/importin/karyopherin β and GTP. In addition, at the nuclear pore there are differences between the mechanisms since nuclear transport of PKC-α cannot be inhibited by wheat germ agglutinin or an antibody to nuclear pore complex protein [Schmalz et al., 1998]. In light of these findings, it will be extremely interesting in the future to determine whether or not also other PKC isozymes require an intact cytoskeleton to be transported into the nucleus.

Recently, however, a computer-assisted analysis was used to search for the presence of bipartite nuclear targeting motifs in PKC isozymes. In cPKC isoforms (-α, -β_I, -β_{II}, -γ) two bipartite motifs were present, while atypical -ι/ λ and -ζ displayed one motif, whereas nPKC did not exhibit any bipartite motif structure [Wooten et al., 1997]. We sought to investigate the nature of the signal directing PKC isoforms to the nucleus analyzing bipartite NLSs in individual PCK isozymes by using a sequence search algorithm. The results are presented in Table I. We have been able to assess the presence of two motifs in cPKCs (-α, -β_I, β_{II}, -γ), and of one motif in both nPKCs (-δ, -ϵ) and aPKCs (-ι/- λ, -ζ). Remarkably, these sequences seem to be highly conserved among different species. Nevertheless, the issue of sequences allowing translocation of PKC isoforms to the nucleus is likely to be extremely more complicated, as underlined by the recent data by Gokmen-Polar and Fields [1998]. Indeed, these authors demonstrated that in K562 erythroleukemia cells a PKC-α/PKC-β_{II} chimera containing only the carboxyl-terminal 13 amino acids from PKC-β_{II} (β_{II} V5) is capable of nuclear translocation and lamin B phosphorylation as wild-type PKC-β_{II}. This sequence does not correspond to any known NLS, but it binds to PG, a potent and selective PKC-

TABLE I. Bipartite Motifs Identified in PKC Isoforms[a]

PKC isoform	Bipartite motif	Amino acids	Species
−α	KR X21 RxKxKxK	76–105	Bo, Hu, Mo, Rb, Rt
	KQ X15 KRxR	141–161	Bo, Hu, Mo, Rb, Rt
−β_I	KR X21 RxKxKxK	76–105	Hu, Mo, Rb
	KR X15 RRxR	141–161	Hu, Mo, Rb
−β_{II}	KR X21 RxKxKxK	76–105	Bo, Hu, Mo, Rb, Rt
	KR X15 RRxR	141–161	Bo, Hu, Mo, Rb, Rt
−γ	RR X21 RxKxKxR	76–105	Bo, Hu, Mo, Rb, Rt
	RR X15 RRxR	141–161	Bo, Hu, Mo, Rb, Rt
−δ	KR X6 RPKxKxP	643–659	Hu, Mo, Rt
−ϵ	KK X5 KPRxKxK	686–699	Hu, Mo, Rb, Rt
−ι/−λ	RR XRK X11 KRxxRR	123–144	Hu, Mo
−ζ	RR XRK X11 KRxxRR	123–144	Hu, Mo, Rb, Rt, Fr

[a]Bo, bovine; Fr, frog; Hu, human; Mo, mouse; Rb, rabbit; Rt, rat.

β_{II} activator present in the nuclear membrane [see later and Murray and Fields, 1998]. This finding underscores the fact that several other unknown nuclear targeting signals are likely to exist because not all the sequenced nuclear proteins contain a NLS [Dingwall and Laskey, 1991]. Moreover, NLS masking can be regulated by phosphorylation as the main mechanism controlling NLS-dependent nuclear localization of different proteins [Jans, 1995; Xiao and Jans, 1998].

Since PKC-δ seems to localize to the nucleolus, the question arises as to whether or not this isoform possesses a nucleolar targeting signal [Hatanaka, 1990]. However, the issue of nucleolar targeting sequences is even more complex and under debate than that of NLS, also because so far it has been proven difficult to identify sequences targeting proteins to the nucleolus clearly separated from those necessary for nuclear import [Schmidt-Zachmann and Nigg, 1993; Russo et al., 1997]. Recently, Das et al. [1998] identified in the Trypanosoma Brucei Nopp44/46 protein a 27 amino acid sequence within the amino terminal region whose deletion abrogated nucleolar but not nuclear localization. Future investigations should be aimed at identifying similar sequence(s) also in PKC-δ.

Subnuclear Localization of PKC

Early reports indicated that PKC translocated to the nuclear envelope of NIH 3T3 cells [e.g., Leach et al., 1989; Fields et al., 1990]. Nevertheless, several subsequent studies have shown that PKC could move from the cytoplasm further inside the nucleus. These studies were facilitated by widespread use of immunofluorescent staining coupled with CLSM, which allowed for an accurate dissection of the involved subnuclear compartments, and supported the data coming from biochemical fractionation experiments. In Swiss 3T3 cells IGF-I, PDGF, or EGF caused an increase in PKC-α within the nucleus, except for nucleoli. Fractionation experiments and immunoblotting analysis were in agreement with morphological findings [Neri et al., 1994a]. As shown in Figure 1, in quiescent cells PKC-α was mainly detected in the cytoplasm and in the perinuclear region. Nuclei were immunolabeled to a much lesser extent (Fig. 1A). Incubation of cells with IGF-I for 30 min produced a striking increase in PKC-α labeling in the nuclear interior (Fig. 1B).

A very detailed analysis of the subnuclear distribution of PKC isoforms was performed in nervous NG-108–15 cells by Beckman et al. [1994]. It was observed that the -α isozyme was located in the nucleus excluding nucleoli, whereas the -δ isotype was found only in the nucleoli. Finally, PKC-ϵ was localized in the pore complexes at the nuclear envelope. Although present in the cytoplasm, the β_{II} isoform was not detectable in nuclei. In nuclei of neuro-2a neuroblastoma cells PKC-α is constitutively present and does not change after

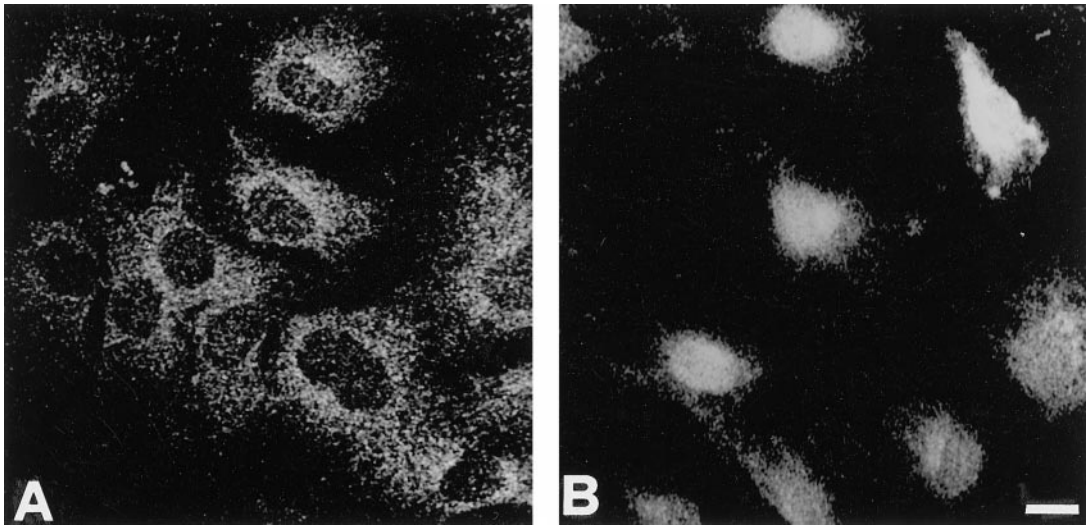

Fig. 1. Single CLSM optical sections showing the subcellular distribution of PKC-α in Swiss 3T3 cells. **A**: Quiescent cells. **B**: 30 min of IGF-I stimulation. A polyclonal antibody to PKC-α was employed, as described by Neri et al. [1998]. Scale bar = 10 μm.

stimulation with phorbol esters. However, as revealed by extraction experiments, phorbol esters lead to a firmer association of the kinase with nuclear components, suggesting that this isotype not only associates with lipids but also with proteins inside the nucleus. The presence of active PKC-α inside the nucleus could allow the enzyme to phosphorylate not only proteins located near the membrane but also further inside in the nucleus, for example chromatin and/or nuclear matrix proteins [Buchner et al., 1997].

An issue that should be emphasized is that contradictory findings have in some cases been reported concerning the nuclear localization of PKC isoforms in a given tissue or cell line. For example, the -δ and -ζ isotypes have been detected in rat liver isolated nuclei, while the -α isozyme was present only in the cell homogenate. The -γ, -β, and -ϵ isoforms were found neither in the homogenate nor in the nuclei [deMoel et al., 1998]. This is in contrast to previous data reported by Rogue et al. [1990] who, in the same rat tissue model, indicated in PKC-β the nuclear isozyme of liver. The discrepancies could be due to the fact that in many cases the results have been obtained with antibodies of different origin or raised against different peptide sequences. There is another likely reason to explain these different results. Indeed, it is well established that PKC is post-translationally or co-translationally phosphorylated. Thr497 and the homologous Thr500 in the α and βII isozymes, respectively, are phosphorylated [Liu and Heckman, 1998]. Also PKC-δ and -ζ isozymes are phosphorylated in their activation loop sites by PDK-1 in a PI 3-K-dependent manner [Le Good et al., 1998]. Therefore, at least some of the aforementioned variations may have occurred because certain antibodies raised against PKC specifically recognize phosphorylated forms of the enzyme, as exemplified by an investigation on the immunological demonstration of PKC-μ in different murine tissues and cell lines [Rennecke et al., 1996]. Indeed, the authors were able to demonstrate that in some tissues PKC-μ was recognized only by monoclonal antibody P26720 and not by the polyclonal antiserum sc-639. It was additionally shown that phosphorylation of activated PKC-μ caused its reduced interaction with antibody sc-639. Thus, it will be desirable in the future to rely on antibodies that distinguish among phosphorylated and unphosphorylated PKC forms. In any case, results of immunocytochemical investigations obtained with peptide antibodies must be interpreted very cautiously, if they were not substantiated by parallel immunoblotting analysis.

In light of the aforementioned problems, alternative techniques to study by immunofluorescence the distribution of PKC isozymes would appear desirable. In this connection, Bastiaens and Jovin [1996] covalently labeled purified recombinant PKC-β_I with the fluorescent sulfoindocyanine succinimide ester, Cy3. After labeling, the lipid/Ca^{++}-dependent activity was fully retained and it was also possible to determine the loci of the Cy3 labels on the protein. The regulatory domain of the kinase exhibited a 3.5-fold higher specific activity than the catalytic domain. Subsequently, the labeled PKC-β_I was microinjected into Balb/c 3T3 cells to follow its time-dependent redistribution in response to phorbol esters. The combined use of CLSM, fluorescence life time imaging microscopy, and novel determinations of fluorescence resonance energy transfer based on photobleaching digital imaging microscopy allowed to assess the localization to the nucleus of the kinase. Simultaneous labeling with a polyclonal antibody recognizing the C-terminal catalytic domain of the kinase, indicated that, after phorbol ester treatment, PKC was fragmented and that the regulatory, but not the catalytic domain, was retained predominantly in the nucleus. Although the amount of work is considerable, it is tempting to think that in the future such a technique might be applied to other PKC isoforms. It may also be worth recalling that intracellular localization of PKC could be analyzed by using fluorescently-tagged PMA in living cells [Godson et al., 1996] or fluorescent derivatives of the bisindolylmaleimide inhibitors [Chen and Poenie, 1993]. However, in these cases no clear information on the isoforms could be obtained, unless the studied cell line already overexpressed one of the isozymes [Chen and Poenie, 1993]. Finally, a promising emerging technique to reveal subcellular localization of PKC isoforms which are dependent on DAG for activation, has recently been utilized by Oancea et al. [1998]. They exploited the fact that cysteine-rich domains of PKC bind lipid membranes in the presence of DAG or phorbol ester. By monitoring the membrane translocation of a GFP-tagged Cys-domain of PKC-γ, it was observed a translocation to the nuclear membranes upon

phorbol ester treatment but not in the presence of arachidonic acid. However, all the PKC isoforms can now be stably overexpressed as hybrids tagged with GFP and this will undoubtedly allow for more accurate investigations on their intracellular localization and redistribution following diverse stimuli [Bertolaso et al., 1998; Almholt et al., 1999].

Since PKC translocates not only to the nuclear membrane but also further inside the nucleus, different groups have examined in greater detail the subnuclear localization of the kinase. The fine localization of PKC-α has been investigated by means of electron microscope/quantitative immunogold labeling in 3T3 mouse fibroblasts mitogenically stimulated by IGF-I. The enzyme, which in untreated cells was mainly present in the cytoplasm, after IGF-I treatment was reduced in this compartment and almost doubled in the nucleus. PKC-α was found not only associated with the nuclear envelope but mainly with the interchromatin domains. By using in situ nuclear matrix preparations, PKC-α appeared to be retained both at the nuclear lamina and at the inner nuclear matrix, suggesting a direct involvement in the phosphorylation of nuclear proteins which are responsible for regulation of different cell responses [Zini et al., 1995]. Other PKC isoforms have been found in association with the nuclear matrix. In pancreatic islet β cells only PKC-δ could be detected in the nucleus, partially associated with the nuclear matrix [Knutson and Hoenig, 1997]. The localization of PKC-ζ was investigated by means of immunoelectron microscopy which revealed the localization of this isoform within the inner nuclear matrix of PC-12 cells [Wooten et al., 1997]. However, there is also a report showing the binding of PKC-β_I isotype to the chromatin in FELC [Mallia et al., 1995].

It should be recalled that the nuclear matrix is thought to be the nuclear equivalent of the cytoskeleton [Berezney et al., 1995]. If the matrix is the site of PKC activation, how do lipid cofactors access the matrix enzyme? There are some studies [Payrastre et al., 1991; Brooksbank et al., 1993] which illustrated that several kinases acting on PI, DAG kinase, and PLC were associated with the cytoskeleton. Moreover, other investigations have demonstrated that several cytoskeletal proteins bind to various lipids, such as PI, PIP_2, and PS [Keenan and Kelleher, 1998]. In this connection, it is of interest to recall that several enzymes of the polyphosphoinositide cycle (including the PLC activity which generates DAG) have been demonstrated to be associated with the nuclear matrix and some nuclear matrix proteins may bind to lipids [D'Santos et al., 1998; Neri et al., 1999]. In Table II we summarize the subnuclear localization of various PKC isoforms.

Finally, we would remind that intriguing results about nuclear PKC localization emerged from a pioneeristic investigation, in which Jurkat and U937 cells were exposed to microgravity during a Space Shuttle flight, and stimulated with a radiolabeled phorbol ester that specifically activates and labels several PKC isoforms. The subcellular distribution of PKC in the cytosol and nuclear fractions appeared to be correlated with the applied acceleration. In both cell types the relative amount of phorbol ester labeling in the nuclear fraction decreased

TABLE II. Subnuclear Localization of PKC Isoforms

Nuclear domain	PKC isoform	Cell line/tissue	Reference
Nuclear envelope	α	NIH 3T3	Leach et al., 1989
			Fields et al., 1990
	β_{II}	HL-60	Hocevar et al., 1993
	γ	2H3 rat leukemia	Oancea et al., 1998
Nuclear interior	α	Swiss 3T3	Neri et al., 1994a
		NG-108-15	Beckman et al., 1994
		Neuro-2a	Buchner et al., 1997
Nucleolus	γ	Epithelial lens cells	Gonzalez-Charneco and Takemoto, 1998
	δ	NG-108-15	Beckman et al., 1994
Nuclear pore complex	ϵ	NG-108-15	Beckman et al., 1994
Inner nuclear matrix	α	Swiss 3T3	Zini et al., 1995
	δ	Pancreatic β cells	Knutson and Hoenig, 1997
	ζ	PC-12	Wooten et al., 1997
Chromatin	β_I	FELC	Mallia et al., 1995

with applied acceleration, whereas the labeling in cytosolic fraction increased with *g* level. IL-1β synthesis by U937 cells was markedly decreased in microgravity when compared to the onboard 1 *g* control. Therefore, the alteration in PKC distribution due to the effect of gravity may be the consequence of a functional impairment whose relevance in signal transduction remains to be established [Schmitt et al., 1996].

PKC-Binding Proteins at the Nuclear Level

In the signal transduction field a relatively new theme has emerged in the past few years concerning the localization of protein kinases by anchoring proteins. It is commonly thought that specific anchoring proteins located at various sites in the cell fulfill the role of compartimentalizing different kinases to their sites of action. As far as PKC is concerned, despite the tremendous potential diversity of lipid-derived second messengers, PKC-lipid interactions alone appear to be insufficient to explain all aspects of PKC regulation and/or localization. In particular, PKC compartmentalization, which may differ significantly depending upon cell type and state of activation, cannot be explained on this basis [Hug and Sarre, 1993]. Various PKC binding proteins have been identified by means of overlay assays in the cytoplasm of REF52 cells, including the two focal contact proteins, vinculin and talin, plus two other major binding proteins with a molecular weight of >200- and 71-kDa, respectively [Hyatt et al., 1994]. Further investigations have revealed that the >200-kDa protein is also a substrate for PKC-α and its amount decreased during carcinogenesis, suggesting that the quantity and location of PKCs isozymes and their downstream targets may play a relevant role in the control of neoplastic transformation [Chapline et al., 1996]. It has recently been demonstrated that this protein is involved in cytoskeletal remodeling [Chapline et al., 1998]. Other studies were carried out using the yeast two-hybrid system to identify proteins that interact with activated PKC-α. It was therefore possible to detect several novel proteins interacting with PKC, named PICKs. One of these PIKCs, designated PICK1, interacts specifically with the catalytic domain of PKC and is an efficient substrate. PICK1 is localized to the perinuclear region and is phosphorylated in response to PKC activation [Staudinger et al., 1995]. Surprisingly, despite the importance of identifying PKC-binding proteins at the nuclear level, no investigations on this issue have so far been published. In the future, it will be important to identify similar proteins residing in the nucleus, because these informations will significantly add to our knowledge about the regulation of nuclear PKC isoforms.

Regulation of PKC at the Nucleus by Lipid Second Messengers

The presence of PKC in the nucleus prompts the question as to how the kinase would be activated by lipid cofactors and Ca^{++}. Substantial evidence has documented that nuclei contain PS [e.g., Capitani et al., 1986]. Furthermore, it has been demonstrated the existence of a canonical nuclear phosphoinositide cycle that includes enzymes for the synthesis and breakdown of inositol lipids, from which DAG can be generated following hydrolysis of PIP_2 by action of a specific PI-PLC [Cocco et al., 1994; Neri et al., 1999]. However, in other cell lines a different source for nuclear DAG might be PC, through the action of a PLD or a PC-PLC [Raben et al., 1994; D'Santos et al., 1998]. We have very recently reported that in Swiss 3T3 cells the IGF-I-dependent increase in nuclear DAG production could be inhibited by the specific PI-PLC inhibitor 1-O-octadeyl-2-O-methyl-sn-glycero-3-phosphocholine or by neomycin sulfate but not by the purported PC-PLC specific inhibitor D609, or inhibitors of PLD-mediated DAG generation. Treatment of cells with 1-O-octadeyl-2-O-methyl-sn-glycero-3-phosphocholine or neomycin sulfate inhibited translocation of PKC-α to the nucleus. Moreover, exposure of cells to 1-O-octadeyl-2-O-methyl-sn-glycero-3-phosphocholine, but not to D609, dramatically reduced the number of cells entering S-phase upon stimulation with IGF-I. Therefore, it is conceivable that the only phospholipase responsible for generation of nuclear DAG after IGF-I stimulation of 3T3 cells is PI-PLC since, when this activity is inhibited, neither DAG rise is seen nor PKC-α translocation to the nucleus occurs. Furthermore, this PI-PLC activity appears to be essential for the G_0/G_1 to S-phase transition [Neri et al., 1998]. Although the generation of DAG in the nucleus is the most obviously appealing way of attracting and activating PKC, it needs not to be the only one. Indeed, since almost all of the PKC isoforms have been described to translocate to the nucleus, other molecules may be responsible for activating

novel or atypical isoforms, such as PKC-ϵ, -ζ, and -λ, which can be alternatively dependent at least in part for their activation on inositides phosphorylated in the D-3 position of the inositol ring, such as PIP_3 [Carpenter and Cantley, 1996; Chou et al., 1998]. In this connection, it should be emphasized that the enzyme responsible for this phosphorylation (i.e., PI 3-kinase) has been found in nuclei [Neri et al., 1994b; Lu et al., 1998]. Moreover, the effect of different fatty acids on the translocation of PKC isozymes cannot be overlooked, as demonstrated by the recent findings by Shirai et al. [1998]. In fact, it is well known that several fatty acids can greatly enhance the DAG-dependent activation of PKC [Nishizuka, 1995].

Both the source and the regulation of nuclear Ca^{++} are currently topics of intensive and controversial debate. Indeed, some reports have indicated that nuclei are able to regulate their Ca^{++} content independently of the cytosol, whereas others have shown that Ca^{++} diffuses freely in and out from the nucleus. Although the question as to whether or not there is independent nuclear Ca^{++} regulation is far from being solved, it is important to emphasize the fact that Ca^{++} concentration can be high enough for activation of Ca^{++}-dependent processes in the nucleus. The activation of PKC is certainly only one aspect of the diverse actions of nuclear Ca^{++} [Santella and Carafoli, 1997].

If DAG, PIP_3, and fatty acids are likely candidates for the attraction and/or activation of nuclear PKC isoforms, very little is known of molecules which might inactivate PKC once it translocates to the nucleus. In cow mammary gland a study was designed to determine whether gangliosides, putative physiological regulators of PKC, and the PKC system were present in cell nuclei. PKC and a wide molecular weight range of substrate proteins (120-, 97-, 56-, 43-, 38-, and 36-kDa) were extracted by Triton X-100 treatment of nuclei. Furthermore, it was found that three gangliosides (GD3 and GM3 but particularly GT1b) were able to inhibit PKC-mediated phosphorylation, thus suggesting that PKC-mediated signal transduction in cow mammary gland nuclei may be regulated by gangliosides [Katoh et al., 1993].

Moreover, isolated nuclei contain neutral sphingomyelinase, an enzyme which generates insoluble ceramide [Albi and Viola Magni, 1996; Micheli et al., 1998]. Interestingly, ceramide has been shown to inhibit PKC-α [albeit not directly, see Lee et al., 1996] or -δ [Huwiler et al., 1998]. However, it should not be forgotten that PKC-α has also been demonstrated to be activated by ceramide [Huwiler et al., 1998]. The discrepancies are likely to depend on the cell types but there is the distinctive possibility this is a system to control PKC activity within the nucleus, and we feel that in the future it should be explored in more depth.

Furthermore, ceramide is converted into sphingosine by means of ceramidase [Perry and Hannun, 1998]. Sphingosine has been reported to act as an inhibitor of PKC by interacting with the regulatory domain of the enzyme [Liu and Heckman, 1998]. Thus, also sphingosine might be involved in the control of nuclear PKC activity.

Expression of PKC at the Nuclear Level in Different Tissues

The central nervous system is the site where PKC was initially discovered and subsequently several isoforms have been mapped to specific regions of it [Tanaka and Nishizuka, 1994]. Some of the isoforms are also present in nuclei. For example, PKC-δ, -ϵ, and -ζ were investigated in isolated cell nuclei from bovine cerebral cortex. Neuronal nuclei had a higher concentration than glial nuclei of both PKC-δ and -ϵ. PKC-ζ was present in the nucleoplasm and at the nuclear envelope [Rosenberger et al., 1995].

Cardiac myocytes were found to express PKC-α, -δ, -ϵ, and -ζ but only the -δ and -ϵ isozymes were detected in isolated nuclei, suggesting that they could be part of the signal transduction pathway involved in the effect elicited by phorbol ester or DAG on opioid gene transcription in isolated nuclei. Conversely, prodynorphin gene transcription was prevented by pretreatment of myocytes or isolated nuclei with different PKC inhibitors such as staurosporine or chelerythrine [Ventura et al., 1995, 1997] suggesting that myocardial opiod gene expression may be regulated through autocrine or paracrine mechanisms by nuclear PKC. Moreover, the expression of PKC-α, -δ, and -ϵ as well as PKC activity were increased in nuclei of cardiomyopathic myocytes compared to nuclei from control cells [Ventura et al., 1997].

In isolated nuclei derived from pregnant mice mammary gland, prolactin was found to stimulate PKC activity. This activity was time- and dose-dependent and was blocked by the protein

kinase inhibitor, staurosporine. This study correlated the possible direct effects of prolactin in the nucleus of mammary cells [Fan and Rillema, 1993].

Three Ca^{++}-independent PKC isoforms (i.e., -δ, -ϵ, and -ζ) were detected in the nuclear fraction of unstimulated rat parotid acinar cells. Treatment with norepinephrine was characterized by down regulation of PKC-δ and the contemporary enhancement of amylase secretion implying that one or more of these isozymes may regulate such processes [Terzian et al., 1996].

The localization of two conventional isoforms of PKC (-α and -γ) was determined in bovine lens epithelial cells. Exposure to 40 mM galactose induced the redistribution of PKC-α to nuclei but PKC-γ was localized in nucleoli. Translocation of these isoforms to the nuclear compartment suggested they may be involved in nuclear events leading to the development of cataract [Gonzalez-Charneco and Takemoto, 1998].

Nuclear PKC and Cell Proliferation

In addition to the report by Neri et al. [1994a] there are several other studies that have tried to link the presence of nuclear PKC isoforms with cell proliferation. These investigations have been performed in a variety of cell lines treated with different mitogenic stimuli.

Exposure to docosahexaenoic acid causes epidermal hyperproliferation in guinea pig skin and this treatment was accompanied by increase of PKC-α and -ζ isozymes at the nuclear level, and enhacement of typical and atypical PKC activity in the same cell compartment. This increase was reversed by a lipoxygense metabolite of linoleic acid, 13-HODE. The increase in nuclear PKC isozymes paralleled a marked increase in the expression of nuclear MAP kinase [Mani et al., 1998a; 1998b]. Astroglial cells are induced to proliferate by subnanomolar concentrations of VIP that, in neonatal cortical astrocytes, induced the nuclear translocation mainly of PKC-α, and to a lesser extent of the -δ and -ζ isoforms [Olah e al., 1994]. Smooth muscle cell differentiation and proliferation are increasingly seen to be intimately tied to the etiology of both atherosclerosis and hypertension [e.g., Alam et al., 1996; Wang et al., 1997]. Cultured vascular smooth muscle cells respond to both PDGF and ANG 2. Haller et al. [1994] demonstrated that these two growth factors act on different specific isozymes. ANG 2 provoked a translocation of PKC-α towards the nucleus after 12 min, while the -β isoform was rapidly translocated by both the agonists to the nucleus. More recently, the same authors extended these early observations by showing by CLSM analysis that exposure of these cells to bFGF resulted in a rapid translocation to the nucleus of the -α and -ϵ isotypes. In contrast, treatment with thrombin did not influence intracellular distribution of PKC-ϵ, but instead induced a rapid nuclear translocation of PKC-ζ and, to a lesser extent, of PKC-α [Haller et al., 1998]. Moreover, they also analyzed the nuclear immunoreactivity of PKC isoforms during the cell cycle. Resting cells were stimulated with serum which translocated to the nuclear compartment the -α and -ϵ isozymes. After 4 h of serum stimulation, the nuclear immunoreactivity for these isoforms was reduced to or below control values. At 8 h, increased nuclear expression of the isoforms -α, -ϵ, and -ζ was observed. Taken together these results demonstrated a complex temporal distribution of PKC isoforms in response to different vasoactive hormones and growth factors.

Treatment of avian cultured myoblasts with vitamin D_3 induces the stimulation of DNA synthesis. This regulation of DNA synthesis was paralleled by increased amounts of PKC-β in the nucleus [Marinissen et al., 1998].

EPO is a hormone, as a well as a hematopoietic growth factor, that specifically regulates proliferation of erythroid progenitor cells. Even though the EPO receptor has no intrinsic kinase activity, it triggers the activation of protein kinases via PLA_2, PLC, and PLD. A cascade of serine/threonine kinases, which include Raf-1, MAP kinase, and PKC is then activated. In the murine target cell line B6Sut.EP, EPO, and IL-3 induced a rapid DAG rise and nuclear translocation of PKC-β_I and -β_{II} [Mallia et al., 1997].

Following a prolactin challenge, Ganguli et al. [1996] monitored PKC activity at short intervals in different cellular fractions of a rat nodal lymphoma cell line (NB2) over a 60 min period. PKC activity in the cytosolic fraction declined rapidly to 50% of its original activity within the first 30 min, while PKC activity in the nuclear fraction increased sharply, reaching its highest level following prolactin challange. The kinase activity declined by 60 min reaching levels close to its basal values suggesting it may be in-

volved in mitogenic signal transduction for prolactin in NB2 cells.

Although all the of previously listed articles are highly suggestive of an important role played by PKC isotypes in the control of cell proliferation, they did not directly address the issue of the step(s) that could be affected by the kinase.

The most direct evidence for a proliferation-linked function of nuclear PKC has come from studies on lamin B phosphorylation carried out on HL-60 cells. Lamin B is rapidly phosphorylated by the -β_{II} isoform of PKC following its translocation to the nucleus after treatment with bryostatin, an activator of PKC. The sites of PKC-mediated phosphorylation lie within the carboxyl-terminal domain of lamin B immediately adjacent to the central α-helical rod domain. Functionally, the phosphorylation of these sites led to the time-dependent solubilization of lamin B. Depletion or inhibition of cdc2 kinase activity had no effect on PKC-mediated lamin B phosphorylation [Hocevar et al., 1993]. Since lamin B phosphorylation occurs during the nuclear envelope breakdown at the G_2-M phase progression of the cell cycle, the possibility existed that it was due to different kinases and not only to p34cdc2/cyclin B kinase, as previously thought [see Moir et al., 1995 and references therein]. Indeed, it was susbsequently demonstrated that both p34cdc2/cyclin B kinase and PKC-β_{II} could phosphorylate purified soluble lamin B at similar rates. However, PKC-β_{II} phosphorylated interphase nuclear envelope lamin B at more than 200 times the rate of cdc2 kinase. Moreover, it was found that PKC-β_{II} translocated to the nucleus during the G_2-M phase concomitantly with the phosphorylation of the β_{II} site Ser^{405}. This site is not a target for p34cdc2/cyclin B kinase, indicating a physiologic role for nuclear PKC-β_{II} in mitotic lamin phosphorylation [Goss et al., 1994]. Additional studies carried out with the selective PKC inhibitor, chelerythrine chloride, demonstrated that cells could be arrested in G_2 phase. The involvement of β_{II} PKC was demonstrated by three lines of evidence. First, chelerythrine caused dose-dependent inhibition of PKC-β_{II}, similar to that for G_2 phase blockade in whole cells. Second, it specifically inhibited PKC-β_{II}-mediated lamin B phosphorylation and mitotic nuclear lamina disassembly, and led to selective loss of PKC-β_{II} during G_2 phase. Third, chelerythrine-mediated G_2 phase arrest resulted from selective inhibition and degradation of PKC-β_{II} [Thompson and Fields, 1996]. Taken together, these data pointed to the relevance that PKC-β_{II} activation is required for nuclear lamin B phosphorylation and disassembly and entry into mitosis. The same authors tried to identify a specific activator of PKC-β_{II} at the G_2-M transition. Recently, they demonstrated that during the G_2-M phase, in parallel with increased phosphorylation of lamin B, there was a striking increase in the levels of nuclear DAG, capable of stimulating nuclear PKC activity in vitro [Sun et al., 1997]. Other data were obtained with nuclear membrane extracts, that were able to reconstitute PKC-β_{II}-selective phosphorylation, indicating the presence of a specific NMAF that selectively activates three- to four-fold kinase activity above the level obtained with optimal concentration of DAG, Ca^{++}, and PS [Murray et al., 1994]. NMAF turned out to be PG, on the basis of several biochemical observations, and in vitro binding studies showed that PG binds to the carboxyl-terminal region of PKC-β_{II}. It should be emphasized that the nuclear PG main function may reside in modulating nuclear PKC-β_{II} translocation. In fact, nuclear PG levels do not change appreciably during the cell cycle. It is therefore possible that PG serves primarily to facilitate and/or enhance the selective binding of PKC-β_{II} to the nuclear mambrane where it can be fully activated in the presence of elevated DAG generated during G_2 phase [Murray and Fields, 1998].

Nevertheless, lamin B phosphorylation by PKC may fulfill yet another role. NLS-dependent nuclear transport of lamin B_2 was inhibited by PKC-mediated phosphorylation at two sites N-terminally adjacent to the NLS ($RS^{410}S^{411}$RGKRRRIE) [Hennekes et al., 1993].

The PKC isoforms which have been linked to cell proliferations have been listed in Table III.

Nuclear PKC and Differentiation

A growing body of evidence suggests that translocation to the nucleus of different PKC isoforms plays an important role in the mechanisms which regulate the differentiation of cells of hemolymphopoietic lineage. In rat splenocytes, two RA analogues caused translocation of PKC to the nucleus and its activation. However, no information on the isoforms and substrates involved was provided. Nevertheless, PKC in-

TABLE III. Nuclear PKC Isoforms Involved in Cell Proliferation

PKC isoform	Agonist	Cell line/tissue	Reference
α	IGF-I	Swiss 3T3	Neri et al., 1994a, 1998
	13-HODE	Guinea pig skin	Mani et al., 1998a,b
	VIP	Astroglial cells	Olah et al., 1994
	ANG 2	Smooth muscle cells	Haller et al., 1994
	bFGF	Smooth muscle cells	Haller et al., 1998
	Thrombin	Smooth muscle cells	Haller et al., 1998
β (subtype not specified)	ANG 2, PDGF	Smooth muscle cells	Haller et al., 1994
	Vitamin D_3	Avian myoblasts	Marinissen et al., 1998
β_I	EPO, IL-3	B6Sut.EP	Mason-Garcia et al., 1998
β_{II}	EPO, IL-3	B6Sut.EP	Mason-Garcia et al., 1998
	Bryostatin	HL-60	e.g., Hocevar et al., 1993
δ	VIP	Astroglial cells	Olah et al., 1994
ϵ	bFGF	Smooth muscle cells	Haller et al., 1998
ζ	13-HODE	Guinea pig skin	Mani et al., 1998a,b
	VIP	Astroglial cells	Olah et al., 1994
	Thrombin	Smooth muscle cells	Haller et al., 1998

hibitors blocked the RA-stimulated nuclear phosphorylation [Zorn and Sauro, 1995].

In the HL-60 human promyelocytic leukemia cell line ATRA is a powerful differentiating agent toward morphologically mature granulocyte-like cells. Other agents, such as vitamin D_3, or chemicals like phorbol esters, can also induce terminal HL-60 differentiation into macrophages [Collins, 1987]. Both ATRA and vitamin D_3 are employed in therapeutic treatment of acute promyelocytic leukemia [Quignon et al., 1997].

The level of PKC catalytic activity has been shown to increase progressively in the nuclei of HL-60 cells starting from 1 h of ATRA treatment, while immunochemical and immunocytochemical investigations indicated that the -α and the -ζ isoforms accumulated within the nucleus [Zauli et al., 1996a]. Also exposure to vitamin D_3 increased the levels of intranuclear PKC-ζ in the same cell line [Bertolaso et al., 1998]. However, the same authors also showed that overexpression of a constitutively active mutated form of PKC-ζ (which still accumulates within the nucleus) was not accompanied by the appearance of a differentiated morphology following ATRA treatment. This suggested that nuclear PKC-ζ is necessary but not sufficient to induce granulocytic differentiation of HL-60 myeloid cells. On the other hand, phorbol ester exposure resulted in an increase in PKC-δ within the nucleus of these cells [Owen et al., 1996].

NB4 cells share with HL-60 cells the ability to differentiate upon ATRA or vitamin D_3 treatment but they are considered a "true" model for human promyelocytic leukemia since they always carry the t (15;17) translocation [e.g., Dyck et al., 1994]. Following exposure to vitamin D_3 a rapid nuclear translocation of both -α and -δ isoforms was seen in NB4 cells, accompanied by a phosphorylation of downstream protein signaling intermediates [Berry et al., 1996].

Another model to study the events coupled to monocytic differentiation is represented by the U937 human promyelocytic cell line which responds to phorbol esters with growth arrest and substratum adherence [Harris and Ralph, 1985]. Upon treatment with phorbol esters, PKC-ζ, which is primarily cytosolic in undifferentiated cells, accumulated inside the nucleus [Kiley and Parker, 1995].

GM-CFC have the potential to differentiate into either macrophages and/or neutrophils when they are treated with cytokines such as M-CSF or SCF [Metcalf and Nicola, 1991]. M-CSF stimulated the translocation of PKC-α to the nucleus and differentiated the cells into macrophage. In contrast, SCF was unable to induce such a translocation. The data indicated a role for PKC-α in M-CSF- but not SCF-stimulated macrophage development of GM-CFC [Whetton et al., 1994]. Additional experiments, carried out by overexpressing a constitutively activated form of PKC-α, showed the protein to be located primarily within the nucleus and caused macrophage development even in the presence of stimuli that normally promote only neutrophilic development. Therefore, M-CSF-stimulated translocation of PKC-α

to the nucleus is a signal associated with macrophage development in primary mammalian hematopoietic progenitor cells [Pierce et al., 1998].

The pluripotent hemopoietic HEL cell line undergoes megakaryocytic differentiation upon phorbol ester treatment [Marks et al., 1987]. Western-blot analysis of phorbol ester-treated cells showed that an early and transient increase of -α, -β_I, -ϵ, - ζ, and -θ isoforms within the nuclei was followed at 72 h (by which time cells had acquired a differentiated megakaryocytic phenotype) by a drastic decline of all but PKC- ζ isoform. Moreover, at 72 h PKC-δ de novo appeared within the nucleus [Zauli et al., 1996b].

In FELC commitment to hemoglobin synthesis in response to HMBA is completed by 24 h and proceeds to terminal differentiation by 96 h. PKC-α was found associated to the nucleus after 24 h of treatement and was absent at 96 h. In PKC-α antisense-transfected cells differentiation was blocked compared to that seen in vector-transfected cells suggesting an important temporal role for nuclear PKC-α localization in differentiating FELC [Mallia et al., 1999].

Phorbol esters are able to induce the differentiation of a human pre-B cell line, KM-3, which expresses TdT [Schneider et al., 1977]. This differentiation was accompanied by an intranuclear translocation of the -β_{II} isoform, via the nuclear pore complex, which associated with interchromatin regions, conceivably corresponding to the nuclear matrix [Trubiani et al., 1995a]. Interestingly, the same authors were subsequently able to demonstrate that TdT is a PKC substrate both in vivo and in vitro, thus suggesting that PKC-dependent TdT phosphorylation could play a key role in the pathway affecting the control of gene transcription and protein synthesis during lymphoid cell differentiation [Trubiani et al., 1995b].

Nevertheless, nuclear PKC isozymes seem to be also involved in the differentiation of cells of different lineage.

B16 melanoma cell line differentiates upon treatment with RA. This differentiation was found to be accompanied by the increase of nuclear-associated PKC-α levels. Moreover, overexpression of PKC-α resulted in a more differentiated phenotype. This relevance of PKC-α in the control of differentiation could be mediated by increased AP-1 activity [Gruber et al., 1995].

Treatment with either vitamin D_3 or phorbol esters of the renal epithelial cell line MDBK induced translocation of PKC-β to the nuclear membrane. The effects of these chemicals appeared to be limited to the Ca^{++}-dependent isozymes since they had no effect on the Ca^{++}-independent isoform, PKC-ζ. These data specifically implicated PKC-β in the control of vitamin D_3-mediated nuclear events at kidney level which result in the expression of calbindin D-28K, a Ca^{++}-binding protein involved in transepithelial Ca^{++} transport [Simboli-Campbell et al., 1994]. Nuclear PKC isoforms have been extensively investigated also in the rat pheochromocytoma cell line PC-12, which can be induced to differentiate into sympathetic neurons upon treatment with NGF [Levi Montalcini, 1987]. In untreated PC-12 cells several isoforms were detected in the nucleus: -α, -β_{II}, -δ, -ϵ, -η, and -ζ. In fully differentiated cells the -α isozyme increased whereas it was observed a complete loss of the -δ isoform at the nuclear level [Borgatti et al., 1996]. Further studies have shown that in nuclei the atypical -ι/ λ isoform is also present [Wooten et al., 1997]. Moreover, a NGF dose-dependent, phorbol ester-insensitive increase in nuclear PKC-ζ was observed, with a concomitant decrease in cytoplasmic immunoreactivity, and conceivably it was required for the NGF-induced differentiation [Wooten et al., 1997]. Since these findings suggested that PKC-ζ might be important for the regulation of nuclear processes, the same investigators explored the presence of specific PKC-ζ substrates in the nucleus of PC-12 cells. They characterized a 106-kDa nuclear protein which is phosphorylated both in vivo and in vitro by PKC-ζ. The amino acid sequence analysis revealed that this protein was homologous to nucleolin, a polypeptide involved in several aspects of RNA metabolism, including pre-rRNA synthesis, rRNA processing, ribosomal assembly and maturation [Tuteja and Tuteja, 1998]. They also documented an enhanced phosphorylation of nucleolin following NGF treatment [Zhou et al., 1997]. In this connection, it is interesting to remind that another specific nuclear substrate for PKC-ζ has been identified as the heterogeneous ribonucleoprotein A1 [Municio et al., 1995]. Taken together, these findings suggest that PKC-ζ could be critically involved in a pathway that connects membrane signaling to nuclear regulatory events at the level of RNA processing and transport.

Sperm maturation can be considered a natural model of terminal cell differentiation [Skinner, 1991].

It can, therefore, represent a valuable system to study the role of various PKC isoforms in differentiation. A first report [Caramelli et al., 1996], in which the authors employed an anti-PKC polyclonal antibody recognizing several canonical isozymes [Martelli et al., 1991], evidentiated that in rat seminiferous tubules the maturation process was paralleled by a striking downregulation of nuclear PKC. Subsequently, a more detailed study was performed by the same group, employing isoform-specific antisera, which indicated a progressive reduction of the isozymes (-α, -β_I, -β_{II}, -ggr;, -δ, -ϵ, -ζ, -θ) present in the early stages of spermatogenesis so that in late spermatids no one of them was found in the nucleus [Zini et al., 1997a]. In contrast, some isoforms were specifically retained in the acrosome (-α,- β_{II}, -γ), in the neck (-β_I, -ζ), or in the tail (-ϵ), suggesting a functional involvement of different isoforms in some events of spermatozoa differentiation. Furthermore, disassembly of the sperm nuclear envelope at fertilization is one of the earliest events in the development of the pronucleus. Nuclear lamina disassembly in interphase sea urchin egg cytosol is a result of lamin B phosphorylation mediated by PKC as demonstrated by immunodepletion of cytosolic PKC, restoration by addition of purified rat brain PKC, and use of specific PKC inhibitors, suggesting that PKC is the major kinase required for interphase disassembly of lamina [Collas et al., 1997].

A list of nuclear PKC isozymes implicated in cell differentiation is reported in Table IV.

Nuclear PKC and Neoplastic Transformation

Many of the articles mentioned in the previous sections deal with phenomena studied in cell lines derived from neoplasms. This fact already underlines the importance of PKC isozymes in the regulation of tumor biology. However, there are papers suggesting a direct relationship between PKC and neoplastic transformation. In this connection, it should be emphasized that much of the interest that has surrounded PKC initially stemmed from the identification of this enzyme as the high affinity intracellular receptor for phorbol esters, a class of powerful tumor promoters [Hug and Sarre, 1993].

Cadmium is a carcinogen with weak genotoxicity, but may be tumor-promoting since it interferes with several steps of cellular signal transduction. In mouse 3T3/10 T ½ fibroblasts cadmium potentiated the effect of phorbol ester on nuclear binding and activation of PKC. Furthermore, in a reconstituted in vitro system, cadmium stimulated PKC binding to a 105-kDa nuclear protein [Beyersmann et al., 1994]. It was hypothesized that cadmium substitutes for zinc in the regulatory domain of PKC thus

TABLE IV. Nuclear PKC Isoforms Involved in Cell Differentiation

PKC isoform	Agonist	Cell line	Reference
α	ATRA	HL-60	Zauli et al., 1996a
	Vitamin D_3	NB4	Berry et al., 1996
	M-CSF	Hematopoietic progenitors	Whetton et al., 1994; Pierce et al., 1998
	HMBA	FELC	Mallia et al., 1999
	ATRA	B16 melanoma	Gruber et al., 1995
	NGF	PC-12	Borgatti et al., 1996
β (subtype not specified)	Vitamin D_3	MDBK	Simboli-Campbell et al., 1994
β_{II}	PMA	KM-3	Trubiani et al., 1995a
	NGF	PC-12	Borgatti et al., 1996
δ	PMA	HL-60	Owen et al., 1996
	PMA	HEL	Zauli et al., 1996b
	Vitamin D_3	NB4	Berry et al., 1996
	NGF	PC-12	Borgatti et al., 1996
ϵ	PMA	HEL	Zauli et al., 1996b
	NGF	PC-12	Borgatti et al., 1996
ζ	Vitamin D_3	HL-60	Bertolaso et al., 1998
	PMA	U937	Kiley and Parker, 1995
	NGF	PC-12	Borgatti et al., 1996
			Wooten et al., 1997

rendering the putative protein-protein site exposed. Interestingly, this 105-kDa protein might correspond to nucleolin, a PKC substrate (see above).

In diethylnitrosamine-induced hepatocarcinogenesis both PKC activity in the nuclear fraction and PKC-β expression were maximal at 60 days post-hepatectomy. As a result, it has been suggested a role for nuclear PKC-β in promoting the selective growth of carcinogen-initiated hepatocytes in rat liver [La Porta and Comolli, 1994]. Moreover, using the same experimental model nuclear PKC-β increased activity and expression were detected in lung metastatic nodules suggesting a possible role of such an isozyme in the development of secondary tumors [La Porta et al., 1997].

A point mutation of PKC-α was discovered in a subpopulation of human pituitary tumors characterized by their invasive phenotype as well as in some thyroid neoplasms. When overexpressed in Rat 6 embryo fibroblasts, after treatment with TPA wild-type PKC-α mainly translocated to the plasma membrane while the mutated form translocated primarily to the perinuclear region and slightly to the nucleus. The mutant form was involved in alterations of the growth properties of these cells that displayed a decreased requirement for serum when compared to cells expressing the normal human PKC-α, and they also formed small colonies in soft agar, a characteristic which was not shared by control cells. These growth alterations may be due to the aberrant intracellular translocation of the mutated isozyme, because the mutated PKC-α displayed the same catalytic activity as the wild-type isoform [Alvaro et al., 1997]. Interestingly, this mutation is located in the V3 hinge region separating the regulatory from the catalytic domain of the enzyme (at position 294 of the amino acid sequence) and leads to the substitution of an aspartic acid by a glycine. The V3 region contains the nuclear targeting sequence originally described by James and Olson [1992]. Therefore, a mutation close to this sequence could result in altered subcellular localization by unmasking targeting sites. Alternatively, it might be that the mutation modifies the affinity of the kinase towards its binding-protein(s), even though it is commonly thought that constant rather than variable regions of PKC contain the primary determinants of the binding activity [e.g., Liao et al., 1994]. Indeed, Prevostel et al. [1998] have recently reported that, although the mutated PKC-α isozyme retains an unaltered intrinsic lipid-dependent catalytic activity, there is a selective loss of substrate recognition towards certain previously cloned substrates, referred to as 35F and 35H proteins, which are predicted to be PKC-α anchoring proteins. In any case, in the future, it will be of extreme interest to analyze in detail whether or not other mutated isoforms of PKC are selectively expressed by different types of neoplasms.

In human SKBR-3 human breast cancer cells, stimulation of c-erb B-2 receptor tyrosine kinase induced a rapid and marked translocation of PKC-β_I and -ζ into the nucleus. In parallel, histone phosphorylation was increased in the particulate fraction. It was concluded that c-erb B-2 signal transduction may involve the activation of specific PKC isozymes in cancer cells [Disatnik et al., 1994].

Cell fractionation of B16a melanoma cells indicated that PKC-α could be detected in several particulate fractions including plasma membrane, cytoskeleton, endosomal compartment, and nuclei and may indicate the existence of a functional signaling complex in the cytoplasm and in the nucleus in addition to the plasma membrane of cancerous cells [Timar et al., 1996]. PKC isozymes conceivably involved in neoplastic transformation are shown in Table V.

The possible involvement of PKC isoforms in neoplastic transformation and progression immediately prompts the question as to whether or not this family of enzymes could be a potential target for one or more anti-tumor drugs.

Treatment of U-373 human glioma cells with human α-2b IFN, a cytokine with anti-proliferating function used for treatment of certain forms of tumors, modulated the subcellular distribution of two PKC isozymes (-α and -β). Nuclear PKC-α decreased by 80% after IFN exposure while PKC-β showed no significant changes. This may indicate a key role for nuclear PKC-α in controlling neoplastic cell growth [Acevedo-Duncan et al., 1995]. In the SKBR-3 human breast carcinoma cell line, phorbol esters increase the cytotoxicity of CDDP, one of the most potent anticancer agents, with activity against solid tumors. After examination of the effect of CDDP per se on PKC isozymes, it resulted that, of different six isoforms present, exposure to 10–100 μmM CDDP for 3 h induced

TABLE V. Nuclear PKC Isozymes Involved in Neoplastic Transformation

Transforming agent	PKC isoform	Effect	Cell line/tissue	Reference
Cadmium	Unknown	Increased PKC binding to 105-kDa protein	3T3/10 T½ fibroblasts	Beyersmann et al., 1994
Diethylnitrosamine	β	Tumor development	Liver tumors	La Porta and Comolli, 1994
		Metastatic progression	Liver tumors	La Porta et al., 1997
?	Mutated α	Altered subcellular localization	Pituitary tumors	Alvaro et al., 1997
?	β_I, ζ	Neoplastic transformation	SKBR-3	Disatnik et al., 1994
?	α	Neoplastic transformation	B16a melanoma	Timar et al., 1996

only PKC-ϵ translocation from the plasma to the nuclear membrane, as observed by immunoblotting and CLSM. This translocation was also induced by the cisplatin analog carboplatin but not by the anti-cancer agent adriamycin and taxol. These data supported a role for PKC-ϵ translocation in CDDP-mediated cytotoxicity [Ohmori and Arteaga, 1998].

The potent anti-neoplastic agent DNIG displayed a well documented anti-PKC activity and an ability to reverse multi-drug resistance. In FELC DNIG was able to block HMBA-induced differentiation and this was accompanied by an increase in the amount of nuclear PKC-α. These results suggest that DNIG may increase the effect of other chemioterapic drugs, particularly S-phase specific ones by increasing the length of S-phase and decreasing multidrug-resistance [Patterson et al., 1996].

The mechanism of development of multidrug-resistance to a broad spectrum of chemotherapeutic agents is an issue of fundamental importance in the treatment of neoplastic disease. In many cases the resistance has been related to an enhanced drug efflux from cells due to the overexpression of the membrane-associated 170-kDa P-glycoprotein [e.g., Bradley et al., 1989]. Early investigations showed in multidrug-resistant MCF-7 human breast carcinoma cells elevated levels of intranuclear PKC-α, suggesting that altered transcription of this protein can promote the resistance [Lee et al., 1992]. However, Zini et al. [1997b] came to the conclusion that changes in nuclear PKC activity were not linked to the mechanism(s) of multidrug-resistance in Saos-2 human osteosarcoma cells, even though other elements of the nuclear inositide signaling system varied. Somewhat different findings have recently been reported by La Porta et al. [1998] in LoVo human colon adenocarcinoma cells treated with doxorubicin. When wild-type cells were exposed to Go6976, a specific inhibitor of PKC-α and -β isotypes, a significant increased survival was observed. In contrast, resistant LoVo cells did not show significant changes in the survival to doxorubicin exposure when incubated in the presence of Go6976. Moreover, the use of the inhibitor reduced PKC-α activity in both resistant and not resistant cells, accompanied by an increased expression of the isozyme. However, under such conditions increased nuclear activity of PKC-α and enhancement of P-glycoprotein expression occurred only in wild-type cells. Therefore, the data seem to indicate a specific relationship between PKC-α inhibition, increased nuclear PKC-α activity as well as the increased expression of P-glycoprotein, possibly causing the acquisition of a resistant phenotype in LoVo cells. Due to the importance of this issue, additional investigations are undoubtedly necessary to explain the above reported discrepancies.

Nuclear PKC and Apoptosis

Cell death by apoptosis, first described by Kerr et al. [1972], plays a crucial role in mammalian organisms. Indeed, a complete analysis of any population whatsoever of mammalian cells must take into account the possible relevance of active cell death by apoptosis, besides other obvious processes like proliferation and differentiation. Thus, apoptosis plays a fundamental role during development, especially in the central nervous tissues and in the immune system [Williams, 1994], or during aging [Vaux and Strasser, 1996]. Moreover, a growing body of evidence indicates that failure or suppression of apoptosis is most likely to contribute to the initial development of cancer as well as to the appearance of neoplastic cells resistant to chemotherapy [Williams, 1991]. The most dramatic modifications occurring during the apop-

totic process take place in the nucleus. Indeed, chromatin progressively clusters and wide areas of condensed chromatin appear marginated, at first close to the nuclear envelope, and then in well defined cap-shaped formations at the nuclear poles. These morphological changes are accompanied by biochemical modifications, consisting of proteolytic degradation of polypeptides (of which several reside in the nucleus) and internucleosomal DNA degradation [Vaux and Strasser, 1996; Martelli et al., 1997].

Nuclear protein phosphorylation is likely to play an important role in the regulation of apoptosis [Martelli et al., 1997]. There a few reports concerning the phosphorylation of nuclear proteins during apoptosis: in dexamethasone-treated thymocyte cultures phosphorylation and cleavage of 240-kDa NuMA protein are very early events, detectable after a 30 min exposure to the hormone and preceding internucleosomal DNA cleavage [Weaver et al., 1996]. Conceivably, NuMA phosphorylation, that is normally seen in mitotic cells only, might trigger conformational change(s) within the protein, rendering it a target for a proteolytic enzyme. NuMA cleavage might lead to a loss of interactions with other nuclear proteins and depolymerization of the nuclear matrix. In this connection it is worth mentioning that Strasberg-Rieber and Rieber [1992] reported that when A431 human carcinoma cells are exposed to the protein phosphatase inhibitor, okadaic acid, endonuclease accessibility to DNA is increased and there is a concurrent hyperphosphorylation of a 70-kDa nuclear matrix protein.

The very first report linking PKC nuclear translocation to apoptosis was published in 1994 by Trubiani et al. and showed a redistribution of the kinase toward the nucleus in dexamethasone-treated thymocytes. Following this report, a possible role of PKC during apoptosis has been analyzed in other cell models deriving from hemopoietic precursors. In spontaneously apoptotic U937 cells PKC-δ showed a reduced nuclear level of expression while PKC-α was increased in this cell compartment [Pongracz et al., 1995]. A direct link between PKC and apoptosis was very recently demonstrated by Shimizu et al. [1998] in HL-60 cells induced to apoptosis by camptothecin. Indeed, the α isoform of PKC phosphorylated lamin B 1 h after the addition of the drug and this phenomenon preceded both lamin proteolytic degradation and DNA fragmentation. The role of PKC-α was supported by the use of the kinase inhibitor staurosporine, that prevented lamin B phosphorylation. Nevertheless, different PKC isozymes may play a crucial role both in the activation and in the suppression of apoptosis. For example, in an IL-3-dependent hemopoietic cell line, the v-Abl mediated suppression of apoptosis was associated with nuclear translocation of PKC-β_{II} [Evans et al., 1995].

The c-Abl non-receptor tyrosine kinase was identified as the mammalian homolog of the v-Abl oncogene from the Abelson murine leukemia virus. It is well established that ionizing radiations activate c-Abl [Yuan et al., 1998 and references therein]. It has recently been shown that the SH3 domain inteacts directly with PKC-δ [Yuan et al., 1998]. Upon exposure of human MCF-7 breast cancer cells to ionizing radiations PKC-δ was phosphorylated and activated by c-Abl, then it traslocated to the nucleus. Since both c-Abl and PKC-δ have been implicated in the induction of apoptosis, translocation to the nucleus of this particular PKC isozyme might be related to the nuclear changes ocurring during the execution phase of apoptosis.

There is some evidence that a mechanism leading to apoptosis in heart is ischemia, in which PKC seems to play an important role. Indeed, a translocation to the nucleus of PKC-α and -ϵ occurred after 5 to 40 min of ischemia in rat heart, while the -δ isoform moved from plasma membrane to the cytosolic fraction [Yoshida et al., 1996]. In addition, ischemia provoked the translocation of PKC-ζ from cytosol to nucleus that could be inhibited by wortmannin, an inhibitor of PI 3-kinase [Mizukami et al., 1997]. Somewhat different results about the isozymes which translocate to the nucleus following myocardial ischemia have very recently been published by Albert and Ford [1998]. Indeed, they found that the -ϵ, -η, and -ι isoforms associated with rat myocardial nuclei in response to brief (10–20 min) interval of global ischemia as well as reperfusion of ischemic myocardium. Moreover, they were able to demonstrate an increased overall protein phosphorylation in nuclei prepared from ischemic myocardium. If hearts, prior to ischemia, were exposed to the selective PKC inhibitor, BIM I, nuclear protein phosphorylation was inhibited, even though the -ϵ, -η, and -ι isoforms still translocated to the nucleus.

Taken together, these data indicate that apoptosis, or its prevention, induces different patterns of nuclear translocation of PKC isozymes, suggesting marked differences in their functional roles.

In Table VI we list nuclear PKC isoforms involved in apoptosis.

Concluding Remarks

More than 10 years after the presence of PKC was for the first time demonstrated in rat liver nuclei, a wealth of evidence has been collected about the possible involvement of this family of enzymes in the regulation of many events taking place in the nucleus. Conceivably, the key step to understanding the exact roles played by nuclear PKC is the identification of its substrates in this organelle and the analysis of changes in their functions caused by phosphorylation. Although PKC is able to phosphorylate many nuclear proteins in vitro [see Buchner, 1995] only a few of them are substrate also in vivo. The identified in vivo nuclear substrates of PKC include lamin B, DNA topoisomerase I, p53, myogenin, the vitamin D_3 receptor, TdT, and nucleolin [Buchner, 1995; Trubiani et al., 1995b; Zhou et al., 1997], but in some cases it is not clear whether phosphorylation actually takes place in the nucleus or in the cytoplasm. Phosphorylation has been demonstrated to alter the properties of some of these substrates (lamin B, DNA topoisomerase I, myogenin, the vitamin D_3 receptor), therefore presenting a biological relevance [Buchner, 1995].

Conceivably, nuclear PKC is also involved in the regulation of complex signaling pathways at the nuclear level. For example, Topham and coworkers [1998] suggested that PKC can, itself, regulate the levels of nuclear DAG by inhibiting the nuclear entry of DAG kinase-ζ, an enzyme that removes DAG by converting it into phosphatidic acid. Indeed, in EGF-treated A172 glioblastoma cells the level of DAG kinase-ζ in the nucleus is controlled by the PKC-mediated phosphorylation of a MARCKS (myristoylated alanine-rich c-kinase substrate) homology domain, which seems to encode a NLS. Even if it is not known whether DAG kinase-ζ was phosphorylated in the cytosol or in the nucleus, the outcome is a positive-feedback loop, such that PKC-mediated phosphorylation of DAG kinase-ζ leads to an increase in the levels of nuclear DAG. Moreover, Yu et al. [1998] have recently demonstrated that in vitro phosphorylation of histone H1 by PKC (but not by PKA or cdc2 kinase) decreases the amount of PIP_2 bound to the histone. In a Drosophila in vitro transcription system, PIP_2 binding to H1 counteracted the H1-mediated repression of basal transcription by RNA polymerase II. The authors suggested that PIP_2 binding to H1 may contribute to the regulation of transcription in eukaryotic cells and they were also able to extract PIP_2 from chromatin. Since the presence of PIP_2 at the nuclear level has been reported by other investigators [e.g., Mazzotti et al., 1995], translocation of PKC to the nucleus may fulfill yet another role, especially taking into account the fact that histone H1 seems to be a target in vivo for PKC which translocates to the nucleus of Swiss 3T3 cells after a mitogenic stimulus with IGF-I [Martelli et al., 1989].

Although substantial advances have recently been made in the field of nuclear PKC there seems little doubt that a large body of information is still to be discovered. In particular, the functions of the different isozymes identified at the nuclear level, their substrate specificities, and the mechanisms that mediate their translocation to the nucleus remain vague. Therefore, they must be defined. Molecular biological approaches should now make this possible: (i)

TABLE VI. Nucler PKC Isoforms Involved in Apoptosis

Agent	Cell line/tissue	PKC isoform	Reference
Dexamethasone	Thymocytes	Not specified	Trubiani et al., 1994
Spontaneous apoptosis	U937	α, δ	Pongracz et al., 1995
Camptothecin	HL-60	α	Shimizu et al., 1998
IL-3 deprivation	IC.DP	β_{II}	Evans et al., 1995
Ionizing radiations	MCF-7	δ	Yuan et al., 1998
Ischemia	Rat heart	α, ϵ	Yoshida et al., 1996
		ζ	Mizukami et al., 1997
		ϵ, η, ι	Albert and Ford, 1998

stable overexpression of PKC isozyme cDNAs [Goodnight et al., 1995] under the control of constitutive and inducible promoters and (ii) antisense mRNA strategy can increase or decrease, respectively, the levels of individual isozymes leading to a better understanding of their specific functional roles; (iii) overexpression of selectively mutated PKC cDNAs to abolish NLS motifs should definitely clarify the sequences important for nuclear import of PKC; (iv) expression of chimerae of the regulatory and catalytic domains of different isozymes [Walker et al., 1995] should provide the identification of the sequences required for interactions with proteins and lipids [Liao et al., 1994] thereby allowing the designing of new classes of PKC inhibitors with the potential for isotype selectivity.

Finally, the contribution of aberrant expression or regulation of nuclear PKC to human disease ranging from cataract to atherosclerosis and to cancer, must be further investigated and it is a challenging possibility for the future that PKC and its nuclear substrates will provide important targets for development of novel therapeutic agents.

ACKNOWLEDGMENTS

The authors thank Giovanna Baldini for the illustrations. This work was supported by Associazione Italiana per la Ricerca sul Cancro (A.I.R.C.) grants (to A.M.M. and S.C.), Progetto Finalizzato Biotecnologie (CNR; to S.C.), by Italian MURST Cofinanziamento 1998 to the University of Ferrara, and 60% grants to the Universities of Trieste and Ferrara, and a grant for biomedical research Az. Osp. "Arcispedale S.Anna"—University of Ferrara.

L.M.N. is grateful to Paola Ziccone for her continuous support, encouragement, and understanding.

REFERENCES

Acevedo-Duncan M, Collins J, Zhang R, Haller E, Cooper DR. 1995. In situ effects of interferon on human glioma PKC α and β ultrastructural localization. Cell Growth Differ 6:1353–1365.

Alam R, Ktaoka S, Alam S, Yatsu F. 1996. Inhibition of vascular smooth muscle cell proliferation by the calcium antagonist clentiazem: role of protein kinase C. Atherosclerosis 126:207–219.

Albert CJ, Ford DA. 1998. Identification of specific nuclear protein kinase C isozymes and accelerated protein kinase C-dependent nuclear protein phosphorylation during myocardial ischemia. FEBS Lett 438:32–36.

Albi E, Viola Magni MP. 1996. Chromatin neutral sphingomyelinase and its role in hepatic regeneration. Biochem Biophys Res Commun 236:29–33.

Almholt K, Arkhammer POG, Thastrup O, Tullin S. 1999. Simultaneous visualization of the translocation of protein kinase Cα-green fluorescent protein hybrids and intracellular calcium concentrations. Biochem J 337:211–218.

Alvaro V, Prevostel C, Joubert D, Slosberg E, Weinstein BI. 1997. Ectopic expression of a mutant form of PKC α originally found in human tumors: aberrant subcellular translocation and effects on growth control. Oncogene 14:677–685.

Bastiaens PIH , Jovin TM. 1996. Microspectroscopic imaging tracks the intracellular processing of a signal transduction protein: fluorescent labeled protein kinase C β_I. Proc Natl Acad Sci USA 93:8407–8412.

Beckmann R, Lindschau C, Haller H, Hucho F, Buchner K. 1994. Differential nuclear localization of protein kinase C isoforms in neuroblastoma x glioma hybrid cells. Eur J Biol Chem 222:335–343.

Berezney R, Mortillaro M J, Ma H, Wei X, Samarabandu J. 1995. The nuclear matrix: a structural milieu for genomic function. Int Rev Cytol 162A:1–65.

Berry DM, Antochi R, Bhatia M, Meckling-Gill KA. 1996. 1,25-Dihydroxyvitamin D_3 stimulates expression and translocation of protein kinase C α and δ via a non genomic mechanism and rapidly induces phosphorylation of a 33 kDa protein in acute promyelocytic NB4 cells. J Biol Chem 271:16090–16096.

Bertolaso L, Gibellini D, Secchiero P, Previati M, Falgione D, Visani G, Rizzoli R, Capitani S, Zauli G. 1998. Accumulation of catalytically active PKC-ζ into the nucleus of HL-60 cell line plays a key role in the induction of granulocytic differentiation mediated by all-trans-retinoic acid. Br J Haematol 100:541–549.

Beyersmann D, Block C, Malviya AN. 1994. Effects of cadmium on nuclear protein kinase C. Environ Health Perspect 102:177–180.

Borgatti P, Mazzoni M, Carini C, Neri LM, Marchisio M, Bertolaso L, Previati M, Zauli G, Capitani S. 1996. Changes of nuclear PKC activity and isotype composition in PC12 cell proliferation and differentiation. Exp Cell Res 224:72–78.

Bradley G, Naik M, Ling V. 1989. P-glycoprotein expression in multidrug-resistant human ovarian carcinoma cell lines. Cancer Res 49:2790–2796.

Brooksbank CE, Hutchings A, Butcher GW, Irvine RF, Divecha N. 1993. Monoclonal antibodies to phosphatidylinositol 4-phosphate 5-kinase: distribution and intracellular localization of the C isoform. Biochem J 291:77–82.

Buchner K. 1995. Protein kinase C in the transduction of signals toward and within the cell nucleus. Eur J Biochem 228:211–221.

Buchner K, Lindskau C, Hucho F. 1997. Nuclear localization of protein kinase C α and its association with nuclear components in Neuro-2a neuroblastoma cells. FEBS Lett 406:61–65.

Capitani S, Cocco L, Maraldi NM, Papa S, Manzoli FA. 1986. Effect of phospholipids on transcription and ribonucleoprotein processing in isolated nuclei. Adv Enzyme Regul 25:425–438.

Capitani S, Girard PR, Mazzei GJ, Kuo JF, Berezney R, Manzoli FA. 1987. Immunochemical characterization of protein kinase C in rat liver nuclei and subnuclear fractions. Biochem Biophys Res Commun 142:367–375.

Caramelli E, Matteucci A, Zini N, Carini C, Guidotti L, Ricci D, Maraldi NM, Capitani S. 1996. Nuclear phosphoinositide-specific phospholipase C, phosphatidylinositol 4,5-bisphosphate and protein kinase C during rat spermatogenesis. Eur J Cell Biol 71:154–164.

Carpenter CL, Cantley LC. 1996. Phosphoinositide 3-kinase and the regulation of cell growth. Biochim Biophys Acta 1288:11–16.

Chapline C, Mousseau B, Ramsay K, Duddy S, Li Y, Kiley SC, Jaken S. 1996. Identification of a major PKC-binding protein and substrate in rat embryo fibroblasts. J Biol Chem 271:6417–6422.

Chapline C, Cottom J, Tobin H, Hulmes J, Crabb J, Jaken S. 1998. A major, transformation-sensitive PKC-binding protein is also a PKC substrate involved in cytoskeletal remodeling. J Biol Chem 273:19482–19489.

Chen C-S, Poenie M. 1993. New fluorescent probes for protein kinase C. J Biol Chem 268:15812–15822.

Chou MM, Hou W, Johnson J, Graham LK, Lee MH, Chen CS, Newton AC, Schaffausen BS, Toker A. 1998. Regulation of protein kinase C ζ by PI 3-kinase and PDK-1. Curr Biol 8:1069–1077.

Clemens MJ, Trayner WI, Menaya J. 1992. The role of protein kinase C isoenzymes in the regulation of cell proliferation and cell differentiation. J Cell Sci 203:881–887.

Cocco L, Martelli AM, Gilmour RS. 1994. Inositol lipid cycle and the nucleus. Cell Signal 6:481–485.

Collas P, Thompson L, Fields AP, Poccia DL, Courvalin JC. 1997. PKC-mediated interphase lamin B phosphorylation and solubilization. J Biol Chem 272: 21274–21280.

Collins S. 1987. The HL-60 promyelocytic leukemia cell line: proliferation, differentiation, and cellular oncogene expression. Blood 70:1233–1244.

Das A, Park J, Hagen C, Parsons M. 1998. Distinct domains of a nucleolar protein mediate protein kinase binding, interactions with nuclei acids and nucleolar localization. J Cell Sci 111:2615–2623.

de Moel MP, van Emst-de Vries SE, Willems PH, de Pont JJ. 1998. Purification and isotype analysis of protein kinase C from rat liver nuclei. Int J Biochem Cell Biol 30:185–195.

Dingwall C, Laskey RA. 1991. Nuclear targeting sequences—a consensus? Trends Biochem Sci 16:478–481.

Disatnik MH, Winnier AR, Mochly-Rosen D, Arteaga CL. 1994. Distinct responses of PKC isozymes to c-erb-2 activation in SKBR-3 human breast carcinoma cells. Cell Growth Differ 5:873–880.

D'Santos CS, Clarke JH, Divecha N. 1998. Phospholipid signalling in the nucleus [Een DAG uit het leven van de inositide signalering in de nucleus]. Biochim Biophys Acta 1436:201–232.

Dyck JA, Maul GG, Miller WH, Chen JD, Kakizuka A, Evans RM. 1994. A novel macromolecular structure is a target of the promyelocyte-retinoic acid receptor oncoprotein. Cell 76:334–343.

Evans CA, Lord JM, Owen-Linch PJ, Johnson G, Dive C, Whetton D. 1995. Suppression of apoptosis by v-Abl protein tyrosine kinase is associated with nuclear translocation and activation of protein kinase C in an interleukin-3-dependent haemopoietic cell line. J Cell Sci 108: 2591–2598.

Fan G, Rillema JA. 1993. Prolactin stimulation of protein kinase C in isolated mouse mammary gland nuclei. Horm Metab Res 25:564–568.

Fields AP, Tyler G, Kraft AS, May WS. 1990. Role of nuclear protein kinase C in the mitogenic response to platelet-derived growth factor. J Cell Sci 96:107–114.

Ganguli S, Hu L, Menke P, Collier RJ, Jertler A. 1996. Nuclear accumulation of multiple protein kinases during prolactin induced proliferation of Nb2 rat lymphoma cells. J Cell Physiol 167:251–260.

Godson C, Masliah E, Balboa MA, Ellisman MH, Insel PA. 1996. Isoform-specific redistribution of protein kinase C in living cells. Biochim Biophys Acta 1313:63–71.

Gokmen-Polar Y, Fields AP. 1998. Mapping of a molecular determinant for protein kinase C- PKC-βII isozyme function. J Biol Chem 273:20261–20266.

Gonzalez-Charneco K, Takemoto D. 1998. Localization of protein kinase C in normal and galactosemic bovine lens epithelial cells in culture. Histochem Cell Biol 110:89–94.

Goodnight J, Mischak H, Kolch W, Mushinski JF. 1995. Immunocytochemical localization of eight protein kinase C isozymes overexpressed in NIH 3T3 fibroblasts. J Biol Chem 270:9991–10001.

Gordon AS, Yao L, Wu ZL, Coe IR, Diamond I. 1997. Ethanol alters the subcellular localization of δ- and ϵ-PKC in NG108–15 cells. Mol Pharmacol 52:554–559.

Goss VL, Hocevar BA, Thompson LJ, Stratton CA, Burns DJ, Fields AP. 1994. Identification of nuclear β_{II} protein kinase C as a mitotic lamin kinase. J Biol Chem 269: 19074–19080.

Gruber JR, Desai S, Blusztajn JK, Niles RM. 1995. Retinoic acid specifically increases nuclear PKC α and stimulates AP-1 transcriptional activity in B16 mouse melanoma cells. Exp Cell Res 221:377–384.

Haller H, Quass P, Lindschau C, Luft FC, Distler A. 1994. Platelet-derived growth factor and angiotensin II induce different spatial distribution of PKC α and β in vascular smooth muscle cells. Hypertension 23:848–852.

Haller H, Maasch C, Lindschau C, Brachmann M, Buchner K, Luft FC. 1998. Intracellular targeting and protein kinase C in vascular smooth muscle cells: specific effects of different membrane-bound receptors. Acta Physiol Scand 164:599–609.

Harris P, Ralph P. 1985. Human leukemic models of myelomonocytic development: a review of the HL-60 and U937 cell lines. J Leuk Biol 37:407–422.

Hatanaka M. 1990. Discovery of the nucleolar targeting signal. Bioessays 12:143–148.

Hennekes H, Peter M, Weber K, Nigg EA. 1993. Phosphorylation on protein kinase C sites inhibits nuclear import of lamin B2. J Cell Biol 120:1293–1304.

Hicks GR, Raikhel RV. 1995. Protein import into the nucleus: an integrated view. Annu Rev Cell Dev Biol 11:155–188.

Hocevar BA, Burns DJ, Fields AP. 1993. Identification of PKC phosphorylation sites on human lamin B. J Biol Chem 268:7545–7552.

Hug H, Sarre TF. 1993. Protein kinase C isoenzymes: divergence in signal transduction? Biochem J 291:329–343.

Huwiler A, Fabbro D, Pfeilschifter J. 1998. Selective ceramide binding to protein kinase C-α and -δ isozymes in renal mesangial cells. Biochemistry 37:14556–14562.

Hyatt SL, Liao L, Chapline C, Jaken S. 1994. Identification and characterization of α-PKC binding proteins in normal and transformed REF 52 cells. Biochemistry 33:1223–1228.

Jaken S. 1996. Protein kinase C isozymes and substrates. Curr Opin Cell Biol 8:168–173.

James G, Olson EN. 1992. Deletion of the regulatory domain of protein kinase C α exposes regions in the hinge and catalytic domains that mediate nuclear targeting. J Cell Biol 116:863–874.

Jans DA. 1995. The regulation of protein transport to the nucleus by phosphorylation. Biochem J 311:705–716.

Jones T, Courage C, Hubbard A, Gescher A. 1997. Cellular relocalization of protein kinase C-θ caused by staurosporine and some of its analogues. Biochem Pharmacol 53: 1413–1418.

Katoh N, Kira T, Yuasa. 1993. PKC substrates and ganglioside inhibitors in bovine mammary nuclei. J Dairy Sci 76:3400–3409.

Keenan C, Kelleher D. 1998. Protein kinase C and the cytoskeleton. Cell Signal 10:225–232.

Kerr JFR, Wyllie AH, Currie AR. 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239–257.

Kiley SC, Jaken S. 1994. PKC: interactions and consequences. Trends Cell Biol 4:223–227.

Kiley SC, Parker PJ. 1995. Differential localization of PKC isozymes in U937 cells: evidence for distinct isozyme function during cell differentiation. J Cell Sci 108:1003–1016.

Knutson KL, Hoenig M. 1997. Subnuclear localization of protein kinase C δ in beta cells. Biochem Mol Med 62:50–57.

La Porta CA, Comolli R. 1994. Membrane and nuclear PKC activation in the early stages of diethylnitrosamine-induced rat hepatocarcinogenesis. Carcinogenesis 15: 1743–1747.

La Porta CA, Tessitore L, Comolli R. 1997. Changes in PKC α, δ and in nuclear β isoform expression in tumor and lung metastatic nodules induced by diethylnitrosamine in the rat. Carcinogenesis 18:715–719.

La Porta CA, Dolfini E, Comolli R. 1998. Inhibition of protein kinase C-α isoform enhances the P-glycoprotein expression and the survival of LoVo human colon adenocarcinoma cells to doxorubicin exposure. Br J Cancer 78:1283–1287.

Leach KL, Powers EA, Ruff VA, Jaken S, Kaufmann S. 1989. Type III protein kinase C localization to the nuclear envelope of phorbol ester-treated NIH 3T3 cells. J Cell Biol 109:685–695.

Lee SA, Karaszkiewicz JW, Anderson WA. 1992. Elevated level of nuclear protein kinase C in multidrug-resistant MCF-7 human breast carcinoma cells. Cancer Res 52: 3750–3759.

Lee JY, Hannun YA, Obeid LM. 1996. Ceramide inactivates cellular protein kinase C-α. J Biol Chem 271:13169–13174.

Le Good JA, Ziegler WH, Parekh DB, Alessi DR, Cohen P, Parker PJ. 1998. Protein kinase C isotypes controlled by phosphoinositide 3-kinase trough the protein kinase PDK1. Science 281:2042–2045.

Levi Montalcini R. 1987. The nerve growth factor, thirty-five years later. EMBO J 6:1145–1154.

Liao L, Hyatt SL, Chapline C, Jaken S. 1994. PKC domains involved in interactions with other proteins. Biochemistry 33:1229–1233.

Liu WS, Heckman CA. 1998. The sevenfold way of PKC regulation. Cell Signal 10:529–542.

Livneh E, Fishman DD. 1997. Linking protein kinase C to cell cycle control. Eur J Biochem 248:1–9.

Lu PJ, Hsu AL, Wang DS, Yan HY, Yin HL, Chen CS. 1998. Phosphoinositide 3-kinase in rat liver nuclei. Biochemistry 37:5738–5745.

Mallia CM, Jeter JR, Fields AP, Wilson RB, Beckman BS. 1995. Protein kinase C β from Friend erythroleukemia cells is associated with chromatin and DNA. Mol Cell Biochem 151:107–111.

Mallia CM, Smith M, Clejan S, Beckman BS. 1997. Erythropoietin stimulates nuclear localization of diacylglycerol and protein kinase C βII in B6SUt.EP cells. J Lipid Mediat Cell Signal 17:135–150.

Mallia CM, Aguirre V, McGary E, Scandurro A, Noguki C, Beckman BS. 1999. Protein kinase Cα is an effector of hexamethylene-bis-acetamide-induced differentiation of Friend erythroleukemia cells. Exp Cell Res 246:348–354.

Malviya AN, Block C. 1992. A bipartite nuclear targeting motif in protein kinase C? Trends Biochem Sci 17:179–179.

Mani I, Iversen L, Ziboh WA. 1998a. Up regulation of nuclear PKC and MAP-kinase during hyperproliferation of guinea pig epidermis: modulation by 13-(S)-hydroxyoctadecadienoic acid. Cell Signal 10:143–149.

Mani I, Iversen L, Ziboh WA. 1998b. Evidence of nuclear PKC/MAP-kinase cascade in guinea model of epidermal hyperproliferation. J Invest Dermatol 112:42–48.

Marks PA, Sheffery M, Rifkind RA. 1987. Induction of transformed cells to terminal differentiation and the modulation of gene expression. Cancer Res 47:656–666.

Marinissen MJ, Capiati D, Boland R. 1998. 1,25 $(OH)_2$-vitamin D_3 affects the subcellular distribution of PKC isoenzymes in muscle cells. Cell Signal 10:91–100.

Martelli AM, Gilmour RS, Falcieri E, Manzoli FA, Cocco L. 1989. Mitogen stimulated phosphorylation of nuclear proteins in Swiss 3T3 cells: evidence for a PKC requirement. Exp Cell Res 185:191–202.

Martelli AM, Neri LM, Gilmour RS, Barker PJ, Huskisson NS, Manzoli FA, Cocco L. 1991. Temporal changes in intracellular distribution of protein kinase C in Swiss 3T3 cells during mitogenic stimulation with insulin-like growth factor I and bombesin: translocation to the nucleus follows rapid changes in nuclear polyphosphoinositides. Biochem Biophys Res Commun 177:480–487.

Martelli AM, Cocco L, Riederer BM, Neri LM. 1996. The nuclear matrix: a critical appraisal. Histol Histopathol 11:1035–1048.

Martelli AM, Bareggi R, Bortul R, Grill V, Narducci P, Zweyer M. 1997. The nuclear matrix and apoptosis. Histochem Cell Biol 108:1–10.

Mason-Garcia M, Harlan RE, Mallia C, Jeter JR, Steinberg HB, Fermin C, Beckman BS. 1995. Interleukin-3 or erythropoietin induced nuclear localization of PKC beta isoforms in hematopoietic target cells. Cell Prolif 28:145–155.

Mazzotti G, Zini N, Rizzi E, Rizzoli R, Galanzi A, Ognibene A, Santi S, Matteucci A, Martelli AM, Maraldi NM. 1995. Immunocytochemical detection of phosphatidylinositol 4,5-bisphosphate localization sites within the nucleus. J Histochem Cytochem 43:181–191.

Mellor H, Parker PJ. 1998. The extended protein kinase C superfamily. Biochem J 332:281–292.

Metcalf D, Nicola NA. 1991. Direct proliferative actions of stem cell factor on murine bone marrow cells in vitro: effects of combination with colony stimulating factors. Proc Natl Acad Sci USA 88:6239–6243.

Micheli M. Albi E, Leray C, Viola Magni MP. 1998. Nuclear sphingomyelin protects RNA from RNase action. FEBS Lett 431:443–447.

Mizukami Y, Hirata T, Yoshida K. 1997. Nuclear translocation of PKC ζ during ischemia and its inhibition by wortmannin, an inhibitor of phosphatidylinositol 3-kinase. FEBS Lett 401:247–251.

Moir RD, Spann TP, Goldman RD. 1995. The dynamic properties and possible functions of nuclear lamins. Int Rev Cytol 162B:141–182.

Municio MM, Lozano J, Sanchez P, Moscat J, Diaz-Meco MT. 1995. Identification of heterogeneous ribonucleoprotein A1 as a novel substrate for PKC ζ. J Biol Chem 270:15884–15891.

Murray NR, Burns DJ, Fields AP. 1994. Presence of a β_{II} protein kinase C-selective nuclear membrane activation factor in human leukemia cells. J Biol Chem 269:21385–21390.

Murray NR, Fields AP. 1998. Phosphatidylglycerol is a physiological activator of nuclear protein kinase C. J Biol Chem 273:11514–11520.

Neri LM, Billi AM, Manzoli L, Rubbini S, Gilmour RS, Cocco L, Martelli AM. 1994a. Selective nuclear translocation of PKC α in Swiss 3T3 cells treated with IGF-I, PDGF and EGF. FEBS Lett 347:63–68.

Neri LM, Milani D, Bertolaso L, Stroscio M, Bertagnolo V, Capitani S. 1994b. Nuclear translocation of phosphatidylinositol 3-kinase in rat pheochromocytoma PC12 cells after treatment with nerve growth factor. Cell Mol Biol 40:619–626.

Neri LM, Borgatti P, Capitani S, Martelli AM. 1998. Nuclear diacylglycerol produced by phosphoinositide-specific phospholipase C is responsible for nuclear translocation of protein kinase C-α. J Biol Chem 273:29738–29744.

Neri LM , Capitani S, Borgatti P, Martelli AM. 1999. Lipid signaling and cell responses at the nuclear level. Histol Histopathol 14:321–335.

Newton AC. 1997. Regulation of protein kinase C. Curr Opin Cell Biol 9:161–167.

Nishizuka Y. 1992. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science 258:607–614.

Nishizuka Y. 1995. Protein kinase C and lipid signaling for sustained cellular responses. FASEB J 9:484–496.

Oancea E, Teruel MN, Quest AFG, Meyer T. 1998. Green fluorescent protein (GFP)-tagged cysteine-rich domains from protein kinase C as fluorescent indicators for diacylglycerol signaling in living cells. J Cell Biol 140:485–498.

Oehrlein SA, Maelicke A, Herget T. 1998. Expression of protein kinase C gene family members is temporally and spatially regulated during neural development in vitro. Eur J Cell Biol 77:323–337.

Ohmori T, Arteaga CL. 1998. Protein kinase C ϵ translocation and phosphorylation by cis-diamminedichloroplatinum (II) (CDDP): Potential role in CDDP-mediated cytotoxicity. Cell Growth Differ 9:345–353.

Olah Z, Lehel C, Anderson WB, Brenneman DE, Van Agoston D. 1994. Subnanomolar concentration of VIP induces the nuclear translocation of PKC in neonatal rat cortical astrocytes. J Neurosci Res 39:355–363.

Olson EN, Burgess R, Staudinger J. 1993. Protein kinase C as a transducer of nuclear signals. Cell Growth Differ 4:699–705.

Owen PJ, Johnson GD, Lord JM. 1996. Protein kinase C-δ associates with vimentin intermediate filaments in differentiated HL60. Exp Cell Res 225:366–373.

Patterson KK, Beckman BS, Klotz DM, Mallia CM, Jeter JR. 1996. Dexniguldipine hydrochloride, a protein-kinase-C-specific inhibitor, affects the cell cycle, differentiation, P-glycoprotein levels, and nuclear protein phosphorylation in Friend erythroleukemia cells. J Cancer Res Clin Oncol 122:465–475.

Payrastre B, van Bergen en Henegouwen PM, Breton M, den Hartig JC, Plantavid M, Verkleij AJ, Boonstra J. 1991. Phosphoinositide kinase, diacylglycerol kinase, and phospholipase C activities associated to the cytoskeleton: effect of epidermal growth factor. J Cell Biol 115:121–128.

Perry DK, Hannun YA. 1998. The role of ceramide in cell signaling. Biochim Biophys Acta 1436:233–243.

Pierce A, Heyworth CM, Nicholls SE, Spooncer E, Dexter TM, Lord JM, Owen-Lynch PJ, Wark G, Whetton AD. 1998. An activated protein kinase C α gives a differentiation signal for hematopoietic progenitor cells and mimicks macrophage colony-stimulating factor-stimulated signaling. J Cell Biol 140:1511–1518.

Pongracz J, Tuffley W, Johnson GD, Deacon EM, Burnett D, Stockley RA, Lord JM. 1995. Changes in protein kinase C isoenzyme expression associated with apoptosis in U937 myelomonocytic cells. Exp Cell Res 218:430–438.

Prevostel C, Alvaro V, Vallentin A, Martin A, Jaken S, Joubert D. 1998. Selective loss of substrate recognition induced by the tumor-associated D294G point mutation in protein kinase C-α. Biochem J 334:393–397.

Quignon F, Chen Z, de Thè H. 1997. Retinoic acid and arsenic: towards oncogene-targeted treatments of acute promyelocytic leukaemia. Biochim Biophys Acta 1333:53–61.

Raben DM, Jarpe MB, Leach KL. 1994. Nuclear lipid metabolism in NEST: Nuclear Envelope Signal Transduction. J Membrane Biol 142:1–7.

Rennecke J, Johannes FJ, Richeter KH, Kittstein W, Marks F, Gschwendt M. 1996. Immunological demonstration of protein kinase C μ in murine tissues and various cell lines. Differential recognition of phosphorylated forms and lack of down-regulation upon 12-O-tetradecanoylphorbol-13-acetate treatment of cells. Eur J Biochem 242:428–432.

Rogue P, Labourdette G, Masmoudi A, Yoshida Y, Huang FL, Huang KP, Zwiller J, Vincendon G, Malviya AN. 1990. Rat liver nuclei protein kinase C is the isozyme type II. J Biol Chem 265:4161–4165.

Rosenberger U, Shakibaei M, Buchner K. 1995. Localization of non-conventional protein kinase C isoforms in bovine brain cell nuclei. Biochem J 305:269–275.

Rout MP, Wente SR. 1994. Pores for thought: nuclear pore proteins. Trends Cell Biol 4:357–365.

Russo G, Ricciardelli G, Pietropaolo C. 1997. Different domains cooperate to target the human ribosomal L7a protein to the nucleus and to the nucleoli. J Biol Chem 272:5229–5235.

Santella L , Carafoli E. 1997. Calcium signaling in the cell nucleus. FASEB J 11:1091–1109.

Schmalz D, Kalkbrenner F, Hucho F, Buchner K. 1996. Transport of protein kinase C α into the nucleus requires intact cytoskeleton while the transport of a protein containing a canonical nuclear localization signal does not. J Cell Sci 109:2401–2406.

Schmalz D, Hucho F, Buchner K. 1998. Nuclear import of protein kinase C occurs by a mechanism distinct from the mechanism used by proteins with a classical nuclear localization signal. J Cell Sci 111:1823–1830.

Schmidt-Zachmann MS, Nigg EA. 1993. Protein localization to the nucleolus: a search for targeting domains in nucleolin. J Cell Sci 105:799–806.

Schmitt DA, Hatton JP, Emond C, Chaput D, Paris H, Schaffar L. 1996. The distribution of protein kinase C in human leukocytes is altered in microgravity. FASEB J 10:1627–1634.

Schneider U, Schwenk HU, Bornkamm G. 1977. Characterization of EBV genome-negative "null" and "T" cell lines derived fron children with acute lymphoblastic leukemia and leukemic transformed non-Hodgkin lymphoma. Int J Cancer 19:521–526.

Shimizu T, Chun-Xia C, Shao R-G, Pommier Y. 1998. Lamin B phosphorylation by protein kinase C α and proteolysis during apoptosis in human leukemia HL 60 cells. J Biol Chem 273:8669–8674.

Shirai Y, Kashiwagi K, Yagi K, Sakai N, Saito N. 1998. Distinct effects of fatty acids on translocation of γ- and ϵ-subspecies of protein kinase C. J Cell Biol 143:511–521.

Skinner MK. 1991. Cell-cell interactions in the testis. Endocr Rev 12:45–54.

Silver PA. 1991. How proteins enter the nucleus. Cell 64:489–497.

Simboli-Campbell M, Gagnon A, Franks DJ, Welsh J. 1994. 1,25-dihydroxyvitamin D_3 translocates PKC β to the nucleus and enhances plasma membrane association of PKC α in renal epithelial cells. J Biol Chem 269:3257–3264.

Staudinger J, Zhou J, Burgess R, Elledge SJ, Olson EN. 1995. PICK1: a perinuclear binding protein and substrate for protein kinase C isolated by the yeast two-hybrid system. J Cell Biol 128:263–271.

Strasberg-Rieber M, Rieber M. 1992. Accessibility to DNA in carcinoma chromatin is promoted by nanomolar okadaic acid; effect on AT-rich DNA binding proteins. Cancer Res 52:6397–6399.

Sun B, Murray NR, Fields AP. 1997. A role for nuclear phosphatidylinositol-specific phospholipase C in the G_2/M phase transition. J Biol Chem 272:26313–26317.

Tanaka C, Nishizuka Y. 1994. The protein kinase C family for neuronal signaling. Annu Rev Neurosci 17:551–567.

Terzian AR, Zhang X, Rubin RP. 1996. Differential modulation of protein kinase C isozymes in rat parotid acinar cells. Biochem Pharmacol 52:569–577.

Timar J, Liu B, Bazaz R, Honn KW. 1996. Association of PKC α with cytoplasmic vesicles in melanoma cells. J Histochem Cytochem 44:177–182.

Thompson LJ, Fields AP. 1996. β_{II} protein kinase C is required for the G_2/M phase transition of the cell cycle. J Biol Chem 271:15045–15053.

Topham MK, Bunting M, Zimmerman GA, McIntyre TM, Blackshear PJ, Prescott SM. 1998. Protein kinase C regulates the nuclear localization of diacylglycerol kinase-ζ. Nature 394:697–700.

Trubiani O, Borgatti P, Di Primio R. 1994. Protein kinase C modulation in apoptotic rat thymocytes: an ultrastructural analysis. Histochemistry 102:311–316.

Trubiani O, Rana RA, Stuppia L, Di Primio R. 1995a. Nuclear translocation of β_{II} PKC isoenzyme in phorbol ester-stimulated KM-3 pre-B human leukemic cells. Exp Cell Res 221:172–178.

Trubiani O, Bollum FJ, Di Primio R. 1995b. Terminal deoxynucleotidyl transferase is a nuclear PKC substrate. FEBS Lett 374:367–370.

Tuteja R, Tuteja N. 1998. Nucleolin: a multifunctional major nucleolar phosphoprotein. Crit Rev Biochem Mol Biol 33:407–436.

Vaux DL, Strasser A. 1996. The molecular biology of apoptosis. Proc Natl Acad Sci USA 93:2239–2244.

Ventura C, Pintus G, Vaona I, Bennardini F, Pinna G, Tadolini B. 1995. Phorbol ester regulation of opioid peptide gene expression in myocardial cells. J Biol Chem 270:30115–30120.

Ventura C, Pintus G, Fiori MG, Bennardini F, Pinna G, Gaspa L. 1997. Opioid peptide gene expression in the primary hereditary cardiomyopathy of the Syrian hamster. J Biol Chem 272:6685–6692.

Walker SD, Murray NR, Burns DJ, Fields AP. 1995. PKC chimeras catalytic domains of α and β_{II} PKC contain determinants for isotype-specific functions. Proc Natl Acad Sci USA 92:9156–9160.

Wang S, Desai D, Wright G, Niles RM, Wright GL. 1997. Effects of protein kinase Cα on A7r5 smooth muscle cell proliferation and differentiation. Exp Cell Res 236:117–126.

Weaver VM, Carson CE, Walker PR, Chaly N, Lach B, Raymond Y, Brown DL, Sikorska M. 1996. Degradation of nuclear matrix and DNA cleavage in apoptotic thymocytes. J Cell Sci 109:45–56.

Whetton AD, Heyworth CM, Nicholls SE, Evans CA, Lord JM, Dexter TM, Owen-Linch PJ. 1994. Cytokine mediated PKC activation is a signal for lineage determination in bipotential granulocyte-macrophage colony forming cells. J Cell Biol 125:651–659.

Williams GT. 1991. Programmed cell death: apoptosis and oncogenesis. Cell 65:1097–1098.

Williams GT. 1994. Apoptosis in the immune system. J Pathol 173:1–4.

Wooten MW, Zhou G, Wooten MC, Seibenhener ML. 1997. Transport of PKC isoforms to the nucleus of PC12 cells by nerve growth factor: association of atypical ζ PKC with the nuclear matrix. J Neurosci Res 49:393–403.

Xiao CY, Jans DA. 1998. An engineered site for protein kinase C flanking the SV40 large T-antigen NLS confers phorbol ester-inducible nuclear import. FEBS Lett 436: 313–317.

Yuan Z-M, Utsugisawa T, Ishiko T, Nakada S, Huang Y, Kharbanda S, Weichselbaum R, Kufe D. 1998. Activation of protein kinase C δ by the c-Abl tyrosine kinase in response to ionizing radiation. Oncogene 16:1643–1648.

Yoshida K, Hirata T, Akita Y, Mizukami Y, Yamaguchi K, Sorimaki Y, Ishihara T, Kawashama S. 1996. Translocation of protein kinase C-α, -δ and -ϵ isoforms in ischemic rat heart. Biochim Biophys Acta 1317:36–44.

Yu H, Fukami K, Watanabe Y, Ozaki C, Takenawa T. 1998. Phosphatidylinositol 4,5-bisphosphate reverses the inhibition of RNA transcription caused by histone H1. Eur J Biochem 251:281–287.

Zauli G, Visani G, Bassini A, Caramelli E, Ottaviani E, Bertolaso L, Bertagnolo V, Borgatti P, Capitani S. 1996a. Nuclear translocation of protein kinase C-α and -ζ isoforms in HL-60 cells induced to differentiate along the granulocytic lineage by all-trans-retinoic acid. Br J Haematol 93:542–550.

Zauli G, Bassini A, Catani L, Gibellini D, Celeghini C, Borgatti P, Caramelli E, Guidotti L, Capitani S. 1996b. PMA induced magakaryocytic differentiation of HEL cells is accompanied by striking modifications of PKC catalytic activity and isoform composition at the nuclear. Br J Haematol 92:530–536.

Zhou G, Seibenhener ML, Wooten MW. 1997. Nucleolin is a protein kinase C-ζ substrate. J Biol Chem 272:31130–31137.

Zorn RE, Sauro MD. 1995. Retinoic acid induces translocation of PKC and activation of nuclear PKC in rat splenocytes. Int J Immunopharmacol 17:303–311.

Zini N, Martelli AM, Neri LM, Bavelloni A, Sabatelli P, Santi S, Maraldi NM. 1995. Immunocytochemical evaluation of PKC translocation to the inner nuclear matrix in 3T3 mouse fibroblasts after IGF-I treatment. Histochemistry 103:447–457.

Zini N Matteucci A, Sabbatelli P, Valmori A, Caramelli E, Maraldi NM. 1997a. PKC isoforms undergo quantitative variations during rat spermatogenesis and are selectively retained at specific spermatozoon sites. Eur J Cell Biol 72:142–150.

Zini N, Neri LM, Ognibene A, Scotlandi K, Baldini, Maraldi NM. 1997b. Increase of nuclear phosphatidylinositol 4,5-bisphosphate and phospholipase C β1 is not associated to variations of protein kinase C in multidrug-resistant Saos-2 cells. Microsc Res Techniq 36:172–178.