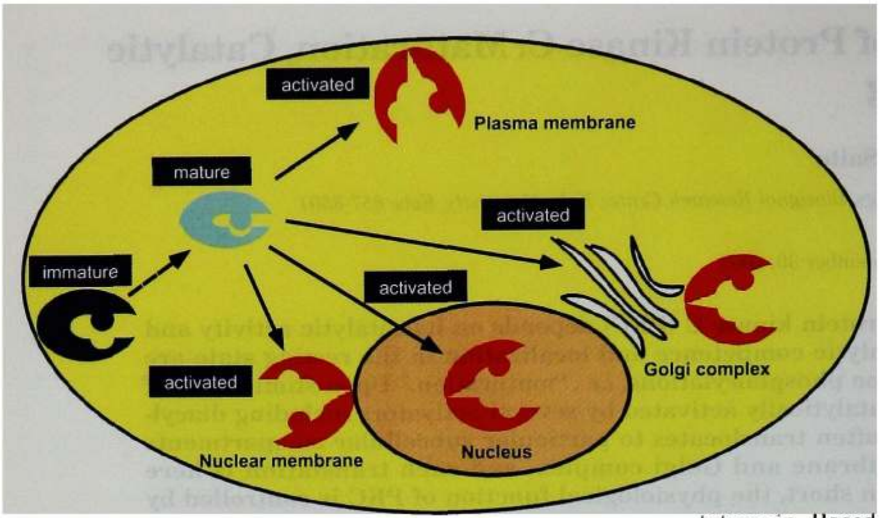
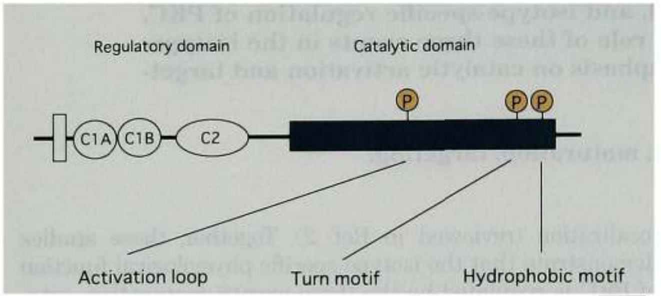


Fig. 1. **Overview of PKC activation.** The physiological function of PKC is controlled by three events: maturation, catalytic activation and targeting. Newly translated "immature" PKC (shown in navy) cannot be catalytically activated. It is serine/threonine-phosphorylated at three distinct sites, thus maturing into a form that is localized in the cytosol (light blue) and is sensitive to physiological stimuli. Upon stimulation of various receptors, this "mature" PKC can be catalytically activated by several activators and targeted to specific subcellular compartments including plasma membrane, Golgi complex, nuclear membrane and nucleus (shown in red).



	Activation loop	Turn motif	Hydrophobic motif
α	497T	638T	657S
βI	500T	642T	661S
βII	500T	641T	660S
γ	514T	655T	674T
δ	505T	643S	662S
ε	566T	710T	729S
η	512T	655T	674S
θ	538T	676S	695S
ζ	410T	560T	(579E)
ι/λ	403T	555T	(574E)

Number shows position of serine / threonine.

Fig. 2. **Conservative serine/threonine residues in the activation loop, turn motif, and hydrophobic motif of 10 mammalian PKCs.**

the catalytic competence and correct intracellular localization of PKCs in the resting state. Therefore, it represents one of the rate-limiting steps for PKC activation.

3. Catalytic activation

3-1. Phosphatidylserine. Phosphatidylserine (PS) is necessary for catalytic activity of all PKCs. It is thought to bind to either the C1 or C2 domains but the specific binding site has not been identified. The crystal structure of the C2 domain of PKCα in the presence of Ca^{2+} and PS reveals that a short chain of PS is coordinated to the C2 domain (*10*). In contrast, the binding of PS to the C1B domain of PKCβII has also been reported (*11*). Additional studies are necessary to determine how PS interacts with each isotype.

3-2. Calcium ions. Calcium ions (Ca^{2+}) regulate the activity of the cPKCs via the C2 domain. The Ca^{2+}-binding, C2 domain of the cPKCs is homologous to that of synaptotagmin. Based on crystallographic analysis of the synaptotagmin C2 domain in the presence and absence of Ca^{2+} (*12*), it appears that Ca^{2+}-binding changes the conformation of the C2 domain. Although this has not yet been demonstrated for PKC, by analogy one can propose that Ca^{2+}-binding to the cPKC-C2 domain also induces a conformational change (*13, 14*). As the C2 domains of PKCα and β have been crystallized (*15, 16*), it is likely that these studies are under way.

In addition to its putative role in modulating the conformation of PKC, Ca^{2+} also increases the affinity of the enzyme for PS. The Ca^{2+}-induced increase in the affinity of PKC-C2 domain for PS was suggested by initial studies using lyposomes (*17*), and directly demonstrated by Newton and coworkers (*18, 19*). Interestingly, they also suggested that each isotype of cPKCs is differentially regulated by calcium (*19*).

3-3. Lipid mediators. *3-3a. Diacylglycerol (DAG) and phorbol ester.* DAG and phorbol ester activate the cPKC and nPKCs by binding to the C1 domain (*1, 20*). The C1 domain of the cPKC and nPKCs has two cysteine-rich domains (C1A and C1B), each containing ~50 amino acids, including six cysteine and two histidine residues arranged in a zinc finger motif. The aPKCs lack one of the cysteine-rich domains and thus are insensitive to activation by these compounds. The C1B domain of PKCδ has been crystallized and shown to contain a phorbol ester–binding pocket (*21*).

Two types of DAG appear to be important for the physiological activation of PKC. One is rapidly produced from phosphatidylinositol 4,5-bisphosphate (PIP_2) by phospholipase C upon stimulation of G protein-coupled receptors. The other is thought to result from hydrolysis of phosphatidylcholine (PC). This latter DAG production occurs slowly and is more sustained. In addition to the temporal difference, the fatty acid composition of the DAG derived from PC differs from that released from PIP_2. Interestingly, PKC isotypes are differentially sensitive to the fatty acid composition of DAG (*22*). 1-Steroyl-2-arachidonoyl-*sn*-glycerol (SAG) stimulates PKCα and δ more effectively than do 1-steroyl-2-docosahexaenoyl-*sn*-glycerol (SDG) and 1-steroyl-2-eicosapentaenoyl-glycerol (SEG). In contrast, activation of PKCβI by SDG and SEG is higher than that by SAG. Thus, the composition of the DAG and its temporal release may regulate isotype-selective activation.

3-3b. Fatty acid. Fatty acids are known to activate PKC in an isotype-specific manner (*4*). For example, saturated

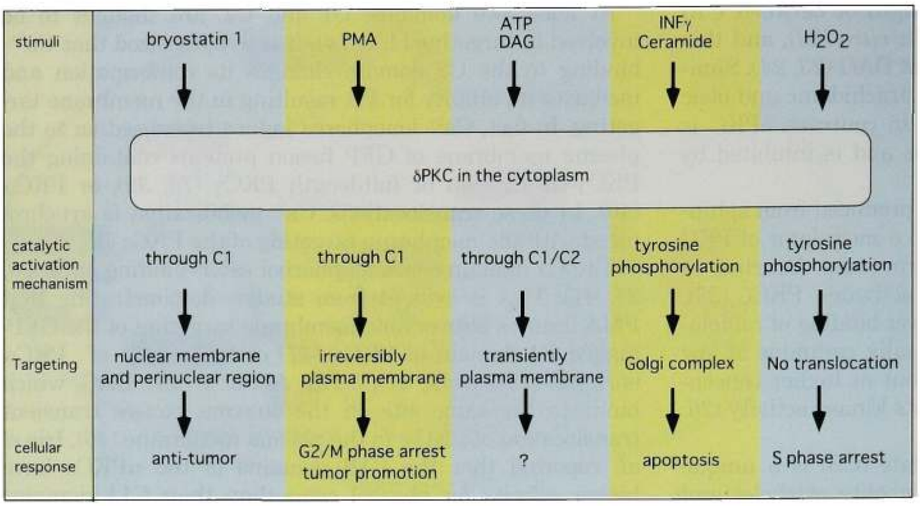


Fig. 3. **Diverse cellular response resulting from distinct targeting of δPKC.** Irreversible membrane-targeting of δPKC by PMA induces G2/M arrest or tumor promotion, while anti-tumor ligands such as bryostatin 1 translocate δPKC to the perinuclear region including nuclear membrane. Ceramide-induced targeting to the Golgi complex results in apoptosis and activation of δPKC by hydrogen peroxide treatment, which does not change the localization, increases the number of cells in S phase. ATP causes transient targeting to the plasma membrane, but the significance of this response is not known.

effects similar to those described above. Carbacol and angiotensin II induce transient translocation to the plasma membrane of α and βII PKC, respectively (*40, 42*). Similarly, PKCγ, δ, and ε are targeted to the plasma membrane in response to purinergic stimulation (*50*). We also found that INFγ stimulation, which generates ceramide, causes translocation of PKCδ to the Golgi complex, in a similar way to treatment of cells with the ceramide analogue (*51*). Additionally, PAF stimulation, which mobilizes AA in addition to DAG production and Ca^{2+} increase, localized ζPKC to the nucleus (our unpublished data).

Physiologically, targeting PKC to the plasma membrane is necessary for phosphorylation of membrane-associated substrates. This is clearly illustrated by our experiments demonstrating that PMA-induced targeting of PKCδ to the plasma membrane results in phosphorylation of MARCKS on the membrane, whereas PKCδ activation by hydrogen peroxide, which does not elicit translocation, fails to phosphorylate this substrate (*52*). Other experiments revealed that ceramide-induced targeting of PKCδ to the Golgi complex resulted in apoptosis (unpublished data), while PMA-induced activation and plasma membrane targeting of PKCδ increased the number of cells in G2/M phase (*43*). Thus, differential targeting leads to distinct cellular responses (some of which are summarized in Fig. 3).

The diversity in the number and effects of lipid mediators on PKC localization and cellular responses reflect the importance of targeting in the physiological activation and function of the different PKC isotypes. Thus, targeting plays a major role in regulating isotype-specific signal transduction.

5. Perspective and conclusion

The importance of targeting in isotype-specific activation of PKC has become apparent, but a question regarding the correlation between targeting and catalytic activation remains to be solved: does PKC translocate after it becomes catalyticaly active or is it activated after its translocation? To address this issue, PKC inhibitors and a kinase-negative mutant have been used. PMA induces translocation of PKCγ in the presence of a PKC inhibitor, staurosporine (*39*), and the kinase-dead PKC βII shows translocation, although its re-translocation is inhibited (*53*). Moreover, PKCδ can translocate to the Golgi complex without catalytic activation in response to ceramide (*51*). These results indicate that translocation of PKC is independent of its catalytic activity. However, we can not conclude that PKC becomes catalytically active after translocation, because hydrogen peroxide activates PKCδ without translocation (*51*). Development of a fluorescent substrate which will enable us to spatio-temporally visualize PKC phosphorylation would be useful to evaluate the correlation between translocation and catalytic activation.

Although the molecular mechanism of PKC targeting is not well understood, a scaffold protein such as AKAP or RACKS may determine the localization of PKC when activated (reviewed in Refs. *54* and *55*). Lipid mediators can be also candidates as targeting modulator.

In conclusion, the physiological function of PKC is regulated by maturation, catalytic activation and targeting. Catalytic activation and targeting are temporally and spatially orchestrated, contributing to isotype-specific activation of PKC under physiological conditions. In these events, lipid mediators play important roles.

REFERENCES

1. Nishizuka, Y. (1988) The molecular heterogeneity of protein kinase C and implications for cellular regulation. *Nature* **334**, 661–665
2. Newton, A.C. (2001) Protein kinase C: Structural and spatial regulation by phosphorylation, cofactor, and macromolecular interactios. *Chem. Rev.* **101**, 2353–2364
3. Kishimoto, A., Takai, Y., Mori, T., Kikkawa, U., and Nishizuka, Y. (1980) Activation of calcium and phospholipid-dependent protein kinase by diacylglycerol, its possible relation to phosphatidylinositol turnover. *J. Biol. Chem.* **255**, 2273–2276
4. Nishizuka, Y. (1995) Protein kinase C and lipid signaling for sustained cellular response. *FASEB* **9**, 484–496
5. Ohno, S. (1997) The distinct biological potential of PKC isotypes in *Protein Kinase C*, pp. 179–188, Springer-Verlag, Heidelberg
6. Keranen, L.M. Dutil, E.M., and Newton, A. (1995) Protein kinease C is regulated in vivo by three functionally distinct phosphorylation. *Curr. Biol.* **5**, 1394–1403
7. Dutil, E.M., Toker, A., and Newton, A. (1998) Regulation of conventinal protein kinase C isozymes by phosphoinositide-dependent kinase 1 (PDK-1). *Curr. Biol.* **8**, 1366–1375