Epac: effectors and biological functions

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Chapter

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Abstract

Epac1 (also known as cAMP-GEF-I) and Epac2 (also known as cAMP-GEF-II) are cyclic AMP-activated guanine nucleotide exchange factors for Ras-like GTPases. Since their discovery about ten years ago, it is now accepted that Epac proteins are novel cAMP sensors that regulate several pivotal cellular processes, including calcium handling, cell proliferation, cell survival, cell differentiation, cell polarization, cell-cell adhesion events, gene transcription, secretion, ion transport, and neuronal signaling. Recent studies even indicated that Epac proteins might play a role in the regulation of inflammation and the development of cardiac hypertrophy. Meanwhile, a plethora of diverse effectors of Epac proteins have been assigned, such as Ras and Rho GTPases, phospholiase C-ε, phospholipase D, mitogen-activated protein kinases, protein kinase B/Akt, ion channels, secretory-granule associated proteins and regulators of the actin-microtubule network, the latter probably involved in the spatiotemporal dynamics of Epac-related signaling. This review highlights multi-faceted effectors and diverse biological functions driven by Epac proteins that might explain certain controversial signaling properties of cAMP.

Introduction

Members of the Ras superfamily of small GTPases are now considered to be pivotal regulators of fundamental cellular processes, such as cell proliferation, cell differentiation, cell maturation, cell migration, apoptosis, cytoskeletal rearrangements and contraction [1-3]. Based on sequence homology the Ras superfamily is divided now into five different branches, namely Ras (including Rap1 and Rap2), Rho, Rab, Ran and Arf, and each branch is characterized by distinct signaling properties [1-3]. Small GTPases are activated upon stimulation of signaling pathways by extracellular stimuli and function as molecular switches, cycling between an inactive GDP-bound state and an active GTP-bound state. Guanine nucleotide exchange factors (GEFs) stimulate the GDP/GTP exchange activity of Ras-like GTPases and activate the switch, whereas GTPase-activating proteins (GAPs) accelerate the slow intrinsic GTPase activity of Ras-like GTPases and inactivate the switch [4-7]. Spatiotemporal dynamics of small GTPases, GEFs and GAPs likely provide a mechanism for the generation of distinct biological net outcomes of Ras signaling [8-10]. Several members of the GEF family sense the universal second messengers calcium and cyclic AMP (cAMP) [7, 11-15]. Since their discovery about ten years ago, much attention has been focused on Epac (exchange protein directly activated by cAMP) proteins, GEFs specific for Ras-like GTPases directly activated by cAMP [13, 14, 16]. Epac proteins seem to control several effector proteins and to regulate pivotal processes, including calcium handling, cell proliferation, cell survival, gene transcription, vesicle trafficking, secretion, exocytosis, ion transport, and neuronal signaling. Recent studies indicate that Epac proteins might play a role in inflammation and in the development of

cardiac hypertrophy [17-20]. Epac proteins seem to exert their biological function either alone and/or in concert with the major cAMP regulated effector protein kinase A (PKA), and A-kinase anchoring proteins (AKAPs), known to interact with PKA and cAMP phosphodiesterases (PDEs), seem to coordinate Epac-driven signaling [14, 21] (Fig. 1). It has been reported that Epac1 interacts with PDE4D3 within a cAMP-responsive multiprotein complex, which contains mAKAP, PKA, PDE4D3 and Epac1 [22, 23]. In this review, we will discuss our current knowledge regarding effectors and biological functions of Epac proteins. The reader is also referred to several recent excellent reviews on this topic [13, 14, 16, 24, 25].

Epac proteins

Two isoforms of Epac, namely Epac1 (also known as cAMP-GEF-1) and Epac2 (also known as cAMP-GEF-II), have been identified so far. Epac1 and Epac2 exhibit a distinct expression pattern in mature and developing tissues. In particular, Epac1 mRNA is expressed ubiquitously, whereas Epac2 mRNA is predominantly expressed in the brain and endocrine tissues [26]. Epac2 is currently undetectable in all hematopoietic cell types [27], while Epac1 has been found to be expressed in human monocytes and macrophages [28], B and T cells [27], eosinophils, neutrophils, platelets and CD34-positive hematopoietic cells [17]. Epac proteins are localized to the plasma membrane, perinuclear regions, the nuclear membrane and mitochondria, and subcellular redistribution of Epac proteins appears to be tightly regulated during the cell cycle [29]. These findings indicate that cellular compartmentalization might play a prominent role to exert the diverse signaling properties of Epac proteins. Epac1 and Epac2 are multi-domain proteins consisting of an N-terminal regulatory region and a C-terminal catalytic region (Fig. 1). The N-terminal regulatory domain bears a Dishevelled, Egl-10, Pleckstrin (DEP) domain implicated in membrane association and a high-affinity cAMP-binding domain (cAMP-B). Recently the cAMP-B domain of Epac1 has been found to interact with microtubules [30], while the entire N-terminal region might serve to direct Epac to mitochondria [29]. Epac2 additionally contains a second low-affinity cAMP-A domain of uncertain biological function. A Ras-exchange motif (REM) domain acts as an intramolecular bridge between the regulatory and the catalytic regions to stabilize the GEF domain. Epac proteins also bear a Ras-associating (RA) domain which is present in several Ras-interacting proteins. Until now, however, only Epac2 has been shown to interact with GTP-bound Ras [31], while no interaction partners for the RA domain of Epac1 have been found yet. A CDC25 homology domain (CDC25HD) in the C-terminal catalytic domain exhibits GEF activity for Ras-like GTPases. X-ray crystallography studies of full-length Epac2, solved in the absence of cAMP, indicated the presense of autoinhibitory properties in the C-terminal region being relieved upon binding of cAMP [32]. Thus, cAMP binding to Epac might induce conformational changes releasing Epac proteins from their inhibitory intramolecular constraint to enable interaction with their effectors [14, 32].

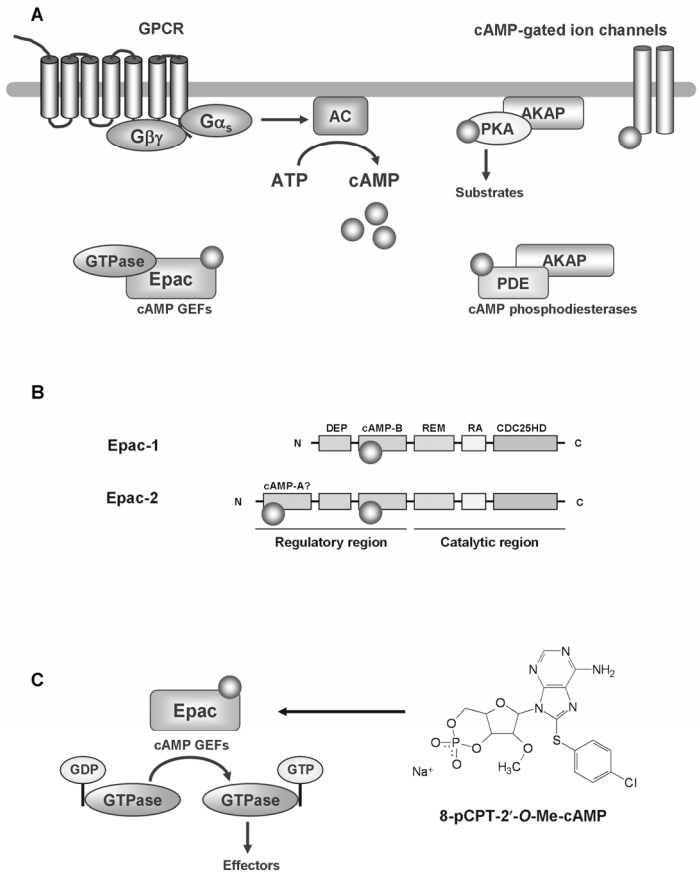

Fig. 1. Epac: A novel cAMP sensor. A, The generation of the second messenger molecule cAMP upon stimulation of G_s-coupled and cAMP-elevating receptors is well established. It has generally been assumed that cAMP acts via protein kinase A (PKA) or cyclic nucleotide gated ion channels to elicit its cellular effects. The identification of the Epac proteins (also known as cAMP-GEFs) has profoundly changed this PKA/cAMP dogma. Recent studies indicate that such diverse signaling properties are coordinated by members of the A-kinase anchoring family. GPCRs, G protein-coupled receptors; AKAP, A-kinase anchoring protein; GEFs, guanine nucleotide exchange factors. **B,** Schematic representation of the domain organization of Epac proteins; DEP, Dishevelled, Egl-10, Pleckstrin domain; REM, Ras-exchanger motif; RA, Ras-associating domain. **C,** Activation of Epac by the membrane permeable and Epac-specific cAMP analog 8-pCPT-2'-*O*-Me-cAMP leads to activation of effectors.

Initial studies claimed that a higher concentration of cAMP is required to activate Epac rather than PKA. Recently, however, it has been reported that the binding affinity of cAMP for PKA and Epac is very similar (k_d ~2,9 μM) [33]. Cellular compartmentalization of cAMP formation and effector protein availability may

decide whether Epac and/or PKA are activated in response to moderate cellular cAMP increases [33]. Indeed, using Epac-based fluorescence resonance energy transfer cAMP sensors, spatiotemporal dynamics of Epac signaling have been identified at the plasma membrane, cytoplasm, mitochondria and the nucleus in cell models, indicating that cellular cAMP concentrations rise to a level sufficient to activate Epac proteins [34-36]. Furthermore, spatiotemporal cAMP signaling has been reported to involve members of the AKAP family [21], and indeed a cAMP-responsive multiprotein complex, including mAKAP-PKA-PDE43-Epac1 has been identified to be important for cAMP signaling in the heart [22, 23]. Meanwhile, membrane-permeable cAMP analogs have been characterized, such as 8-(4-chlorophenylthio)-2'-*O*-methyl-cAMP (8-pCPT-2'-*O*-Me-cAMP) (Fig. 1), and these analogs are used as pharmacological tools to study cAMP effects driven by Epac independent of PKA [24, 37, 38]. To further validate Epac-dependent cAMP signaling, PKA inhibitors might be used, as Epac proteins are insensitive to PKA inhibitors [24]. Inhibition of Epac activation by the cAMP analog Rp-cAMPS has been reported *in vitro*, but this compound failed to inhibit Epac signaling in cell models (reviewed in [24]). Thus, specific pharmacological antagonists of Epac proteins are currently not available.

Epac effectors and cellular functions

Epac proteins were initially characterized as cAMP-activated GEFs for Rap1 and Rap2 [26, 39]. However, Epac proteins seem also to represent a molecular link between different members of the Ras superfamily [18, 31, 40, 41]. In addition, direct binding to and activation of R-Ras by Epac1 has been reported [42]. Epac proteins not only bind to Ras-like GTPases, but also interact with regulators of calcium-induced exocytosis and with the microtubule cytoskeleton network [43-45]. So far, Epac proteins are implicated in several diverse cellular responses, including secretion [41, 46], integrin-mediated cell adhesion [43, 47], formation of cell-cell junctions [48-51], cellular calcium handling [52-57], apoptosis [58], cardiac hypertrophy [18, 20], cell proliferation [59, 60], cell differentiation [28, 61] and gene expression [19, 20, 62]. In particular, Epac2 is implicated in calcium-induced insulin secretion from pancreatic β-cells [63, 64]. Together these findings confirm the importance of Epac proteins for cellular functioning. Epac proteins probably exert such distinct signaling properties upon signaling to a plethora of effector proteins (Fig. 2).

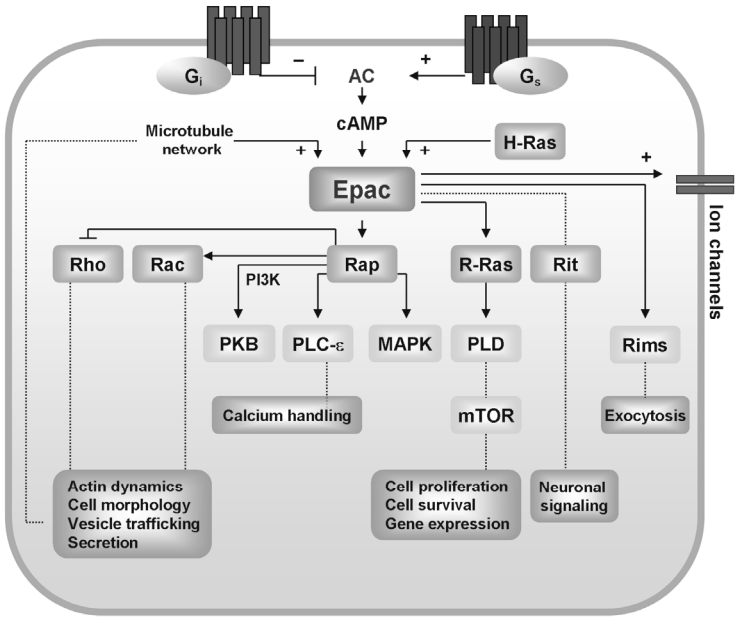

Fig.2. Epac: effectors and biological functions. (a) Schematic representation of the Epac signaling pathway. Second messenger-generating receptors involved in activation of Epac are indicated; small GTPases are shown in orange, other effectors are shown in blue, and distinct cellular responses are indicated in salmon. AC, adenylyl cyclase; PLD, phospholipase D; PLC, phospholipase C; mTOR, mammalian target of rapamycin; PKB, protein kinase B. Whereas Epac2 is recruited by activated H-Ras to the plasma membrane, the GEF activity of Epac1 is regulated by LC2 MAP1A, a microtubule-associated protein. Signaling of various GPCRs, receptor tyrosine kinases and integrin receptors may converge on the level of the diverse effectors of Epac proteins (not shown).

Rap1

Initially Rap1 has been reported to antagonize Ras signaling, but Rap1 is now implicated in the regulation of integrin-mediated cell adhesion and cadherin-mediated formation of cell junctions [13, 65-68]. Rap1 is activated by different members of the GEF family including Epac1 and Epac2 [7, 26, 39, 69]. As reported for Epac proteins, Rap1 is localized to the plasma membrane and intracellular compartments, including the Golgi, lysosomes, endosomes and perinuclear regions [65, 70-72]. Initial studies in ovarian carcinoma OVCAR-3 cells indicated that Epac1-Rap1 signaling modulates integrin-mediated cell adhesion, as activation of Epac by 8-pCPT-2'-*O*-Me-cAMP enhanced cell adhesion to fibronectin [47]. Meanwhile, it has been shown that Epac-Rap signaling controls integrin-mediated cell adhesion in several cell types, including monocytic U937 cells and freshly isolated human monocytes [17]. Rap1 might regulate integrin-mediated cell

adhesion via cellular redistribution of integrins and/or via conformational alterations of integrins, but the exact molecular mechanisms are currently still under investigation. Epac1-Rap1 signaling has also been implicated in the regulation of cell-cell adhesion and endothelial barrier functioning, cellular processes known to involve members of the cadherin family [73]. In Madin-Darby canine kidney cells, Epac1-Rap1 signaling has been reported to regulate E-cadherin-mediated cell-cell adhesion [74]. Recent studies in endothelial cells indicated that Epac1-Rap1 signaling enhanced endothelial barrier functioning, a process probably involving redistribution of E-cadherin and recruitment of other cell junction molecules to cell-cell contacts [49, 50]. Of interest, it has been shown that the level of Epac1 increased 3-fold during differentiation of monocytes into mature macrophages and that the Epac1-Rap1 pathway was operational in mature macrophages, but not in monocytes [28]. Recently, it has been reported that G_s-coupled and cAMP-elevating receptors regulate inflammation through activation of Epac1-Rap1 signaling in monocytes [17]. In addition, Epac-Rap1 has been reported to modulate inflammatory processes in vascular endothelial cells upon induction of the "suppressor of cytokine signalling 3" [19]. Thus, evidence has been provided that Epac1-Rap1 signaling might not only control cell-cell contact events, but also cell differentiation and inflammatory processes.

Rap2

Activation of Rap2 by Epac1 and Epac2 has been reported. In addition, it has been shown that Rap2 is localized to the plasma membrane and intracellular compartments [7, 26, 39, 69]. Until now, however, our current knowledge about Rap2 effectors and Rap2-driven cellular responses is rather limited. Importantly, we reported that G_s-coupled and cAMP-elevating receptors stimulate a novel member of the phosphatidylinositol 4,5-bisphosphate (PIP_2)-specific phospholipase C family, namely PLC-ε, through activation of Rap2 via Epac. Activation of PLC-ε by Epac and Rap2 then results in the generation of inositol-1,4,5-trisphosphate, leading to increases in cellular calcium [52, 55]. Based on these findings we have shown that Epac-Rap2-PLC-ε-induced activation of H-Ras and extracellular signal-regulated kinases 1 and 2 (ERK1/2) by a calcium-regulated Ras-GEF [75]. G_i-coupled and cAMP-reducing receptors inhibited the Epac-Rap2-PLC-ε-driven cellular responses [76]. Interestingly, PLC-ε seems to represent a molecular link between the universal second messengers calcium and cAMP and members of the Ras superfamily of small GTPases, including Ras, Rap and Rho (reviewed in [77]) (Fig. 3). The novel PLC family member PLC-ε is characterized by the presence of a N-terminal CDC25 domain possessing GEF activity for some Ras-like GTPases and two C-terminal RA domains, providing upstream and downstream interactions and potential feedback mechanisms with Ras-like GTPases [78-80]. PLC-ε is abundantly expressed in different tissues, including the heart [78, 79, 81], and as reported for Epac proteins, PLC-ε exhibits a perinuclear localization in cardiac myocytes [18, 22, 81].

Intriguingly, recent studies in mice deficient for the Epac effector PLC-ε indicated that stimulation of calcium-induced calcium release by the typical G_s-coupled β-adrenoceptor required Epac-Rap-PLC-ε in cardiac myocytes [53, 56]. These findings are the first indication that Epac driven PLC-ε might be important for cardiac functioning, but the physiological impact of Epac-driven calcium dynamics in the heart still requires further investigation. Whether Epac1 and Epac2 induce the cAMP-dependent PLC-ε stimulation not only upon activation of Rap2, but rather also upon activation of Epac-regulated Rap1 and R-Ras remains to be studied. Beside PLC-ε, Rap2 might activate the c-Jun N-terminal kinase (JNK) via mitogen-activated protein kinase kinase kinase kinase 4, and might regulate the actin cytoskeleton via Traf2- and Nck-interacting kinase [82, 83]. In addition, it has been shown that Rap2 is required for Wnt/β-catenin signalling pathway in *Xenopus* early development [84]. As developmental changes of Epac expression has been reported as well [20], these findings might indicate that Epac-Rap2 signaling modulate cellular growth via diverse effector proteins.

R-Ras

As member of the Ras superfamily of small GTPases, R-Ras interacts with several different GEFs and GAPs [7, 15]. Importantly, we have shown recently that Epac1 directly binds to and activate R-Ras [42]. R-Ras has been reported to play a role in the regulation of various aspects of integrin-mediated cell adhesion, and thus R-Ras might modulate integrin signaling in concert with Rap1 [66, 85, 86]. Recently, R-Ras has been implicated in the regulation of cellular calcium handling as well. It has been reported that R-Ras triggers alterations in cellular calcium handling probably upon decreasing the calcium content of the endoplasmatic reticulum [87]. In addition, it has been shown that activation of PLC-ε by R-Ras altered the cellular calcium content [88]. As Epac is capable to both activate R-Ras and PLC-ε, such Epac-driven signaling properties might converge on the level of PLC-ε to modulate cellular calcium handling (Fig. 3). We reported recently that activation of R-Ras by Epac induces stimulation of phospholipase D (PLD) [42]. PLD enzymes hydrolyze phosphatidylcholine of cell membranes to phosphatidic acid (PA) in response to stimuli, and are considered to be involved in a large variety of early and late cellular responses, including calcium mobilization, secretion, exocytosis, cytoskeleton rearrangement, cell proliferation and apoptosis [89]. Importantly, PLD-generated PA is implicated in the regulation of the mammalian target of rapamycin (mTOR) [90], a phosphatidylinositol 3-kinase (PI 3-kinase)-regulated effector protein known to be involved in the regulation of gene transcription and cell-cycle progression [91-93] (Fig. 2). Epac has been reported to induce R-Ras-dependent PLD stimulation [42], and it has been shown that Epac modulates cellular PI 3-kinase responses [60, 94]. These findings suggest that Epac signaling might control gene expression and cell proliferation through activation of PLD, a process probably involving R-Ras and PI 3-kinase.

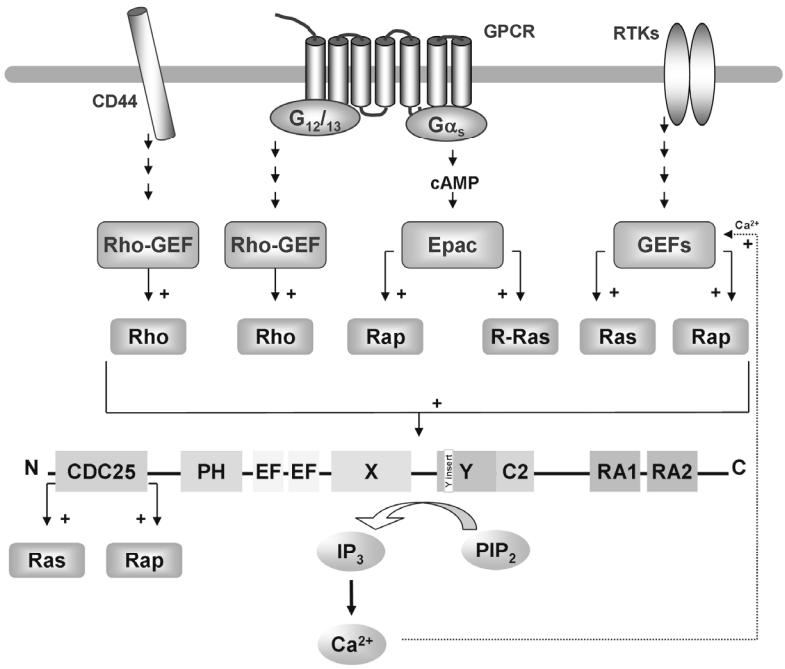

Fig.3. Regulation of the Epac effector PLC-ε by second messengers and members of the Ras superfamily. Activation of Epac proteins upon stimulation of G_s-coupled and cAMP-elevating receptors (e.g. β_2-adrenergic receptors, E1 prostanoid receptor and M_3 muscarinic receptors) induce PLC-ε-dependent elevations in cellular calcium upon GTP-loading of the Ras-like GTPase Rap2B. As Epac 1 directly binds to and activate R-Ras, Rap2B might act in concert with R-Ras to modulate PLC-ε signaling. Activation of PLC-ε by receptor tyrosine kinase (RTKs) (e.g. platelet-derived growth factor receptor and epidermal-derived growth factor (EGF) receptor) has been shown to involve Rap (Rap1 and Rap2B) and Ras. In particular, activation of Rap2 by EGF seems to involve a calcium-regulated GEF. Activation of PLC-ε upon stimulation of $G_{12/13}$-coupled receptors (e.g. receptor for lysophosphatidic acid) or the surface protein CD44 has been shown to involve Rho, the latter being activated by p115 Rho-GEF or LARG, respectively. Wherase activation of PLC-ε by Rho seems to involve a unique Y insert in the cataytic core, activation of PLC-ε by Rap and Ras seems to involve the two C-terminal RA domains. In addition, PLC-ε bears a N-terminal CDC25 domain thereby exhibiting GEF activity to Rap1 and Ras. These mechanisms probably provide upstream and downstream interactions with small GTPases. PLC-ε-driven calcium elevations might contribute to the activation of calcium-regulated GEFs. However, the impact of such diverse feed-forward and/or feed-back regulatory mechanisms between PLC-ε and members of the Ras superfamily and second messengers remains to be studied. PH, pleckstrin homology; EF, EF-hands; X and Y, catalytic core; C2, C2 domain; RA, Ras-associating domain.

Mitogen-activated protein kinases

Several studies indicate that Rap1 modulates mitogen-activated protein kinase (MAPK) responses, in particular of ERK1/2 [95]. Rap1 induces ERK1/2 stimulation or inhibition depending on specific cell types, and cellular compartmentalization might define signaling specificity. Meanwhile, Epac-Rap signaling triggered upon stimulation of various receptors has been reported to activate ERK1/2 in several cell types, including FRTL5 cells [96], native rat kidney cells [97], cultured rat hippocampal neurons and PC12 cells [98], HEK-293 cells and N1E-115 neuroblastoma cells [75], osteoblast [96, 99], and human umbilical vein endothelial cells [100]. In addition, it has been reported that even productive coupling between Epac-Rap did not result in activation of ERK1/2 [38, 101]. It has been proposed that Epac-triggered activation of ERK1/2 by Rap1 is dependent on the mode of Rap activation, i.e. a plasma membrane pool of Rap1 colocalized with Epac must be activated in order to trigger ERK1/2 responses [101]. In addition, it has been shown that Epac activates JNK, a member of the MAPK family [102, 103]. Strikingly, activation of JNK by Epac was independent of its GEF activity [102]. Activation of MAPKs by Epac has not only been implicated in the regulation of cell proliferation and cell-cycle regulation, but rather also in the modulation of ion channels [97] and regulation of gene expression [62].

Rho GTPases

Activation of Rho GTPases in response to diverse extracellular stimuli is known to play a pivotal role in the regulation of actin dynamic-dependent processes, including cell polarization, cell migration and contractility [104, 105]. Interaction of Epac proteins with the microtubule cytoskeleton network has been described to control their GEF activity [43], indicating that actin dynamics might play a central role in the regulation of Epac signaling properties. Regulation of the Epac effector PLC-ε by members of the Ras superfamily, namely R-Ras and most importantly Rho GTPases, has been shown to modulate actin-driven processes such as the formation of membrane protrusions [88] and cell migration [106]. The Epac effector Rap1 has been reported to increase the GAP activity of ARAP3 towards RhoA and to inhibit RhoA-dependent formation of membrane protrusions [40]. Such process may present a molecular link between Rap1 and RhoA. Recently, it has been shown that Epac-Rap1 enhances endothelial barrier functioning in human pulmonary artery endothelial cells, a process requiring activation of Rac by a Tiam1/Vav2-dependent pathway [48]. Recruitment of Rac via Tiam1/Vav2 signaling to defined membrane areas at the cell periphery seems to trigger endothelial barrier functioning by Epac-Rap1. Rac might be also important for the regulation of integrin-mediated cell-cell adhesion events by Epac-Rap1, as Rac recruitment to the plasma membrane is under control of integrins [107, 108]. Strikingly, evidence has been provided that secretion of the amyloid precursor protein by Epac-Rap1 requires Rac in various cell types,

including primary cultures of mouse cortical neurons [41]. Meanwhile, it has been reported that the α-secretase-induced cleavage or "shedding" of the amyloid precursor protein ectodomain by members of the matrix metalloproteinase family, namely ADAMs (a disintegrin and metalloproteases), involves Epac proteins [46]. Matrix metalloproteinases are now considered to be pivotal regulators of actin-driven processes, including cell proliferation and cell migration [109, 110]. As Epac driven signaling to Ras and Rho GTPases seems to modulate actin dynamics, it is reasonable to assume that regulation of matrix metalloproteinases by Epac proteins might contribute to such cellular events.

Ion channels, ion transporters and Ca^{2+} signaling

It is generally accepted that cAMP profoundly modulates ion channel functioning [11]. Several recent studies point to a major role of Epac proteins in the cAMP-dependent regulation of ion channels [63]. It has been reported that Epac2 inhibits the ATP-sensitive potassium channel (K_{ATP}-channel) in human pancreatic β-cells [111]. Such process involved direct interaction of Epac2 with the sulphonylurea receptor-1 (SUR-1) subunit of the K_{ATP} channel and has been now implicated in the regulation of insulin release from pancreatic β-cells [45, 63, 64, 111]. Of interest, it has been shown recently that the PLC-substrate PIP_2 stimulates the activity of K_{ATP}-channels upon reducing their sensitivity to ATP [112]. Strikingly, PLC-ε has been reported to cleave PIP_2 upon stimulation through cAMP-dependent Epac proteins [52, 53, 55, 56, 76]. Thus, Epac-driven PLC-ε responses might contribute to the inhibitory effects of cAMP on K_{ATP} channel activity. Activation of Ca^{2+} and Cl^- channels by glucagon in rat hepatocytes seems also to involve Epac proteins, probably Epac2 known to be expressed in these cells [113]. Whether Epac-dependent regulation of Ca^{2+} and Cl^- channels is mediated by PLC signals remains to be determined. As reported for the ATP-dependent H^+-K^+ transporter in rat kidney cortical collecting duct cells [97], and for the Na^+-H^+ exchanger isoform 3 in kidney cells [114], Epac proteins regulate the activity of ion transporters, but the underlying molecular mechanisms are unknown yet. Recent findings indicate that cAMP-regulated Epac proteins positively regulate cellular calcium increases. Stimulation of calcium-induced calcium release (CICR) by the Epac activator 8-pCPT-2'-*O*-Me-cAMP induced insulin secretion from human pancreatic β-cells and insulin-secreting cell lines, a process being sensitive to dominant negative Epac2 [64]. Epac proteins might regulate CICR through K_{ATP} channels, ryanodine receptors and Rap1-sensitive sarco-endoplasmic reticulum Ca^{2+}-ATPases [63, 64, 115]. A recent report indicates that Epac-Rap1 activates p38 MAPK in cerebellar neurons, a process enhancing cellular calcium through modulation of calcium-dependent K^+ channels [116]. Such mechanism might indicate a role for Epac proteins in the regulation of neuronal excitability. Finally, it is now accepted that the novel Epac-Rap-PLC-ε pathway regulates cellular calcium handling [52, 53, 55, 76]. Indeed, cardiac myocytes from mice deficient for PLC-ε exhibited a diminished CICR in response to G_s-coupled

and cAMP-elevating receptors [53, 56]. Epac proteins probably acting in concert with PKA might not only regulate normal cardiomyocytes physiology [53], but might contribute also to developmental and pathophysiological alterations in the heart, as reported recently [20].

Exocytosis

Stimulus-secretion coupling is an important event in several secretory cells to control the exocytosis machinery [25, 117]. Epac proteins regulate exocytotic processes in non-neuronal and neuronal cell types, including insulin secretion from pancreatic β-cells [118] and the release of glutamate release at crayfish neuromuscular junctions [119]. Interaction of Epac proteins with secretory-granule associated proteins, including Rim (Rab3-interacting molecule) and Rim2 [45, 120], might play a role in the regulation of exocytosis. In pancreatic β-cells interaction of Epac2 with a secretory-granule membrane-associated pool of the SUR-1 subunit of K_{ATP} channels induces opening of ClC-3 Cl^- cloride channels causing Cl^- influx into secretory granules creating an electromotive force facilitating ATP-dependent H^+ uptake by a v-type H^+-ATPase [121, 122]. Such acidification stimulates the "priming" of secretory granules, thereby rendering them release-competent. It has even been hypothesized that Epac2 might act in a more direct manner to up-regulate the activity of the v-type H^+-ATPase channels [63]. Based on our current knowledge, Epac proteins seem to regulate exocytosis either through interaction with secretory-granule associated proteins or regulation of ion channels.

Microtubules

Microtubules are believed to act in concert with the actin cytoskeleton to regulate diverse cellular responses, including cell polarization, cell division and cell migration, and contribution of cAMP-dependent processes is organized through members of the AKAP family [123-125]. Recently, interaction of Epac proteins with microtubules and/or microtubule-associated proteins has been identified [44, 126], suggesting that Epac proteins participate in the cAMP-dependent regulation of the microtubule network. In particular, Epac1 has been shown to bind to the light chain 2 (LC2) of the microtubule-associated protein MAP1A, the latter known to interact with the actin-microtubule network by catalyzing polymerization and stabilization of microtubules [43, 127]. It has also been reported that LC2 enhances activation of Rap1 by Epac1 [43], suggesting that the GEF activity of Epac proteins is under control of the microtubule network. As MAP1 proteins are multi-subunit protein complexes known to modulate cellular responses, LC2 may serve as an anchor to promote the interaction of Epac proteins with the microtubule network [128]. Strikingly, it has been reported that the Rho-specific nucleotide exchange factor GEF-H1 connect microtubule network alterations to Rho-dependent signaling [129]. As binding of LC2 to Epac proteins probably enhances the affinity of the cAMP-

binding domains for cAMP [126], it might seem reasonable to suggest that the stability of the microtubule network define the ability of Epac proteins to activate Rap. Since Epac proteins have been reported to associate with the microtubules of the mitotic spindle [29], LC2 might also maintain Epac activity during cell division.

Others

Promiscuity is presumably the most striking feature of Epac driven signaling. As novel cAMP sensors Epac proteins modulate multi-faceted effectors and diverse biological functions. In order to explain signaling properties of cAMP still being unclear and controversial, Epac related research has attracted much attention over the last ten years. Of particular interest in this context, a recent study indicating that Epac proteins mediate the cAMP-dependent activation of the small GTPase Rit, the latter known to be important for neurotrophin signaling [61, 130]. It has been shown that Epac1 is required for the activation of Rit by the G_s-coupled and cAMP-elevating pituitary adenylate cyclase-activating polypeptide receptor, a process independent of Rap and independent of direct activation of Rit by Epac1 or Epac2. As pituitary adenylate cyclase-activating polypeptide receptors are known to regulate neuronal processes, including neuronal differentiation, neuronal proliferation and neuronal survival [131], these findings suggest that Epac proteins might regulate learning and memory processes. An additional new research line represents the relation between Epac and protein kinase B (PKB)/Akt, the latter known to control many vital processes, including cell proliferation and cell survival upon modulation of gene transcription [132]. The role of cAMP in the regulation of cell proliferation is still a matter of debate: cAMP stimulates proliferation in certain cell types, while it inhibits proliferation in other cell types [11, 95]. Recent studies implicate that Epac proteins might be involved in these opposing effects of cAMP on cell proliferation. Activation of PI 3-kinase-dependent Akt signaling by Epac proteins has been shown in WRT cells [59]. Epac-dependent activation of Akt increased gene expression and proliferation of macrophages, a process depending on Rap1. In addition, it has been hypothesized that activation of Akt by Epac requires its recruitment to the plasma membrane [60]. However, depending on the stimuli the cells are exposed to, cAMP-induced Epac activation can either stimulate or inhibit Akt phosphorylation probably through interaction with distinct effectors [94, 133, 134]. For example, it has been reported that Epac proteins induced a decrease in Akt phosphorylation in adipocytes presumably upon modulation of cAMP-reducing phosphodiesterases 3B and E4 [135]. These results implicate that cell type specificity determines the crosstalk between Epac and PKB/Akt, a process probably involving distinct spatiotemporal dynamics.

Future directions

In the last decade, Epac proteins have been recognized to play a central role in the regulation of cAMP-driven cellular processes upon signaling to diverse effectors. Several properties of Epac proteins still need to be elucidated. Additional studies on the structures of Epac1 and Epac2 in the absence and presence of cAMP will certainly extent our current knowledge of their mode of activation and regulation. Studies on the molecular nature of the interaction of Epac proteins with their diverse effectors are also warranted. Without doubt, the development of Epac specific pharmacological antagonists and mice deficient in Epac proteins would be very helpful. Of particular interest is the relationship between Epac and PLC-ε-calcium signaling, which represents a molecular link between the two universal second messengers calcium and cAMP. Epac proteins are known to activate Ras-like GTPases, which in turn bind and activate PLC-ε, thereby modulating cellular calcium handling. Strikingly, PLC-ε bears beside the typical PLC-defining catalytical domains a CDC25 domain, which exhibits GEF activity to the identical subset of small GTPases known to activate PLC-ε (reviewed in [77]) (Fig. 3). Indeed, it has been reported that the PLC-ε-specific CDC25 homology domain activate Rap1 leading to sustained Rap1 signaling [136], indicating that PLC-ε acts both downstream and upstream of Ras-like GTPases. In this context, also activation of Rap1 has been proposed to occur downstream of PLC-ε-dependent activation of R-Ras [85, 88]. It should be noted, however, that PLC-ε-driven PIP_2 hydrolysis might alter cellular responses by mechanisms independent of calcium, as alterations in the cellular PIP_2 content are known to regulate several cellular processes as well [137]. To decipher the role of the diverse effectors mediating Epac responses and to understand the impact of the spatiotemporal dynamics of Epac signaling are the important challenges for future research in this exciting field of signal transduction.

Concluding remarks

Our current knowledge about effectors and biological functions of Epac proteins is still incomplete and controversial. Studies on the developmental expression pattern of Epac and changes in expression under pathophysiological circumstances are still rather limited. However, initial studies indicate that Epac proteins might be important for the regulation of inflammatory processes and the development of cardiac hypertrophy. The interaction of Epac proteins with matrix metalloproteinases implicates that Epac signaling might play a key role in several aspects of tissue remodeling. The involvement of Epac in insulin secretion and amyloid precursor protein secretion might indicate that Epac signals are relevant for future treatment of several devastating disorders, including Type II diabetes and Alzheimer's disease.

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Within the last ten years our knowledge about the impact of Epac-driven signaling routes has increased remarkably, and novel Epac related signaling properties are published every week. Unfortunately, as for space limitations we were not able to include all recent work on Epac. We thank Dr. Oude Weernink for critical reading of the manuscript. Sara S. Roscioni is recipient of an Ubbo Emmius Fellowship from the School of Behavioral and Cognitive Neurosciences (BCN), University of Groningen, and Martina Schmidt is a Rosalind Franklin Fellow at the University of Groningen.

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