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*MINIREVIEW*

# Cell-Type Specific Integration of Cross-Talk between Extracellular Signal-Regulated Kinase and cAMP Signaling

MILES D. HOUSLAY and WALTER KOLCH

*Molecular Pharmacology Group, Division of Biochemistry & Molecular Biology, Institute of Biomedical & Life Sciences, University of Glasgow, Glasgow, Scotland, United Kingdom (M.D.H., W.K.); and The Beatson Institute for Cancer Research, CRC Beatson Laboratories, Bearsden, Glasgow, Scotland, United Kingdom (W.K.)*

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A myriad of signaling networks control the host of processes necessary for the functioning of cells in health and disease. The range of specialized cell types and the requirements for growth and differentiation have caused ubiquitously expressed signal transduction systems to be adapted to meet the specific requirements of particular cells at particular times. The integration of various signaling systems demands that such processes communicate with each other in feedforward and feedback regulatory loops. To allow for this, cellular differentiation specifies an appropriate array of components whose signaling can be integrated in time and space. A common means of achieving this is for key components in one signaling system to be expressed as isoforms that differ in their ability to couple to other regulatory signaling systems. This may occur either by virtue of their being differentially susceptible to modification or because their expression is targeted to a specific intracellular location allowing for compartmentalized signaling.

The mitogen-activated protein kinase (MAPK) pathway seems to be one of the most ancient signal transduction modules conserved in all eukaryotes (Lewis et al., 1998; English et al., 1999; Schaeffer et al., 1999) and of similar versatility and importance as the cyclic AMP (cAMP) signaling system. When MAPK was cloned about a decade ago, the cDNAs revealed high homologies to two yeast kinases, Fus3 and Kss1, both known to function in a pathway that induces cell cycle arrest in response to mating factor. Taking account of this unexpected relationship and the never ending discovery of yet another external signal that activated MAPK, the enzyme was rechristened "extracellular signal-regulated kinase" (ERK). Since then molecular cloning has unveiled an ever growing family of related genes and made the once biochemical entity MAPK the founder of a large family of kinases that share common regulatory motifs, but seem to serve different functions. This is testimony to nature's economy of using a basic kinase module for an amazing variety of tasks, but unfortunately it also generated a bewildering nomenclature. Although MAPK is often used interchangeably to designate either the whole family or the ERK branch, in this review we will use MAPK to refer to the whole family and use the specific names, such as ERK, for the subgroups.

Cyclic AMP has served as a paradigm for an intracellular second messenger (Houslay and Milligan, 1997). It is involved in mediating the action of a host of processes in specialized cells, ranging from control of various metabolic events, muscle contraction, secretion, and memory, for example. In most cells cAMP serves to inhibit cell growth. However, to confound this, in certain cell types, such as those from the thyroid and pituitary, it can actually stimulate cell growth. Over the years it has proved extremely frustrating and problematic to try and resolve the role of cAMP in regulating cell growth and, perhaps crucially, the pathway controlling ERK activation. Undoubtedly, the interaction between these two signaling systems is of pivotal importance. However, the very complexity of these signaling systems means that it is going to be extremely challenging to resolve the connections that serve to integrate cellular responses through these pathways.

In this review we propose that specific biological effects are achieved on a combinatorial basis. Depending on cell- and situation-specific expression patterns and activity profiles, a small set of basic signaling modules can be linked in various ways to achieve a multitude of specific biological responses. The realization of any potential linkage pattern is determined by spatial and temporal components, like a game of chess where an infinite variety of combinations is spawned

**ABBREVIATIONS:** MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; MAPKK, MAPK kinase; MAPKKK, MAPKK kinase; MEK, ; JNK, ; KIM, kinase interaction motif; PDE, phosphodiesterase; PKA, protein kinase A; RBD, Ras-binding domain; NGF, nerve growth factor; EGF, epidermal growth factor; $PLA_2$, phospholipase $A_2$; COX, cyclooxygenase; $PGE_2$, prostaglandin $E_2$; CRE, cAMP responsive element; $\beta_2$AR, $\beta_2$-adrenergic receptor; 7-TM, 7-transmembrane.

by the temporal and spatial positioning of an otherwise identical set of pieces. Thus, there are at least five layers of regulation: i) the expression of regulators, modulators, and targets; ii) the subcellular localization or compartmentalization of these molecules; iii) their activation status; iv) their mutual interactions; and v) the order and timing of changes in parameters i through iv. In this review we will try to illustrate this concept of dynamic signaling networks using the multiple layers of cross-talk between the cAMP and ERK signaling systems as a paradigm.

## MAPK Signaling Pathways

The core module of MAPK pathways (Lewis et al., 1998; English et al., 1999; Schaeffer et al., 1999) consists of three kinases, MAPK, a serine/threonine-specific kinase, which is activated via phosphorylation by MAPK kinase (MAPKK). MAPKK in turn is activated by a MAPKK kinase (MAPKKK). Typically, MAPKKKs integrate the input signals, and hence often exhibit complex regulation, which usually involves interaction with small GTPases of the Ras or Rho families. MAPKKs appear to be highly specific for their respective MAPKs, and phosphorylate only a restricted subset of MAPKs. In contrast, MAPKs have multiple substrates and constitute the effector end of the kinase cascade. The phosphorylation steps between the kinases are facilitated by physical interactions between the components (English et al., 1999; Schaeffer et al., 1999). They not only serve to amplify the input signals, but also provide regulatory interfaces for tuning the activity (Yeung et al., 1999). In this review we will focus on the ERK pathway, which features Raf family kinases as MAPKKK, MEK-1 and -2 (MAPK/ERK kinase) as MAPKK, and ERK-1, -2 (often designated p44 and p42 MAPK) as MAPK.

The Raf kinase family comprises three isoforms, Raf-1, the cellular homolog of the v-raf oncogene that is ubiquitously expressed, as well as A-raf and B-raf, which show a more restricted expression pattern. Raf-1 is activated in response to a multitude of growth factors and tumor-promoting phorbol esters. A crucial step in Raf-1 activation is the binding to activated, GTP-loaded Ras, which recruits Raf-1 from the cytosol to the plasma membrane where activation takes place. The exact mechanism of activation is still incompletely understood, but involves changes in phosphorylation, the rearrangement of protein associations and binding to lipids. Although both A-raf and B-raf can bind to Ras/GTP and are responsive to activation by Ras, important differences in the activation mechanisms exist. Raf-1 and A-raf require secondary signals for full activation in addition to Ras, whereas B-raf is fully activated by Ras alone. All three Raf isoforms can activate MEK, but the different phenotypes of Raf-1, A-raf and B-raf knock-out mice suggests that the function of Raf family kinases is not redundant.

At present at least six MAPK subgroups can be distinguished in mammalian cells (Fig. 1). The classical ERK pathway is mainly responsive to mitogens and governs such fundamental processes as cell proliferation, neoplastic transformation, differentiation, and survival. The physiological role of the stress-activated JNK and p38 pathways is more obscure, but they seem to be more involved in the control of cell cycle checkpoints, apoptosis, and differentiation (Schaeffer et al., 1999).

Although the diverse MAPK pathways respond to different external cues and exert distinct biological functions, there seems to be considerable potential for an extensive overlap not only with regard to the activating stimuli, but also in regard to downstream targets. Although it is largely unknown how the specificity and fidelity of distinct biological responses is secured, recent proposals indicate that MAPK substrates contain docking sites that serve to define fidelity of interaction by enhancing the binding between specific MAPK and their substrates. Thus, while both ERK and JNK prefer PX(S/T)P as a consensus phosphorylation motif the presence of such a motif is insufficient in itself to define a protein as genuine substrate in vivo. Rather, it has been suggested that authentic substrates contain additional

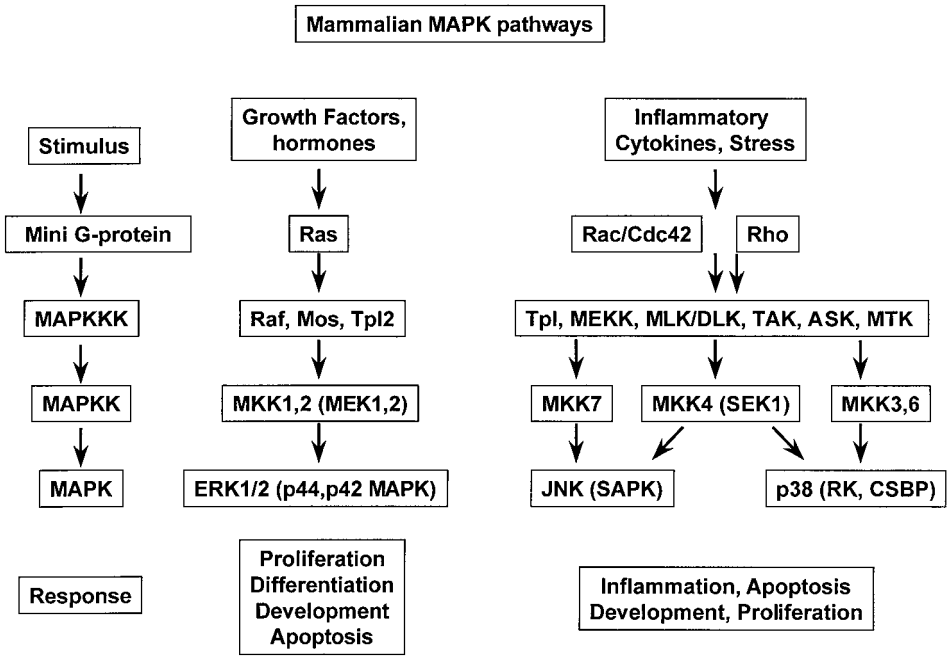


**Fig. 1.** Schematic for MAP kinases. This shows the various mammalian mitogen activated protein (MAP) kinase pathways that have been elucidated. Shown on the left is the generalized scheme of a stimulus serving to activate a mini-guanine nucleotide binding protein (G-protein) by allowing GTP to replace GDP in its active site. The GTP-bound G-protein then activates a kinase cascade comprising a MAPKKK, a MAPKK, and, finally, MAPK itself. The components of the growth factor-stimulated ras pathway to ERK activation are shown together with the two cytokine/stress-activated pathways.

"docking" motifs that enhance interaction of potential substrate proteins with either or both ERK and JNK (Kallunki et al., 1996). Thus ERK substrates appear to be modular systems containing the target phosphorylation site and either one or two docking sites (Gavin and Nebreda, 1996; Yang et al., 1998; Jacobs et al., 1999; Saxena et al., 1999; Smith et al., 1999; Zuniga et al., 1999). Two such docking sites have been identified (Fig. 2a). One is provided by a FxF motif that is located some 5 to 30 amino acids carboxyl-terminal to the ERK phosphorylation site and where the intervening residue (x) is most often Q. In the case of the transcription factor LIM-1 an adjacent proline residue, as in the motif FQFP, also appears to be important. However, this proline is clearly not needed in all instances. The other docking site is the so-called kinase interaction motif (KIM) that is located approximately 120 to 150 amino acids N-terminal to the target for ERK phosphorylation. At the core of the KIM region is a LxL motif located some 3 to 6 amino acids C-terminal to a pair of basic residues (R/K, R/K). These are usually, but not in all instances, located two residues C-terminal to an aliphatic residue (L/V). Interestingly, some substrates, such as the transcription factor Elk-1 contain both such sites while others, such as the STEP and the PTP tyrosyl phosphatases, contain only the KIM site.

## Cyclic AMP Signaling

cAMP provided the paradigm for the second messenger concept and spawned the field of intracellular signaling. Intriguingly, however, there remains a dearth of knowledge concerning the role of the many isoforms of components related to its generation, detection, and degradation (Houslay and Milligan, 1997).

Why, for example, are there at least 10 forms of adenylyl cyclase able to generate cAMP? These are not evidence of redundancy, but relate to requirements to define regulation and intracellular localization (Sunahara et al., 1996). Thus, while all these isoenzymes can be activated by the GTP-bound $\alpha$-subunit of $G_s$, they are differentially sensitive to regulation by $Ca^{2+}$, by phosphorylation and by G-protein $\beta\gamma$- and inhibitory, $\alpha$-subunits (Smit and Iyengar, 1998). And the AC-3 isoform is apparently restricted to specialized regions of the cell surface plasma membrane in olfactory neurons (Houslay et al., 1998; Schwencke et al., 1999).

(a)

(L/V)-X-X-(K/R)-(K/R)-$(X)_{3\text{-}6}$-L-X-L-$(X)_{120\text{-}150}$-**S**-P-$(X)_{5\text{-}30}$-FQF

KIM FQF

(b)

| *PKA motif in KIM regions* | | *KIM regions lacking PKA motif* | |
|---|---|---|---|
| PTP-SL: | RRGSN | Elk-1: | RKPRD |
| STEP: | RRGSN | rsk: | RRVRK |
| He-PTP: | RRGSS | SAP-1: | KKPKG |
| LC-PTP: | RRGSN | SAP-2: | KKPKG |
| JIP-1: | KRPTT | | |
| PDE4: | KKVTS | | |

**Fig. 2.** Docking motifs for ERK. a, this shows the consensus motif for the KIM and FQF docking sites and their spatial organization relative to the target site for phosphorylation. b, these show a range of proteins having KIM docking sites that either do or do not contain a consensus PKA phosphorylation site associated with the core basic residues found in their KIM docking site. The sequence associated with this core region is shown.

The complexity of the cAMP synthetic machinery is, surprisingly, outdone by that which degrades cAMP (Conti and Jin, 1999; Soderling and Beavo, 2000). For, there are at least 15 genes encoding over 30 different phosphodiesterase (PDE) isoforms, each of which is able to hydrolyze cAMP! The importance of this diversity is clearly evident from the actions of selective inhibitors that have been and are being developed for a variety of therapeutic uses. Nascent analyses of this multitude of isoenzymes shows that they are poised to play a pivotal role in integrating cAMP signaling to that of other signaling systems. In addition, PDE enzymes can be found both as soluble cytosolic species and also targeted to interact with particular subcellular membranes and other proteins (Houslay and Milligan, 1997; Houslay et al., 1998), indicating that they play a fundamental role in defining compartmentalized signaling reactions.

Protein kinase A (PKA) is an effector system that responds functionally to changes in intracellular cAMP. This is a heterotetrameric protein that is, again, found as isoforms. Of particular note are the RI and RII forms of the regulatory (R) subunits. While the RI isoform is found in the cell cytosol, the RII isoform is predominately localized at discrete intracellular sites. This anchoring is achieved by the dimerization interface of the RII subunit binding to specific proteins called AKAPs (Colledge and Scott, 1999). Indeed, there is a large family of such AKAPs and these are, seemingly, expressed in a cell-type specific fashion. Thus the anchored RII form of PKA is able to sample gradients of cAMP that have been established through the action of either or both anchored PDE isoforms and adenylyl cyclase isoforms localized to discrete subdomains of the cell surface plasma membrane. This provides the cellular machinery that underpins compartmentalized cAMP signaling for which the notion was originally established convincingly in studies done on cardiac myocytes (Brunton et al., 1981).

It is now apparent, however, that cells have additional cAMP detection systems. Cyclic nucleotide-gated ion channels provide one of these. Such channels are found at specific intracellular sites and thus, like RII-PKA are able to sample intracellular gradients of cAMP. The other is a family of recently discovered cAMP-stimulated GTPase exchange proteins, called EPACs or cAMP-GEFs that, seemingly, serve to activate the small G-proteins Rap1a and Rap1b (de Rooij et al., 1998; Kawasaki et al., 1998). The functional significance of these GEFs remains to be fully established in terms of their intracellular localization and the processes that they regulate.

## Cross-Talk between the cAMP Second Messenger System and the ERK Pathway

When the charting of the ERK pathway was successfully accomplished some 8 years ago, it was one of the first pathways that laid open a seamless connection between extracellular growth factors and nuclear transcription factors, and in addition revealed a plethora of novel regulatory motifs.

**Inhibition of the Raf-1 Kinase by PKA.** A seminal discovery was that the elevation of cAMP in fibroblasts and vascular smooth muscle cells induced a profound inhibition of

ERK activation by growth factors (Burgering et al., 1993; Cook and McCormick, 1993; Graves et al., 1993; Sevetson et al., 1993; Wu et al., 1993; Hafner et al., 1994; Hordijk et al., 1994; Russel et al., 1994). This inhibition was mapped to occur at a point downstream of Ras and upstream of Raf-1 and to require the action of PKA. The target identified was Raf (Fig. 3).

In vitro, PKA phosphorylates Raf-1 on three sites, serines 43, 259, and 621 (Hafner et al., 1994). These sites are constitutively phosphorylated in quiescent cells, but their phosphorylation is enhanced when fibroblasts are treated with cAMP agonists. Notably, the constitutive phosphorylation is not sensitive to a PKA inhibitor suggesting it is maintained by other kinases. PKA phosphorylation of Raf-1 on Ser-43, located in the regulatory domain just upstream of the Ras-Binding Domain (RBD) reduces the affinity of Raf-1 to Ras/GTP, and hence interferes with Raf-1 activation (Wu et al., 1993). However, the observation (Mischak et al., 1996) that Raf kinase mutants lacking Ser-43 were still susceptible to cAMP inhibition indicated that PKA phosphorylation can, in addition, directly suppress the catalytic activity of Raf-1.

Ser-259 is also phosphorylated by akt/PKB, and since cAMP can activate akt/PKB (Filippa et al., 1999), the cAMP-mediated increase in Ser-259 phosphorylation could occur indirectly by this route. Surprisingly, mutation of Ser-259 to negatively charged amino acids that often function as phosphorylation surrogates renders Raf-1 constitutively active but still receptive to further stimulation by growth factors (Dent et al., 1995). Nevertheless, such a mutant is largely refractory to inhibition by PKA, consistent with a negative regulatory function of Ser-259 phosphorylation. This may be reconciled if this action is exerted by the binding of an inhibitor to the negatively charged phosphoserine rather than by a direct negative effect on catalytic activity. Indeed, both Raf-1 phosphoserines 259 and 621 have been shown to serve as docking sites for 14-3-3 proteins. These are dimeric adapter proteins that bind to phosphoserine consensus motifs. It has been proposed that the binding of a 14-3-3 dimer stabilizes Raf-1 in an inactive conformation, and that Ras initiates Raf-1 activation by displacing 14-3-3 from phosphorylated Ser-259 (Tzivion et al., 1998). According to this model the mutation of Ser-259 should generate a transition state that facilitates activation by Ras, a proposal that has yet to be tested.

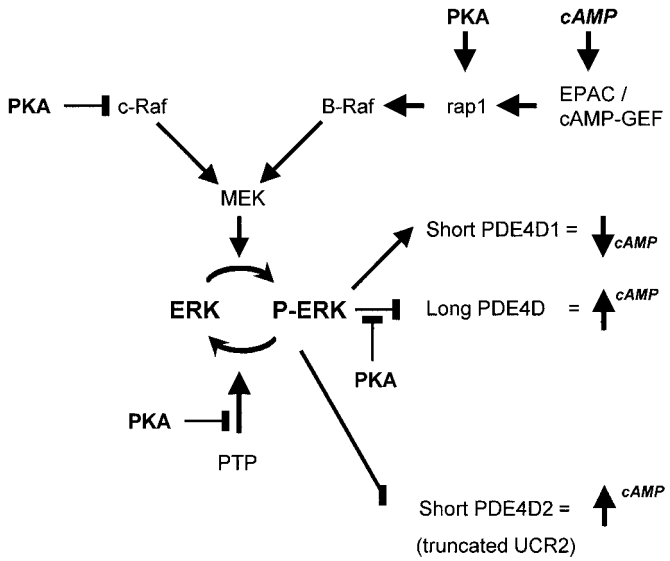


**Fig. 3.** Potential sites of cross-talk between the ERK and cAMP signaling systems: I. This highlights the range of connections where cAMP exerts controlling effects on the ERK signaling pathway. It is crucial to note that these connections will be formulated in a cell-type specific fashion. Thus different cell types will express different complements of, for example, PDE4 isoforms, Raf isoforms, and ERK-regulating protein tyrosyl phosphatases (PTP).

The role of Ser-621 phosphorylation in Raf-1 is less clear (Mischak et al., 1996). Understanding has not been aided by the fact that mutation of Ser-621 to a variety of different amino acids has deleterious consequences for the catalytic activity of Raf-1, thus prohibiting informative studies through mutational analyses. Biochemical experiments with the isolated Raf-1 kinase domain lacking Ser-43 and Ser-259, suggested that although Ser-621 is required for structural integrity, its phosphorylation suppresses catalytic activity (Mischak et al., 1996). In contrast, other studies have found no effect of Ser-621 phosphorylation or even observed a positive correlation with an increase in activity (Sprenkle et al., 1997; Thorson et al., 1998). The reasons for these discrepancies are unknown, but may be due to variations in 14-3-3 association and the assortment of other post-translational modifications. An example for the context-dependent effects of phosphorylation is provided by the observation that the loss of Ras binding by Ser-43 phosphorylation can be partially restored by protein kinase C activation of Raf-1 (Hafner et al., 1994). Given that PKA can antagonize the activity of the isolated Raf-1 kinase domain, where Ser-621 is the only detectable PKA target site (Mischak et al., 1996), it is likely that at least in this context Ser-621 phosphorylation participates in an inhibitory process.

**Complex Interactions between B-raf and the cAMP Signaling System.** Cyclic AMP, however, seemingly promotes ERK activity in several cell types, including 3T3-F442A preadipocytes, ovarian granulosa cells, melanoma, pituitary cells, and neuronal cells, where ERK is intimately involved in cAMP-regulated processes such as long-term potentiation and circadian gene expression.

The basis for this phenomenon has been most extensively investigated in PC12 cells, a rat pheochromocytoma cell line that is widely used as a model for neuronal differentiation. Differentiation requires sustained ERK activation and ERK nuclear translocation, which mainly appears to be mediated by cAMP through stimulation of MEK (Yao et al., 1998). PC12 cells express Raf-1 and B-raf, both of which are activated in response to the differentiation factor nerve growth factor (NGF). Stork's group recently reported (Vossler et al., 1997) that, in PC12 cells, cAMP shuts off Raf-1 but stimulates ERKs through the activation of B-raf by the Ras-related G-protein Rap1. Rap1 was originally isolated as suppressor of Ras transformation, probably because it can bind to and sequester Raf-1 in an inactive complex when overexpressed. In contrast, Rap1 can bind to and activate B-raf (Ohtsuka et al., 1996). Interestingly, cAMP and NGF are two of the various growth factors among the physiological activators of Rap1 (Vossler et al., 1997).

The way in which cAMP is able to activate Rap1 still requires elucidation. It has been convincingly shown that PKA can phosphorylate Rap1, and this was assumed to provide the underlying molecular mechanism for activation. However, more recently cAMP-activated GDP/GTP exchange

factors have been identified (de Rooij et al., 1998; Kawasaki et al., 1998). These possess both cAMP binding domains and GTPase exchange factor domains and have been called EPACs or cAMP-GEFs. Such proteins can, seemingly, serve to activate Rap1 without any requirement for PKA action. It may be that both PKA-dependent and independent pathways can allow for Rap1 activation and that these are selected in a cell-type specific manner (Fig. 3).

Nevertheless, Stork's group went on to show (York et al., 1998) that NGF uses Ras/Raf-1 to elicit rapid but, due to Raf-1 suppression, transient ERK activation, and Rap1/B-raf to maintain the prolonged phase needed for neuronal differentiation. EGF does not stimulate Rap1, and hence cannot sustain ERK activity and fails to support differentiation. This clever model neatly rationalizes how a specific biological response linked to a specific kinetic response within the ERK pathway is orchestrated by cAMP switching signaling from Raf-1 to B-raf. It is also supported by evidence from other cell systems suggesting that the expression of B-raf determines whether cAMP inhibits or activates ERK (Gao et al., 1999; Seidel et al., 1999). Despite its appeal, this model is at odds with previous results from other groups showing that both Raf-1 and B-raf were inhibited by PKA and that cAMP must bypass Raf to activate MEK (Vaillancourt et al., 1994; Erhardt et al., 1995; Peraldi et al., 1995). The most likely explanation for these discrepancies may be the use of different PC12 cell clones, which are equipped with a different set of MEK activators. For instance, NGF also sustains A-raf activation (Wixler et al., 1996), and at least in hematopoetic cells A-raf activation is resistant to cAMP (Sutor et al., 1999). Alternatively, PKA could play a more direct role by affecting ERK inactivation and subcellular localization as discussed below.

**Cyclic AMP and the Control of ERK Deactivation.** KIM motifs appear to play a pivotal role in defining the ability of ERKs to interact with other proteins. Intriguingly, certain of these motifs (Fig. 2b) contain a putative site (RRxS/T) for phosphorylation by PKA. This offers the potential for PKA to modify the KIM docking site for ERK substrates by introducing a negative charge near to the pair of positive charges shown to be essential for ERK docking. Very recently this has been examined with regard to the PTP and PTP-SL tyrosyl phosphatases (Blanco-Aparicio et al., 1999; Saxena et al., 1999). These two enzymes show a highly restricted, cell-type specific distribution and both serve as negative regulators of ERK. Indeed PTP is a cytosolic tyrosyl phosphatase that specifically regulates ERK and not JNK. Such specificity is seemingly conferred because ERK can bind to these tyrosyl phosphatases through their KIM motifs. This allows these phosphatases to dephosphorylate the critical tyrosine residue that MEK phosphorylates in the activation loop of ERK, thus causing ERK deactivation. Such an interaction, and the ensuing dephosphorylation of ERK, prevents the nuclear translocation of ERK-2 (Blanco-Aparicio et al., 1999) and thus the delivery of activated ERK to the site where it can affect transcriptional factors.

Intriguingly, however, it has been shown recently that PKA phosphorylation within the KIM region of these two tyrosyl phosphatases prevents their binding to ERK. Thus in cells where these or similar phosphatases act as important regulators of ERK, then PKA activation can be expected to synergize and even sustain ERK activation by inhibiting its dephosphorylation. Indeed, if there is a tonic stimulation of ERK in cells, which is normally kept in check by dephosphorylation, then PKA might even serve to achieve ERK activation. In this regard, it has been noted (Englaro et al., 1995) that increased levels of cAMP in melanoma cells led to ERK activation without activation of either Raf or MEK kinase. It is possible that this could be an example of an effect mediated by the uncoupling of a tyrosyl phosphatase.

The restricted expression of these tyrosyl phosphatases means that PKA can be expected to exert highly cell-type specific effects through modifying the ability of these enzymes to bind to and thus dephosphorylate ERK. For example, such a system may help to explain an effect of sustained ERK activation in PC12 cells (see above). However, clearly, if many ERK substrates also require binding to KIM regions then the relative concentrations and affinities of both substrates and any such phosphatases for ERK binding will be of importance in appreciating rates of activation and deactivation of this key regulatory protein kinase.

Nevertheless, that KIM motifs appear to be specific for ERKs and do not allow JNK binding, indicates a means of conferring enhanced specificity to this system and directing potential regulation by PKA in a cell-type specific fashion.

**Regulation of cAMP Levels Directed by ERK-2 Docking and Phosphorylation of PDE4 cAMP Phosphodiesterases.** Very recently it has been shown that members of the multigene PDE4 cAMP-specific phosphodiesterases can serve as ERK-2 substrates and thus provide a direct means whereby ERK-2 activation can lead to changes in intracellular cAMP levels (Hoffmann et al., 1999; MacKenzie et al., 2000). PDE4 enzymes are closely related to the drosophila *dunc* PDE whose disruption causes memory and learning defects (Houslay et al., 1998). They are characterized by their ability to be inhibited by the compound rolipram, which serves as the paradigm for a PDE4 selective inhibitor. There is currently great interest in these enzymes because PDE4-selective inhibitors are currently being developed for treating inflammatory disease, in particular chronic obstructive pulmonary disease and asthma, and also have potential as antidepressant and antileukemic agents (Houslay et al., 1998; Rogers and Giembycz, 1998; Torphy et al., 1999).

Four genes encode over 16 different PDE4 splice variants that are divided into two groupings (Houslay et al., 1998). The so-called long isoenzymes possess two conserved regions, called UCR1 and UCR2, that are unique to the PDE4 enzyme family and are located between the catalytic unit and the extreme N-terminal domain, while short isoenzymes lack UCR1 (Fig. 3). Such regions can interact with each other and serve to mediate the stimulatory effect of PKA phosphorylation of UCR1 on enzyme activity (Beard et al., 2000). Individual isoenzymes are each distinguished by unique N-terminal regions. These are encoded by distinct exons, for which a major role appears to be to define intracellular targeting (Houslay et al., 1998; McPhee et al., 1999; Yarwood et al., 1999). In many cells PDE3 and PDE4 isoenzymes provide the major PDE activities. Nevertheless, selective inhibitors generate very different effects on cellular function, being consistent with compartmentalized cAMP signaling determined by the very different intracellular localization of these enzymes (Houslay et al., 1998).

Studies on the PDE4D enzyme family have shown (MacKenzie et al., 2000) that not only does ERK-2 phosphorylate

these isoenzymes at a single serine residue in their catalytic unit but that functional KIM and FQF docking sites flank this target residue (Fig. 2). These sites allow ERK-2 to form a tight complex with PDE4D enzymes in cells, such that they can be co-immunoprecipitated. Mutation of either of these sites destroys this interaction and negates phosphorylation in vivo.

PDE4D isoenzymes are expressed in a cell-type specific fashion with the most commonly expressed species being the long PDE4D3 and PDE4D5 isoenzymes. Phosphorylation by ERK-2 causes these isoenzymes to become markedly inhibited, thus leading to an increase in cAMP levels in cells where they provide a major fraction of the total PDE activity or in compartmentalized cAMP systems that they dominate (Bolger et al., 1997; Hoffmann et al., 1999; MacKenzie et al., 2000). This provides a regulatory system where ERK-2 activation could, potentially, lead to an increase in cAMP levels. However, ERK regulation of these long PDE4 enzymes is in itself subject to feedback control. This is because PKA can phosphorylate a single site within UCR1 that ablates the inhibitory effect of ERK phosphorylation (Figs. 3 and 4) (Hoffmann et al., 1999; MacKenzie et al., 2000). The underpinning mechanism appears to be the disruption of the interaction between UCR1 and UCR2 by PKA-mediated phosphorylation of UCR1. Thus these long PDE4D isoenzymes can determine a transient increase in cAMP levels that is triggered by them being phosphorylated by ERK. For the initial inhibitory phosphorylation of the PDE4D3/5 catalytic unit by ERK can be expected to lead to an increase in cAMP, whereupon PKA is activated causing phosphorylation of the regulatory UCR1 region. This ablates ERK-2 inhibition, causing the increased PDE4D activity to lower cAMP levels. Clearly the form of the ERK-mediated change in cAMP levels will depend on a variety of factors including the magnitude of PDE4D activity and its location within the cell, the availability of PKA and the regulation of ERK activity itself. This system defines a novel way of regulating the interactions between these two signaling systems.

It should be noted that while the KIM docking region of PDE4D contains a putative PKA phosphorylation site (Fig. 2) these long PDE4D isoenzymes, when expressed in COS cells, were not phosphorylated at this site upon PKA activation (Hoffmann et al., 1998, 1999). This would be consistent with these isoenzymes being in a tight complex with ERK-2 such that access of PKA to this site was prevented. This tight complex is likely to result from PDE4D enzymes having both KIM and FQF docking sites (MacKenzie et al., 2000). This is unlike the tyrosyl phosphatases that just have a KIM site (Blanco-Aparicio et al., 1999; Saxena et al., 1999) and where a presumed lower affinity of interaction will allow competition with and thus access to PKA.

*PDE4* genes also encode short isoenzymes (Houslay et al., 1998). These differ from the long isoenzymes in that they lack the UCR1 component of the N-terminal regulatory module (Fig. 3). Not only does this remove the site for stimulatory regulation by PKA, but the lone UCR2 component serves to switch the effect of ERK-2 phosphorylation from causing inhibition to causing activation (MacKenzie et al., 2000). Thus, ERK phosphorylation of the short PDE4D1 isoenzyme with a lone, intact UCR2 causes a small activation that is able to decrease cAMP levels (Figs. 3 and 4), at least in any compartment regulated by such isoenzymes.

The *PDE4* gene also encodes a truncated or "supershort" form, called PDE4D2, that not only lacks UCR1 but also has an N-terminally truncated UCR2. Intriguingly, this form is also inhibited by ERK phosphorylation, albeit to a lesser extent than the PDE4D long isoenzymes (MacKenzie et al., 2000). This then provides a PDE4 isoenzyme that is not only ERK inhibited but, unlike the long isoenzymes, is not subject to feedback regulation by PKA as it lacks the UCR1 and hence a site for PKA phosphorylation (Figs. 3 and 4). It also indicates that the paired UCR1 and UCR2, found in long isoenzymes, serve to amplify an inherent inhibitory action that ERK phosphorylation exerts upon the PDE4D catalytic unit. In addition, it is evident from these observations that it is the N-terminal portion of UCR2 that, in the absence of

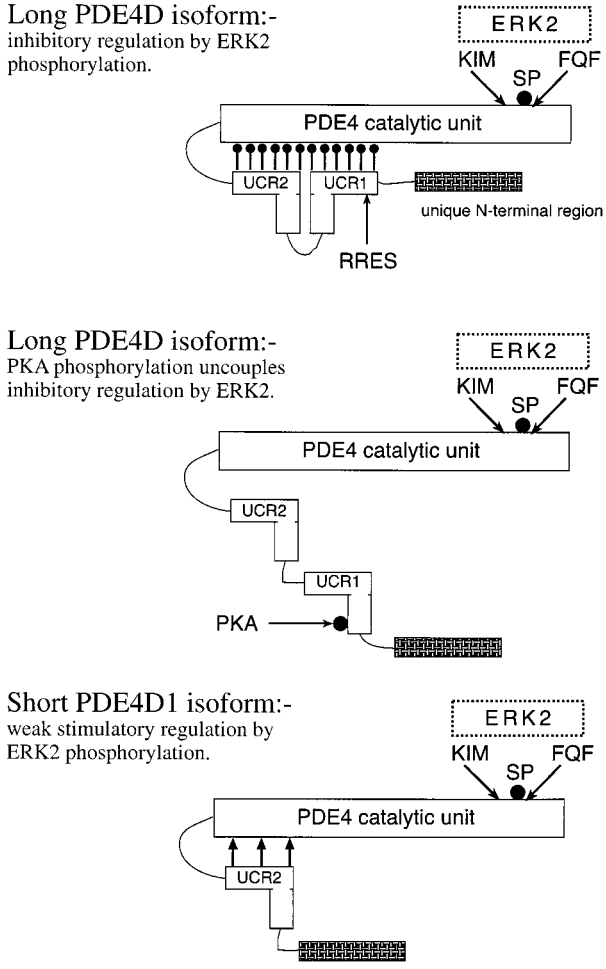


**Fig. 4.** Regulation of PDE4 cAMP-specific phosphodiesterases. This schematically shows the long PDE4 isoenzymes that have both UCR1 and UCR2 domains and the short isoenzymes that lack UCR1. Studies on PDE4D gene products have shown that, in the long PDE4D3 and PDE4D5 isoenzymes, UCR1 and UCR2 form a regulatory module that orchestrates inhibition of PDE4 activity consequent to ERK phosphorylation of the enzyme catalytic unit. UCR1 serves to direct the regulatory effect of PKA phosphorylation by uncoupling the interaction between UCR1 and UCR2, causing enzyme activation and ablation of any ERK-mediated inhibition. In the short PDE4D1 isoenzyme, which has a lone, intact UCR2 region, this directs enzyme activation to occur consequent upon ERK phosphorylation. However, in the short PDE4D2 isoenzyme, which has a lone, N-terminally truncated UCR2 region, this directs enzyme inhibition consequent upon ERK phosphorylation. However, unlike with the long isoenzymes, ERK-mediated inhibition of PDE4D2 cannot be ablated by PKA as there is no UCR1 with its PKA phosphorylation motif.

UCR1, serves to direct activation of the PDE4D1 short forms as a consequence of ERK phosphorylation.

Thus *PDE4* genes appear to encode a family of isoforms that i) are expressed in a cell-type specific fashion; ii) show targeted intracellular localization; and iii) for the PDE4B, -4C, and -4D isoenzymes share an ability to be regulated by ERK-2 phosphorylation. Indeed, it may well be that the underlying reason for generating short and long PDE4 isoenzymes is related to governing the response of these enzymes to ERK phosphorylation and integrating a response to PKA action.

**ERK Activation Can Elevate cAMP Levels in Smooth Muscle Cells through an Autocrine Loop.** Challenge of certain smooth muscle cells with growth factors such as platelet-derived growth factor and bradykinin has been shown to elicit ERK activation and an increase in cAMP levels without growth stimulation (Pyne et al., 1997; Bornfeldt and Krebs, 1999). The prime reason for the increased accumulation of cAMP in these cells is, seemingly, not driven primarily by any direct effect of ERK-2 on either PDE activity or the tyrosyl phosphorylation of $G_s$. Rather it appears to be an indirect autocrine action that is elicited via the activation of cytosolic phospholipase $A_2$ ($PLA_2$). This is caused through its phosphorylation elicited most probably by the action of ERK (Lin et al., 1993) although, as seen in thyroid cells, possibly also via PKC action (Ekokoski et al., 2000). Such activation of $PLA_2$ generates arachidonic acid, a precursor for prostaglandin synthesis through the cyclooxygenase (COX) pathway. Thus the generation and export of prostaglandin $E_2$ ($PGE_2$) causes, in an autocrine fashion, the receptor-stimulated activation of adenylyl cyclase activation and increased cAMP levels (Fig. 5). It is this increase in cAMP that leads to negative regulation of cell growth as treatment with the cyclooxygenase inhibitor, indomethacin, releases cells from this inhibition and allows growth factors to cause cell proliferation. This feedback system appears to be physiologically relevant for some cell types as evidenced by the observation that the ability of ERK to drive the proliferation of smooth muscle cells is subverted by the expression of COX-2. In COX-2-expressing cells, ERK leads to growth inhibition caused by $PGE_2$-mediated cAMP accumulation and activation of PKA (Bornfeldt and Krebs, 1999).

**7-Transmembrane (7-TM) G-Protein-Linked Receptors That Mediate ERK Activation: Constitutive and PKA-Switched Controls.** The provocative proposal has been made (Daaka et al., 1997; Maudsley et al., 2000) that the 7-TM receptors that normally serve to activate adenylyl cyclase can, under certain circumstances, be switched to elicit ERK activation (Fig. 4). It has long been established that the $\beta_2$-adrenergic receptor ($\beta_2$AR), upon agonist occupancy, binds to the heterotrimeric G-protein, $G_s$. This causes the $G_s$ $\beta$-subunit to bind GTP and then dissociate from the complex to stimulate adenylyl cyclase. The subsequent hydrolysis of GTP to GDP provides a turn-off reaction, allowing reassociation of the GDP-bound $G_s$ $\beta$-subunit with the $\beta\gamma$ pair. The $\beta_2$AR is also subject to rapid desensitization due to the dual action of both receptor-specific kinases (G-protein receptor kinases) and PKA that phosphorylate the C-terminal tail of the receptor and prevent productive coupling to $G_s$. Long-term desensitization results from the internalization of the receptor through coated pits. However, it has been suggested that the PKA phosphorylated form of the $\beta_2$AR is not only unable to couple productively to $G_s$ but switches its allegiance to allow coupling to the $G_i$ family of proteins, some of whom serve to inhibit adenylyl cyclase (Daaka et al., 1997). $G_i$ proteins are usually found in excess of $G_s$ and their activation releases not only GTP-bound $\alpha$-$G_i$ but also a large pool of $\beta\gamma$-subunits. It is the release of such $\beta\gamma$-subunits that is believed to lead to ERK activation (Fig. 4). The details of this process remain to be unequivocally defined. A possibility is a direct stimulatory effect on SRC kinase that allows it to couple with SOS, triggering Ras, and hence ERK, activation.

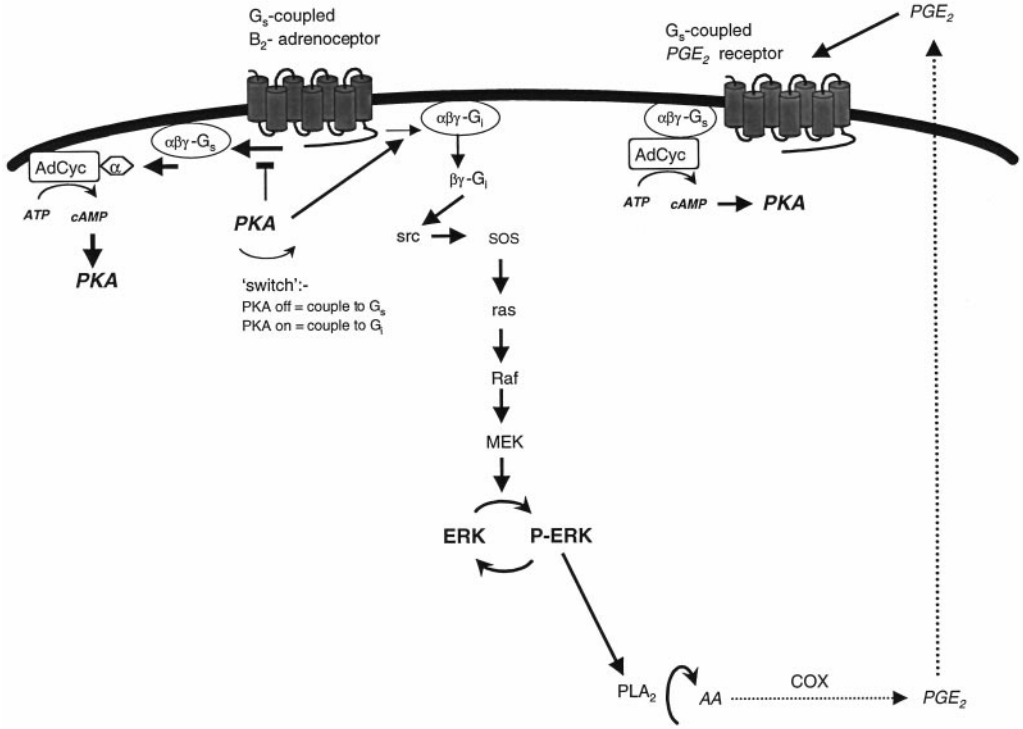


**Fig. 5.** Potential sites of cross-talk between the ERK and cAMP signaling systems: II. This shows two further scenarios that integrate PKA and ERK signaling. The schematic on the left side of this figure highlights the proposed ability of $G_s$-coupled receptors, such as the $\beta_2$-adrenoceptor, to switch to coupling to $G_i$ and thereby causing ERK activation. Increased cAMP levels, causing PKA activation and phosphorylation of the $\beta_2$-adrenoceptor, operate this switch. Shown on the right side of this schematic is the proposal that, in smooth muscle cells, ERK can cause $PLA_2$ activation to generate an autocrine effect that leads to an increase in cAMP levels by $PGE_2$ stimulation of adenylyl cyclase.

The corollary of this is that receptors that have been shown to inhibit adenylyl cyclase by coupling to $G_i$ proteins, such as $\alpha_2$-adrenoceptors, may also serve to elicit ERK activation through such a $\beta\gamma$-subunit-mediated pathway.

These are intriguing and provocative suggestions that open up a range of important questions. Thus it remains unclear as to the range of $G_s$- and $G_i$-linked 7-TM/GPC receptors that can elicit such effects and whether their ability to do this is cell-type specific. It also remains to be defined whether all three $G_i$ isoforms are equally capable of eliciting coupling to ERK and whether their relative expression levels and associated form of $\beta\gamma$ subunits is important. Indeed, as $G_s$ activation also generates $\beta\gamma$-subunits this suggests a threshold or specificity effect in allowing ERK activation. Furthermore, it needs to be identified whether PKA phosphorylation of the $\beta_2$AR and coupling to $G_i$ is subject to interference by G-protein receptor kinase phosphorylation and the subsequent recruitment of the cytosolic protein, arrestin, to this plasma membrane signaling complex, which normally serves to desensitize by inhibiting $G_s$ coupling. These issues suggest that such a process is very likely to be regulated in a highly cell-type specific fashion.

**Tyrosyl Kinase Receptor-Stimulated Adenylyl Cyclase.** Occupancy of the EGF receptor tyrosyl kinase leads to ERK activation. However, it has been suggested that in a highly restricted range of cell types it can also lead to adenylyl cyclase activation (Sun et al., 1997). This is believed to occur through the direct tyrosyl phosphorylation of the $\alpha$-subunit of $G_s$. However, the site of tyrosyl phosphorylation and the mechanism that underpins the proposed activation of $G_s$ by this effect is unclear, as is why this has only been seen in a few instances (example and/or reference). It is certainly possible to discriminate between EGF effects on adenylyl cyclase and on PDE activity by either including (selective) PDE inhibitors in cell-based assays or by isolating membranes for adenylyl cyclase-based assays.

**CREB: A Target for Both cAMP and ERK Signaling Pathways.** CREB is a 43-kDa transcription factor that binds to the conserved cAMP response element (CRE) (Shaywitz and Greenberg, 1999). This consists of an 8-base pair palindromic sequence (TGACGTCA). There are two additional CREB family members, namely CREM and ATF-1. All contain a single consensus phosphorylation site for PKA and all bind to DNA as dimers through a basic carboxyl terminal leucine zipper (bZIP) motif. The single PKA site in CREB is found at Ser-133. To be active CREB requires the phosphorylation of serine 133 enabling it to recruit p300/CBP, CREB binding protein, an essential cofactor that interacts with the basic transcription machinery (Shaywitz and Greenberg, 1999). Current evidence suggests that PKA activation in the extranuclear region of the cell leads to the translocation of the free PKA catalytic unit to the nucleus, whereupon it can phosphorylate CREB family members. However, there is also evidence indicative of the presence of PKA binding proteins (AKAPs) and PKA regulatory (RII) subunits associated with the nuclear membrane and even within the nucleus itself (Colledge and Scott, 1999). This implies that compartmentalized effects involving both extra- and intranuclear changes in cAMP concentrations may regulate CREB phosphorylation.

Ser-133, however, also provides a target for p90rsk (Shaywitz and Greenberg, 1999). This protein kinase is an authentic substrate for ERK, with which it binds through a KIM docking site that does not contain a PKA consensus motif (Gavin and Nebreda, 1999). Ironically then, while CREB activation is often regarded as an index of PKA action it can also provide an index of ERK activation. Thus both the cAMP and ERK pathway actually share a common output in CREB activation. Indeed, an important readout may be the cumulative effect of phosphorylation of Ser-133 in response to ERK and PKA action. Certainly the absence of a PKA site in the ERK-binding KIM domain of p90rsk (Gavin and Nebreda, 1999) clearly uncouples this from any inhibitory regulation through PKA phosphorylation. This contrasts with the ability of PKA to phosphorylate the KIM region of PTP tyrosyl phosphatases. This serves to uncouple them from interacting with and thus deactivating ERK. Thus, in cells that express PTP tyrosyl phosphatases, PKA activation will promote ERK activation of p90rsk and hence CREB phosphorylation.

Interestingly, phosphorylation of Ser-133 in CREB also serves to prime Ser-129 for phosphorylation by GSK3 (Shaywitz and Greenberg, 1999). However, the significance of this has yet to be fully appreciated, although there is some indication that this further enhances transcriptional activation.

Interestingly, BAD, a proapoptotic member of the Bcl2 family, also provides a substrate that is shared by PKA and p90rsk (Chao and Korsmeyer, 1998). BAD dimerizes with either Bclx or Bcl2 and in so doing ablates their protective function to induce apoptosis (Yang et al., 1995). However, phosphorylation of BAD on Ser-136 by akt/PKB and Ser-112 by either p90rsk or PKA redirects the binding specificity of BAD toward 14-3-3 releasing its deadly grip on Bcl2 and Bclx and thus promoting cell survival (Bonni et al., 1999; Datta et al., 1999; Harada et al., 1999; Tan et al., 1999). The significance of the overlap between p90rsk and PKA is unclear. However, it seems clear that it relates to compartmentalized signaling effects as it is an RII form of PKA anchored at the mitochondrial membrane that is responsible for PKA-mediated BAD phosphorylation.

## Conclusion

No signaling pathway functions in isolation. However, we are only at the start of understanding the range of possible connections that allow integration between them. The cAMP and ERK pathways are ubiquitous and pivotal to cellular growth, development, and function. We have tried in this review to highlight some new and exciting processes that provide connections between these pathways. In doing so it is quite clear that the modular design of proteins and their expression patterns are pivotal to the wiring systems that are unique to each cell-type. Thus, in analyzing systems it is crucial to take account of the set of players involved, their isoforms and intracellular location. In trying to reconcile studies performed in different cell types it is thus crucial to appreciate the identify of the isoforms present and, in addition, whether the intracellular organization of components of specific signaling systems generates compartmentalized responses. In addition, as these can be coupled to feedback and feedforward loops then analyses must be done over appropriate temporal windows. Thus changes in individual components of signaling systems are transient and geared to trigger a defined cascade of responses over time as they pass over set activation thresholds. An understanding of the cross-talk processes that interlink signaling systems in defined cell

types is crucial to our understanding of cell function in health and disease and thus in developing new diagnostic methodologies and new therapeutics.

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**References**

Beard MB, Olsen AE, Jones RE, Erdogan S, Houslay MD and Bolger GB (2000) UCR1 and UCR2 domains unique to the cAMP-specific phosphodiesterase (PDE4) family form a discrete module via electrostatic interactions. *J Biol Chem* **275:** 10349–10358.

Blanco-Aparicio C, Torres J and Pulido R (1999) A novel regulatory mechanism of MAP kinase activation and nuclear translocation mediated by PKA and the PTP-SL tyrosine phosphatase. *J Cell Biol* **147:**1129–1135.

Bolger GB, Erdogan S, Jones RE, Loughney K, Scotland G, Hoffmann R, Wilkinson I, Farrell C and Houslay MD (1997) Characterisation of five different proteins produced by alternatively spliced mRNAs from the human cAMP-specific phsohodiesterase PDE4D gene. *Biochem J* **328:**539–548.

Bonni A, Brunet A, West AE, Datta SR, Takasu MA and Greenberg ME (1999) Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and independent mechanisms [See comments]. *Science (Wash DC)* **286:**1358–1362.

Bornfeldt KE and Krebs EG (1999) Crosstalk between protein kinase A and growth factor receptor pathways in arterial smooth muscle. *Cell Signal* **11:**456–477.

Brunton LL, Hayes JS and Mayer SE (1981) Functional compartmentation of cyclic AMP and protein kinase in heart. *Adv Cyclic Nucleotide Res* **14:**391–397.

Burgering BM, Pronk GJ, van Weeren PC, Chardin P and Bos JL (1993) cAMP antagonizes p21ras-directed activation of extracellular signal-regulated kinase 2 and phosphorylation of mSos nucleotide exchange factor. *EMBO J* **12:**4211–4220.

Chao DT and Korsmeyer SJ (1998) BCL-2 family: Regulators of cell death. *Annu Rev Immunol* **16:**395–419.

Colledge M and Scott JD (1999) AKAPs: From structure to function. *Trends Cell Biol* **9:**216–221.

Conti M and Jin SLC (1999) The molecular biology of cyclic nucleotide phosphodiesterases. *Prog Nucleic Acid Res* **63:**1–38.

Cook SJ and McCormick F (1993) Inhibition by cAMP of ras-dependent activation of raf. *Science (Wash DC)* **262:**1069–1072.

Daaka Y, Luttrell LM and Lefkowitz RJ (1997) Switching off the coupling of the beta2-adrenergic receptor to different G-proteins by protein kinase A. *Nature (Lond)* **390:**88–91.

Datta SR, Brunet A and Greenberg ME (1999) Cellular survival: A play in three Akts. *Genes Dev* **13:**2905–2927.

Dent P, Reardon DB, Morrison DK and Sturgill TW (1995) Regulation of Raf-1 and Raf-1 mutants by Ras-dependent and Ras-independent mechanisms in vitro. *Mol Cell Biol* **15:**4125–4135.

de Rooij J, Zwartkruis FJ, Verheijen MH, Cool RH, Nijman SM, Wittinghofer A and Bos JL (1998) Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature (Lond)* **396:**474–477.

Ekokoski E, Dugue B, Vainio M, Vainio PJ and Tornquist K (2000) Extracellular ATP-mediated phospholipase A(2) activation in rat thyroid FRTL-5 cells: Regulation by a G(i)/G(o) protein, Ca(2+) and mitogen-activated protein kinase. *J Cell Physiol* **183:**155–162.

Englaro W, Rezzeonico R, Durand-Clement M, Lallemand D, Ortonne JP and Ballotti R (1995) Mitogen-activated protein kinase pathway and AP-1 are activated during cAMP-induced melanogenesis in B-16 mealanoma cells. *J Biol Chem* **270:** 24315–24320.

English J, Pearson G, Wilsbacher J, Swantek J, Karandikar M, Xu S and Cobb MH (1999) New insights into the control of MAP kinase pathways. *Exp Cell Res* **253:**255–270.

Erhardt P, Troppmair J, Rapp UR and Cooper GM (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.

Filippa N, Sable CL, Filloux C, Hemmings B and Van Obberghen E (1999) Mechanism of protein kinase B activation by cyclic AMP-dependent protein kinase. *Mol Cell Biol* **19:**4989–5000.

Gao Z, Chen T, Weber MJ and Linden J (1999) A2B adenosine and P2Y2 receptors stimulate mitogen-activated protein kinase in human embryonic kidney-293 cells. Cross-talk between cyclic AMP and protein kinase C pathways. *J Biol Chem* **274:**5972–5980.

Gavin AC and Nebreda AR (1999) A MAP kinase docking site is required for phosphorylation and activation of p90(rsk)/MAPKAP kinase-1. *Curr Biol* **9:**281–284.

Graves LM, Bornfeldt KE, Raines EW, Potts BC, Macdonald SG, Ross R and Krebs EG (1993) Protein kinase A antagonizes platelet-derived growth factor-induced signaling by mitogen-activated protein kinase in human arterial smooth muscle cells. *Proc Natl Acad Sci USA* **90:**10300–10304.

Hafner S, Adler HS and Mischak H (1994) Mechanism of inhibition of Raf-1 by protein kinase A. *Mol Cell Biol* **14:**6696–6703.

Harada H, Becknell B, Wilm M, Mann M, Huang LJ, Taylor SS, Scott JD and Korsmeyer SJ (1999) Phosphorylation and inactivation of BAD by mitochondria-anchored protein kinase A. *Mol Cell* **3:**413–422.

Hoffmann R, Baillie GS, MacKenzie SJ, Yarwood SJ and Houslay MD (1999) The MAP kinase ERK2 inhibits the cyclic AMP-specific phosphodiesterase, HSPDE4D3 by phosphorylating it at Ser579. *EMBO J* **18:**893–903.

Hoffmann R, Wilkinson IR, McCallum JF, Engels P and Houslay MD (1998) cAMP-specific phosphodiesterase HSPDE4D3 mutants which mimic activation and changes in rolipram inhibition triggered by protein kinase A phosphorylation of Ser-54: Generation of a molecular model. *Biochem J* **333:**139–149.

Hordijk PL, Verlaan I, Jalink K, Van Corven EJ and Moolenaar WH (1994) cAMP abrogates the p21(ras)-mitogen-activated protein kinase pathway in fibroblasts. *J Biol Chem* **269:**3534–3538.

Houslay MD and Milligan G (1997) Tailoring cAMP signalling responses through isoform multiplicity. *Trends Biochem Sci* **22:**217–224.

Houslay MD, Sullivan M and Bolger GB (1998) The multi-enzyme PDE4 cyclic AMP specific phosphodiesterase family: Intracellular targeting, regulation and selective inhibition by compounds exerting anti-inflammatory and anti-depressant actions. *Adv Pharmacol* **44:**225–342.

Jacobs D, Glossip D, Xing H, Muslin AJ and Kornfeld K (1999) Multiple docking sites on substrate proteins form a modular system that mediates recognition by ERK MAP kinase. *Genes Dev* **13:**163–175.

Kallunki T, Deng T, Hibi M and Karin M (1996) c-Jun can recruit JNK to phosphorylate dimerization partners via specific docking interactions. *Cell* **87:**929–939.

Kawasaki H, Springett GM, Mochizuki N, Toki Mochizuki, Nakaya M, Matsuda M, Housman DE and Graybiel AM (1998) A family of cAMP-binding proteins that directly activate Rap1. *Science (Wash DC)* **282:**2275–2279.

Lewis TS, Shapiro PS and Ahn NG (1998) Signal transduction through MAP kinase cascades. *Adv Cancer Res* **74:**49–139.

Lin LL, Wartmann M, Lin AY, Knopf JL, Seth A and Davis RJ (1993) cPLA2 is phosphorylated and activated by MAP kinase. *Cell* **72:**269–278.

MacKenzie SJ, Baillie GS, McPhee I, Bolger GB and Houslay MD (2000) ERK2 MAP kinase binding, phosphorylation and regulation of PDE4D cAMP specific phosphodiesterases: The involvement of C-terminal docking sites and N-terminal UCR regions. *J Biol Chem* **275:**16609–16617.

Maudsley S, Pierce KL, Zamah AM, Miller WE, Ahn S, Daaka Y, Lefkowitz RJ and Luttrell LM (2000) The beta(2)-adrenergic receptor mediates extracellular signal-regulated kinase activation via assembly of a multi-receptor complex with the epidermal growth factor receptor. *J Biol Chem* **275:**9572–9580.

McPhee I, Yarwood SJ, Scotland G, Huston E, Beard MB, Ross AH, Houslay ES and Houslay MD (1999) Association with the src family tyrosyl kinase lyn triggers a conformational change in the catalytic region of human cAMP-specific phosphodiesterase HSPDE4A4B: Consequences for rolipram inhibition. *J Biol Chem* **274:** 11796–11810.

Mischak H, Seitz T, Janosch P, Eulitz Janosch, Steen H, Schellerer M, Philipp A and Kolch W (1996) Negative regulation of Raf-1 by phosphorylation of serine 621. *Mol Cell Biol* **16:**5409–5418.

Ohtsuka T, Shimizu K, Yamamori B, Kuroda S and Takai Y (1996) Activation of brain B-Raf protein kinase by Rap1B small GTP-binding protein. *J Biol Chem* **271:**1258–1261.

Peraldi P, Frodin M, Barnier JV, Calleja V, Scimeca JC, Filloux C, Calothy G and Van Obberghen E (1995) Regulation of the MAP kinase cascade in PC12 cells: B-Raf activates MEK-1 (MAP kinase or ERK kinase) and is inhibited by cAMP. *FEBS Lett* **357:**290–296.

Pyne NJ, Tolan D and Pyne S (1997) Bradykinin stimulates cAMP synthesis via mitogen-activated protein kinase-dependent regulation of cytosolic phospholipase A2 and prostaglandin E2 release in airway smooth muscle. *Biochem J* **328:**689–694.

Rogers DF and Giembycz MA (1998) Asthma therapy for the 21st century. *Trends Pharmacol Sci* **19:**160–164.

Russell M, Winitz S and Johnson GL (1994) Acetylcholine muscarinic m1 receptor regulation of cyclic AMP synthesis controls growth factor stimulation of Raf activity. *Mol Cell Biol* **14:**2343–2351.

Saxena M, Williams S, Tasken K and Mustelin T (1999) Crosstalk between cAMP-dependent kinase and MAP kinase through a protein tyrosine phosphatase. *Nat Cell Biol* **1:**305–311.

Schaeffer HJ and Weber MJ (1999) Mitogen-activated protein kinases: Specific messages from ubiquitous messengers. *Mol Cell Biol* **19:**2435–2444.

Schwencke C, Yamamoto M, Okumura S, Toya Y, Kim SJ and Ishikawa Y (1999) Compartmentation of cyclic adenosine 3′,5′-monophosphate signaling in caveolae. *Mol Endocrinol* **13:**1061–1070.

Seidel MG, Klinger M, Freissmuth M and Holler C (1999) Activation of mitogen-activated protein kinase by the A(2A)-adenosine receptor via a rap1-dependent and via a p21(ras)-dependent pathway. *J Biol Chem* **274:**25833–25841.

Sevetson BR, Kong X and Lawrence Jr JC (1993) Increasing cAMP attenuates activation of mitogen-activated protein kinase. *Proc Natl Acad Sci USA* **90:**10305–10309.

Shaywitz AJ and Greenberg ME (1999) CREB: A stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu Rev Biochem* **68:**821–861.

Smit MJ and Iyengar R (1998) Mammalian adenylyl cyclases. *Adv Second Messenger Phosphoprotein Res* **32:**1–21.

Smith JA, Poteet-Smith CE, Malarkey K and Sturgill TW (1999) Identification of an extracellular signal-regulated kinase (ERK) docking site in ribosomal S6 kinase, a sequence critical for activation by ERK in vivo. *J Biol Chem* **274:**2893–2898.

Soderling SH and Beavo JA (2000) Regulation of cAMP and cGMP signaling: New phosphodiesterases and new functions. *Curr Opin Cell Biol* **12:**174–179.

Sprenkle AB, Davies SP, Carling D, Hardie DG and Sturgill TW (1997) Identification of Raf-1 Ser621 kinase activity from NIH 3T3 cells as AMP-activated protein kinase. *FEBS Lett* **403:**254–258.

Sun H, Chen Z, Poppleton H, Scholich K, Mullenix J, Weipz GJ, Fulgham DL, Bertics PJ and Patel TB (1997) The juxtamembrane, cytosolic region of the epidermal growth factor receptor is involved in association with alpha-subunit of Gs. *J Biol Chem* **272:**5413–5420.

Sunahara RK, Dessauer CW and Gilman AG (1996) Complexity and diversity of mammalian adenylyl cyclases. *Annu Rev Pharmacol Toxicol* **36:**461–480.
Sutor SL, Vroman BT, Armstrong EA, Abraham RT and Karnitz LM (1999) A phosphatidylinositol 3-kinase-dependent pathway that differentially regulates c-Raf and A-Raf. *J Biol Chem* **274:**7002–7010.
Tan Y, Ruan H, Demeter MR and Comb MJ (1999) p90(RSK) blocks bad-mediated cell death via a protein kinase C-dependent pathway. *J Biol Chem* **274:**34859–34867.
Thorson JA, Yu LW, Hsu AL, Shih NY, Graves PR, Tanner JW, Allen PM, Piwnica-Worms H and Shaw AS (1998) 14-3-3 proteins are required for maintenance of Raf-1 phosphorylation and kinase activity. *Mol Cell Biol* **18:**5229–5238.
Torphy TJ, Barnette MS, Underwood DC, Griswold DE, Christensen SB, Murdoch RD, Nieman RB and Compton CH (1999) Ariflo (SB 207499), a second generation phosphodiesterase 4 inhibitor for the treatment of asthma and COPD: From concept to clinic. *Pulm Pharmacol Ther* **12:**131–135.
Tzivion G, Luo Z and Avruch J (1998) A dimeric 14-3-3 protein is an essential cofactor for Raf kinase activity. *Nature (Lond)* **394:**88–92.
Vaillancourt RR, Gardner AM and Johnson GL (1994) B-Raf-dependent regulation of the MEK-1/mitogen-activated protein kinase pathway in PC12 cells and regulation by cyclic AMP. *Mol Cell Biol* **14:**6522–6530.
Vossler MR, Yao H, York RD, Pan MG, Rim CS and Stork PJ (1997) cAMP activates MAP kinase and Elk-1 through a B-Raf- and Rap1-dependent pathway. *Cell* **89:**73–82.
Wixler V, Smola U, Schuler M and Rapp U (1996) Differential regulation of Raf isozymes by growth versus differentiation inducing factors in PC12 pheochromocytoma cells. *FEBS Lett* **385:**131–137
Wu J, Dent P, Jelinek T, Wolfman A, Weber MJ and Sturgill TW (1993) Inhibition of the EGF-activated MAP kinase signaling pathway by adenosine 3′,5′-monophosphate. *Science (Wash DC)* **262:**1065–1069.
Yang E, Zha J, Jockel J, Boise LH, Thompson CB and Korsmeyer SJ (1995) Bad, a heterodimeric partner for Bcl-XL and Bcl-2, displaces Bax and promotes cell death. *Cell* **80:**285–291.
Yang SH, Whitmarsh AJ, Davis RJ and Sharrocks AD (1998) Differential targeting of MAP kinases to the ETS-domain transcription factor Elk-1. *EMBO J* **17:**1740–1749.
Yao H, York RD, Misra-Press A, Carr DW and Stork PJ (1998) The cyclic adenosine monophosphate-dependent protein kinase (PKA) is required for the sustained activation of mitogen-activated kinases and gene expression by nerve growth factor. *J Biol Chem* **273:**8240–8247.
Yarwood SJ, Steele MR, Scotland G, Houslay MD and Bolger GB (1999) The RACK1 signaling scaffold protein selectively interacts with the cAMP-specific phosphodiesterase PDE4D5 isoform. *J Biol Chem* **274:**14909–14917.
Yeung K, Seitz T, Li S, Janosch P, McFerran B, Kaiser C, Fee F, Katsanakis KD, Rose DW, Mischak H, Sedivy JM, and Kolch W (1999) Suppression of Raf-1 kinase activity and MAP kinase signaling by RKIP. *Nature (Lond)* **401:**173–177.
York RD, Yao H, Dillon T, Ellig CL, Eckert SP, McCleskey EW and Stork PJ (1998) Rap1 mediates sustained MAP kinase activation induced by nerve growth factor. *Nature (Lond)* **392:**622–626.
Zuniga A, Torres J, Ubeda J and Pulido R (1999) Interaction of mitogen-activated protein kinases with the kinase interaction motif of the tyrosine phosphatase PTP-SL provides substrate specificity and retains ERK2 in the cytoplasm. *J Biol Chem* **274:**21900–21907.

**Send reprint requests to:** Dr. Miles D. Houslay, Division of Biochemistry & Molecular Biology, University of Glasgow, Davidson Building, Glasgow G12 8QQ, Scotland, UK. E-mail: M.Houslay@bio.gla.ac.uk