GEORGE FOX UNIVERSITY

Digital Commons @ George Fox University

Faculty Publications - Department of Biology and Chemistry

Department of Biology and Chemistry

2002

Crosstalk between cAMP and MAP Kinase Signaling in the Regulation of Cell Proliferation

Philip J. S. Stork

John M. Schmitt
George Fox University, jschmitt@georgefox.edu

Follow this and additional works at: https://digitalcommons.georgefox.edu/bio_fac

Part of the Biology Commons

Recommended Citation

Stork, Philip J. S. and Schmitt, John M., "Crosstalk between cAMP and MAP Kinase Signaling in the Regulation of Cell Proliferation" (2002). *Faculty Publications - Department of Biology and Chemistry*. 54.
https://digitalcommons.georgefox.edu/bio_fac/54

This Article is brought to you for free and open access by the Department of Biology and Chemistry at Digital Commons @ George Fox University. It has been accepted for inclusion in Faculty Publications - Department of Biology and Chemistry by an authorized administrator of Digital Commons @ George Fox University. For more information, please contact arolfe@georgefox.edu.

Crosstalk between cAMP and MAP kinase signaling in the regulation of cell proliferation

Philip J. S. Stork and John M. Schmitt

Hormonal stimulation of cyclic adenosine monophosphate (cAMP) and the cAMP-dependent protein kinase PKA regulates cell growth by multiple mechanisms. A hallmark of cAMP is its ability to stimulate cell growth in many cell types while inhibiting cell growth in others. In this review, the cell type-specific effects of cAMP on the mitogen-activated protein (MAP) kinase (also called extracellular signal-regulated kinase, or ERK) cascade and cell proliferation are examined. Two basic themes are discussed. First, the capacity of cAMP for either positive or negative regulation of the ERK cascade accounts for many of the cell type-specific actions of cAMP on cell proliferation. Second, there are several specific mechanisms involved in the inhibition or activation of ERKs by cAMP. Emerging new data suggest that one of these mechanisms might involve the activation of the GTPase Rap1, which can activate or inhibit ERK signaling in a cell-specific manner.

Philip J. S. Stork*
John M. Schmitt
Vollum Institute and the Dept of Cell and Developmental Biology, Oregon Health and Science University, Portland, OR 97201, USA.
*e-mail: stork@ohsu.edu

Since its discovery in the 1960s, the cyclic nucleotide cyclic adenosine monophosphate (cAMP) and its principal target, the cAMP-dependent protein kinase (PKA), have been intimately involved in studies of hormone action in the metabolic pathways of the mammalian cell. Over the years, a growing appreciation of their role in cell growth and proliferation has also emerged [1]. That cAMP is important is undisputed. Yet how it functions is the subject of repeated speculation: this remarkable regulator seems to both activate and inhibit cell proliferation, and much research has been dedicated to determining what sort of mechanisms could foster such opposite effects.

One target of cAMP that is associated with cell proliferation is the mitogen-activated protein (MAP) kinase – also called extracellular signal-regulated kinase, or ERK – cascade. cAMP regulation of the ERK cascade provides important crosstalk between hormones and growth factor signaling. Significantly, ERK can be activated or inhibited by cAMP, in a cell-specific manner, to dictate the growth effects of cAMP (see Figs 1 and 2). ERK signaling couples growth factors to cell proliferation through the GTPase Ras. Active Ras binds to and activates the MAP kinase kinase kinase (MAPKKK), Raf-1. Activated Raf-1 phosphorylates and activates the MAPKK, MEK, which in turn phosphorylates and activates ERK. cAMP activates another GTPase of the Ras superfamily, Rap1, whose effects on ERKs seem to parallel those of cAMP. Recent reports suggesting that cAMP and Rap1 act independently of PKA have further complicated the picture of how cAMP might regulate ERKs.

Thus, in this review we examine the multiple pathways that have been proposed for cAMP to either inhibit or activate ERK signaling pathways. We also address the roles of Ras, Rap1 and PKA in cAMP signaling. We demonstrate that cAMP uses a variety of pathways to inhibit ERKs and that these mechanisms share common features including a dependence on PKA and the inhibition of Ras-dependent signals to Raf-1. We discuss additional mechanisms by which cAMP activates ERKs that involve the activation of either Ras or Rap1, as well as both PKA-dependent and -independent pathways.

The inhibition of cell proliferation by cAMP

It has long been appreciated that cAMP can inhibit cell growth by blocking growth factor activation of ERKs. The antiproliferative actions of hormones, cAMP and PKA have been linked to inhibition of the ERK kinase cascade in many cell types. Examples are provided in Table 1.

Hormones increase intracellular cAMP levels through G-protein-coupled receptors (GPCRs) that link hormones to the heterotrimeric G protein $G\alpha_s$. Constitutively activated mutants of $G\alpha_s$ can block Ras-dependent proliferation of NIH3T3 fibroblasts, suggesting that hormones coupled to $G\alpha_s$ proteins inhibit cell growth by inhibiting ERKs (see Table 1). cAMP has recently been shown to activate multiple intracellular signaling cascades independently of its activation of PKA [2]. However, most studies examining cAMP inhibition of ERKs show a requirement for PKA (see Table 1). Various mechanisms whereby cAMP can inhibit ERK activity and cell proliferation are described in the following sections.

cAMP inhibition of cell proliferation: ERK-independent mechanisms

In cells where ERK activation is required for cell proliferation, the finding that cAMP inhibits ERKs has often suggested that cAMP inhibition of ERKs mediates the antiproliferative effects of cAMP. However, cAMP can inhibit cell proliferation without inhibiting ERKs [3,4] or while activating ERKs [5]. Consistent with these studies, genetic ablation of the gene for Raf-1 does not effect proliferation or ERK activation in embryonic fibroblast cells although further development is blocked, due to an increase in apoptosis [6]. In addition, it is important to point out the risk of overinterpreting experiments that show cAMP inhibition of ERKs at selected times. Examination of single time points showing complete inhibition might miss more subtle effects. For example, in studies using CCL39 fibroblast cells,

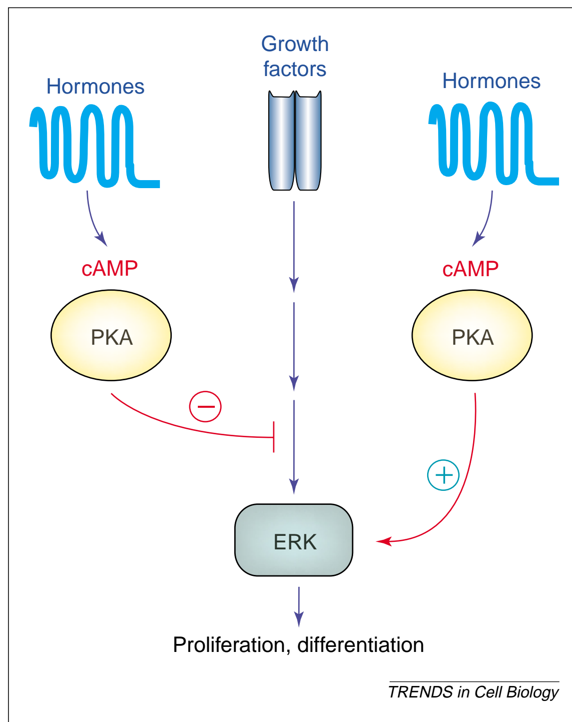

Fig. 1. cAMP regulation of extracellular signal-regulated kinases (ERKs) and cell proliferation. Stimulation of cells with growth factors results in activation of ERK. ERK can stimulate either proliferation or differentiation depending on the stimulus and cell type. Hormonal stimulation of cells can activate $G\alpha_s$ and adenylyl cyclases to stimulate the production of cAMP. cAMP activates the cAMP-dependent protein kinase, PKA. In some cells, PKA activation inhibits growth factor-dependent activation of ERKs (minus sign). In other cells, PKA activation stimulates ERKs (plus sign).

McKenzie and Pouyssegur noted that growth factor activation of ERKs cells is not inhibited by cAMP but instead is delayed [3]. In vascular smooth muscle cells, cAMP has been reported to inhibit either early or late ERK activation, depending on the stimuli [7]. In one study, cAMP was able to block early activation while enhancing late activation of ERKs [8]. Similarly, c-jun N-terminal kinase (JNK) activation is not blocked by cAMP but is delayed [9]. For this reason, we have included detailed information of ERK time points in Tables 1 and 2.

cAMP inhibition of cell-cycle progression

Many mechanisms have been proposed to explain the antiproliferative effects of cAMP, increasing cell-cycle inhibitor proteins $p21^{cip1}$ [10] or $p27^{kip1}$ [7,11,12], as well as decreasing the levels of cyclin D1 [12] and cyclin D3 [11,13]. $p27^{kip1}$ mediates the cAMP-dependent block in G1 in some cells [14]. Paradoxically, cAMP-dependent increases in $p27^{kip1}$ protein levels have been reported in thyroid cells, where cAMP stimulates proliferation [15], consistent with a positive function for this protein [16,17]. Other growth-inhibiting effects of cAMP include the stimulation of apoptosis [18–21] and the stimulation of differentiation [22]. In thyroid cells, where cAMP stimulates proliferation, the onset of S phase is delayed, and cAMP levels must be reduced to allow propagation [23]. Therefore, it is likely that cAMP exerts growth inhibitory signals that coexist with proliferative signals in some cells.

cAMP inhibition of ERKs through PKA and Rap1

Landmark molecular and biochemical studies by Krebs [24], Sturgill [25], Bos [26], McCormick [27] and colleagues demonstrated that the target of cAMP action is downstream of Ras and upstream of Raf-1. The inhibition of Ras-dependent signals by cAMP requires PKA [28].

PKA can block Ras-dependent signals to ERKs by blocking Raf-1 activation [27], and PKA phosphorylation seems to inhibit Raf-1 activity directly [29]. For example, phosphorylation of serine 621 inhibits Raf-1 kinase domains but might be required to activate full-length Raf-1 through interaction with the protein 14-3-3 [86]. Other PKA sites have also been proposed as negative regulators (see Fig. 3). Phosphorylation of serine 259 by either PKA [30] or Akt [31] is inhibitory, and mutation of this site to alanine results in constitutive membrane association [30,32]. Previous studies proposed that phosphorylation of serine 43 on Raf-1 by PKA inhibits Raf-1 in fibroblasts by preventing Raf-1 from binding to Ras [25]. Yet mutagenesis of this site failed to block PKA from inhibiting ERKs in both the fibroblast cell line NIH3T3 and the human embryonic kidney cell line Hek293 [33]. Additional mechanisms might therefore account for the ability of PKA to uncouple Raf-1 from Ras.

One possible mechanism involves Rap1. This GTPase was first identified as an antagonist of Ras-induced cell transformation in NIH3T3 cells [34], and as an inhibitor of ERKs in Rat-1 fibroblasts [35]. Rap1 is activated by PKA [36] and is therefore a potential mediator of the inhibition of Ras-dependent signaling to ERKs by cAMP (see Fig. 2a). Interestingly, although Rap1 can block signals from constitutively activated Ras, it cannot block signals from constitutively active Raf-1 [35]. Thus, like cAMP, the actions of Rap1 manifest themselves upstream of Raf-1 and downstream of Ras [26]. Activated, GTP-loaded Rap1 antagonizes Ras activation of Raf-1 and ERKs by binding to and sequestering Raf-1 away from Ras – an action that is triggered by PKA [37]. The requirement for Rap1 in cAMP inhibitory effects on both ERKs and cell growth has been demonstrated recently in NIH3T3 cells (see Fig. 2a) [37]. Although no studies examining activation of endogenous Rap1 by extracellular stimuli have shown an inhibition of endogenous Raf-1 kinase activity directly (see Ref. [38] and references therein), it has been shown that the activation of endogenous Rap1 does block Ras binding to Raf-1 [37].

Regulation of Rap1 and ERKs by PKA activation of Src kinase

The activation of Rap1 by PKA has been demonstrated in a wide variety of cells [39], including neurons

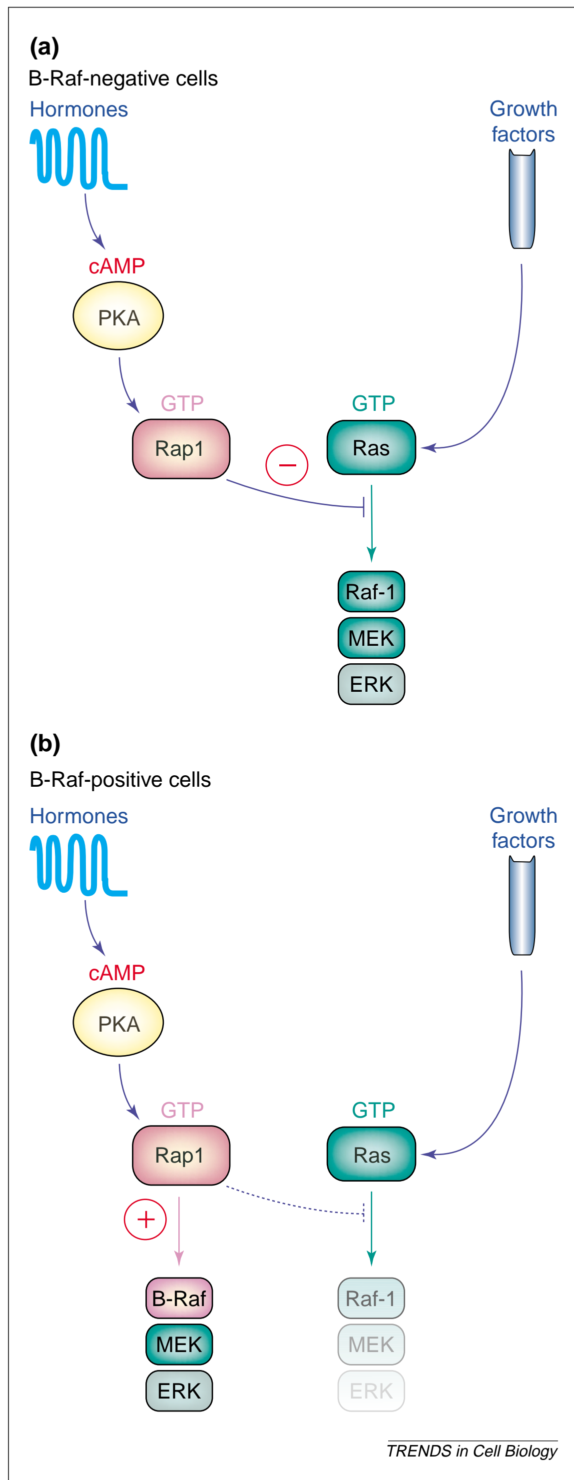

Fig. 2. A model showing how Rap1 activation by cAMP might regulate extracellular signal-regulated kinases (ERKs) in a cell type-specific manner. (a) Rap1 activation by cAMP inhibits ERKs. Hormonal stimulation of a $G\alpha_s$/cAMP/PKA module leads to Rap1 activation (GTP loading). Many cells express Raf-1 as the major Raf isoform. In these cells, GTP-loaded Rap1 blocks Ras activation of Raf-1, thereby inhibiting growth factor activation of ERKs and cell proliferation. (b) Some cells express B-Raf as well as Raf-1. In these cells, GTP-loaded Rap1 can activate B-Raf and the mitogen-activated protein (MAP) kinase cascade and hormonal stimulation of cAMP/PKA/Rap1 in these cells activates ERKs. Rap1 might also antagonize Ras activation of Raf-1, as in (a). Rap1 activation of B-Raf often predominates over the inhibition of Raf-1, resulting in a net effect of ERK activation.

[40,41], glia [42] and fibroblasts [37,43]. Although Rap1 can be phosphorylated directly by PKA [44], this phosphorylation step is not required for the activation of Rap1 by cAMP [36]. It is possible that phosphorylation of Rap1 by PKA plays a role in influencing effector pathways of Rap1 that are distinct from Raf-1, as suggested by Altschuler and coworkers who have recently identified a function for this phosphorylation in thyroid cell proliferation [45]. Additional evidence suggests that the activation of Rap1 by PKA is indirect [46] and upstream guanine nucleotide exchange factors (GEFs) might be involved.

One GEF, C3G [Crk Src homology domain 3 (SH3) guanine nucleotide exchange factor], specifically activates Rap1 [47], and is a potential target of the effects of PKA. For example, expression of an interfering mutant of C3G blocks the activation of Rap1 by PKA in fibroblasts [43,46]. PKA recruits C3G to the plasma membrane in a complex with the adaptor Crk-L and the scaffold protein Cbl. C3G and Crk-L are recruited to Cbl following the phosphorylation of a specific tyrosine residue on Cbl that serves as a docking site for the SH2 domain of Crk-L [46]. This tyrosine phosphorylation of Cbl requires Src [46], and PKA activates Src by phosphorylating serine 17 directly [1] to induce the formation of the Cbl/Crk-L/C3G complex. Phosphorylation of Src at serine 17 is required for PKA to activate Rap1, and to inhibit ERKs and cell growth in both NIH3T3 cells and mouse embryonic fibroblasts [46]. The requirement of PKA for Src to inhibit ERKs identifies a novel antiproliferative function for Src, one that is distinct from the well-studied proliferative actions of this proto-oncogene. It is likely that Src activation by PKA, like that of Rap1, will display cell-specific actions on ERK signaling.

Rap1 activation can regulate intracellular signals independently of ERKs

Stimuli that activate Rap1 are not always associated with regulation of ERK signaling [48] and non-Ras pathways have been proposed [49–52]. Many recent studies point to a positive role for C3G [53] and Rap1 in cell adhesion [38] in a variety of cell types including macrophages [50] and lymphocytes [52,54,55]. In many studies, the regulation of ERKs by Rap1 was ruled out [52,56], while one study supported a role for ERK inhibition in Rap1 actions [57]. Increased cell adhesion by Rap1 can promote proliferation indirectly [52]. Therefore, it is possible that cAMP might also regulate Rap1-dependent integrin pathways to regulate proliferation in selected situations.

cAMP uses multiple mechanisms to inhibit ERKs

The activation of Rap1 by PKA disrupts Ras/Raf-1 signaling in multiple cell types. However, other mechanisms by which PKA inhibits ERKs have been proposed (Fig. 3). Interestingly, many of these mechanisms identify additional targets of PKA

Table 1. Examples of the inhibition of ERKs and proliferation by cAMP[a]

Cell type	Stimulus of proliferation	ERK timepoints	Stimulus of cAMP	PKA	Refs
Adipocytes	Insulin	5 min	Glucagon, isoproterenol, forskolin, cAMP	Yes	[28]
Endothelial cells	VEGF, FGF	5, 10, 15 min	Isoproterenol, cAMP	Yes	[113]
NIH 3T3 cells	EGF, PDGF	1, 3, 5, 10, 20, 30, 60 min	Isoproterenol, Gαs, forskolin, PGE1	Yes	[37,45,114]
Rat-1 fibroblasts	EGF	5 min	Forskolin, cAMP	Yes	[25,27]
Smooth muscle cells	PDGF, thrombin, EGF	5, 10, 15, 20, 30, 60, 90 min	Forskolin, cAMP, phosphodiesterase inhibitors	Yes	[115,116]
Hepatocytes	EGF	2.5, 5, 10, 20, 30, 60 min	Glucagon, cAMP	ND	[117]
Pancreatic acinar cells (AR42J)	CCK, EGF	3, 30 min	Forskolin, cAMP	ND	[118]
Bone cells (MG63)	Serum	10 min	Parathyroid hormone	No	[125]

[a]cAMP inhibition of ERKs and proliferation in a variety of cell types, together with the method of elevating ERK/proliferation and the method of raising cAMP levels. The requirement of PKA for these effects is indicated when known.
Abbreviations: CCK, cholecystokinin; EGF, epidermal growth factor; FGF, fibroblast growth factor; ND, not determined; PDGF, platelet-derived growth factor; PKA, protein kinase A; VEGF, vascular endothelial growth factor.

that, like Rap1, directly or indirectly inhibit Ras-dependent activation of Raf-1. For example, activation of ERKs by growth factors is blunted in nonadherent cells, and a role for PKA in this block has been demonstrated [58]. This block might be achieved through an inhibitory phosphorylation of the p21-associated kinase (PAK) [58] by PKA, which might be required for Raf-1 to be fully activated by Ras [59]. However, recent studies question the physiological significance of PAK in these actions [60].

Other kinase targets for PKA have also been proposed. Recent studies have identified Akt (also termed protein kinase B, or PKB) as a potent negative regulator of Raf-1 [31]. This action has been identified primarily in myoblast cells [61], as well as in the breast cancer cell line MCF-7 [31].

Rap1 might activate Akt by activating the phosphoinositol-3 kinase (PI3-K) [62]. Direct phosphorylation of Akt by PKA has also been suggested [63]. However, cAMP does not activate PKB in all cells, and in some cells it inhibits PKB activation by growth factors [64]. Either mechanism of activation of Akt by cAMP might limit activation of Raf-1 and ERKs (see Fig. 3). Finally, the serum- and glucocorticoid-inducible kinase, SGK, also inhibits Raf kinase signaling [65], and can be activated by cAMP and PKA [66]. Therefore, SGK might also mediate inhibitory signals from cAMP/PKA to Raf kinases.

ERKs can be inhibited by the family of dual specificity MAP kinases phosphatases (MKPs), which can be transcriptionally activated by cAMP [67,68]. This induction by cAMP limits the activation of ERK in selected systems [68–70] and might be one of several mechanisms that could account for the delayed inhibition of ERKs following cAMP treatment that occurs in a time frame compatible with transcriptional regulation.

Table 2. Examples of the activation of ERKs by cAMP[a]

Cell type	Stimulus of cAMP	ERK timepoints	Effect	PKA	Refs
Rat thyroid cells (FRTL-5)	TSH	3, 10, 15, 30, 60 min	P	No	[82]
Bone cells (ATDC5, MC4)	Parathyroid hormone	1, 2, 5, 10, 15, 30 min	P	No	[125]
Polycystic kidney epithelium	Forskolin, cAMP, secretin, VIP, vasopressin, PGE2	5, 15, 30, 60, 120 min	P	Yes	[119]
Human prostate tumor cells (LnCaP)	Epinephrine, forskolin	5, 10, 20 min	P, D	Yes	[77]
Sertoli cells	FSH	5, 15, 30, 60 min and 1, 2, 4, 6, 8 hrs	P, D	Yes	[92]
Cardiac myocytes	Isoproterenol	8 and 10 min	D	Yes	[120]
Granulosa cells	LH, FSH, forskolin, cAMP	5, 10, 20, 60 min	D	Yes	[121]
Pre-adipocytes	Catecholamine, forskolin, cAMP, isoproterenol	0.5, 1, 2, 5, 10, 20, 30, 60 min	D	Yes	[122,123]
Pituitary (GH_4C_1/GH_3 cells)	VIP, PACAP38, forskolin, cAMP	5, 10, 20, 30, 60, 120 min	D	Yes	[78,124]
PC12 cells	Adenosine, norepinephrine, forskolin, cAMP	5, 10, 15, 30, 60, 90, 120 min	D	Yes	[8,41]

[a]cAMP activation of ERKs in a variety of cell types, together with the method of elevating cAMP levels, and the effect of that elevation (proliferation, differentiation, or both). The requirement of PKA for these effects is indicated when known.
Abbreviations: D, differentiation; FSH, follicle-stimulating hormone; LH, leuteinizing hormone; P, proliferation; PGE2, prostaglandin E2; PKA, protein kinase A; TSH, thyroid-stimulating hormone; VIP, vasoactive intestinal peptide.

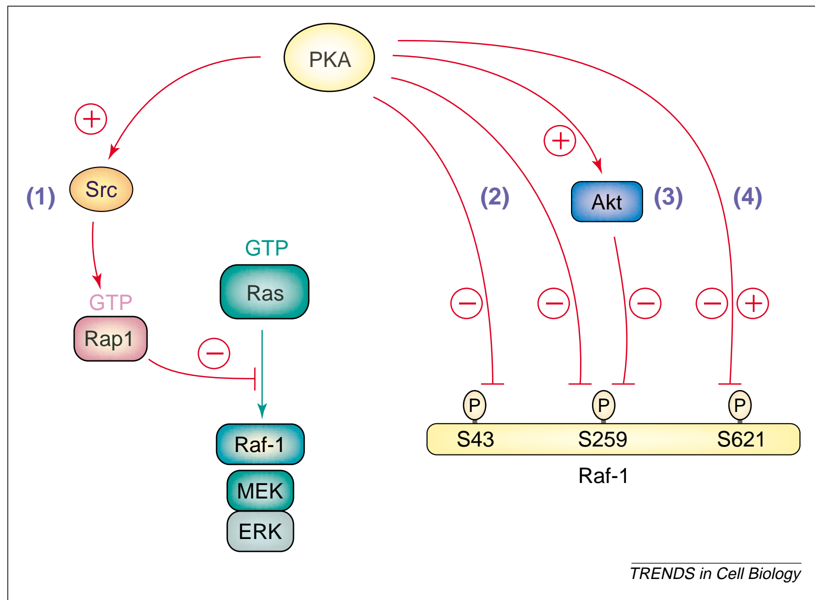

Fig. 3. Possible mechanisms of cAMP/PKA inhibition of ERK activation. cAMP can activate Rap1 to antagonize Ras signaling to Raf-1. cAMP activation of PKA activates Rap1 via an Src-dependent pathway (1). PKA might also inhibit Raf-1 by direct phosphorylation at serines 43, 259 and 621. PKA phosphorylation of serine 43 can inhibit the ability of Raf-1 to bind to GTP-loaded Ras (2). cAMP and PKA might interfere with the activation of Raf-1 by activating the serine/threonine kinase Akt, which can also inhibit Raf-1 by direct phosphorylation on serine 259 (3). PKA phosphorylation at serine 621 can inhibit isolated kinase domains, but might potentiate the activity of full-length Raf-1 through 14-3-3 binding (4).

The stimulation of cell proliferation and differentiation by cAMP

cAMP does not only inhibit cell proliferation, it can also stimulate cell proliferation by stimulating ERKs in diverse cell types. In all known examples, cAMP-mediated cell proliferation is induced by hormonal activation of GPCRs that are coupled to Gα_s (see Table 2).

When cAMP activates ERK, it can stimulate cell differentiation as well as proliferation [22,41]. Interestingly, the ERK cascade can mediate both proliferation and differentiation within the same cell. This has been well documented in the pheochromocytoma cell line PC12, where transient activation of ERKs by epidermal growth factor (EGF) triggers proliferation, whereas sustained activation of ERKs by nerve growth factor and fibroblast growth factor triggers differentiation sympathetic-like neurons [47]. Moreover, in Schwann cells, low concentrations of cAMP activate proliferation through ERK, whereas higher concentrations of cAMP induce sustained activation of ERKs, as well as markers of differentiation [71]. Similar dosage effects of cAMP have been seen in kidney cells [72].

The ERK-dependence of cAMP-induced cellular differentiation has been well studied in neuronal cells. However, the coupling of cAMP/PKA to ERKs in these cells might depend on the development stage [73]. In neurons, neuronal activity and depolarization induce changes in synaptic plasticity that have been shown to require both PKA and ERKs [40,74–76]. Neuroendocrine differentiation of prostatic tumor cells by cAMP also requires ERKs [77].

cAMP-mediated cell differentiation is characterized by the induction of specific genes through phosphorylation of the transcription factor CREB by PKA. Although there is no known requirement for ERKs in this phosphorylation of CREB, ERKs seem to be required for cAMP transcriptional effects, possibly through phosphorylation of a protein downstream of CREB [74]. Additional examples of the requirement of ERKs for the transcriptional effects of cAMP include induction of the prolactin gene in pituitary cells [78], and of the dopamine beta-hydroxylate gene in PC12 cells [79].

Mechanisms by which cAMP stimulates ERK signaling

Rap1 activation of B-Raf

Rap1 has another action in certain cell types – it can activate the Raf isoform B-Raf. This action is independent of Ras and provides a pathway for cAMP to activate ERKs. Early studies in PC12 cells determined that the target of the activation of ERKs by cAMP was upstream of MEK [22]. The Ras independence of the effects of cAMP was suggested by studies examining the regulation of ERKs by parathyroid hormone and cAMP in Chinese hamster ovary cells [80] and by forskolin in PC12 cells [41]. A role for Rap1 in cAMP activation of ERKs was first demonstrated using interfering mutants of Rap1, and it was confirmed later using a genetic approach [18].

For Rap1 to activate ERKs, the Raf isoform B-Raf must be expressed. B-Raf is the major Raf isoform in the brain [81], and is expressed in a wide variety of cell types, including endocrine cells [78,82] and cells of neural crest origin [41,83], as well as endothelial cells [84], prostate cells [85] and selected fibroblasts [37,43,57]. Low levels of B-Raf protein have also been detected in kidney, lung, liver, heart and thymus [87]. In cells that do not express B-Raf, transfection of B-Raf converts cAMP from an inhibitor to an activator of ERKs [41,42].

B-Raf is highly homologous to Raf-1 within both its kinase and Ras-binding domains, and, like Raf-1, it has only one known substrate: the MAPKK, MEK. Although both B-Raf and Raf-1 bind to Rap1, only B-Raf is activated [41]. Studies in B-Raf-expressing cells have shown that cAMP activation of ERKs requires Rap1 and B-Raf [18,40,42,43], and a role for the B-Raf binding partner 14-3-3 in Rap1 activation of B-Raf by cAMP has been demonstrated [39].

cAMP activation of Rap1 does not always result in B-Raf activation. For example, in a study in PC12 cells, cAMP did not activate B-Raf despite activating Rap1 [88]. This is consistent with earlier studies [89], but contrasts with others [39,41,42,90]. Full-length B-Raf proteins seem to be regulated by cAMP distinctly from shorter forms [91] and PC12 cells differ in the expression of full-length and shorter splice forms of B-Raf [41,89]. Therefore, it is possible that these differences reflect clonal variation in PC12

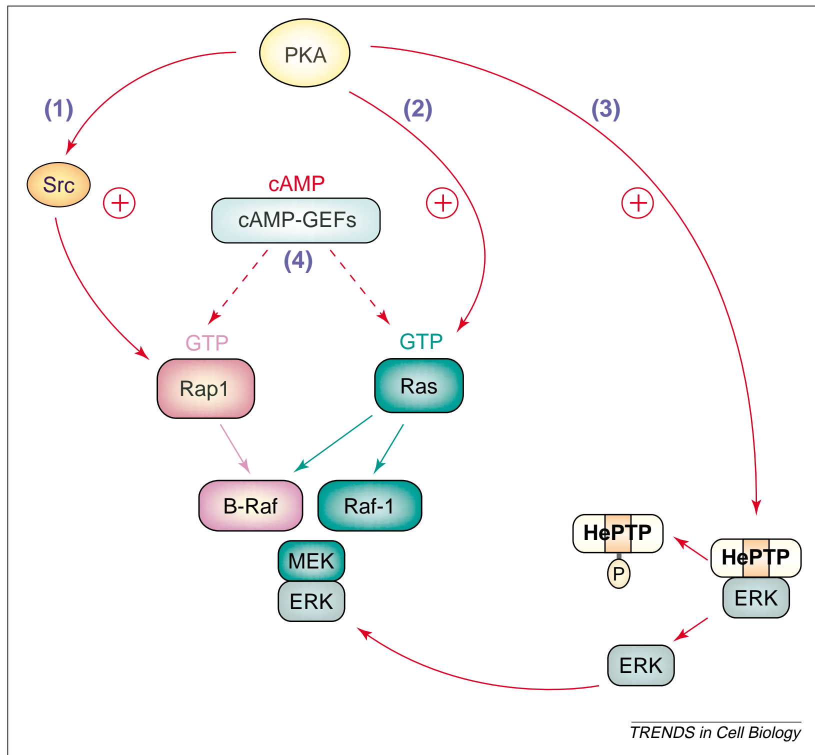

Fig. 4. Possible mechanisms by which cAMP/PKA can activate ERKs. (1) In B-Raf-expressing cells, hormonal stimulation of PKA might activate ERKs by the Src-dependent activation of Rap1, which can activate ERKs through B-Raf. (2) PKA might also stimulate Ras activation in response to G-protein-coupled receptor stimulation, which results in ERK activation through Ras activation of either B-Raf or Raf-1. (3) PKA might activate ERKs by releasing ERKs from inactivating phosphatases. PKA phosphorylation of the ERK phosphatase HePTP (as well as the related phosphatases PTP-SL and STEP) releases ERK from inhibition by the phosphatase. (4) cAMP might also activate Ras and Rap1 through PKA-independent pathways involving cAMP-GEFs for Rap1 and Ras, respectively.

isolates of the expression of full-length and shorter splice forms of B-Raf. The level of expression of 14-3-3 also seems to be a critical variable that might dictate whether or not Rap1 activation by cAMP can activate endogenous B-Raf proteins [39].

The contrasting actions of Rap1 on Raf-1 and B-Raf provide an explanation for the cell type specificity of cAMP regulation of ERKs. In this model, the consequence of cAMP activation of Rap1 depends on the level of B-Raf expression (see Fig. 2). One important area for future study is to determine whether changes in B-Raf expression levels account for the reversal of cAMP regulation of ERKs and cell growth seen in certain developmental and pathophysiological systems.

A role for Src in PKA activation of ERKs through Rap1/B-Raf

A pathway linking Src to PKA activation of Rap1 has been demonstrated recently in B-Raf-negative cells (see Fig. 3). It is likely that Src also mediates PKA activation of Rap1 in B-Raf-positive cells (see Fig. 4), providing a potential role for Src in the activation of ERKs by PKA in these cells. Indeed, previous studies have shown that ERK activation by PKA requires members of the Src family [92–94]. It is possible that these and other examples represent novel uses of the PKA–Src–Rap1 pathway. However, at least one additional mechanism of PKA activation of ERKs through Rap1 and B-Raf has been identified in striatal neurons, where Rap1 activation is primed through PKA-induced calcium release [40]. The availability of selective inhibitors of Src family kinases will allow the requirement of Src in ERK regulation by cAMP to be tested in these and other systems.

PKA-independent activation of Rap1 by cAMP

Although cAMP uses PKA to exert most of its effects, cAMP might also have actions that are independent of PKA, including the activation of Rap1. In thyroid cells, Rap1 can be activated by cAMP by both PKA-dependent and PKA-independent mechanisms [62,95]. Studies in leukemic cells and kidney cells have also identified a PKA-independent activation of Rap1 [96–98]. These actions might be mediated by a family of cAMP-binding proteins termed cAMP-GEFs [99] or Epacs [100]. These proteins show increased GEF activity towards Rap1 upon cAMP binding.

A recent paper has identified a role for Epac I in the PKA-independent activation of ERKs and H,K-ATPase regulation. In that study, roles for Epac I, Rap1 and B-Raf were demonstrated using microinjection of neutralizing antibodies [96]. However, for many studies, there are no convenient molecular or pharmacological tools to interfere with the function of endogenous cAMP-GEFs, and their requirement has been inferred in situations where cAMP activation of Rap1 or ERK has been shown to be independent of PKA [2]. This will certainly change as new tools become available.

PKA-independent actions of cAMP that regulate cell growth have been identified in selected cell types including thyroid cells and ovarian cells where cAMP promotes proliferation [2]. In both cases, a role for cAMP regulation of PI3-K, rather than ERKs, has been proposed [2]. There are few examples of PKA-independent action of cAMP to inhibit ERK activation and cell growth [125]. It is possible that this reflects the expression pattern of Epac proteins, although our understanding of the role of Epacs in cAMP-dependent growth control is likely to grow as new systems are examined critically.

Alternatively, PKA might participate in directing Rap1 function along specific effector pathways, as suggested by recent studies [45]. For example, it is interesting to speculate that PKA phosphorylation of Src induces the assembly of a large protein complex that includes Cbl, Crk and C3G that not only activates Rap1 but also directs its actions towards Raf isoforms.

cAMP activation of ERKs can require Ras

Although early studies indicated that Ras is not required in the activation of ERKs by PKA, it is now clear that Ras participates in cAMP signaling under certain circumstances. In selected neurons, cAMP and PKA require Ras to activate ERK [101,102]. In thyroid cells, thyroid-stimulating hormone (TSH) stimulates Ras in thyroid cells using a cAMP-dependent but PKA-independent mechanism [103].

Although TSH uses a Ras-dependent pathway to stimulate thyroid cell proliferation, ERK-independent effectors of Ras have been postulated [104]. It is possible that Rap1 activation by cAMP enhances Ras binding to other effectors. Indeed, a model of cAMP function in thyroid cells has been proposed in which sequestration of Raf-1 by Rap1 redirects Ras signaling to non-Raf-1 effectors, including PI3-K [105].

A Ras-dependence of cAMP signaling has been revealed in other cells, such as melanocytes. In these cells, cAMP activation of ERKs requires both Ras and B-Raf but does not require Rap1 or PKA [83]. Recently, a Ras-specific GEF, CnrasGEF, was identified that, like other cAMP-GEFs, is activated by cAMP in a PKA-independent fashion [106]. This or related GEFs might play a role in the PKA-independent activation of Ras by cAMP (see Fig. 4).

β-Adrenergic receptor uses multiple pathways to activate ERKs

Classically, G-protein signaling to Ras uses signals generated from the βγ subunits of the heterotrimeric G proteins [107]. However, a role for $G\alpha_s$ and PKA in Gβγ signaling to Ras has been identified for the activation of the β-adrenergic receptor by the agonist isoproterenol [108]. This action of isoproterenol has been shown to involve a PKA-dependent switch of the β-adrenergic receptor coupling from $G\alpha_s$ to $G\alpha_i$, and subsequent activation of Ras through the βγ subunits of G_i [108].

Because agonist binding to GPCRs can activate both $G\alpha_s$ and βγ, Gβγ activation of Ras might proceed concurrently with Gα activation of Rap1. In Hek293 cells, isoproterenol can activate Rap1 with $G\alpha_s$/cAMP, and can activate Ras through βγ [43]. In COS-7 cells, isoproterenol can inhibit ERKs through cAMP, but can activate ERKs through βγ and Ras [109]. The role of Rap1 in cAMP inhibition of ERKs in these cells, however, was not examined.

PKA can activate ERKs by additional mechanisms

Additional targets of PKA might potentiate ERK signaling. PKA phosphorylation of a common kinase interaction motif (KIM) within a family of ERK-directed phosphotyrosine phosphatases (PTPases), including HePTP [110], PTP-SL and STEP [111], results in the release of bound ERK and subsequent increase in ERK activity (see Fig. 4). Two other mechanisms for activating ERKs by cAMP have been described in neuronal cells. In neuronal hippocampal cells, PKA activation of brain-derived neurotrophic factor (BDNF) signaling contributes to cAMP activation of ERKs [76]. In addition, PKA potentiates the activation of ERKs through the GTPase Rheb [112]. Rheb is expressed within hippocampal cells and its ability to activate ERKs is potentiated by PKA phosphorylation of Raf-1 [112].

Concluding remarks

Numerous distinct mechanisms exist that allow cAMP to regulate ERK signaling. It will be critical to determine whether any specific mechanism has broad applicability to a variety of cell types or is limited to selected cells and stimuli. Most mechanisms explaining cAMP inhibition of ERKs involve the uncoupling of Raf-1 from Ras activation, either by direct actions of PKA on Raf-1 or through the actions of PKA on the GTPase Rap1. Other mechanisms, including the activation of selected PTPases, might be limited to specific cell types. Models to explain the activation of ERKs by cAMP are more diverse, and include the involvement of either Rap1 or Ras, and might include PKA-independent actions of cAMP.

Although it is clear that distinct models operate in cell types where cAMP either stimulates or inhibits ERKs, one model, the activation of Rap1 by cAMP, might be used in both cell types, either to stimulate or to inhibit ERKs. This specificity can result from the combined effects of the actions of Rap1 actions on both Raf-1 and B-Raf (see Fig. 2). Because cAMP can activate Rap1 in a variety of cells, it will be important to assess the expression level of B-Raf when evaluating the mechanism of action of cAMP in specific cell types.

References

1 Gottesman, M.M. and Fleischmann, R.D. (1986) The role of cAMP in regulating tumour cell growth. *Cancer Surv.* 5, 291–308

2 Richards, J.S. (2001) New signaling pathways for hormones and cyclic adenosine 3′,5′-monophosphate action in endocrine cells. *Mol. Endocrinol.* 15, 209–218

3 McKenzie, F.R. and Pouyssegur, J. (1996) cAMP-mediated growth inhibition in fibroblasts is not mediated via mitogen-activated protein (MAP) kinase (ERK) inhibition. cAMP-dependent protein kinase induces a temporal shift in growth factor-stimulated MAP kinases. *J. Biol. Chem.* 271, 13476–13483

4 Kahan, C. *et al.* (1992) Coordinate, biphasic activation of p44 mitogen-activated protein kinase and S6 kinase by growth factors in hamster fibroblasts. Evidence for thrombin-induced signals different from phosphoinositide turnover and adenylylcyclase inhibition. *J. Biol. Chem.* 267, 13369–13375

5 Hu, Z.Y. *et al.* (1998) cAMP inhibits linoleic acid-induced growth by antagonizing p27(kip1) depletion, but not interfering with the extracellular signal-regulated kinase or AP-1 activities. *Biochim. Biophys. Acta* 1405, 139–146

6 Mikula, M. *et al.* (2001) Embryonic lethality and fetal liver apoptosis in mice lacking the c-raf-1 gene. *EMBO J.* 20, 1952–1962

7 Cospedal, R. *et al.* (1999) Differential regulation of extracellular signal-regulated protein kinases (ERKs) 1 and 2 by cAMP and dissociation of ERK inhibition from anti-mitogenic effects in rabbit vascular smooth muscle cells. *Biochem. J.* 342, 407–414

8 Arslan, G. and Fredholm, B.B. (2000) Stimulatory and inhibitory effects of adenosine A(2A) receptors on nerve growth factor-induced phosphorylation of extracellular regulated kinases 1/2 in PC12 cells. *Neurosci. Lett.* 292, 183–186

9 Hsueh, Y-P. and Lai, M-Z. (1995) c-jun N-terminal kinase but not mitogen-activated protein kinase is sensitive to cAMP inhibition in lymphocytes. *J. Biol. Chem.* 270, 18094–18098

10 Lee, T.H. *et al.* (2000) Induction of p21WAF1 expression via Sp1-binding sites by tamoxifen in estrogen receptor-negative lung cancer cells. *Oncogene* 19, 3766–3773

11 van Oirschot, B.A. *et al.* (2001) Protein kinase A regulates expression of p27(kip1) and cyclin D3 to suppress proliferation of leukemic T cell lines. *J. Biol. Chem.* 276, 33854–33860

12 L'Allemain, G. *et al.* (1997) Cyclin D1 expression is a major target of the cAMP-induced inhibition of cell cycle entry in fibroblasts. *Oncogene* 14, 1981–1990

13 Naderi, S. *et al.* (2000) cAMP-mediated growth inhibition of lymphoid cells in G1: rapid down-regulation of cyclin D3 at the level of translation. *Eur. J. Immunol.* 30, 1757–1768

14 Kato, J.Y. *et al.* (1994) Cyclic AMP-induced G1 phase arrest mediated by an inhibitor (p27Kip1) of cyclin-dependent kinase 4 activation. *Cell* 79, 487–496
15 Depoortere, F. *et al.* (1996) Paradoxical accumulation of the cyclin-dependent kinase inhibitor p27kip1 during the cAMP-dependent mitogenic stimulation of thyroid epithelial cells. *J. Cell Sci.* 109, 1759–1764
16 LaBaer, J. *et al.* (1997) New functional activities for the p21 family of CDK inhibitors. *Genes Dev.* 11, 847–862
17 Cheng, M. *et al.* (1999) The p21(Cip1) and p27(Kip1) CDK 'inhibitors' are essential activators of cyclin D-dependent kinases in murine fibroblasts. *EMBO J.* 18, 1571–1583
18 Wan, Y. and Huang, X.Y. (1998) Analysis of the Gs/mitogen-activated protein kinase pathway in mutant S49 cells. *J. Biol. Chem.* 273, 14533–14537
19 Chen, T.C. *et al.* (1998) Up-regulation of the cAMP/PKA pathway inhibits proliferation, induces differentiation, and leads to apoptosis in malignant gliomas. *Lab. Invest.* 78, 165–174
20 Boucher, M.J. *et al.* (2001) cAMP protection of pancreatic cancer cells against apoptosis induced by ERK inhibition. *Biochem. Biophys. Res. Commun.* 285, 207–216
21 Myklebust, J.H. *et al.* (1999) Activation of the cAMP signaling pathway increases apoptosis in human B-precursor cells and is associated with downregulation of Mcl-1 expression. *J. Cell. Physiol.* 180, 71–80
22 Young, S.W. *et al.* (1994) Differentiation of PC12 cells in response to a cAMP analogue is accompanied by sustained activation of mitogen-activated protein kinase. Comparison with the effects of insulin, growth factors and phorbol esters. *FEBS Lett.* 338, 212–216
23 Feliciello, A. *et al.* (2000) The localization and activity of cAMP-dependent protein kinase affect cell cycle progression in thyroid cells. *J. Biol. Chem.* 275, 303–311
24 Graves, L.M. *et al.* (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. U. S. A.* 90, 10300–10304
25 Wu, J. *et al.* (1993) Inhibition of the EGF-activated MAP kinase signaling pathway by adenosine 3′,5′-monophosphate. *Science* 262, 1065–1068
26 Burgering, B.M. *et al.* (1993) cAMP antagonizes p21ras-directed activation of extracellular signal-regulated kinase 2 and phosphorylation of mSos nucleotide exchange factor. *EMBO J.* 12, 4211–4220
27 Cook, S.J. and McCormick, F. (1993) Inhibition by cAMP of Ras-dependent activation of Raf. *Science* 262, 1069–1072
28 Sevetson, B.R. *et al.* (1993) Increasing cAMP attenuates activation of mitogen-activated protein kinase. *Proc. Natl. Acad. Sci. U. S. A.* 90, 10305–10309
29 Mischak, H. *et al.* (1996) Negative regulation of Raf-1 by phosphorylation of serine 621. *Mol. Cell. Biol.* 16, 5409–5418
30 Dhillon, A.S. *et al.* (2002) Regulation of Raf-1 activation and signalling by dephosphorylation. *EMBO J.* 21, 64–71
31 Zimmermann, S. and Moelling, K. (1999) Phosphorylation and regulation of Raf by Akt (protein kinase B). *Science* 286, 1741–1744
32 Kubicek, M. *et al.* (2002) Dephosphorylation of Ser-259 regulates Raf-1 membrane association. *J. Biol. Chem.* 277, 7913–7919
33 Sidovar, M.F. *et al.* (2000) Phosphorylation of serine 43 is not required for inhibition of C-Raf kinase by the cAMP-dependent protein kinase. *J. Biol. Chem.* 275, 28688–28694
34 Kitayama, H. *et al.* (1989) A ras-related gene with transformation suppressor activity. *Cell* 56, 77–84
35 Cook, S.J. *et al.* (1993) RapV12 antagonizes Ras-dependent activation of ERK1 and ERK2 by LPA and EGF in Rat-1 fibroblasts. *EMBO J.* 12, 3475–3485
36 Altschuler, D.L. *et al.* (1995) Cyclic AMP-dependent activation of Rap1b. *J. Biol. Chem.* 270, 10373–10376
37 Schmitt, J.M. and Stork, P.J.S. (2001) Cyclic AMP-mediated inhibition of cell growth requires the small G protein rap1. *Mol. Cell. Biol.* 21, 3671–3683
38 Bos, J.L. *et al.* (2001) Rap1 signalling: adhering to new models. *Nat. Rev. Mol. Cell. Biol.* 2, 369–377
39 Qiu, W. *et al.* (2000) Cell type-specific regulation of B-Raf kinase by cAMP and 14-3-3 proteins. *J. Biol. Chem.* 275, 31921–31929
40 Zanassi, P. *et al.* (2001) cAMP-dependent protein kinase induces cAMP-response element-binding protein phosphorylation via an intracellular calcium release/ERK-dependent pathway in striatal neurons. *J. Biol. Chem.* 276, 11487–11495
41 Vossler, M. *et al.* (1997) cAMP activates MAP kinase and Elk-1 through a B-Raf- and Rap1-dependent pathway. *Cell* 89, 73–82
42 Dugan, L.L. *et al.* (1999) Differential effects of cAMP in neurons and astrocytes. Role of B-raf. *J. Biol. Chem.* 274, 25842–25848
43 Schmitt, J.M. and Stork, P.J.S. (2000) β_2-adrenergic receptor activates extracellular regulated kinases (ERKs) via the small G protein Rap1 and the serine/threonine kinase B-Raf. *J. Biol. Chem.* 275, 25342–25350
44 Quilliam, L.A. *et al.* (1991) Rap1A is a substrate for cyclic AMP-dependent protein kinase in human neutrophils. *J. Immunol.* 147, 1628–1635
45 Ribeiro-Neto, F. *et al.* (2002) On the mitogenic properties of Rab1b: cAMP-induced G1/S entry requires phosphorylation of Rap1b. *Proc. Natl. Acad. Sci. U. S. A.* 99, 5418–5423
46 Schmitt, J.M. and Stork, P.J.S. (2002) PKA phosphorylation of Src mediates cAMP's inhibition of cell growth via Rap1. *Mol. Cell* 9, 85–94
47 York, R.D. *et al.* (1998) Rap1 mediates sustained MAP kinase activation induced by nerve growth factor. *Nature* 392, 622–625
48 Zwartkruis, F.J. and Bos, J.L. (1999) Ras and Rap1: two highly related small GTPases with distinct function. *Exp. Cell Res.* 253, 157–165
49 Wang, L. *et al.* (2001) Cyclic AMP inhibits extracellular signal-regulated kinase and phosphatidylinositol 3-kinase/Akt pathways by inhibiting Rap1. *J. Biol. Chem.* 276, 37242–37249
50 Caron, E. *et al.* (2000) The GTPase Rap1 controls functional activation of macrophage integrin alphaMbeta2 by LPS and other inflammatory mediators. *Curr. Biol.* 10, 974–978
51 Arai, A. *et al.* (2001) Rap1 is activated by erythropoietin or interleukin-3 and is involved in regulation of beta 1 integrin-mediated hematopoietic cell adhesion. *J. Biol. Chem.* 276, 10453–10462
52 Sebzda, E. *et al.* (2002) Rap1A positively regulates T cells via integrin activation rather than inhibiting lymphocyte signaling. *Nat. Immunol.* 3, 251–258
53 Ohba, Y. *et al.* (2001) Requirement for C3G-dependent Rap1 activation for cell adhesion and embryogenesis. *EMBO J.* 20, 3333–3341
54 Tsukamoto, N. *et al.* (1999) Rap1 GTPase-activating protein SPA-1 negatively regulates cell adhesion. *J. Biol. Chem.* 274, 18463–18469
55 Reedquist, K.A. *et al.* (2000) The small GTPase, Rap1, mediates CD31-induced integrin adhesion. *J. Cell Biol.* 148, 1151–1158
56 Katagiri, K. *et al.* (2002) Rap1 functions as a key regulator of T-cell and antigen-presenting cell interactions and modulates T-cell responses. *Mol. Cell. Biol.* 22, 1001–1015
57 Barberis, L. *et al.* (2000) Distinct roles of the adaptor protein shc and focal adhesion kinase in integrin signaling to ERK. *J. Biol. Chem.* 275, 36532–36540
58 Howe, A.K. and Juliano, R.L. (2000) Regulation of anchorage-dependent signal transduction by protein kinase A and p21-activated kinase. *Nat. Cell Biol.* 2, 593–600
59 Mason, C.S. *et al.* (1999) Serine and tyrosine phosphorylations cooperate in Raf-1, but not B-Raf activation. *EMBO J.* 18, 2137–2148
60 Chiloeches, A. *et al.* (2001) S338 phosphorylation of Raf-1 is independent of phosphatidylinositol 3-kinase and Pak3. *Mol. Cell. Biol.* 21, 2423–2434
61 Rommel, C. *et al.* (1999) Differentiation stage-specific inhibition of the Raf–MEK–ERK pathway by Akt. *Science* 286, 1738–1741
62 Tsygankova, O.M. *et al.* (2001) Coordinated regulation of Rap1 and thyroid differentiation by cyclic AMP and protein kinase A. *Mol. Cell. Biol.* 21, 1921–1929
63 Filippa, N. *et al.* (1999) Mechanism of protein kinase B activation by cyclic AMP-dependent protein kinase. *Mol. Cell. Biol.* 19, 4989–5000
64 Kim, S. *et al.* (2001) Cyclic AMP inhibits Akt activity by blocking the membrane localization of PDK1. *J. Biol. Chem.* 276, 12864–12870
65 Zhang, B.H. *et al.* (2001) Serum- and glucocorticoid-inducible kinase SGK phosphorylates and negatively regulates B-Raf. *J. Biol. Chem.* 276, 31620–31626
66 Gonzalez-Robayna, I.J. *et al.* (2000) Follicle-stimulating hormone (FSH) stimulates phosphorylation and activation of protein kinase B (PKB/Akt) and serum and glucocorticoid-induced kinase (Sgk): evidence for A kinase-independent signaling by FSH in granulosa cells. *Mol. Endocrinol.* 14, 1283–1300
67 Misra-Press, A. *et al.* (1995) A novel mitogen-activated protein kinase phosphatase: structure, expression and regulation. *J. Biol. Chem.* 270, 14587–14596
68 Burgun, C. *et al.* (2000) Cyclic AMP-elevating agents induce the expression of MAP kinase phosphatase-1 in PC12 cells. *FEBS Lett.* 484, 189–193
69 Plevin, R. *et al.* (1997) Cyclic AMP inhibitors inhibits PDGF-stimulated mitogen-activated protein kinase activity in rat aortic smooth muscle cells via inactivation of c-Raf-1 kinase and induction of MAP kinase phosphatase-1. *Cell. Signal.* 9, 323–328
70 Wellbrock, C. *et al.* (2002) Activation of p59Fyn leads to melanocyte dedifferentiation by influencing MKP-1-regulated mitogen-activated protein kinase signaling. *J. Biol. Chem.* 277, 6443–6454
71 Mutoh, T. *et al.* (1998) Differential signaling cascade of MAP kinase and S6 kinase depends on 3′,5′-monophosphate concentration in Schwann cells: correlation to cellular differentiation and proliferation. *Brain Res.* 810, 274–278

72 Cole, J.A. (1999) Parathyroid hormone activates mitogen-activated protein kinase in opossum kidney cells. *Endocrinology* 140, 5771–5779

73 Vogt Weisenhorn, D.M. *et al.* (2001) Coupling of cAMP/PKA and MAPK signaling in neuronal cells is dependent on developmental stage. *Exp. Neurol.* 169, 44–55

74 Grewal, S.S. *et al.* (2000) Calcium and cAMP signals differentially regulate cAMP-responsive element-binding protein function via a Rap1-extracellular signal-regulated kinase pathway. *J. Biol. Chem.* 275, 34433–34441

75 Grewal, S.S. *et al.* (1999) Extracellular-signal-regulated kinase signalling in neurons. *Curr. Opin. Neurobiol.* 9, 544–553

76 Patterson, S.L. *et al.* (2001) Some forms of cAMP-mediated long-lasting potentiation are associated with release of BDNF and nuclear translocation of phospho-MAP kinase. *Neuron* 32, 123–140

77 Deeble, P.D. *et al.* (2001) Interleukin-6- and cyclic AMP-mediated signaling potentiates neuroendocrine differentiation of LNCaP prostate tumor cells. *Mol. Cell. Biol.* 21, 8471–8482

78 Kievit, P. *et al.* (2001) Analysis of the role of the mitogen-activated protein kinase in mediating cyclic-adenosine 3′,5′-monophosphate effects on prolactin promoter activity. *Mol. Endocrinol.* 15, 614–624

79 Swanson, D.J. *et al.* (1998) AP1 proteins mediate the cAMP response of the dopamine beta-hydroxylase gene. *J. Biol. Chem.* 273, 24065–24074

80 Verheijen, M.H. and Defize, L.H. (1997) Parathyroid hormone activates mitogen-activated protein kinase via a cAMP-mediated pathway independent of Ras. *J. Biol. Chem.* 272, 3423–3429

81 Morice, C. *et al.* (1999) Raf-1 and B-Raf proteins have similar regional distributions but differential subcellular localization in adult rat brain. *Eur. J. Neurosci.* 11, 1995–2006

82 Iacovelli, L. *et al.* (2001) Thyrotropin activates mitogen-activated protein kinase pathway in FRTL-5 by a cAMP-dependent protein kinase A-independent mechanism. *Mol. Pharmacol.* 60, 924–933

83 Busca, R. *et al.* (2000) Ras mediates the cAMP-dependent activation of extracellular signal-regulated kinases (ERKs) in melanocytes. *EMBO J.* 19, 2900–2910

84 Wojnowski, L. *et al.* (1997) Endothelial apoptosis in Braf-deficient mice. *Nat. Genet.* 16, 293–297

85 Chen, T. *et al.* (1999) Elevation of cyclic adenosine 3′,5′-monophosphate potentiates activation of mitogen-activated protein kinase by growth factors in LNCaP prostate cancer cells. *Cancer Res.* 59, 213–218

86 Thorson, J.A. *et al.* (1998) 14-3-3 proteins are required for maintenance of Raf-1 phosphorylation and kinase activity. *Mol. Cell. Biol.* 18, 5229–5238

87 Barnier, J.V. *et al.* (1995) The mouse B-raf gene encodes multiple protein isoforms with tissue-specific expression. *J. Biol. Chem.* 270, 23381–23389

88 Zwartkruis, F.J. *et al.* (1998) Extracellular signal-regulated activation of Rap1 fails to interfere in Ras effector signalling. *EMBO J.* 17, 5905–5912

89 Vaillancourt, R.R. *et al.* (1994) B-Raf dependent regulation of the MEK-1/MAP kinase pathway in PC12 cells and regulation by cAMP. *Mol. Cell. Biol.* 14, 6522–6530

90 Kao, S. *et al.* (2001) Identification of the mechanisms regulating the differential activation of the MAPK cascade by epidermal growth factor and nerve growth factor in PC12 cells. *J. Biol. Chem.* 276, 18169–18177

91 MacNicol, M.C. and MacNicol, A.M. (1999) Nerve growth factor-stimulated B-Raf catalytic activity is refractory to inhibition by cAMP-dependent protein kinase. *J. Biol. Chem.* 274, 13193–13197

92 Crepieux, P. *et al.* (2001) The ERK-dependent signalling is stage-specifically modulated by FSH, during primary Sertoli cell maturation. *Oncogene* 20, 4696–4709

93 Kobierski, L.A. *et al.* (1999) Cyclic AMP-dependent activation of the proenkephalin gene requires phosphorylation of CREB at serine-133 and a Src-related kinase. *J. Neurochem.* 73, 129–138

94 Lindquist, J.M. *et al.* (2000) β_3- and α_1-adrenergic Erk1/2 activation is Src- but not G_i-mediated in brown adipocytes. *J. Biol. Chem.* 275, 22670–22677

95 Dremier, S. *et al.* (2000) Activation of the small G protein Rap1 in dog thyroid cells by both cAMP-dependent and -independent pathways. *Biochem. Biophys. Res. Commun.* 267, 7–11

96 Laroche-Joubert, N. *et al.* (2002) PKA-independent activation of ERK and H,K-ATPase by cAMP in native kidney cells: role of Epac I. *J. Biol. Chem.* 10.1074/jbc.M201868200 (www.jbc.org)

97 von Lintig, F.C. *et al.* (2000) Quantitative determination of Rap 1 activation in cyclic nucleotide-treated HL-60 leukemic cells: lack of Rap 1 activation in variant cells. *Oncogene* 19, 4029–4034

98 Mei, F.C. *et al.* (2002) Differential signaling of cyclic AMP. Opposing effects of exchange protein directly activated by cAMP and cAMP-dependent protein kinase A on protein kinase B activation. *J. Biol. Chem.* 277, 11497–11504

99 Kawasaki, H. *et al.* (1998) A family of cAMP-binding proteins that directly activate Rap1. *Science* 282, 2275–2279

100 de Rooij, J. *et al.* (1998) Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature* 396, 474–477

101 Ambrosini, A. *et al.* (2000) cAMP cascade leads to Ras activation in cortical neurons. *Mol. Brain Res.* 75, 54–60

102 Iida, N. *et al.* (2001) Requirement of Ras for the activation of mitogen-activated protein kinase by calcium influx, cAMP, and neurotrophin in hippocampal neurons. *J. Neurosci.* 21, 6459–6466

103 Tsygankova, O.M. *et al.* (2000) Cyclic AMP activates Ras. *Oncogene* 19, 3609–3615

104 Cass, L.A. and Meinkoth, J.L. (2000) Ras signaling through PI3K confers hormone-independent proliferation that is compatible with differentiation. *Oncogene* 19, 924–932

105 Ciullo, I. *et al.* (2001) cAMP signaling selectively influences Ras effectors pathways. *Oncogene* 20, 1186–1192

106 Pham, N. *et al.* (2000) The guanine nucleotide exchange factor CNrasGEF activates ras in response to cAMP and cGMP. *Curr. Biol.* 10, 555–558

107 Crespo, P. *et al.* (1994) Ras-dependent activation of MAP kinase pathway mediated by G-protein beta gamma subunits. *Nature* 369, 418–420

108 Daaka, Y. *et al.* (1997) Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. *Nature* 390, 88–91

109 Crespo, P. *et al.* (1995) Dual effect of beta-adrenergic receptors on mitogen-activated protein kinase. Evidence for a beta gamma-dependent activation and a G alpha s-cAMP-mediated inhibition. *J. Biol. Chem.* 270, 25259–25265

110 Saxena, M. *et al.* (1999) Crosstalk between cAMP-dependent kinase and MAP kinase through a protein tyrosine phosphatase. *Nat. Cell Biol.* 1, 305–311

111 Blanco-Aparicio, C. *et al.* (1999) A novel regulatory mechanism of MAP kinases activation and nuclear translocation mediated by PKA and the PTP-SL tyrosine phosphatase. *J. Cell Biol.* 147, 1129–1136

112 Yee, W.M. and Worley, P.F. (1997) Rheb interacts with Raf-1 kinase and may function to integrate growth factor- and protein kinase A-dependent signals. *Mol. Cell. Biol.* 17, 921–933

113 D'Angelo, G. *et al.* (1997) cAMP-dependent protein kinase inhibits the mitogenic action of vascular endothelial growth factor and fibroblast growth factor in capillary endothelial cells by blocking Raf activation. *J. Cell. Biochem.* 67, 353–366

114 Chen, J. and Iyengar, R. (1994) Suppression of Ras-induced transformation of NIH 3T3 cells by activated G alpha s. *Science* 263, 1278–1281

115 Scott, P.H. *et al.* (1996) A regulatory role for cAMP in phosphatidylinositol 3-kinase/p70 ribosomal S6 kinase-mediated DNA synthesis in platelet-derived-growth-factor-stimulated bovine airway smooth-muscle cells. *Biochem. J.* 318, 965–971

116 Osinski, M.T. and Schror, K. (2000) Inhibition of platelet-derived growth factor-induced mitogenesis by phosphodiesterase 3 inhibitors: role of protein kinase A in vascular smooth muscle cell mitogenesis. *Biochem. Pharmacol.* 60, 381–387

117 Thoresen, G.H. *et al.* (1999) Effects of cAMP on ERK mitogen-activated protein kinase activity in hepatocytes do not parallel the bidirectional regulation of DNA synthesis. *Cell Biol. Int.* 23, 13–20

118 Piiper, A. *et al.* (2000) Pertussis toxin inhibits cholecystokinin- and epidermal growth factor-induced mitogen-activated protein kinase activation by disinhibition of the cAMP signaling pathway and inhibition of c-Raf-1. *Mol. Pharmacol.* 58, 608–613

119 Yamaguchi, T. *et al.* (2000) cAMP stimulates the *in vitro* proliferation of renal cyst epithelial cells by activating the extracellular signal-regulated kinase pathway. *Kidney Int.* 57, 1460–1471

120 Zou, Y. *et al.* (1999) Both Gs and Gi proteins are critically involved in isoproterenol-induced cardiomyocyte hypertrophy. *J. Biol. Chem.* 274, 9760–9770

121 Seger, R. *et al.* (2001) The ERK signaling cascade inhibits gonadotropin-stimulated steroidogenesis. *J. Biol. Chem.* 276, 13957–13964

122 Greenberg, A.S. *et al.* (2001) Stimulation of lipolysis and hormone-sensitive lipase via the extracellular signal-regulated kinase pathway. *J. Biol. Chem.* 276, 45456–45461

123 Yarwood, S.J. *et al.* (1996) Cyclic AMP stimulates the phosphorylation and activation of p42 and p44 mitogen-activated protein kinases in 3T3-F442A preadipocytes. *Biochem. Biophys. Res. Commun.* 224, 734–739

124 Yonehara, T. *et al.* (2001) Involvement of mitogen-activated protein kinase in cyclic adenosine 3′,5′-monophosphate-induced hormone gene expression in rat pituitary GH(3) cells. *Endocrinology* 142, 2811–2819

125 Fujita, T. *et al.* (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.* 10.1074/jbc.M110364200 (www.jbc.org)