Cell. Mol. Life Sci. 63 (2006) 637–641
1420-682X/06/060637-5
DOI 10.1007/s00018-005-5541-1

Cellular and Molecular Life Sciences

Visions & Reflections

Functional interactions of protein kinase A and C in signalling networks: a recapitulation

E. Maioli*, C. Torricelli and V. Fortino

Department of Physiology University of Siena, via Aldo Moro 8, 53100 Siena (Italy), Fax: +39 0577 234219, e-mail: maioli@unisi.it

Received 10 November 2005; received after revision 28 December 2005; accepted 3 January 2006
Online First 23 February 2006

Abstract. On the basis of evidence collected from the literature, we propose a general model by which protein kinase (PK) A and the different PKC isoforms can inversely affect cell growth. Molecular switches, which are able to direct the signal towards antiproliferative or mitogenic pathways, are the different isoforms of Raf and PKC. Conflicting data are also reported and discussed in an attempt to reconcile them.

Keywords. PKA, Raf-1, Raf B, RKIP, PKC isoforms, ERKs, p21.

Introduction

The prototypical mammalian mitogen-activated protein kinase (MAPK) cascade, the Ras/Raf/MAPK/extracellular signal-regulated kinase (ERK) kinase (MEK)/ERK1/2 pathway, serves to link growth factor receptors (GFRs) at the plasma membrane to changes in gene expression in the nucleus. The MAPK cascade can be also modulated by several hormones, regulatory peptides and ions through G-protein-coupled receptors (GPCRs). This process involves the release of the G$\beta\gamma$ dimer that, via phosphatidyl inositol 3-kinase (PI3-kinase), may regulate the phosphorylation of the protein Shc. Upon activation, Shc binds to the adapter protein Grb2. Grb2 can then associate with the guanine nucleotide exchange factor Sos, which in turn activates Ras, anchored to the plasma membrane, by exchange of GTP for GDP. GTP-bound Ras then binds directly to a serine-threonine kinase, Raf, which in turn phosphorylates MEK, leading to the subsequent activation of ERKs [1].

The GFR and GPCR transduction pathways, which largely overlap, are among the best-known mechanisms involved in the control of cell growth. However, GPCR signalling is much more versatile. In fact, the Gα subunit normally couples with the adenylate cyclase (AC)-PKA and phospholipase C (PLC)-PKC pathways, which, acting at the level of Ras-Raf crossover point, further modulate the MAPK family [2–3].

This article focuses on the possible opposite effects of the PKA and PKC pathways on ERK activity [4], and the possible opposite effects on cell growth of a same second messenger [5–8]. However, it is important to note that PKA and PKC may have other common targets inside a cell. For example, in lymphocytes, nuclear factor of activated T cells (NFAT) is another key pathway that can be inversely regulated by the two kinases [9–10]. This suggests the general concept that PKA/PKC is functionally active in a yin/yang fashion.

The PKA/Raf-1/Ras-MEK-ERK cascade

As far as the action of cAMP/PKA on cell growth is concerned, a stimulatory effect has been described, for example, in PC12 cells [6] and in rat enterocytes [11], whereas growth arrest is evoked in skin fibroblasts [4] and in MCF-7 breast cancer cells [12].

* Corresponding author.

The MAPK cascade usually transduces signals from GPCRs or GFRs to two members of the Ras family of small G proteins, Ras and Rap-1, which then stimulate the sequential activation of Raf, MEK and ERK 1/2. Ras functions principally to activate isoform Raf-1, whereas Rap-1 stimulates isoform Raf-B [13]. As schematically illustrated in Figure 1, the molecular shunt of cyclic AMP (cAMP) occurs at the level of the different isoforms of Raf: Raf-1 and/or Raf-B.

In Raf-1-expressing cells, phosphorylation by PKA of this isoform reduces the affinity of Raf-1 binding to Ras, resulting in the inhibition of ERK activation and cell proliferation [14]. Moreover, the activation of Rap-1 by PKA not only uncouples Raf-1 from Ras but also promotes the association of Rap-1 with Raf-1. Since Rap-1/Raf-1 is an inactive complex [15], both mechanisms converge on blockage of ERK phosphorylation and growth arrest.

Conversely, in Raf-B-expressing cells, the stimulation of ERK activity by cAMP can be achieved by promotion of the Rap-1/Raf-B complex formation and then MEK/ERK activation [6]. No study has ever demonstrated an inhibitory effect of the cAMP/PKA pathway on Raf-B, which is even more effective than Raf-1 in activating MEK [16]. Intriguingly, oncogenic mutants of Raf-B, although inactive as kinases, can signal to ERK by activating Raf-1 [17].

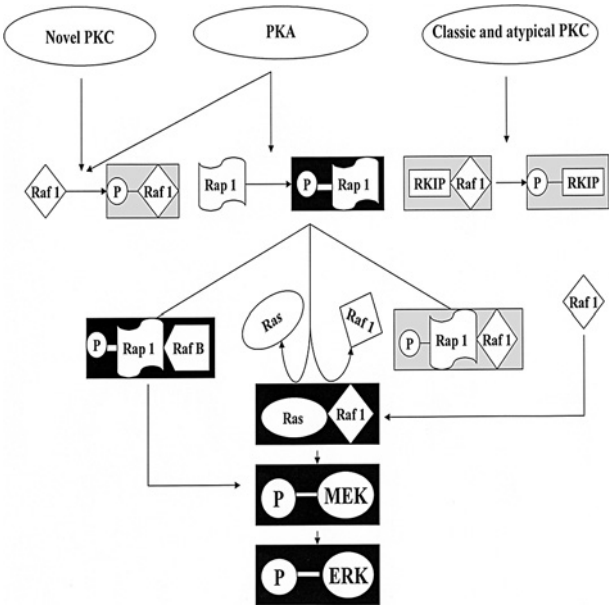

Figure 1. Schematic representation of the opposite regulation of MEK/ERK activity by PKA and PKC. PKA phosphorylates the Raf-1 isoform and the small G protein Rap-1; novel PKCs phosphorylate the Raf-1 isoform; classic and atypical PKCs phosphorylate the Raf-1 inhibitory protein RKIP. *Pathways leading to ERK inhibition:* (i) Raf-1 phosphorylation, which reduces the affinity of Raf-1 binding to Ras; (ii) Rap-1 phosphorylation, which results both in Raf-1 sequestration in an inactive complex and in Raf-1/Ras complex dissociation. *Pathways leading to ERK activation:* (i) Rap-1 phosphorylation followed by Raf-B binding in an active complex; (ii) RKIP phosphorylation, which results in Raf-1 release from the Raf-1/RKIP inactive complex. Omitted from this model is the indirect activation of Raf-B by classical/atypical PKCs, through phosphorylation of RKIP.

The PKC-Raf-1/Ras-MEK-ERK cascade

Proliferative behavior following PKC activation can also be opposite in different cell types. It is generally held that the sequential activation of PKC-Raf-1/Ras-MEK-ERK leads to cell proliferation [18–19]. However, as recently discussed [20], since both PKA and PKC are serine/threonine kinases able to phosphorylate Raf-1 at the same sites, they could not be activating and inhibiting at the same time. In fact, Raf-1, phosphorylated in Ser 259 by both PKs [21], is a target for 14.3.3 protein, whose binding maintains Raf-1 in an inactive conformation [22]. Therefore, PKC stimulation can also result in the blockage of ERK phosphorylation and growth arrest [23].

In the case of PKCs, the molecular switch, able to direct the signal towards antiproliferative or mitogenic pathways, is the PKC isoform itself (Fig. 1). While novel PKC isoforms (δ, ε, η, θ) directly phosphorylate (then inhibit) Raf-1, classical (α, βI, βII, γ) and atypical (ζ, λ, ι) PKCs phosphorylate Raf kinase inhibitory protein (RKIP), causing its dissociation from Raf-1 and thereby enhancing downstream signaling to ERK [24]. Different PKCs act at different levels in blocking and activating Raf-1. Novel PKCs work in the cytoplasm, before Raf-1 membrane translocation; classical and atypical PKCs remove RKIP from Raf-1 after its membrane recruitment, allowing Raf-1 phosphorylation in Ser338 and Tyr 341 by p21-activated kinase (PAK) and Src family kinases, respectively, and its fully activation [25].

Since an analogous mechanism is also operative on Raf-B isoform, for example, in melanoma cancer cells [25], a general rule could be drawn by which classical and atypical PKCs should always stimulate ERKs phosphorylation via RKIP/Raf, regardless of the Raf isoform. In the generalization of this last concept, however, caution is mandatory. Indeed, the biochemical properties of Raf-B are not completely known, and there is no consensus about the impact of RKIP on Raf-B activity [26].

Evidence supporting the rule

Theoretically, the PKC isoforms able to phosphorylate RKIP by allowing activation of the Ras/Raf-1/MEK/ERK cascade should promote cell growth, while PKC isoforms directly targeting Raf-1 should induce cell cycle arrest by preventing ERK activation. The literature provides ample evidence for each scenario. For instance, PMA induces proliferation in cells expressing mainly PKC α, such as fibroblasts [4] and the breast cancer cell line MDA-MB

231 [27], while it is antiproliferative in glioma cells [28], where the ε is the prevalent PKC isoform [29]. Analogously, overexpression of PKC α stimulates growth in MCF-7 and in glioma cells, while PKC δ overexpression exerts the opposite effect in the latter [30–31].

Exceptions to the rule

Some published data seem to confute the rule exposed above. Here are four notable examples:

1) In transfected cells, forced expression of PKC η or δ (both novel isoforms) has opposite effects on cell growth [32].
2) The inhibition of PKC δ (a novel isoform) can result in reduced ERK phosphorylation [33]. In apparent agreement, PKC δ overexpression promotes sustained ERK activation [7] but,
3) Paradoxically, induces growth arrest [7].
4) Pharmacological activation of PKC promotes growth arrest in pancreatic cancer cells expressing almost exclusively the PKC α isoform [34].

Exceptions do not prove the rule wrong

Indeed, each apparent discrepancy has a possible explanation:

1) It has been documented that forced expression of an individual PKC isoform can alter the expression of the others [30, 35] and lead to misleading results. Therefore, the general rule, illustrated in Figure 1, can find confirmation mainly in native cells.
2) It has been recently demonstrated that the δ isoform of PKC can activate ERK in an Raf-1- independent way, by directly targeting the exchange factor for Ras/Rap1, RasGRP3 [36]. Moreover, the widely use of phorbol esters (such as PMA) to stimulate PKCs can lead to no specific responses, due to known PKC-unrelated targets of phorbol esters themselves. In particular, we suspect that many negative or positive effects on ERK phosphorylation/proliferation, which have been ascribed to PKC δ, may be due to the PKC-independent activation of chimerins or RasGRP, respectively, by PMA and/or analogues [37].
3) The final determinant of the proliferative response is the degree of ERK activation: a weak activation can lead to a proliferative response; robust stimulation can result in cell cycle arrest in the G1 phase [7]. This latter effect has been ascribed to PKC δ-mediated $p21^{WAF1/CIP1}$ induction in MCF-7 breast cancer cells [7]. The authors suggested that the increase in p21 expression is consequent to enhanced ERK phosphorylation by superphysiological PKC δ activity.

Recently, an elegant study confirmed the cause-effect relationship between pharmacologically activated PKC δ and p21 in lung adenocarcinoma cells [38]. However, the authors did not detect phospho-ERK levels; hence, no mechanistic information has been provided linking PKC δ activation to p21 induction.

Unfortunately, the essential role of ERK in mediating the PKC δ induction of p21 has so far not been unequivocally demonstrated. In this regard, we recently found that the δ isoform of PKC can induce p21 expression in an MCF-7 cell line through pathways (not identified yet) that are both ERK- and p53-independent [unpublished observations].

4) Consistent with the model that we propose, Detjen's group found elevated ERK levels following PKC α activation, but the final response was growth arrest. In this case, too, the growth arrest evoked by PKC α is linked to p21 induction. Again, it is not clear whether p21 induction is ERK dependent. Interestingly, the authors observed a nuclear migration of PKC α that largely exceeded on its membrane translocation.

Since we still lack information on the functional consequences of nuclear localization of PKCs, it is impossible to understand the respective contribution of the two pools of the enzyme. Nevertheless, we would like to suggest that both in MCF-7 cells (personal experience) and in pancreatic cancer cells [34], p21 is induced (at least in part) by a direct effect of PKC δ and α, respectively, on some unidentified nuclear target, in an ERK-independent way.

Concluding remarks

The central role of the Ras/Raf/MEK/MAPK cascade in regulating tumor cell growth – providing a framework for pharmacological intervention – has attracted much interest in recent years. The practical implications of our novel scenario are evident.

Attempts have been made to develop anticancer drugs against Raf-1 [39]. However, this strategy is not free of risks since Raf-1 blockage could remove a key substrate in the antiproliferative pathway of PKA and some PKC isoforms. On the contrary, the positive control of the cAMP/PKA axis on Raf-B, together with its possible activation by classical and atypical PKCs (through RKIP), suggests that inhibitors of this Raf isoform, in tumors expressing high levels of either native or mutant Raf-B, may harbor more therapeutic potential than drugs targeting its homologue Raf-1.

Moreover, despite the controversial and incomplete mechanistic data about the role of individual PKC isoforms on cell cycle control, anticancer strategies targeting PKC isoforms have been developed [19]. However,

the molecular basis of these therapeutic approaches is just beginning to be clarified. So far, we can say nothing but that the final net effect of the upstream targets on MEK/ERK activity derives from a balance between stimulating and inhibiting kinase cascades and ultimately depends on the contingent PKA/PKC ratio, combined with the cell-specific expression of PKC and Raf isoforms. The inclusion of p21 among the PKCs effectors confers on PKCs an additive ability in controlling not only cell growth but also apoptotic cell death. This opens a further area of debate about the role of PKCs in the control of (cancer) cell life. In fact, as a consequence of the conflicting effects of p21 on apoptosis [40], discordant findings have been reported also on PKCs and cell survival [41].

We believe that reflections and further investigation are needed before any pharmacological manipulation of cancer can be generalized.

In future, a close inspection of the literature and an integration effort is to be hoped and recommended to authors presenting new data.

1 Clapham D. E. and Neer E. J. (1997) G protein beta gamma subunits. Annu. Rev. Pharmacol. Toxicol. **37:** 167–203

2 Sugden P. H. and Clerk A. (1997) Regulation of the ERK subgroup of MAP kinase cascades through G protein-coupled receptors. Cell. Signal. **9:** 337–351

3 Liebmann C. (2001) Regulation of MAP kinase activity by peptide receptor signalling pathway: paradigm of multiplicity. Cell. Signal. **13:** 777–785

4 Fortino V., Torricelli C., Gardi C., Valacchi G., Rossi Paccani S. and Maioli E. (2002) ERKs are the point of divergence of PKA and PKC activation by PTHrP in human skin fibroblasts. Cell. Mol. Life Sci. **59:** 2165–2171

5 Wu J., Dent P., Jelinek T., Wolfman A., Weber M. J. and Sturgill T. W. (1993) Inhibition of the EGF-activated MAP kinase signaling pathway by adenosine 3′,5′-monophosphate. Science **262:** 1065–1069

6 Erhardt P., Troppmair J., Rapp U. R. and Cooper G. M. (1995) Differential regulation of Raf-1 and B-Raf and Ras dependent activation of Mitogen-Activated Protein Kinase by Cyclic AMP in PC12 cells. Mol. Cell. Biol. **15:** 5524–5530

7 Shanmugam M., Krett N. L., Maizels E. T., Murad F. M., Rosen S. T. and Hunzicker-Dunn M. (2001) A role for Protein Kinase C δ in the differential sensitivity of MCF-7 and MDA-MB 231 human breast cancer cells to phorbol ester-induced growth arrest and $p21^{WAF1/CIP1}$ induction. Cancer Lett. **172:** 43–53

8 Huang S., Maher V. M. and McCormick J. (1999) Involvement of intermediary metabolites in the pathway of extracellularCa2+-induced mitogen-activated protein kinase activation in human fibroblasts. Cell. Signal. **11:** 263–274

9 Grumont R., Lock P., Mollinari M., Shannon F. M., Moore A. and Gerondakis S. (2004) The mitogen-induced increase in T cell size involves PKC and NFAT activation of Rel/NF-kappaB-dependent c-myc expression. Immunity **21:** 19–30

10 Chow C. W. and Davis R. J. (2000) Integration of calcium and cyclic AMP signaling pathways by 14-3-3. Mol. Cell. Biol. **20:** 702–712

11 Gentili C., Morelli S., Boland R. and de Boland A. R. (2001) Parathyroid hormone activation of map kinase in rat duodenal cells is mediated by 3′,5′-cyclic AMP and Ca(2+). Biochim. Biophys. Acta **1540:** 201–212

12 Maioli E. and Fortino V. (2004) PTHrP on MCF-7 breast cancer cells: a growth factor or an antimitogenic peptide? Br. J. Cancer **90:** 1293–1294

13 York R. D., Yao H., Dillon T., Ellig C. L., Eckert S. P., McCleskey E. W. et al. (1998) Rap-1 mediates sustained MAP kinase activation induced by nerve growth factor. Nature **392:** 622–626

14 Wu J., Dent P., Jelinek T., Wolfman A., Weber M. J. and Sturgill T. W. (1993) Inhibition of the EGF-activated MAP kinase signaling pathway by adenosine 3′,5′-monophosphate. Science **262:** 1065–1069

15 Fujita T., Meguro T., Fukuyama R., Nakamuta H. and Koida M. (2002) New signaling pathway for parathyroid hormone and cyclic AMP action on extracellular-regulated kinase and cell proliferation in bone cells. Checkpoint of modulation by cyclic AMP. J. Biol. Chem. **277:** 22191–22200

16 Papin C., Denouel-Galy A., Laugier D., Calothy G. and Eychene A. (1998) Modulation of kinase activity and oncogenic properties by alternative splicing reveals a novel regulatory mechanism for B-Raf. J. Biol. Chem. **273:** 24939–24947

17 Wan P. T., Garnett M. J., Roe S. M., Lee S., Niculescu-Duvaz D., Good V. M. et al. (2004) Cancer Genome Project. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell **116:** 855–867

18 Akashi M., Osawa Y., Koeffler H. P. and Hachiya M. (1999) $p21^{WAF1}$ expression by an activator of protein kinase C is regulated mainly at the post-transcriptional level in cells lacking p53: important role of RNA stabilization. Biochem. J. **337:** 607–616

19 Mackay H. J. and Twelves C. J. (2003) Protein Kinase C: a target for anticancer drugs? Endocr. Relat. Cancer **10:** 389–396

20 Maioli E. and Fortino V. (2004) Protein kinase C: a target for anticancer drugs? Endocr. Relat. Cancer **11:** 161–162

21 Kolch W., Heldecker G., Kochs G., Hummel R., Vahidi H., Mischak H. et al. (1993) Protein kinase Cα activates RAF-1 by direct phosphorylation. Nature **364:** 249–252

22 Dumaz N. and Marais R. (2003) Protein Kinase A blocks Raf-1 activity by stimulating 14-3-3 binding and blocking Raf-1 interaction with Ras. J. Biol. Chem. **32:** 29819–29823

23 Vucenik I., Ramakrishna G., Tantivejkul K., Anderson L. M and Ramljak D. (2005) Inositol hexaphosphate (IP6) blocks proliferation of human breast cancer cells through a PKCdelta-dependent increase in p27Kip1 and decrease in retinoblastoma protein (pRb) phosphorylation. Breast Cancer Res. Treat. **91:** 35–45

24 Corbit K. C., Trakul N., Eves E. M., Diaz B., Marshall M. and Rosner M. R. (2003) Activation of Raf-1 signaling by protein kinase C through a mechanism involving Raf kinase inhibitory protein. J. Biol. Chem **278:** 13061–13068

25 Park S., Yeung M. L., Beach S., Shields J. M. and Yeung K. C. (2005) RKIP downregulates B-Raf kinase activity in melanoma cancer cells. Oncogene **24:** 3535–3540

26 Trakul N., Menard R. E., Schade G. R., Qian Z. and Rosner M. R. (2005) Raf kinase inhibitory protein regulates Raf-1 but not B-Raf kinase activation. J. Biol. Chem. **280:** 24931–24940

27 Jarzabek K., Laudanski P., Dzieciol J., Dabrowska M. and Wolczynski S. (2002) Protein kinase C involvement in proliferation and survival of breast cancer cells. Folia Histochem. Cytobiol. **40:** 193–194

28 Tsai J. C., Teng L. J., Chen C. T., Hong T. M., Goldman C. K. and Gillespie G. Y. (2003) Protein kinase C mediates induced secretion of vascular endothelial growth factor by human glioma cells. Biochem. Biophys. Res. Commun. **309:** 952–960

29 Sharif T. R. and Sharif, M. (1999) Overexpression of protein kinase C epsilon in astroglial brain tumor derived cell lines and primary tumor samples. Int. J. Oncol. **15:** 237–243

30 Ways D. K., Kukoly C. A., deVente J., Hooke L., Bryant O., Posekany K. J. et al. (1995) MCF-7 breast cancer cells transfected with Protein Kinase C-alpha exhibit altered expression of other Protein Kinase C isoforms and display a more aggressive neoplastic phenotype. J. Clin. Invest. **95:** 1906–1915

31 Mandil R., Ashkenazi E., Blass M., Kronfeld I., Kazimirsky G., Rosenthal, G. et al. (2001) Protein kinase Calpha and protein

kinase Cdelta play opposite roles in the proliferation and apoptosis of glioma cells. Cancer Res. **61:** 4612–4619
32 Fima E., Shtutman M., Libros P., Missel A., Shahaf G., Kahana G. et al. (2001) PKCeta enhances cell cycle progression, the expression of G1 cyclins and p21 in MCF-7 cells. Oncogene **20:** 6794–6804
33 Liu J. F., Crepin M., Liu J. M., Barritault D. and Ledoux D. (2002) FGF-2 and TPA induce matrix metalloproteinase-9 secretion in MCF-7 cells through PKC activation of the Ras/ERK pathway. Biochem. Biophys. Res. Commun. **293:** 1174–1182
34 Detjen K. M., Brembeck F. H., Welzel M., Kaiser A., Haller H., Wiedenmann, B. et al. (2000) Activation of protein kinase Calpha inhibits growth of pancreatic cancer cells via p21(cip)-mediated G(1) arrest. J. Cell. Sci. **113:** 3025–3035
35 Romanova L. Y., Alexandrov I. A., Nordan R. P., Blagosklonny M. V. and Mushinski J. F. (1998) Cross-talk between protein kinase C-alpha (PKC-alpha) and -delta (PKC-delta): PKC-alpha elevates the PKC-delta protein level, altering its mRNA transcription and degradation. Biochemistry **37:** 5558–5565
36 Brodie C., Steinhart R., Kazimirsky G., Rubinfeld H., Hyman T., Ayres J. N. et al. (2004) PKCdelta associates with and is involved in the phosphorylation of RasGRP3 in response to phorbol esters. Mol. Pharmacol. **66:** 76–84
37 Kazanietz M. G. (2002) Novel 'nonkinase' phorbol ester receptors: the C1 domain connection. Mol. Pharmacol. **61:** 759–776
38 Nakagawa M., Oliva J. L., Kothapalli D., Fournier A., Assoian R. K. and Kazanietz M. G. (2005) Phorbol ester-induced G1 phase arrest selectively mediated by protein kinase Cdelta-dependent induction of p21. J. Biol. Chem. **280:** 33926–33933
39 Rudin C. M., Holmlund J., Fleming G. F., Mani S., Stadler W. M., Schumm P. et al. (2001) Phase I Trial of ISIS 5132, an antisense oligonucleotide inhibitor of c-raf-1, administered by 24-hour weekly infusion to patients with advanced cancer. Clin. Cancer Res. **7:** 1214–1220
40 Gartel A. L. (2005) The conflicting roles of the cdk inhibitor p21(CIP1/WAF1) in apoptosis. Leuk. Res. **29:** 1237–1238
41 Jackson D. N. and Foster D. A. (2004) The enigmatic protein kinase Cdelta: complex roles in cell proliferation and survival. FASEB J. **18:** 627–636