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PHARMACOLOGICAL REVIEWS

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Exchange Protein Directly Activated by cAMP (epac): A Multidomain cAMP Mediator in the Regulation of Diverse Biological Functions

Martina Schmidt, Frank J. Dekker, and Harm Maarsingh

Department of Molecular Pharmacology (M.S., H.M.), Groningen Research Institute for Pharmacy, University of Groningen, The Netherlands; Groningen Research Institute for Asthma and COPD, University of Groningen, University Medical Center Groningen, The Netherlands (M.S., H.M.); and Department of Pharmaceutical Gene Modulation (F.J.D.), Groningen Research Institute for Pharmacy, University of Groningen, The Netherlands (M.S., F.J.D., H.M.)

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Address correspondence to: Dr. Martina Schmidt, Department of Molecular Pharmacology, University Center of Pharmacy, Building 3211, Room 406, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands. E-mail: m.schmidt@rug.nl
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Abstract——**Since the discovery nearly 60 years ago, cAMP is envisioned as one of the most universal and versatile second messengers. The tremendous feature of cAMP to tightly control highly diverse physiologic processes, including calcium homeostasis, metabolism, secretion, muscle contraction, cell fate, and gene transcription, is reflected by the award of five Nobel prizes. The discovery of Epac (exchange protein directly activated by cAMP) has ignited a new surge of cAMP-related research and has depicted novel cAMP properties independent of protein kinase A and cyclic nucleotide-gated channels. The multidomain architecture of Epac determines its activity state and allows cell-type specific protein-protein and protein-lipid interactions that control fine-tuning of pivotal biologic responses through the "old" second messenger cAMP. Compartmentalization of cAMP in space and time, maintained by A-kinase anchoring proteins, phosphodiesterases, and β-arrestins, contributes to the Epac signalosome of small GTPases, phospholipases, mitogen- and lipid-activated kinases, and transcription factors. These novel cAMP sensors seem to implement certain unexpected signaling properties of cAMP and thereby to permit delicate adaptations of biologic responses. Agonists and antagonists selective for Epac are developed and will support further studies on the biologic net outcome of the activation of Epac. This will increase our current knowledge on the pathophysiology of devastating diseases, such as diabetes, cognitive impairment, renal and heart failure, (pulmonary) hypertension, asthma, and chronic obstructive pulmonary disease. Further insights into the cAMP dynamics executed by the Epac signalosome will help to optimize the pharmacological treatment of these diseases.**

I. Introduction

cAMP is one of the most common and universal second messengers, and its production is initiated upon binding of a wide range of extracellular ligands to G_s protein-coupled receptors and subsequent activation of membrane-bound adenylyl cyclases (ACs), which generate cAMP from adenosine triphosphate (Hanoune and Defer, 2001; Beavo and Brunton, 2002). cAMP regulates pivotal physiologic processes including metabolism, secretion, calcium homeostasis, muscle contraction, cell fate, and gene transcription. The impact and complexity of research into the molecular architecture of cAMP signaling are not only reflected by five Nobel awards since the discovery of cAMP in 1957 by Sutherland and colleagues (Beavo and Brunton, 2002) but also by a unique interplay of signaling components that tightly control the cellular content of cAMP. Next to G protein-coupled receptors and ACs, cAMP-specific phosphodiesterases (PDEs; Fig. 1), which degrade cAMP into the inactive 5′-AMP, maintain the spatiotemporal nature of cAMP signaling by shaping a cAMP gradient throughout the cell (Hanoune and Defer, 2001; Conti and Beavo, 2007; McCahill et al., 2008; Houslay, 2010; Keravis and Lugnier, 2010). Subcellular membrane clustering of receptors, ACs, and PDEs to lipid rafts and caveolae and cell compartment-specific (co)localization to distinct cAMP effectors further support the maintenance of compartmentalized cAMP signaling (Patel et al., 2008a,b). Moreover, recent studies demonstrated also that internalized G protein-coupled receptors—until now believed to act as a "loss-of-function" receptor signal—maintain their signaling properties (Calebiro et al., 2009; Nikolaev et al., 2010), and endosomes, in which internalized receptors may end up (Murphy et al., 2009), are now being recognized as an essential site of cellular signaling, providing a novel target for the treatment of diseases.

Subcellular compartmentalized cAMP signaling is also facilitated by members of the A-kinase anchoring protein (AKAP) family by generating spatially discrete signaling complexes that contain different binding partners and create local gradients of cAMP (Fig. 1, A and D), and thereby permit and control specific cellular responses (Wong and Scott, 2004; Beene and Scott, 2007; Zaccolo, 2009, 2011; Skroblin et al., 2010; Tröger et al., 2012). Dysfunction of cAMP-sensing and AKAP-bearing multiprotein complexes seem to alleviate the progression of several diseases, including chronic heart failure, cardiac arrhythmia, Alzheimer's disease, human immunodeficiency virus infection, diabetes mellitus, and cancer (Skroblin et al., 2010; Zaccolo, 2011; Tröger et al., 2012). Ligand-directed signaling or biased agonism, referring to G_s-induced cAMP-dependent versus β-arrestin-mediated signaling, adds another level of complexity of G protein-

ABBREVIATIONS: 8-NBD-cAMP, 8-[2-(7-nitro-4-benzofurazanyl) aminoethyl-thio] cAMP; 8-pCPT, 8-(4-chlorophenylthio); AC, adenylyl cyclase; AKAP, A-kinase anchoring protein; CaMKII, Ca^{2+}/calmodulin-dependent kinase II; cAMP-B, cAMP binding B domain; CDC25HD, CDC25-homology domain; cMyBPC, cardiac myosin-binding protein C; COPD, chronic obstructive pulmonary disease; cTnI, cardiac troponin I; DEP, disheveled-Egl-10-pleckstrin; Epac, exchange protein directly activated by cAMP; ERK, extracellular regulated kinase; ERM, ezrin-radixin-moesin; GAP, GTPase-activating proteins; GEF, guanine exchange factor; GLP-1, glucagon-like peptide-1; GRK, G protein-coupled receptor kinase; HDAC, histone deacetylase; HUVEC, human umbical vein endothelial cells; IQGAP, IQ motif containing GTPase activating protein; JNK, c-Jun N-terminal kinase; mTOR, mammalian targets of rapamycin; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB; PACAP, pituitary adenylate cyclase-activation polypeptide; PDE, phosphodiesterase; PDZ, PSD-95/DlgA/ZO-1; PGE_2, prostaglandin E_2; PIP_2, phosphatidylinositol-4,5-bisphosphate; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PLD, phospholipase D; RA, Ras association domain; REM, Ras exchange motif; RyR2, sarcoplasmic ryanodine receptor; SOCS-3, suppressor of cytokine signaling-3; SUR1, sulfonylurea receptor-1; TGF-β, transforming growth factor-β.

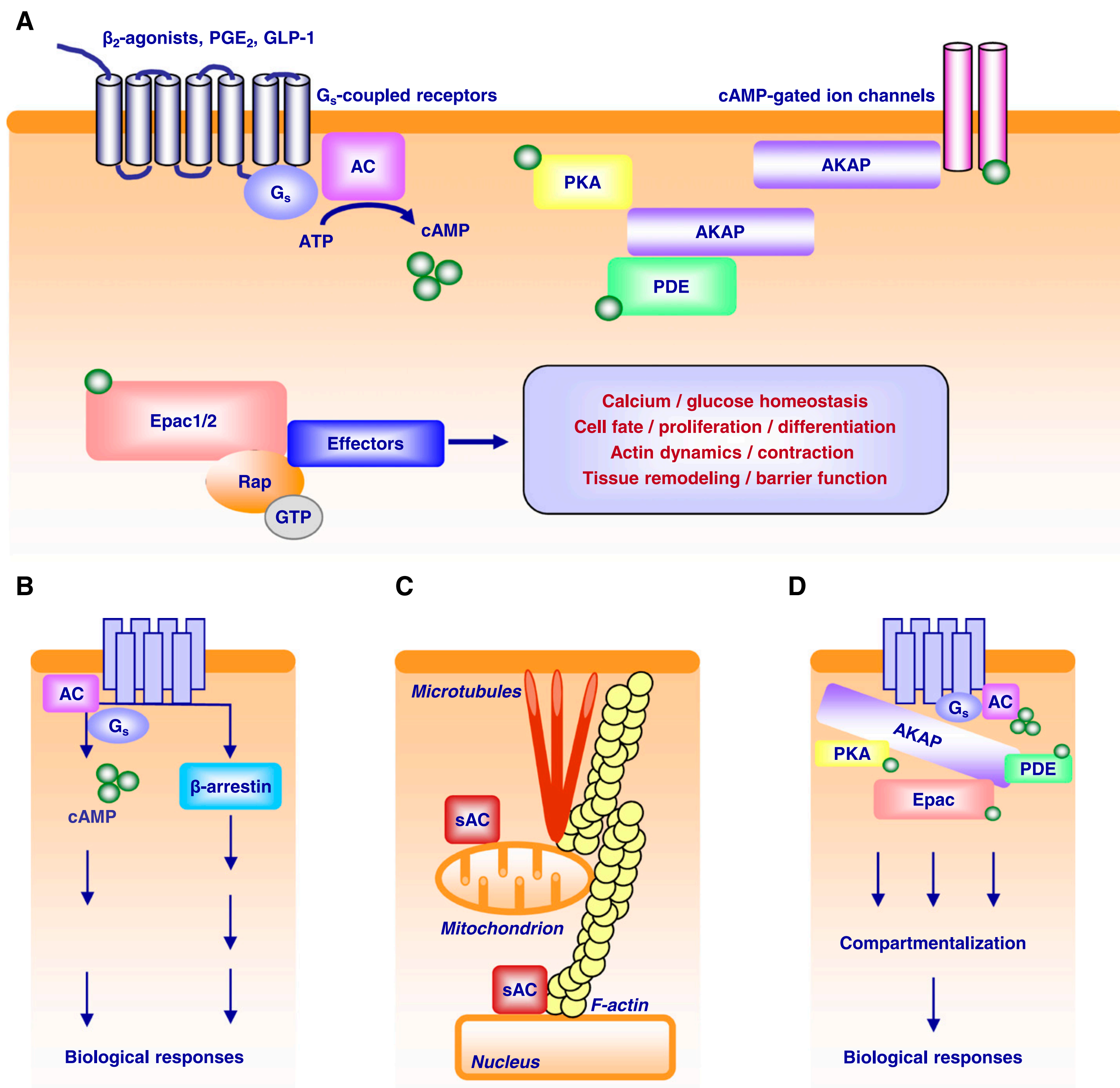


Fig. 1. The novel cAMP target Epac is centered in the sophisticated molecular network of compartmentalized cAMP signaling. (A) Generation of the universal second messenger cyclic AMP (cAMP) is initiated upon stimulation of G_s protein-coupled receptors through binding of appropriate ligands, including β_2-agonists, prostanoids, and GLP-1, and subsequent activation of membrane-bound AC family members. Next to G protein-coupled receptors and ACs, cAMP-specific PDEs shape the cAMP gradient throughout the cell to maintain the spatiotemporal nature of cAMP signaling. Most biologic effects of cAMP are classically assigned to PKA. The recent discovery of Epac profoundly altered the compartmentalized cAMP signaling network. Epac, acting either alone or in concert with PKA, regulates diverse biologic responses by activating members of the Ras superfamily, in particular Rap GTPases, via GTP-loading. Ligand-directed signaling or biased agonism, thus G_s-induced cAMP-dependent versus β-arrestin-mediated signaling (B) and subcellular cAMP microdomains maintained by soluble AC (sAC or AC10) (C) have been linked to cardiac and neuronal signaling properties of Epac. Additionally, cAMP-sensing multiprotein complexes maintained by A-kinase anchoring proteins (AKAPs)control specific biologic functions of Epac. One of many distinct complexes, consisting of different AKAPs, G protein-coupled receptors, and AC and PDE isoenzymes, is depicted in this (D). See text for further details.

coupled receptor signaling (Fig. 1B) and has been comparably reviewed within the context of heart failure, cardiovascular disease, human immunodeficiency virus, obstructive lung disease, and cancer (Whalen et al., 2011; Reiter et al., 2012). Next to fluorescence and bioluminescence resonance energy transfer techniques (Lohse et al., 2012), dynamic mass redistribution allowing label-free real-time analysis of

G protein-coupled receptors were supportive to monitor novel receptor signaling properties (Kenakin, 2010; Schröder et al., 2010). Moreover, subcellular microdomains of cAMP maintained by soluble AC (Fig. 1C), which are distinct from cAMP compartments maintained by membrane-bound ACs, might turn out to be of key importance for the regulation of a variety of physiologic systems (Tresguerres et al., 2011; Chen et al., 2012b). Until recently, most cAMP effects have been attributed to the activation of protein kinase A (PKA) and cyclic nucleotide-gated ion channels (Cohen, 2002; Zambon et al., 2005; Biel, 2009; Biel and Michalakis, 2009). However, several cAMP-mediated cellular events appeared to be insensitive to PKA inhibition and have prompted investigators to define alternative mechanisms and targets for the cellular action of cAMP.

In this review, the discovery and biology of the novel cAMP target Epac (exchange protein directly activated by cAMP) will be described. Identification and characterization of Epac completely altered our current view of the molecular mechanisms of cAMP that permit a tightly controlled fine-tuning of pivotal biologic responses through this "old" second messenger. Several unique signaling properties of Epac are defined by its multidomain architecture that represents the structural basis in the temporal-dynamic control of both autoinhibition and activation of this novel cAMP-regulated exchange factor. Next to the activity state of Epac, the multidomain structure determines cell-type specific protein-protein interactions, thereby defining subcellular localization and subsequently the biologic net outcome of the activation of Epac partly through formation of cAMP-sensing multiprotein complexes. Moreover, this review provides an update on the molecular tools, agonists, and antagonists selective for Epac through both orthosteric and allosteric mechanisms currently used to study the Epac functions. Finally, this review illustrates how Epac-specific signaling properties contribute to the sophisticated molecular signaling network of cAMP that regulate cardiac output, neuronal processes, glucose homeostasis, vascular functioning, and responses in the airways.

A. The Discovery of Epac

In 1998, Epac, also known as cAMP-guanine exchange factor (cAMP-GEF), was identified upon a database search aimed to unravel the mechanism involved in the cAMP-dependent activation of the small GTPase Rap1 that was independent of PKA (de Rooij et al., 1998). Independently, Epac1 (cAMP-GEF-I) and Epac2 (cAMP-GEF-II) were found in a differential display screen for novel cyclic nucleotide-binding domain-bearing proteins, which are enriched in the striatum (Kawasaki et al., 1998). As members of the Ras superfamily of small GTPases, Rap functions as a molecular switch cycling between an inactive GDP-bound state and an active GTP-bound state (Fig. 2A). Guanine nucleotide exchange factors (GEFs) stimulate the GDP/GTP activity of Rap and activate the switch, whereas GTPase-activating proteins (GAPs) exert opposite effects on Rap activity upon acceleration of its slow intrinsic GTPase activity (Quilliam et al., 2002; Bos et al., 2007; Gloerich and Bos, 2011; van Dam et al., 2011). The generation of the distinct biologic net outcomes of Rap signaling relies on the spatiotemporal dynamics of the small GTPases GEFs and GAPs. Repac, which lacks the cAMP-dependent regulatory sequences, has been identified as a homolog of Epac and exhibits constitutive activity toward Rap (de Rooij et al., 2000). Subsequently, a family of Rap-specific GEFs has been identified which is characterized by the presence of a PSD-95/DlgA/ZO-1 (PDZ) domain, a Ras-association (RA) domain, and a region related to the cyclic nucleotide binding domain (Kuiperij et al., 2003).

Epac is a cAMP-regulated GEF that favors GDP/GTP exchange and thereby direct activation of the Ras family members Rap1 and Rap2 (de Rooij et al., 1998; Kawasaki et al., 1998; Figs. 1 and 2). Thus, Epac controls cellular responses initiated by activation of Rap through cAMP. Although Epac signals through its canonical GEF activity to Rap1 and Rap2 in the majority of all studies, activation of the mitogen-activated family member c-Jun N-terminal kinase (JNK; Hochbaum et al., 2003), phospholipase D (PLD; López de Jesús et al., 2006), and the small GTPase Rit (Shi et al., 2006), as well as microtubule growth (Sehrawat et al., 2008), is independent of the Rap-GEF activity of Epac. In addition, although direct protein-protein interactions have not been proven in all studies, Epac proteins serve as a molecular link to distinct members and downstream effectors of the Ras superfamily (Fig. 2), including Rho (Krugmann et al., 2004; Birukova et al., 2010; Roscioni et al., 2011b; Zieba et al., 2011; Jeyaraj et al., 2012), Rac (Maillet et al., 2003; Robert et al., 2005; Roscioni et al., 2011b; Zieba et al., 2011), Ras (Morel et al., 2005; Li et al., 2006; López de Jesús et al., 2006; Métrich et al., 2008), Rit (Shi et al., 2006), as well as Ran and RanBP2 (Liu et al., 2010a; Gloerich et al., 2011).

The two Epac isoforms Epac1 (cAMP-GEF-1) and Epac2 (cAMP-GEF-II) exhibit a broad expression profile both on the mRNA and protein level, albeit with distinct expression levels depending on developmental stages and pathophysiological microenvironmental alterations present under disease settings. Alternative splicing adds another level of complexity to the differential expression profile of Epac (Niimura et al., 2009). Epac1 is most abundant in the heart, kidney, blood vessels, adipose tissue, central nervous system, ovary, and uterus (de Rooij et al., 1998; Kawasaki et al., 1998), whereas Epac2 splice variants (Epac2A

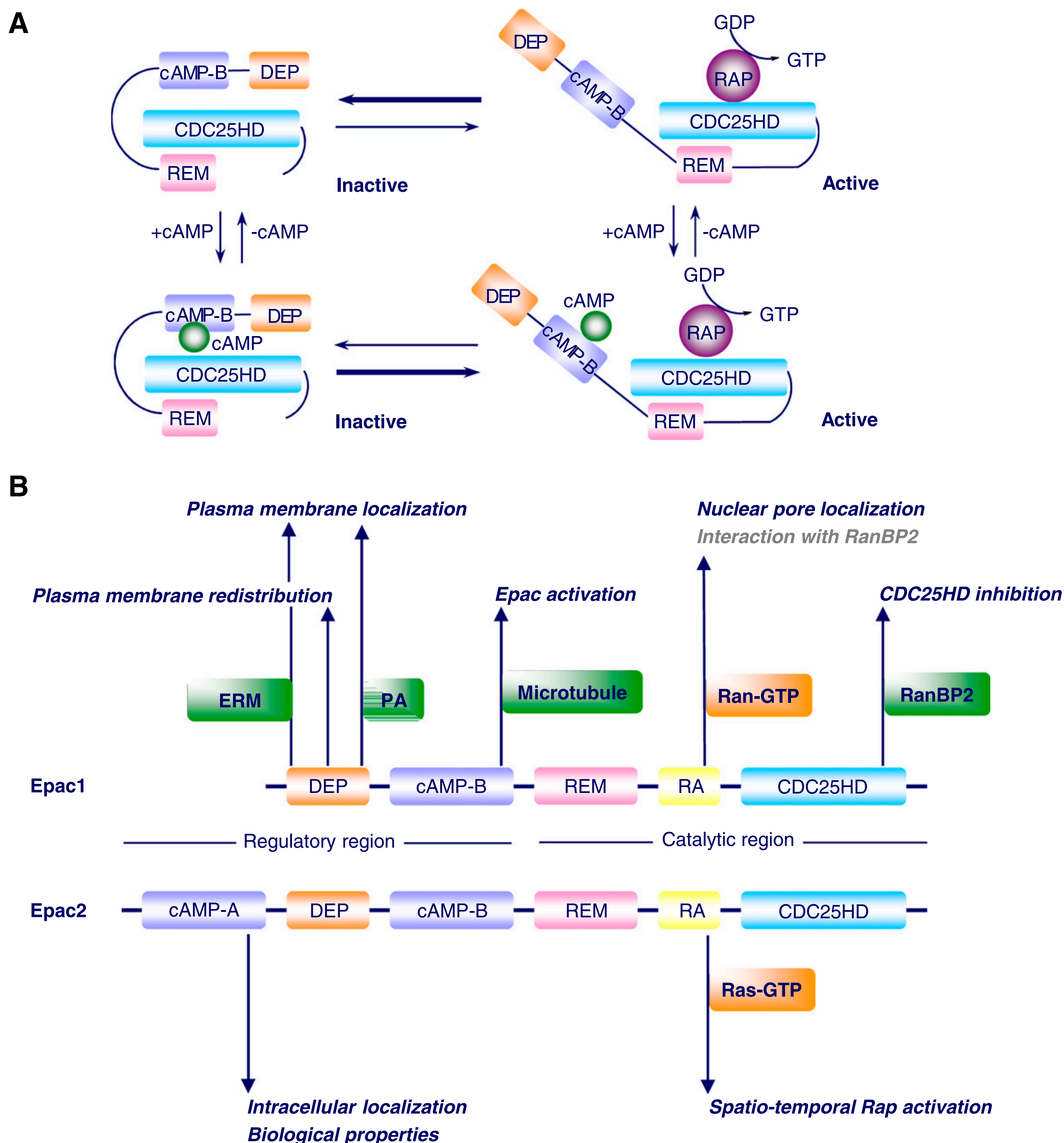


Fig. 2. Architecture of Epac: defining temporal and spatial dynamics. The hypothetical model predicts equlibria between active and inactive states of Epac, both in the cAMP-bound state and nonbound state. Depicted in the model is the cAMP-B domain of Epac1. The model predicts a shift of Epac's equilibrium from the nonbound, predominantly inactive, state to the cAMP-bound, predominantly active, state. (A) Thus, relative orientation between the N-terminal regulatory and C-terminal catalytic regions together with the binding of cAMP induces the equilibrium shift to the active (open) state. (B) Multidomain structure of Epac. Interaction partners that determine both intracellular localization and activity of Epac1 and Epac2 are indicated. cAMP-A, low-affinity cAMP-binding site; PA, phosphatidic acid; RANBP2, RAN-binding protein-2.

and Epac2B) are mostly expressed in the central nervous system (Epac2A), adrenal gland (Epac2B), and pancreas (Epac2A; (de Rooij et al., 1998; Kawasaki et al., 1998; Niimura et al., 2009). Additionally, expression of Epac1 has been found to be present in monocytes, macrophages, B and T lymphocytes, eosinophils, neutrophils, platelets, and in CD34-positive hematopoietic cells (Tiwari et al., 2004; Bryn et al., 2006; Gerlo et al., 2006), whereas Epac2 was undetectable in all studied hematopoietic cell types (Tiwari et al., 2004). Neurite outgrowth and axon regeneration require Epac2 upregulation and Epac1 downregulation in adults (Murray and Shewan, 2008; Murray et al., 2009a). In addition, relative to Epac1, Epac2 becomes dominant in the adult brain and lung compared with fetal organs (Ulucan et al., 2007), suggesting that Epac1 and Epac2 differentially contribute to fetal and adult organ functions. Interestingly, prostaglandin E_2 (PGE_2)-activated Epac1 promotes the intimal cushion formation in rat perinatal ductus arteriosus through stimulating hyaluronan synthesis (Yokoyama et al., 2008b).

TABLE 1
Alterations of the expression of Epac1 and Epac2 mRNA/protein in different (models of) diseases or developmental stages

Condition	Tissue	Epac1	Epac2	mRNA/protein	References
Cardiac hypertrophy					
Patients	Left ventricle	↑	↔	mRNA + protein	Métrich et al., 2008
Catecholamine induced	Mouse left ventricle	↑	↑	mRNA	Ulucan et al., 2007
Pressure overload induced	Mouse/rat left ventricle	↑	↔	mRNA	Ulucan et al., 2007; Métrich et al., 2008
FCS	H9C2 rat cardiomyoblasts	↑	n.d.	mRNA + protein	Yokoyama et al., 2008c
Myocardial infarction					
Ligation left coronary artery	Rat heart, cardiac fibroblasts	↓	n.d.	mRNA + protein	Yokoyama et al., 2008c
TGF-β	Rat cardiac fibroblasts	↓	↔	mRNA + protein	Yokoyama et al., 2008c
	Rat skin and lung fibroblasts	↓	n.d.	mRNA	Yokoyama et al., 2008c
	Mouse hepatic stellate cells	↓	n.d.	mRNA	Yokoyama et al., 2008c
Hypoxia	Human coronary artery endothelial cells	↓		mRNA + protein	www.pa2online.org/abstracts/vol10issue3abst205p.pdf
Alzheimer's disease					
Patients	Frontal cortex, hippocampus	↑	↓	mRNA	McPhee et al., 2005
Anxiety and depression					
Patients		Variants		DNA	Middeldorp et al., 2010
Autism					
Patients			Variants	DNA	Bacchelli et al., 2003
Diabetes					
STZ-induced	Mouse kidney (cortical tubules)	↑[a]	n.d.	mRNA + protein	Sun et al., 2011
D-Glucose	Human kidney HK-2 cells	↑	n.d.	mRNA + protein	Sun et al., 2011
Pulmonary arterial hypertension					
Patients	Pulmonary arterial smooth muscle cells	↓	↓	mRNA + protein	Murray et al., 2009b
MCT induced	Rat lung	↓		mRNA	Murray et al., 2009b
Vascular injury					
Mechanical injury	Mouse femoral artery	↑	↔	mRNA + protein	Yokoyama et al., 2008a
Inflammation					
CFA/peanut oil emulsion	Rat L4-5 ganglia	↑	n.d.	Protein	Wang et al., 2007
COPD					
Patients	Lung tissue	↓[b]	↔	mRNA + protein	Oldenburger et al., 2012
Cigarette smoke induced	Human airway smooth muscle cells	↓	↔	mRNA + protein	Oldenburger et al., 2012
Development					
Fetuses, neonates, 3 and 12 weeks old	Mouse heart	↑	↑	mRNA	Ulucan et al., 2007
	Mouse lung	↑[c]	↑/↓[d]	mRNA	Ulucan et al., 2007
	Mouse kidney	↑	↓	mRNA	Ulucan et al., 2007
	Mouse brain	↑	↑	mRNA	Ulucan et al., 2007

CFA, complete Freund's adjuvant; FCS, fetal calf serum; MCT, monocrotalin; n.d., not determined; STZ, streptozotocin, TGF-β, transforming growth factor-β.
[a] Increases in Epac1 correlate with blood glucose levels.
[b] Epac1 protein is decreased, whereas Epac1 mRNA is unaltered.
[c] Epac1 mRNA is increased in 3-week-old but not 12-week-old mice versus fetus and neonates.
[d] Epac1 mRNA is increased in 3-week-old mice versus neonates but slightly decreased in 12-week-old mice.

In a mouse model of vascular injury, Epac1 is upregulated during neointima formation (Table 1) and promotes vascular smooth muscle migration (Yokoyama et al., 2008a), suggesting that Epac1 regulates vascular remodeling and restenosis upon vascular injury. Although it is presently not known whether Epac is required for the development of cardiac hypertrophy or whether its increased expression is a result of hypertrophy, both Epac proteins are upregulated by chronic catecholamine infusion-induced hypertrophy, and only Epac1 is increased in pressure overload-induced hypertrophy (Ulucan et al., 2007). Transforming growth factor-β_1 (TGF-β_1) is a central mediator of fibrogenesis and is involved in the recruitment and activation of (myo)fibroblasts, which may derive from resident mesenchymal cells, circulating fibrocytes, and/or epithelial-to-mesenchymal transition, a process in which epithelial cells transdifferentiate into mesenchymal cells (Schmierer and Hill, 2007; Biernacka et al., 2011; Nieto, 2011; Kovacic et al., 2012; Small, 2012). Loss of Epac1 by TGF-β1 accelerates the deposition of extracellular matrix proteins including collagenIα1, collagenIα2, and collagenIIIα1, and reduces Epac1-dependent migration of fibroblasts from various tissues (including heart, lung, liver, and skin), whereas overexpression of Epac1 attenuates TGF-β1-induced collagen synthesis (Yokoyama et al., 2008c), suggesting that TGF-β1-controlled Epac1 expression modulates tissue homeostasis and fibrogenesis. Likewise, loss of Epac1 by TGF-β1 in U937 monocytic cells may protect against aberrant transendothelial migration of leukocytes (Basoni et al., 2005). The expression of Epac1 in airway smooth muscle cells is reduced by cigarette smoke extract, a cellular model to study chronic obstructive pulmonary disease (COPD), which may reduce the anti-inflammatory effects of cAMP. The expression of Epac1 is similarly reduced in lung tissue from COPD patients (Oldenburger et al., 2012; Table 1). Although the underlying molecular mechanisms are far from being resolved, fluctuations in Epac

expression seem to reflect and/or sense pathophysiological diseases/stages.

Epac, Epac1, and/or Epac2 signals alone or in concert with PKA, suggesting that cAMP-sensing multiprotein complexes are important to maintain proper control of biologic functions. Several recent studies report on the existence of cAMP-sensing AKAP bearing multiprotein complexes (see section I.C). Most biologic effects of Epac require its exchange activity toward members of the Ras family, thereby inducing activation of a plethora of diverse effectors (Figs. 4–8), including the phospholipases C-ε and D, extracellular signal-regulated kinases (ERK1/2), phosphoinositide 3-kinase-dependent protein-kinase B (PKB)/Akt, the suppressor of cytokine signaling-3 (SOCS-3), the CCAAT/enhancer-binding protein (C/EBP), nuclear factor (NF)-κB (Grandoch et al., 2010). Likewise, Epac signals to several small GTPases, including Rho (Krugmann et al., 2004; Birukova et al., 2010; Roscioni et al., 2011b; Zieba et al., 2011; Jeyaraj et al., 2012), Rac (Maillet et al., 2003; Robert et al., 2005; Roscioni et al., 2011b; Zieba et al., 2011), Ras (Morel et al., 2005; Li et al., 2006; López de Jesús et al., 2006; Métrich et al., 2008), Rit (Shi et al., 2006), and Ran (Gloerich et al., 2011; Liu et al., 2010a). Independent of Rap (Figs. 5 and 7), Epac activates the mitogen-activated family member JNK (Hochbaum et al., 2003), the small GTPase Rit (Shi et al., 2006), and microtubule network dynamics (Sehrawat et al., 2008). Such multiplicity in its signaling properties enables Epac to regulate cardiac and smooth muscle contraction through regulation of cellular calcium and actin-microtubule cytoskeleton dynamics (Figs. 4 and 8). Epac also regulates cell fate and cell proliferation and differentiation (Fig. 8), survival, and apoptosis through the regulation of gene transcription and the activity of several kinases and cellular metabolism through the regulation of insulin secretion and the production of reactive oxygen species (Fig. 6). Dysfunctions of the signaling properties of Epac seem to contribute to disease including cardiac hypertrophy, heart failure, Alzheimer's disease, schizophrenia, autism, atherosclerosis, diabetes, asthma, and COPD (see section II).

B. Architecture of Epac: Temporal Dynamics in Autoinhibition and Activation

Epac 1 and Epac 2 are multidomain proteins that consist of a C-terminal catalytic and a autoinhibitory N-terminal regulatory region (de Rooij et al., 2000; Bos et al., 2003; Rehmann et al., 2003a; Bos, 2006; Fig. 2). The exchange activity of Epac is located in the C-terminal region of the CDC25-homology domain (CDC25HD), a catalytic domain stabilized by the Ras-exchange motif (REM) domain that is responsible for the GDP-GTP exchange. The CDC25HD and REM domains are separated by a RA domain. The RA domain in Epac2 allows its interaction with GTP-bound Ras and thereby controls spatiotemporal activation of Rap by cAMP (Li et al., 2006, 2008). A high-affinity cAMP-binding domain (cAMP-B) and a membrane-anchoring DEP (disheveled-Egl-10-pleckstrin) domain are located in the N-terminal regulatory region (Fig. 2). The DEP domain in Epac1 is required for its dynamic redistribution to the plasma membrane (Ponsioen et al., 2009), and it has been hypothesized that cAMP, as shown for Epac2 (Li et al., 2011), might induce additional conformational changes of the DEP domain to further promote plasma membrane localization of Epac1 (Consonni et al., 2012). Epac2 harbors an additional low-affinity cAMP-binding domain (cAMP-A), which determines the intracellular localization and biologic properties of Epac2A and Epac2B (Niimura et al., 2009; Fig. 2B).

The Epac architecture defines its mode of activation. Isolated fragments containing cAMP-B domains of either Epac1 and Epac2, but not the cAMP-A domain from Epac2, exhibit an autoinhibitory function on the regulatory region that is relieved by binding of cAMP, supporting the idea that binding of cAMP induces a conformational change that opens the catalytic CDC25HD domain from autoinhibitory restraints and thereby permits GTP-loading of Rap (de Rooij et al., 2000; Bos et al., 2003; Rehmann et al., 2003a; Bos, 2006; Fig. 2A). X-ray crystallography of Epac2 yielded novel insights into the dynamic equilibrium of critical conformational switches from a closed, auto-inhibited state, in which the N-terminal regulatory region sterically blocks the C-terminal catalytic region, to a completely different, open, and catalytically active state (Rehmann et al., 2006, 2007, 2008). The highly informative snapshots of the CDC25HD domain in the open and closed conformations reveal no significant differences (Rehmann et al., 2006), substantiating the concept that cAMP regulates the activity of Epac by lifting an autoinhibition, rather than by inducing an allosteric change, in the Rap-binding site (Fig. 2A). A coupled equilibrium model supposes equilibria between active and inactive states of Epac, both in the cAMP-bound state and in the unbound state, and rationalizes activation of Epac by shifting the equilibrium from the unbound, predominantly inactive state to the cAMP bound, predominantly active state of Epac (Fig. 2A). The conformational changes upon Epac activation depend on the relative orientation between the N-terminal regulatory and C-terminal catalytic regions. This is, among others, determined by an ionic latch, which consists of ionic interaction between the CDC25HD and the N-terminal helical bundle of the cyclic nucleotide binding domain, the lid of the Epacs' regulatory cyclic nucleotide binding domain forms part of a β-sheet (the switchboard) that is anchored to the REM of the catalytic region (Fig. 2A). Another important contributor is the so-called "hinge region,"

which is located between the cyclic nucleotide binding domain and the REM domain (Rehmann et al., 2007; Tsalkova et al., 2009; Li et al., 2011). Upon activation, the ionic latch is broken, and a conformation change in the hinge region stabilizes the open conformation, which is stabilized by the bound cyclic nucleotide (Rehmann et al., 2008). As well as inducing closure in the cyclic nucleotide binding domain, there is evidence that cAMP also affects the ionic latch as major contact point with the regulatory region. Rather than causing a significant change in average structure, it appears that cAMP loosens the structure around key ionic latch residues, making the closed inactive conformation less favored for entropic reasons (Das et al., 2008). Thus, apart from conformational changes, changes in internal protein dynamics also play an important role in Epac activation.

It has been described that Epac activators mainly act by modulation of the proteins dynamics, which proved to be the key allosteric modulator for Epac activation (Das et al., 2008, 2009; Gavina et al., 2009). Molecular dynamic simulations by NMR corroborate the experimental trends in cAMP binding domain dynamics and uncover unanticipated dynamic attributes, rationalizing previously unexplained aspects of Epac activation and autoinhibition (Selvaratnam et al., 2011; Van-Schouwen et al., 2011; Fig. 2A). Hampered by application of lower resolution techniques (Yu et al., 2006; Brock et al., 2007), the dynamics of the critical conformational switch in Epac1 are now resolved by NMR spectroscopy (Mazhab-Jafari et al., 2007; Das et al., 2008; Harper et al., 2008; Selvaratnam et al., 2012). The cAMP-B domain of Epac1 binds cAMP and translates the initial binding event to changes in the outer parts of the domain (Harper et al., 2008). Autoinhibition of Epac1 is still not fully understood. In addition to the so-called hydrophobic hinge hypothesis in which a steric clash between the side chain of two conserved residues opposes the inactive-to-active conformational transition in the absence of cAMP, Epac1 autoinhibition might involve entropic losses caused by quenching of dynamics that occurs if the inactive-to-active transition takes place in the absence of cAMP (Fig. 2A). Recently, it was reported that complete activation necessitates the simultaneous suppression of multiple autoinhibitory mechanisms (Selvaratnam et al., 2012).

X-ray crystallography and NMR spectroscopy allow state-of-the art insights into the highly dynamic equilibrium of autoinhibition and activation of Epac in vitro. Epac-based fluorescence resonance energy transfer cAMP sensors or GFP/Flag-tagged Epac proteins are applied to monitor temporal dynamics of Epac activation in vivo (Willoughby and Cooper, 2008; Börner et al., 2011). Studies with Epac-based probes to illustrate cellular cAMP dynamics show that concentrations of cAMP rise to a level sufficient to activate both PKA and Epac (Dao et al., 2006). In addition, Epac-based cAMP probes illustrate the rapid onset of Epac activation and its cellular (re)distribution, which seem to be highly cell type specific (DiPilato et al., 2004; Nikolaev et al., 2004; Ponsioen et al., 2004, 2009; Leroy et al., 2008; Liu et al., 2008). Epac-based fluorescence resonance energy transfer biosensors are essential tools to detect cellular cAMP dynamics in various restricted subcellular microdomains that have crucial roles in regulation of cellular functions by localized changes in cAMP concentrations. The imaging of cellular cAMP by Epac-based probes open unexpected insights into cellular responses of cardiomyocytes and neurons in health and disease (Nikolaev et al., 2006, 2010; Shafer et al., 2008; Calebiro et al., 2009; Mironov et al., 2009; Jacobs et al., 2010). A novel mTurquoise-based cAMP sensor for time lapses in living cells by fluorescence lifetime imaging and recording of sensitized emission further improves initial applications (Klarenbeek et al., 2011).

In conclusion, X-ray crystallography and NMR spectroscopy provide the first molecular clues of Epacs' dynamic equilibrium between closed (autoinhibits or inactive) and open (active) states. Together with novel fluorescence imaging techniques a more complete understanding about Epacs' dynamic under cellular conditions in vivo can be obtained.

C. Spatial Regulation of Epac

Epac1 and Epac2 regulate calcium homeostasis in myocyte contraction, insulin secretion from pancreatic β-cells, barrier integrity of tight and adherens junctions in vascular endothelial cells, extracellular matrix deposition from fibroblasts, cell fate in various myocytes and immune cells, pre- and postsynaptic neuronal excitability, migration and integrin-dependent adhesion in various cell types, and gene transcription of a variety of chemo/cytokines as well as hypertrophic markers (see section II). Epac1 and Epac2 (differentially) regulate the wide range of biologic processes. Epac1 induces cardiac excitation-contraction coupling, hypertrophy, and fibrosis and reduces permeability of the vascular endothelium (see section II). Epac2 regulates the release of insulin from pancreatic β-cells and learning and memory processes. Few studies indicate that Epac1 and Epac2 cooperate in the regulation of biologic responses (Roscioni et al., 2009; Oldenburger et al., 2012; Yang et al., 2012). Although complete phenotypes of Epac1 and Epac2 knockout mice have not been reported (Shibasaki et al., 2007; Suzuki et al., 2010), limited functional studies in pancreatic β-cells and primary cortical neurons from these mice suggest that knockout mice of either Epac1 or Epac2 produced viable littermates, probably due to their functional interchangeability. Indeed, both Epac1 and Epac2 are localized to a number of subcellular regions, including the plasma membrane, cytosol, nuclear membrane, and mitochondria (Grandoch et al.,

2010). This cell type-specific colocalization at the plasma membrane and cytoplasm indicates that Epac1 and Epac2 presumably act in concert to modulate cellular responses.

Epac1 and Epac2 are both multidomain proteins consisting of an autoinhibitory N-terminal regulatory region that contains the membrane-anchoring DEP domain and a high-affinity cAMP-B domain, as well as C-terminal catalytic region bearing a CDC25 domain stabilized by the REM domain and a RA domain (de Rooij et al., 2000; Bos et al., 2003; Bos, 2006; Fig. 2B). The low-affinity cAMP-A domain targets Epac2A to the plasma membrane of pancreatic cells and is lacking in the adrenal gland-specific Epac2 splice variant Epac2B, suggesting that the cAMP-A domain accounts for distinct biologic properties of Epac2 (Niimura et al., 2009; Fig. 2B). In addition to inducing a conformational change that relieves the CDC25 domain from autoinhibitory restraints of the N-terminal region (Rehmann et al., 2006, 2007, 2008), cAMP also induces the translocation of Epac1 to the plasma membrane, a process dependent on its DEP domain (Ponsioen et al., 2009). It has been proposed that a cAMP-dependent conformational change in the DEP domain of Epac1 facilitates its interaction with phosphatidic acid at the plasma membrane (Consonni et al., 2012). Indeed, phosphatidic acid binds to Ras and PDE family members (Grange et al., 2000; Baillie et al., 2002; Huston et al., 2006; Zhang and Du, 2009) and thereby contributes to compartmentalized cellular signaling (van Meer et al., 2008). Thyroid-stimulating hormone-mediated and cAMP-dependent mitogenesis requires membrane targeting of Epac1 via its DEP domain, supporting the concept that proper cellular localization of Epac1 profoundly determines its biologic properties (Hochbaum et al., 2008). Interaction of Epac2 with Ras-GTP via its RA domain does not require allosteric activation of Epac2 by Ras but the compartmentalization of Epac2 on Ras-containing membranes and both cAMP and Ras thus control the spatiotemporal activation of Rap (Li et al., 2006, 2008). In addition, Epac1 interacts via its RA domain with Ran-GTP, a small GTPase known to regulate nuclear transport through its interaction with RanBP2 at the nuclear pore (Liu et al., 2010a). Differential subcellular RA domain-dependent targeting of Epac1 to the nuclear envelope and Epac2 to the plasma membrane is expected to activate different cellular pools of Rap1, as has been extensively studied for ERK activation (Wang et al., 2006; Fig. 2B).

Direct RA domain-dependent interaction of Epac1 with Ran-GTP may also control its nuclear localization and its ability to modulate cell fate and gene transcription. Indeed, recent findings indicate that Epac promotes nuclear export of the DNA damage-responsive DNA-protein kinase, a process being opposed by PKA-regulated DNA-protein kinase nuclear import and controlled by diverse PDE4 subtypes (Huston et al., 2008). Thus, nuclear/cytoplasmic trafficking of DNA damage-responsive DNA-protein kinase by Epac and PKA requires distinct cAMP gradients being shaped and generated through a specific PDE subset. In addition, Epac controls nuclear export of histone deacetylase (HDAC)-4 and HDAC5, the latter requiring the presence of HDAC4 (Huston et al., 2008; Métrich et al., 2010). Another interaction partner of Epac1 is RanBP2 (Liu et al., 2010a) via an direct interaction with the zinc fingers of RanBP2 and thereby anchoring Epac1 to the nuclear pore (Gloerich et al., 2011). Direct binding of RanBP2 to the CDC25 domain of Epac1 inhibits its exchange activity toward Rap1 and establishes an inactive pool of Epac1 at the nuclear pore (Gloerich et al., 2011). Post-translational modifications, including phosphorylation, release RanBP2 from Epac1, suggesting that other proteins— in addition to the off-rate regulation by GAPs—may control the Rap-exchange activity of Epac1 (Ohba et al., 2003). Although it has been proposed that Epac1 interacts via its RA domain with the nuclear pore-associated small GTPase Ran (Liu et al., 2010a), the recent studies of Gloerich et al. (2011) indicate that RanBP2 acts as the anchor for Epac1 at the nuclear pore independent of Ran. Shuttling of Epac1 between the plasma membrane and the nuclear envelope may involve the nucleoskeleton, an intricate network of actin polymers, intermediate filaments, and microtubules that links the cell surface to the nuclear envelope (Simon and Wilson, 2011; Jaspersen and Ghosh, 2012). Direct interaction of Epac1 with microtubule-associated protein MAP1 and microtubulin enhances its exchange activity toward Rap1 (Magiera et al., 2004; Gupta and Yarwood, 2005; Mei and Cheng, 2005; Borland et al., 2006; Fig. 2B). The nucleoskeleton component RanBP2 inhibits the Rap1-exchange activity of Epac1, whereas microtubulin enhances its activity, suggesting that exchange activity of Epac1 is tightly controlled by the nucleoskeleton. Different anchors may target Epac1 to distinct subcellular compartments and thereby regulate various biologic functions of Epac1.

Epac-dependent regulation of the cell cycle, migration, and endothelial cell barrier function requires a tightly controlled network between the actin and microtubule cytoskeleton acting in concert with the adhesion molecules of the tight junctions and adherens junctions (Gourlay and Ayscough, 2005; Pannekoek et al., 2009; Bernstein and Bamburg, 2010; Olson and Nordheim, 2010; Simon and Wilson, 2011). In addition, members of the ezrin-radixin-moesin (ERM) family represent a molecular link between transmembrane proteins and the cytoskeleton, which upon strengthening of the cell cortex regulate cellular signaling involved in cellular processes, including endocytosis, exocytosis, motility, and adhesion (Bretscher et al., 2002; Niggli and Rossy, 2008; Fehon et al., 2010;

Neisch and Fehon, 2011). In addition to the cAMP-dependent plasma membrane translocation of Epac1 that involves its DEP domain (Ponsioen et al., 2009), direct binding of Epac1 with its N-terminal 49 amino acids to ERM proteins in their open conformation (Bretscher et al., 2002) also induces plasma membrane recruitment of Epac1 (Gloerich et al., 2010). Binding to phosphatidylinositol-4,5-bisphosphate (PIP_2) and threonine phosphorylation of the C-terminal actin binding domain is required for the interaction between the N-terminal and ERM proteins (Bretscher et al., 2002; Fig. 2B). Membrane translocation of Epac1 by ERM proteins cooperates with its DEP domain-mediated translocation (Gloerich et al., 2010) and is required for Rap-induced cell adhesion and spreading (Ross et al., 2011). The DEP domain targets Epac1 uniformly to the plasma membrane, whereas ERM targets Epac1 to plasma membrane clusters (Gloerich et al., 2010), implicating different biologic outcomes of Epac1 targeting by either the DEP domain or ERM. Direct interaction of Epac1 with either ezrin, radixin, or moesin may play a role in cell adhesion per se, because deletion of the N-terminal 49 amino acid of Epac1 and Epac1 displacement from ERM with the actin binding domain of radixin reduce Epac1-dependent cell adhesion (Gloerich et al., 2010; Ross et al., 2011). Additional recent studies indicate that thyroid-stimulating hormone-mediated and cAMP-dependent mitogenesis requires assembly of both Epac and PKA to a radixin scaffold, a process dependent on the DEP domain of Epac1 (Hochbaum et al., 2008, 2011).

Recent studies unravel the molecular entity of the cAMP-induced and DEP domain-dependent translocation of Epac1 to the plasma membrane (Ponsioen et al., 2009). Epac1 binds directly to phosphatidic acid (Fig. 2B), a process being regulated by cAMP and requiring the DEP domain of Epac1 (Consonni et al., 2012). Cellular depletion of phosphatidic acid by pharmacological inhibition of PLD1 prevents cAMP-induced Epac1 translocation and subsequent Rap activation at the plasma membrane. Although binding of phosphatidic acid to Epac1 still requires further characterization, evidence is provided that phosphatidic acid is the cAMP-regulated DEP domain anchor for Epac1 at the plasma membrane (Consonni et al., 2012). As phosphatidic acid seems to contribute to compartmentalized cellular signaling (van Meer et al., 2008) through binding to Ras and PDE family members (Grange et al., 2000; Baillie et al., 2002; Huston et al., 2006; Zhang and Du, 2009), Epac1 should be added to the growing list of phosphatidic acid-interaction partners. Since phosphatidic acid also activates distinct PDE4 subtypes (Grange et al., 2000; Baillie et al., 2002; Huston et al., 2006), such process most likely limits Epac1 activation by phosphatidic acid. PLD1 also seems to represent the missing molecular link between the small GTPase Rap2A and Ezrin in the process of intestinal cell polarity to brush border formation (Gloerich et al., 2012). Recruitment of PLD1 to PIP_2-enriched apical membrane compartments elevates polarized phosphatidic acid accumulation and subsequent activation of Rap2A and ezrin (Gloerich et al., 2012). Phosphatidic acid-producing PLD1 activates PIP_2-producing phosphatidyl-4-phosphate 5-kinases, a process involving Rho-Rho-kinase (Oude Weernink et al., 2000, 2007; Weernink et al., 2004). In addition, PLD1 is a downstream effector of Epac and activated by actin cytoskeleton components including phosphocofilin (López de Jesús et al., 2006; Han et al., 2007). Thus, molecular networks may contribute to the release of ERM by PIP_2 to further improve membrane targeting of Epac1, a process that should be further supported by PLD1 activation by Epac1.

Different anchors may target Epac1 to distinct subcellular compartments and thereby regulate alternative functions of Epac1 that are not linked to (processes at) the plasma membrane. It is interesting to note that Epac proteins are able to exert their diverse biologic functions either alone or in concert with PKA (Grandoch et al., 2010). Cellular processes that involve the activation of both Epac and PKA suggest interconnectivity between these two major cAMP effectors. Formation of cAMP-sensitive multiprotein complexes maintained by distinct AKAP family members might turn out to be of key importance in such processes (see section II for further details). The first cAMP-responsive multiprotein complexes that encompass Epac1 and Epac2 have been identified in the heart and the brain (see sections II.A and II.B; Figs. 4 and 5). It is interesting to note that the Epac-bearing cAMP-multiprotein complexes identified so far possess a rather distinct composition. The cardiac-specific cAMP-responsive complex is maintained by the nuclear envelope-associated muscle AKAP (mAKAP), PKA, PDE4D3, and Epac1 (see section II.B; Fig. 4) and controls cardiomyocyte hypertrophy through regulation of ERK5 activity (Dodge-Kafka et al., 2005). The neuronal cAMP-sensing complex is maintained by the plasma membrane-associated AKAP79/150 (also known as AKAP5), PKA, Epac2, and PKB/Akt (see section II. B; Fig. 5), and regulates the activity of the survival PKB/Akt pathway (Nijholt et al., 2008). Interestingly, impairments in the AKAP-dependent spatiotemporal compartmentalization of cAMP correlated with heart and brain disease pathogenesis (Nikolaev et al., 2010; Ostroveanu et al., 2010; Aye et al., 2012). In human umbilical vein endothelial cells (HUVECs), Epac1 complexed to AKAP450 (also known as AKAP9) promotes microtubule growth, integrin adhesion at cell-cell borders, and enhancement of the endothelial barrier function (see section II.D; Fig. 7), a process requiring Epac1-dependent Rap1 activation and subsequent elevations in cortical actin and VE-cadherin (Sehrawat et al., 2011). In addition, Maurice and

colleagues reported that PDE4D tethers Epac1 to a VE-cadherin-based signaling complex and thereby regulates vascular permeability (Rampersad et al., 2010). In addition, several recent studies ascribe Epac (particularly Epac1) a pivotal role in the regulation of cell-cell adhesion and integrin-extracellular matrix interactions (Netherton et al., 2007; Raymond et al., 2007; Rampersad et al., 2010).

As indicated above, direct interaction of Epac1 with all three members of the ERM family is now evident (Gloerich et al., 2010; Ross et al., 2011). Ezrin belongs to the dual-specific AKAP binding proteins known to interact with PKA (Dransfield et al., 1997; Skroblin et al., 2010; Tröger et al., 2012), suggesting that ERM proteins might create multiprotein complexes consisting of Epac and PKA. The assembly of a complex composed of radixin, Epac, and PKA has indeed been described (Hochbaum et al., 2011). Tethering of PDE4D5 to the AKAP family member AKAP79 (also known as AKAP5) may contribute to cAMP compartmentalization, a process being modulated by β-arrestin (Lynch et al., 2005). The formation of a β-arrestin/calcium-calmodulin kinase II-Epac1 complex has been recently reported (Mangmool et al., 2010). Elevation of cellular cAMP by the β_1-adrenergic receptor, but not by the β_2-adrenergic receptor, induces the formation of the β-arrestin-calmodulin kinase II-Epac1 complex, allowing its recruitment to the plasma membrane, whereby interaction with cAMP leads to calmodulin kinase II activation. Binding of β-arrestin to the C-terminal tail of the β_1-adrenergic receptor induces a β-arrestin conformation that stabilizes the β-arrestin-calmodulin kinase II-Epac1 interaction (see section II.A), thereby permitting the activation of Epac by cAMP and subsequent calmodulin kinase II activation most likely via a Rap-PLC-ε-protein kinase C-ε mechanism (Mangmool et al., 2010). The recent studies further support the concept that compartmentalized Epac signaling either maintained by different anchor proteins or members of the AKAP family may turn out to be of utmost importance to determine its regulation biologic functions.

D. Epac-Selective Agonists and Antagonists

In the recent years, our knowledge on the impact of Epac in the cAMP signaling pathway has increased due to the development of pharmacological tools to specifically target Epac. In this section we will discuss the available tools to study components of the cAMP signaling pathway, such as ACs, PDEs, AKAPs, PKA, and particularly Epac. As indicated, stimulation of various G_s protein-coupled receptors initiates the formation of cAMP through coupling to membrane-bound ACs (Hanoune and Defer, 2001; Beavo and Brunton, 2002). In addition, cAMP is generated by soluble AC, which represents a complete different cellular entity being predominantly regulated by intracellular levels of bicarbonate and calcium (Tresguerres et al., 2011; Chen et al., 2012b). Next to G protein-coupled receptors and ACs, cAMP-specific PDEs degrade cAMP and thereby shape the cellular cAMP gradient to its spatiotemporal nature (Conti and Beavo, 2007; Houslay, 2010, Keravis and Lugnier, 2010). Pharmacological tools are available to modulate AC and PDE activities, enabling receptor-independent interference of the cellular cAMP gradient. The cell membrane-permeable diterpene forskolin from the Indian plant *Coleus forskolhlii* directly activates membrane-bound ACs and thereby elevates cellular cAMP, whereas P-site AC inhibitors reduce the cellular cAMP content (Hanoune and Defer, 2001; Beavo and Brunton, 2002). The activity of soluble AC is unaffected by these pharmacological tools (Kamenetsky et al., 2006; Tresguerres et al., 2011; Chen et al., 2012b). The superfamily of PDEs can be subdivided into 11 subfamilies characterized by distinct substrate specificity (cAMP and/or cGMP) and regulatory mechanisms (Conti and Beavo, 2007). Most commonly used PDE inhibitors are the isoform nonselective inhibitors, including theophylline and IBMX; however, cilastazol (PDE3), rolipram (PDE4), and roflumilast (PDE4) provide subtype specificity (Conti and Beavo, 2007). Compartmentalized cAMP signaling maintained by AKAP family members can be studied using the AKAP-PKA-binding blocking peptides, including st-Ht31 and RIAD, which disrupt anchoring of PKA either to its RI and/or RII domain (Skroblin et al., 2010; Tröger et al., 2012).

Because both Epac and PKA represent important biologic targets for cAMP, compounds have been developed that, in contrast to cAMP, selectively activate one or both proteins. Direct activation of PKA can be achieved using the highly cell membrane-permeable 8-bromo-cAMP and the prodrug N^6,2′-*O*-dibutyryl cAMP. The prodrug itself is known to be inactive as PKA activator and needs hydrolysis to the active product monobutyryl cAMP. However, the results should be interpreted cautiously, because the released butyrate from N^6,2′-*O*-dibutyryl cAMP exhibits several unwanted side effects (Holz et al., 2006, 2008; Poppe et al., 2008). Moreover, 8-bromo-cAMP is known to activate not only PKA but also Epac (Enserink et al., 2002; Christensen et al., 2003; Rehmann et al., 2003c; Holz et al., 2008). Modifications at the N^6-position of cAMP diminish the ability to activate Epac but not PKA (Christensen et al., 2003; Rehmann et al., 2003b,c), supporting the notion that N^6-derivatives of cAMP specifically activate PKA (Christensen et al., 2003; Holz et al., 2008; Poppe et al., 2008). Therefore, novel N^6-derivatives of cAMP, including 6-Bnz-cAMP, are used to specifically activate PKA, whereas selective inhibition of PKA can be achieved using compounds such as Rp-8-CPT-cAMPS, Rp-cAMPS, and Rp-8-bromo-cAMPS (Christensen et al., 2003; Rehmann et al., 2003c; Poppe et al., 2008). Such PKA inhibitors are used to show that

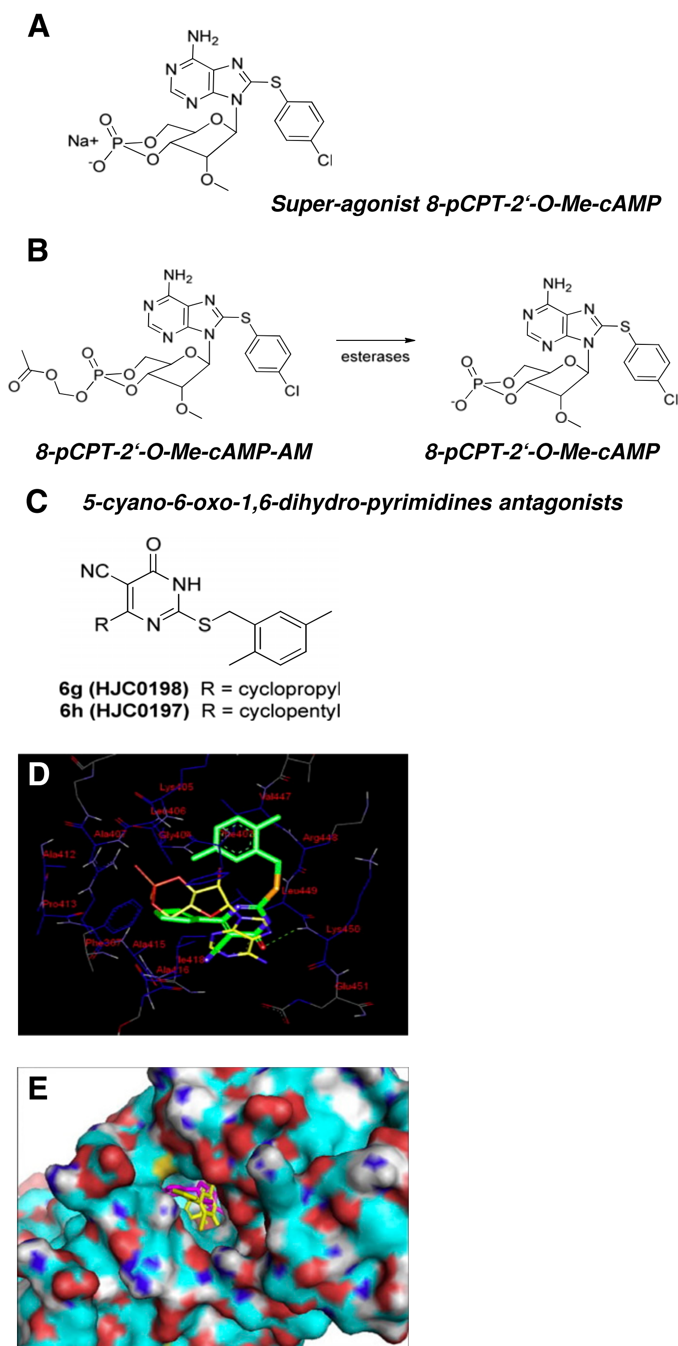


Fig. 3. Epac-selective agonists and antagonists. (A) Structure of 8-pCPT-2′-*O*-Me-cAMP, superagonist for Epac1 and Epac2. (B) Structure of the prodrug acetoxymethyl 8-pCPT-2′-*O*-Me-cAMP (8-pCPT-2′-*O*-Me-cAMP-AM), which is cleaved into the active compound 8-pCPT-2′-*O*-Me-cAMP by the action of esterases. (C) Structure of 5-cyano-6-xo-1,6-dihydro-pyrimidine derivatives, antagonists for Epac. 6g (HJC0196), R=cyclopropyl, antagonist for Epac2; 6h (HJC0197), R= cyclopentyl, antagonist for Epac1. (D and E) Predicted binding and molecular docking of HCJ0197 into see cAMP-binding domain of Epac2. [Panels D and E are reproduced from Chen H, Tsalkova T, Mei FC, Hu Y, Cheng X, and Zhou J (2012) 5-Cyano-6-oxo-1,6-dihydro-pyrimidines as potent antagonists targeting exchange proteins directly activated by cAMP. *Bioorg Med Chem Lett* **22:** 4038–4043. Copyright © 2012 Elsevier. Used with permission.]

Epac-specific analogs indeed act via Epac and independently of PKA.

During the development of Epac-specific compounds, using diverse approaches including fluorescence-based assays and isothermal titration calorimetry (Rehmann et al., 2003b,c; Poppe et al., 2008), it was noted that a 2′-*O*-methyl substitution on the ribose ring of cAMP confers selectivity toward Epac, a process favored by modifications at the 8-position of the base (Fig. 3A). The 2′-*O*-methyl group shifts the conformational equilibrium of cAMP-bound Epac more efficiently to the active state, combined with the increased affinity of the 8-(4-chlorophenylthio) (8-pCPT) group; both modifications result in selectivity for Epac (Enserink et al., 2002; Rehmann et al., 2003b,c). The cAMP analog 8-pCPT-2′-*O*-Me-cAMP, also known as 007, activates Epac with a K_d of 2.9 μM compared with 45 μM for cAMP as endogenous ligand. In addition to the more than tenfold higher efficiency to activate Epac, the more than threefold increased maximal activity of Epac1 defines 8-pCPT-2′-*O*-Me-cAMP as a superagonist for Epac1 (Rehmann et al., 2003b,c). In addition, 8-pCPT-2′-*O*-Me-cAMP activates both Epac1 and Epac2 (Enserink et al., 2002; Rehmann et al., 2003b,c, 2008). Definitely, the development of cAMP compounds selective for Epac has revolutionized research into the signaling properties of cAMP (de Rooij et al., 1998; Enserink et al., 2002; Rehmann et al., 2003a). Subtle modifications of cAMP nucleotides change not only their binding affinity toward Epac1 but also their ability to increase the maximal activity of Epac1, indicating that their individual binding affinity is not the only determinant for the activation of Epac. Such findings can be explained by the induction of different conformational changes of Epac by different cAMP analogs, which causes differential allosteric regulation of its activity. Recent studies with X-ray crystallography and NMR spectroscopy point to the existence of critical conformational switches encompassing a closed, autoinhibited state in which the N-terminal regulatory region sterically blocks the C-terminal catalytic region to a completely different, open, and catalytically active state (Rehmann et al., 2006, 2007, 2008), further supporting the concept of a highly dynamic equilibrium of autoinhibition and activation of Epac in vitro.

The prodrug of 8-pCPT-2′-*O*-Me-cAMP, acetoxymethyl 8-pCPT-2′-*O*-Me-cAMP-AM, activates Epac1 approximately 100-fold as efficient as the parent drug with kinetics comparable with forskolin-induced Epac activation, thus mimicking the signaling onset of natural compounds without any loss of biologic selectivity for Epac-specific signaling under the given conditions (Vliem et al., 2008). Apparently, the prodrug of 8-pCPT-2′-*O*-Me-cAMP is trapped within the cell after the cleavage reaction (Fig. 3B), which results in the parent compound that is poorly cell permeable (Vliem et al., 2008). Measurements with Epac-based fluorescence resonance energy transfer biosensors support this concept of distinct permeability of the Epac-specific cAMP analogs in intact cells (Börner et al., 2011). Thus, the prodrug of 8-pCPT-2′-*O*-Me-cAMP is a very effective tool to

investigate Epac signaling properties and provides a concept for targeted drug delivery to specific cells.

The superagonist 8-pCPT-2′-*O*-Me-cAMP is the most commonly used Epac selective activator; however, it is a good substrate for PDE5 and PDE10 and can act as inhibitor of PDE1, PDE2, and PDE6 (Poppe et al., 2008). The later developed nonhydrolyzable thiophosphate Epac activator Sp-8-pCPT-2′-*O*-Me-cAMPS is not a PDE substrate, which addresses concerns about analog stability (Laxman et al., 2006; Poppe et al., 2008); however, this analog does inhibit all PDEs being tested (Poppe et al., 2008). This indicates that alternative mimics of the phosphodiester are needed.

Studies in human platelets indicate that various cyclic nucleotide analogs used to selectively activate Epac might, in addition to their primary effects, also cause elevation of cAMP or cGMP upon PDE inhibition. This could be an explanation for the observation that in certain cell systems inhibition of PKA also slightly reduces the effects of the Epac activator and does not necessarily mean that PKA and Epac act in concert. Given the impact of cyclic nucleotides to profoundly regulate platelet homeostasis and thrombosis (Ruggeri, 2002, 2009), these findings may have far reaching consequences. Studies in *Trypanosoma brucei* indicated that 8-pCPT-2′-*O*-Me-cAMP might act via its 5′-AMP derivative upon inhibition of PDEs (Laxman et al., 2006). In addition, cAMP analogs and their metabolites induce cortisol synthesis, steroid hydroxylases, as well as $Ca_v3.2$ Ca^{2+} channels and inhibit bTREK-1 K^+ channels (Enyeart and Enyeart, 2009; Liu et al., 2009, 2010b; Enyeart et al., 2010, 2011); such compounds also inhibit the equilibrative nucleoside transporter 1 and activate A_{2A} adenosine receptors (Waidmann et al., 2009). 8-pCPT-2′-*O*-Me-cAMP also exerts antagonistic properties for the thromboxane A2 receptor (Sand et al., 2010). Thus, it is essential to analyze novel findings with these analogs being careful to exclude potential limitations by their off-target effects. The most commonly used cAMP compounds with selectivity for Epac induce orthosteric activation through binding to the cAMP-B domain. As PKA bears a cAMP-binding domain with significant homology to the cAMP-B domain of Epac (de Rooij et al., 1998, 2000), the current searches for small molecules able to selectively interact and modulate cellular Epac activity for therapeutic intervention is probably hampered due to its focus on the orthosteric mode of action.

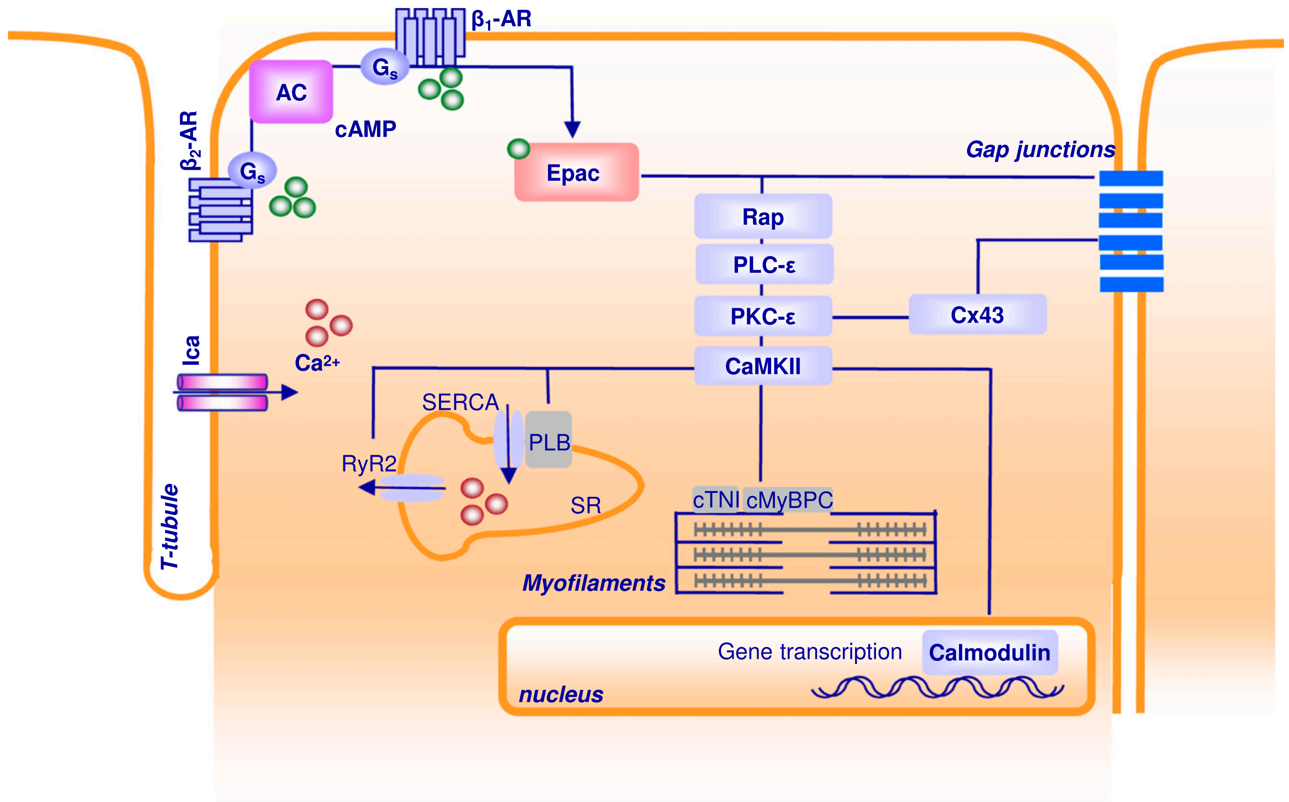
In addition to the orthosteric activation of Epac by cAMP, allosteric activation of Epac through sulfonylureas, widely used as antidiabetic drugs (Verspohl, 2012), has been reported (Zhang et al., 2009). By using full-length Epac2 as a fluorescence resonance energy transfer sensor (termed C-Epac2-Y), it is shown that sulfonylureas activate Epac2 and subsequently promote incretin-stimulated insulin, leading to blood glucose lowering through activation of Rap1. Sulfonylurea-stimulated insulin secretion was reduced both in vitro and in vivo in mice deficient for Epac2 as well as the glucose-lowering effect of the sulfonylurea tolbutamide, which suggests that these drugs exert their the antidiabetic properties via selective activation of Epac2 (Zhang et al., 2009; Fig. 5). Binding of [^{3}H]glibenclamide to Epac2 is inhibited by unlabeled tolbutamide or glibenclamide, another sulfonylurea derivative, with IC_{50} values of 25 nM and 240 μM, respectively, indicating that sulfonylureas bind to Epac2 at a site distinct from the cAMP binding site and with lower affinities for binding Epac2 than for the classic sulfonylurea receptor-1 (SUR1), a subunit of ATP-sensitive K^+-channels (Zhang et al., 2009). Studies with a distinct subset of sulfonylureas indicate that the structure and/or size of the side chain on the urea group modulate their ability to interact with Epac2 and that the core structure of sulfonylureas is essential for their binding to Epac2 (Zhang et al., 2009). Importantly, arginine 447 of Epac2 determines the isoform-selective activation by sulfonylureas (Herbst et al., 2011), strongly suggesting that a direct interaction underlies the molecular link between sulfonylureas and Epac2. Indeed, several early and recent studies demonstrate that Epac2 participates in glucose-, glucagon-, and incretin-enhanced insulin secretion from pancreatic β-cells through multiple mechanisms including inhibition of ATP-sensitive K^+-channels, activation of the ryanodine-sensitive Ca^{2+}-channel Ryr2 and subsequently Ca^{2+}-induced Ca^{2+}-release from the endoplasmic reticulum, recruitment of insulin granules to the plasma membrane, and Rap1-regulated activation of PLC-ε (Ozaki et al., 2000; Kang et al., 2001, 2003; Holz et al., 2006; Shibasaki et al., 2007; Kelley et al., 2009; Dzhura et al., 2011; Leech et al., 2010). Despite this evidence, experts in the field judge the relevance of this phenomenon controversial (Tsalkova et al., 2011; Rehmann, 2012). By using full-length Epac2 and Rap1 combined with isothermal titration calorimetry, direct binding of sulfonylurea to Epac2 and subsequent activation of Rap1 are not found in a series of in vitro studies (Tsalkova et al., 2011), and these findings are confirmed by a similar set of independent experiments (Rehmann, 2012). Although arginine 447 of Epac2 seems to mediate the direct interaction of Epac2 and sulfonylurea, the currently available data strongly suggest that the molecular link between sulfonylurea and Epac2 still has to be defined. Additional studies add another puzzling outcome to the potential link between sulfonylurea and Epac. It is shown by NMR spectroscopy and by Epac1-based fluorescence resonance energy transfer measurements that tolbutamide binds to the cAMP-B domain of Epac1 and subsequently promotes conformational changes in Epac1 (Parnell et al., 2012). Thus, the current findings regarding the

Epac activating properties of sulfonylureas are at least inconclusive and require further studies.

The discovery of Epac-selective cAMP compounds revolutionized our current view on the signaling properties of cAMP (de Rooij et al., 1998; Enserink et al., 2002; Rehmann et al., 2003a). Since then, the identification of small molecules able to act as antagonists for Epac1 and Epac2 has been the ultimate challenge in this research field. To define novel starting points for drug discovery, a novel high-throughput screen based on the displacement of [^{3}H]cAMP from the Epac cAMP-B domain enabled the identification of small molecule hits from the Scottish Biomedical Lead Generation Library (McPhee et al., 2005). These compounds are structurally different from cAMP and selectively bind to the cAMP-binding sites of Epac1 and Epac2, but not to PKA; however, their limited solubility hampered their experimental accessibility (McPhee et al., 2005). Initially identified as an inhibitor of GEFs for the small GTPase ADP ribosylation factor (Morinaga et al., 1996; Chardin and McCormick, 1999), inhibition of Epac signaling has been reported for brefeldin A—although direct inhibition of Epac was not studied (Zhong and Zucker, 2005). In search for leading compounds for drug development, modifications of CH3 at C15 with COOH and H at C2 with OH in brefeldin A show the best docking results on the basis of protein-drug interaction parameters (Bala et al., 2011). However, it seems rather unlikely the brefeldin A-based small molecules may exert the potential to directly interact and subsequently to inhibit Epac. Thus, the development of novel agonists and antagonists for Epac remains an important challenge, in particular as the cAMP signaling network has turned out to be much more complex and dynamic than initially anticipated.

Importantly, Cheng and colleagues recently developed a robust fluorescence-based high-throughput assay for the screening of Epac specific antagonists (Tsalkova et al., 2012a). The fluorescent labeled 8-[2-(7-nitro-4-benzofurazanyl) aminoehyl-thio]cAMP (8-NBD-cAMP) induced a more than hundredfold increase in 8-NBD-cAMP fluorescent signal upon titration with saturating concentrations of purified full-length Epac2, a process reversed by addition of an excess of unlabeled cAMP. Binding of 8-NBD-cAMP to an isolated fragment of the cAMP-B domain of Epac1 induced a maximal sixfold increase in fluorescence. The very large reversible fluorescence change of 8-NBD-cAMP upon its binding to Epac2 (and to a lesser extent to Epac1) serves as an excellent readout for a robust high-throughput assay for Epac antagonists in a NCI diversity set library that contains 1990 carefully selected small molecules. Three compounds, NCS45576, NCS119911, and NCS68636, inhibited the Rap1 exchange activity of Epac1 and Epac2, but not the activity of PKA, at a concentration of 25 μM in the presence of equal molar concentration of cAMP (Tsalkova et al., 2012a). Additional studies showed that high-throughput screening hit ESI-08 acts as Epac antagonist and subsequent structural-activity relationship analysis identified three 5-cyano-6-oxo-1,6-dihydro-pyrimidines (6b, 6g, and 6h) as potential Epac antagonists (Chen et al., 2012a). Replacement of 6-cyclohexyl in 6b by 6-cyclo-propoyl in 6g (HJCO198) and 6h (HJCO197) enhanced their inhibitory activity to IC_{50} values of 4.0 and 5.9 μM, respectively, compared with an IC_{50} value of 7.0 μM of 6b (Fig. 3C). In addition, 6h (HJCO197) inhibited GDP-GTP loading of Rap1 through Epac1, whereas 6g (HJCO198) is more specific for Epac2. Both compounds were added at 25 μM concentration in the presence of equal molar concentration of cAMP and do not inhibit PKA compared with the PKA inhibitor H89. Studies in HEK293/Epac1 and HEK293/Epac2 cells showed that in addition to inhibiting Epac1 and Epac2 biochemically, 6h (HJCO197) and 6g (HJCO198) suppress 8-pCPT-2′-*O*-Me-cAMP-AM-induced PKB/Akt activation. Representative docking studies with 6h (HJCO197) confirm that the C-6 and C-2 positions of the pyrimidine scaffold are crucial to target Epac (Fig. 3, D and E) and that further modifications at the C-6 position may even improve their Epac antagonistic properties (Chen et al., 2012a). On the basis of these very promising initial results, further studies of a systematic lead optimization on the C-6 position and 5-cyano of the pyrimidine scaffold by appropriate modifications of length, size, and chemical nature of the substituent are expected to generate small molecules with even further improved antagonistic properties toward Epac and to limit their potential of unwanted off-target effects. Interestingly, further studies revealed that high-throughput screening hits ESI-05 and ESI-07 are inhibitors of Epac2, but not Epac1 (Tsalkova et al., 2012b), demonstrating that subtype selective inhibition is possible. Clearly, the development of the first antagonists with (subtype) selectivity for Epac represents another research milestone to ascribe biologic responses to Epac.

Alternative opportunities for therapeutic intervention in Epac directed signaling lie in exploiting the often cell type- and function-specific protein-protein interactions (Fig. 2B), including PDZ domains of Rim2 and Piccolo (Ozaki et al., 2000; Fujimoto et al., 2002), microtubule-associated protein MAP1 and microtubulin (Magiera et al., 2004; Gupta and Yarwood, 2005; Mei and Cheng, 2005; Borland et al., 2006), Ras (Li et al., 2006; López de Jesús et al., 2006; Liu et al., 2008), Ran and RanBP2 (Liu et al., 2010a; Gloerich et al., 2011), and ERM proteins (Gloerich et al., 2010; Ross et al., 2011). The cAMP-sensing multiprotein complexes maintained by distinct members of the AKAP family are valuable to add to the growing list of Epac-interacting proteins (see section II.A.4), including mAKAP-PKA-PDE4D3-Epac1 (Dodge-Kafka et al., 2005), AKAP79/150 (AKAP5)-PKA-Epac2-PKB/Akt (Nijholt et al., 2008), and AKAP450 (AKAP9)-Epac1-VE-cadherin-cortical-actin



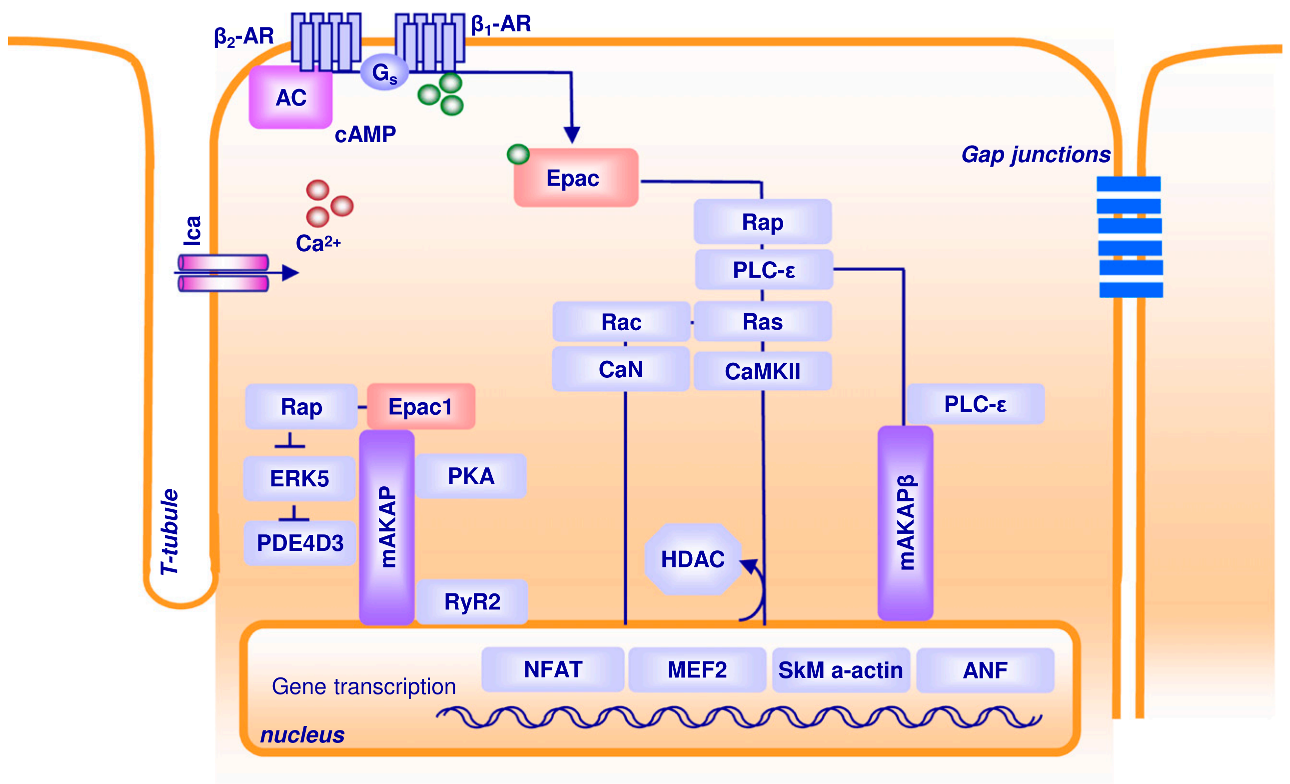


Fig. 4. Epac and the heart. Epac provokes excitation-contraction coupling in response to β_1-adrenergic receptor stimulation through an Epac-Rap-PLC-ε-PKC-ε-CaMKII pathway and subsequent CaMKII-mediated phosphorylation of RyR2 and PLB. In addition, Epac-stimulated CaMKII directly phosphorylates cTnI and cMyBPC and thereby directly regulates myofilament function, a process supported by the induction of calmodulin. (A) Next to the accumulation of Cx43 through Epac at cardiac gap junctions, phosphorylation of Cx43 through PKCε enhances cardiac intercellular communication. Upon sustained cAMP elevation under cardiac stress, Epac induces gene transcription of prohypertrophic transcription factors

(Sehrawat et al., 2011). The small PKA-binding blocking peptides st-Ht31 and RIAD are used to disturb AKAP complexes and thereby potentially interfere with biologic effects of Epac (Skroblin et al., 2010; Tröger et al., 2012). Newly developed AKAP complex disruptors modulate even more efficiently compartmentalized cAMP signaling in the heart (Patel et al., 2010; Christian et al., 2011). Recent studies indicate that the cellular localization of Ras determines its activity and signaling output (Dekker and Hedberg, 2011). Development of small molecule inhibitors that interfere with the membrane localization of Ras is a potentially novel approach in drug discovery (Dekker et al., 2010). Next to cAMP, translocation of the Rap1 exchange factor Epac1 to the plasma membrane requires its DEP domain, and both GDP/GTP-loading of Rap1 as well as Epac1 plasma membrane targeting are biologic processes that are highly dynamic in nature (Bivona et al., 2004; Ponsioen et al., 2009). Interestingly, even protein-phospholipid interactions may further define biologic effects of Epac. Binding of cAMP induces a conformational change of Epac1, which enables its DEP domain to bind to phosphatidic acid in the plasma membrane (Consonni et al., 2012). Thus, cAMP-mediated membrane localization of Epac1 enables the selective activation of Rap at the plasma membrane. PLD1-mediated phosphatidic acid formation may lead to enhanced PIP_2 generation and subsequent to alterations in the protein-protein interactions of Epac1 and ERM proteins (Consonni et al., 2012; Gloerich et al., 2012). This raises the idea to explore small molecule inhibitors of the DEP domain-phosphatic acid interaction as an alternative strategy to antagonize the activity of Epac.

II. Biologic Functions of Epac

Since Epac proteins interact with a plethora of intracellular signaling molecules, Epac1 and Epac2 regulate a wide variety of biologic responses and functions. Alterations in the Epac signalosome have been implicated in the pathophysiology of a number of diseases. In addition, specific targeting of the Epac signaling pathway may open new therapeutic strategies in the pharmacological treatment of these diseases. In this section, we will focus on the role of Epac in regulating physiological responses in health and disease. We will describe novel aspects of the Epac signalosome, such as protein-protein interactions, protein-lipid interactions, and (AKAP-mediated) signal compartmentalization.

A. Epac and the Heart

1. Regulatory Role of Epac in Cardiac Calcium Homeostasis. Acute stimulation of β-adrenergic receptors and subsequent generation of cAMP regulate beneficial cardiac functions including cardiac contractility, relaxation, and heart rate (Rockman et al., 2002; Bers, 2008). Sustained elevation of cAMP, however, leads to hypertrophy and ventricular dysfunction and ultimately to the development of heart failure (Lohse et al., 2003; El-Armouche and Eschenhagen, 2009). Classically, most biologic effects of cAMP in the heart have been assigned to PKA, known to control excitation-contraction coupling through phosphorylation of L-type calcium channels, sarcoplasmic ryanodine receptors (RyR2), and the sarcoplasmic reticulum Ca^{2+}-ATPase 2a regulator protein phospholamban, and thereby subsequently increases cardiac contractility (Rockman et al., 2002; Bers, 2007, 2008; Maier and Bers, 2007; Fig. 4). Increases in Ca^{2+} transients and cardiac contractility are generated upon enhanced myocardial relaxation and sacroplasmic reticulum loading through PKA-dependent phosphorylation of phospholamban, which in turn relieves phospholamban from its inhibition of sarcoplasmic reticulum Ca^{2+}-ATPase 2a. In addition, myofilament relaxation and decreased Ca^{2+} sensitivity are accelerated through PKA-dependent phosphorylation of cardiac troponin I (cTnI) at Ser23/24 and cardiac myosin-binding protein C (cMyBPC) at Ser283 (Maier and Bers, 2007; Bers, 2008). Cardiac excitation-contraction coupling also encompasses activation of L-type calcium channels through cardiomyocyte depolarization, which in turn activates the sacroplasmic RyR2, resulting in cardiac contractility. Fine tuning of excitation-contraction coupling is achieved by Ca^{2+}/calmodulin-dependent kinase II (CaMKII) also known to phosphorylate phospholamban and RyR2 and thereby to enhance sacroplasmic Ca^{2+} uptake and spontaneous Ca^{2+} release (Maier and Bers, 2007; Bers, 2008). From the three β-adrenergic subtypes expressed in the mammalian heart, regulation of cardiac functions is ascribed to the β_1- and β_2-adrenergic receptor subtypes (Lompré et al., 2010). Both receptor subtypes regulate distinct physiologic and pathophysiological responses. The β_1-adrenergic receptor, but not the β_2-adrenergic receptor, stimulates PKA-dependent phosphorylation of phospholamban and cardiac contractile proteins, induce hypertrophy upon moderate overexpression, and promote CaMKII-mediated cardiomyocyte apoptosis (Lompré et al., 2010; Fig. 4).

including SkM α-actin, NFAT, MEF2, and ANF. Scaffolding of the Epac effector PLC-ε to the nuclear envelope-bound mAKAPβ induces ANF. Moreover, Epac induces NFAT, ANF, and SkM α-actin through Rap2B-PLC-ε-Rac-CaN and MEF2 through Rap2B-PLC-ε-Ras-CaMKII as well as nuclear export of HDAC. (B) Epac inhibits prohypertrophic gene transcription through Epac1-mAKAP-Rap1-mediated ERK5 inhibition and PDE4D3 activation. ANF, atrial natriuretic factor; β-AR, β-adrenergic receptors; CaN, calcineurin, Cx43, connexin43; I_{ca}, L-type calcium channels; MEF2, myocyte enhancer factor2; PDE4D3, phosphodiesterase 4D3; PLB, phospholamban; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; SkM α-actin, skeletal muscle α-actin; T-tubule, transverse tubule.

Given the importance of cAMP and Ca^{2+} in cardiac physiology and pathophysiology, several studies aim to define a function of Epac in excitation-contraction coupling. Initial studies in rat neonatal cardiomyocytes show that Epac-selective activation using 8-pCPT-2′-*O*-Me-cAMP triggers a dramatic increase in spontaneous Ca^{2+} oscillations, leaving the amplitude of the Ca^{2+} spikes unaffected (Morel et al., 2005). Adult ventricular cardiomyocytes from mice deficient for the Epac effector PLC-ε possess a decreased electrically evoked Ca^{2+} transient (intracellular Ca^{2+} concentration) in response to β-adrenergic receptor stimulation (Wang et al., 2005). Additional studies show that β-adrenergic receptor stimulation regulates sacroplasmic reticulum Ca^{2+} release through activation of a Epac-Rap- PLC-ε-protein kinase Cε-CaMKII pathway and subsequent CaMKII-dependent phosphorylation of RyR2 and phospholamban (Oestreich et al., 2007, 2009). In rat ventricular cardiac myocytes, however, activation of Epac by 8-pCPT-2′-*O*-Me-cAMP increase the Ca^{2+} oscillations despite a decrease in sacroplasmic reticulum Ca^{2+} load and a reduction of Ca^{2+} transients, processes being accompanied by CaMKII-dependent phosphorylation of RyR2 (Pereira et al., 2007; Fig. 4). Thus, although recent studies report on the identical CaMKII-dependent phosphorylation site on RyR2 (Ser 2815), the net outcome on Ca^{2+} transients remains controversial. Recent studies in adult rat ventricular myocytes indicate that excitation-contraction coupling rely on spatiotemporal compartmentalization of cAMP signaling through the β-adrenergic receptor and both PDE3 and PDE4 (Leroy et al., 2008), implicating that such mechanisms may contribute to distinct biologic net outcomes. In the murine heart, increases of spontaneous Ca^{2+} oscillations by the Epac activator 8-pCPT-2′-*O*-Me-cAMP results in ventricular arrhythmogenesis, a process depending on CaMKII (Hothi et al., 2008), indicating that elevation of spontaneous Ca^{2+} oscillations by Epac and subsequent CaMKII-dependent RyR2 phosphorylations profoundly alter Ca^{2+} homeostasis and thus excitation-contraction coupling.

Clearly, Epac controls cardiac excitation-contraction coupling through phosphorylation of the myofilament proteins cTnI and cMyBPC (Cazorla et al., 2009). In rat ventricular cardiac myocytes, Epac and PKA have opposite effects on Ca^{2+}-activated myofilament force and myofilament Ca^{2+} sensitivity. Epac enhances myofilament Ca^{2+} sensitivity and phosphorylates cTnI and cMyBPC through phospholipase C (PLC)-protein kinase C (PKC)-CaMKII-dependent pathways; however, the specific Epac-dependent phosphorylation sites in cTnI and cMyBPC still have to be defined (Cazorla et al., 2009; Fig. 4A). In addition, Epac induces the expression of calmodulin (Ruiz-Hurtado et al., 2012), providing a feed-forward mechanisms to maintain phosphorylation by CaMKII. As cardiac myofilament function is altered in hypertrophy and heart failure (Layland et al., 2005), these findings suggest that Epac, in a PKA-independent fashion, contributes to these pathologies. Neoformation of cardiac gap junction is another prerequisite to maintain cardiac contractility and to control excitation-contraction coupling. Initial studies in rat neonatal cardiomyocytes show that Epac-selective 8-pCPT-2′-*O*-Me-cAMP induces accumulation of the predominant cardiac gap junction protein connexin43, whereas PKA enhances the gating of connexin43-bearing gap junctions. In addition, upregulation of the adherens junction protein N-cadherin by Epac promotes gap junction formation (Somekawa et al., 2005; Fig. 4A). Recent studies in rat neonatal ventricular cardiomyocytes confirm the potential link between Epac and connexin43. Activation of Epac by 8-pCPT-2′-*O*-Me-cAMP induces phosphorylation of connexin43 through PKCε and subsequently elevates gap junction intercellular communication studied by fluorescence recovery after photobleaching, providing evidence that Epac does not only alter subcellular localization of connexin43 but also its function (Duquesnes et al., 2010). In Langendorff-perfused isolated hearts submitted to global ischemia, however, activation of Epac does not induce preconditioning, a phenomenon known to induce cardioprotection (Duquesnes et al., 2010; Fig. 4A).

2. Epac and Cardiac Hypertrophy. Cardiac hypertrophy serves as a short-term adaptive process to increase the contractile mass of the heart; however, growth signals responsible for hypertrophic growth seem to induce cardiomyocyte apoptosis (Adams and Brown, 2001; Chen and Frangogiannis, 2010; Insel et al., 2012). Recent studies indicate that Epac induces apoptosis in several cell types including cardiomyocytes, albeit it acts cell type dependent in concert with PKA (Kwak et al., 2008; Suzuki et al., 2010). In H9c2 cells and neonatal rat cardiomyocytes, the PDE4 inhibitor roflumilast protects against nitric oxide-induced apoptosis through cAMP-PKA-CREB (cAMP response element-binding protein) and Epac-PKB/Akt pathways (Kwak et al., 2008); in primary cultures of neonatal mouse cardiomyocytes, the inability of Epac to induce apoptosis is correlated with the lack of expression of the apoptosis inducer Bim (Suzuki et al., 2010), suggesting that induction of apoptosis by Epac requires the engagement of complex signaling networks known to determine cell fate. Indeed, apoptosis seems to generate a functional network to autophagy, which sense under certain circumstances stress adaptation to avoid cell death and thus suppresses apoptosis, whereas in other cellular settings it promotes an alternative cell death pathway (Maiuri et al., 2007; Rubinsztein et al., 2007). Autophagy is regulated by mammalian targets of rapamycin (mTOR1 and mTOR2) through the action of phosphoinositide 3 kinase-dependent PKB/Akt signaling (Rubinsztein et al., 2007), the latter known to represent an effector

of Epac. Intriguingly, recent studies indicate that Epac regulates autophagy in response to α-hemolysin (Misra and Pizzo, 2012) and that Epac is part of multiprotein complexes bearing mTOR1 and mTOR2 (Kelly et al., 2010), implicating that Epac regulates autophagy through mTOR1 and mTOR2 pathways. Excessive deposition of extracellular matrix during cardiac fibrosis contributes to cardiac dysfunction (Yokoyama et al., 2008c; Chen and Frangogiannis, 2010; Insel et al., 2012). In cardiac fibroblasts, Epac inhibits migration and synthesis of extracellular matrix components, including collagen I and III. Loss of Epac1 by fibrinogenic TGF-β1 accelerates collagen deposition and reduces Epac1-dependent migration of fibroblasts (Yokoyama et al., 2008c; Table 1). The fibrogenesis mediator TGF-β1 recruits and activates (myo)fibroblasts, which may derive from resident mesenchymal cells, circulating fibrocytes, and/or epithelial-to-mesenchymal transition, a process in which epithelial cells transdifferentiate into mesynchymal cells (Schmierer and Hill, 2007; Biernacka et al., 2011; Nieto, 2011; Kovacic et al., 2012; Small, 2012; Fig. 8). Next to fibroblast-myofibroblast transformation, recent studies indicate that Epac inhibits, either alone or in concert with PKA, the induction of epithelial-to-mesenchymal transition by TGF-β1 in Madin-Darby kidney cells. Activation of Epac reversed the inhibitory effect of TGF-β1 on the epithelial marker E-cadherin and normalized the expression of α-smooth muscle actin (Insel et al., 2012). Epithelial-to-mesenchymal transition has been proposed as a mechanism for tissue fibrosis in numerous settings including development and cancer (Krenning et al., 2010; Chaffer and Weinberg, 2011). Cancer associated fibroblasts are linked to mesenchymal to mesenchymal transition, bone marrow mesenchymal stem cell transition, and endothelial to mesenchymal transition (Cirri and Chiarugi, 2011). Recent studies link Epac to central biologic responses underlying such cell transition processes including cell migration, cell adhesion, cell homing, and (neo)vascularization (Carmona et al., 2008; Baljinnyam et al., 2009, 2010, 2011; Jang et al., 2012; Jia et al., 2012; Pullamsetti et al., 2012; Tang et al., 2012). The Epac effector Rap2 is involved in Wnt/β-catenin signaling by stabilizing β-catenin (Choi and Han, 2005) and could in this contribute to fibrotic processes. Epac strengthens tethering of catenin to adherens junctions and tight junctions (Netherton et al., 2007; Raymond et al., 2007; Noda et al., 2010; Rampersad et al., 2010; Suh and Han, 2010). Intriguingly, the secreted lysophospholipase D autotaxin promotes cancer invasion through a cAMP-Epac-Rac1 pathway in response to lysophosphatidic acid receptor 4 stimulation, a process supported by blood vessel formation through autotaxin (van Meeteren et al., 2006; Harper et al., 2010). Lysophosphatidic acid bound to its receptor can also elevate the phospholipase D product phosphatidic acid (Samadi et al., 2011), which has been recently shown to regulate subcellular localization and functions of Epac (Consonni et al., 2012; Gloerich et al., 2012; Fig. 2).

Expression of Epac is increased in patients with cardiac hypertrophy as well as in different animal models of myocardial hypertrophy and heart failure (Table 1); however, at present it is not known whether this is a cause or consequence of cardiac hypertrophy. Both Epac mRNAs are upregulated by chronic catecholamine infusion-induced hypertrophy, and only Epac1 is increased in pressure overload-induced hypertrophy (Ulucan et al., 2007). Expression of Epac1 protein is upregulated at the early phase of cardiac pressure overload-induced hypertrophy in rat left ventricular myocardium and in left ventricular samples from patients with heart failure (Métrich et al., 2008; Table 1). Activation of Epac by 8-pCPT-2′-*O*-Me-cAMP increases the cell surface area, protein synthesis, and expression of cardiac hypertrophy markers, including atrial natriuretic factor, c-fos, skeletal muscle α-actin, and nuclear factor of activated T cells (NFAT) (Morel et al., 2005; Métrich et al., 2008). Importantly, silencing of Epac1 expression strongly reduces the induction of the hypertrophic markers by β-adrenergic receptor stimulation (Métrich et al., 2008). Epac induces cardiac hypertrophic responses through activation of a Rap2B, Rac, H-Ras, PLC, the phosphatase calcineurin, and CaMKII (Morel et al., 2005; Métrich et al., 2010; Pereira et al., 2012; Fig. 4B). Sustained activation of Epac correlates with the induction of calmodulin (Ruiz-Hurtado et al., 2012); together with the induction of skeletal muscle α-actin Epac seems to fulfill central compensatory cardiac functions during the initiation of cardiac hypertrophy, favoring contraction but also risking the development of arrhythmias (Morel et al., 2005; Ruiz-Hurtado et al., 2012).

3. Epigenetic Regulation by Epac. Intriguingly, it is reported for the first time that Epac activation induces nuclear export of HDAC5, a process involving HDAC4 in a cell-type specific manner (Métrich et al., 2010) and subsequent induction of prohypertrophic transcription factors including NFAT and myocyte enhancer factor 2 in primary cardiac myocytes (Métrich et al., 2010; Pereira et al., 2012). These novel findings can be added to the growing list of nuclear-associated signaling properties of Epac, including nuclear translocation of the DNA damage-responsive DNA-PK (Huston et al., 2008), cell cycle-dependent localization of Epac to the mitotic spindle and centrosomes (Qiao et al., 2002), and targeting of Epac to the nuclear pore via Ran and/or RanBP2 (Liu et al., 2010a; Gloerich et al., 2011; Fig. 2). In addition, microtubules and RanBP2 seem to modulate the exchange activity of Epac at the nuclear envelope (Magiera et al., 2004; Gupta and Yarwood, 2005; Mei and Cheng, 2005; Borland et al., 2006; Gloerich et al., 2011), a process potentially being

supported by components of the nucleoskeleton (Simon and Wilson, 2011; Jaspersen and Ghosh, 2012). Induction of skeletal muscle α-actin and calmodulin by Epac requires its interaction with myocardin-related transcription factors (Parmacek, 2007; Braun and Gautel, 2012; Small, 2012), a process being tightly controlled by the actin and microtubule cytoskeleton (Olson and Nordheim, 2010). The discovery that Epac regulates nuclear export of a specific subset of HDAC family members adds another puzzling aspect to its complex signaling. Although the detailed mechanisms of Epac-induced nuclear export of HDACs remain to be identified (Métrich et al., 2010; Pereira et al., 2012; Fig. 4B), the reports place Epac not only into the context of epigenetic regulation of gene expression (Arrowsmith et al., 2012), but also in post-transcriptional modification of cytosolic proteins via (de)acetylation. Histone modifications, such as acetylation and phosphorylation, play an important role in the regulation of gene transcription (Dekker and Haisma, 2009; Ghizzoni et al., 2011). Next to HDACs, histone acetylation is modulated by histone acetyltransferases. Whereas histone acetyltransferases promote the opening of the chromatin structure by acetylating lysine residues in histone proteins leading to gene activation, HDACs promote the reverse process. Interestingly, the class II HDAC family members HDAC4 and HDAC5 are reported to act as signal-responsive suppressors of the transcriptional program governing cardiac hypertrophy and heart failure (Zhang et al., 2002), which is in line with the previous reported role of Epac in the development of these diseases. Cardiac stress signals activate the phosphatase calcineurin and subsequently induce kinase activities directed at the class II HDACs including HDAC4 and HDAC5. Phosphorylation of HDAC4 and HDAC5 triggers their cytosolic interaction with the 14-3-3 scaffold proteins and thus promotes nuclear export. Due to the nuclear export of HDAC4 and HDAC5, transcription factors favor binding to HATs known to facilitate gene transcription (Zhang et al., 2002). In light of the growing number of Epac-interacting proteins future studies should focus on a potential link between Epac and the scaffold protein 14-3-3.

4. The Cardiac Epac Signalosome. Despite the compelling evidence that links Epac to the induction of cardiac hypertrophy, the current studies remain controversial. In neonatal rat cardiomyocytes, initial studies report that multiprotein complex composed of nuclear envelope-associated mAKAP, PKA, PDE4D3, and Epac1 control cardiomyocyte hypertrophy through regulation of ERK5, a process tightly regulated by cAMP (Dodge-Kafka et al., 2005). It is shown that high cAMP attenuates cardiac hypertrophy through Epac1-Rap1-dependent inhibition of ERK5 and subsequent activation of PDE4D3; however, low cAMP enhances cardiac hypertrophy through ERK5-mediated inactivation of PDE4D3 and subsequent increased PKA-mediated RyR2 phosphorylation as well as NFAT induction (Dodge-Kafka et al., 2005; Fig. 4B). Likewise, mice deficient for the Epac effector PLC-ε show increased cardiac hypertrophy in response to chronic stress (Wang et al., 2005), further substantiating the notion that Epac inhibits cardiac hypertrophy. However, earlier studies demonstrate that RyR2 binds to mAKAP and induce cardiac hypertrophy in response to β-adrenergic receptor stimulation (Pare et al., 2005), suggesting that CaMKII-dependent phosphorylation of RyR2 by Epac also modulates cardiac hypertrophy (Morel et al., 2005; Oestreich et al., 2007, 2009). Indeed, in neonatal rat ventricular cardiomyocytes depletion of the Epac effector PLC-ε using siRNA reduces hypertrophic growth and induction of atrial natriuretic factor. PLC-ε binds to the striated myocytes exclusively expressed mAKAPβ, which promotes scaffolding of PLC-ε to the nuclear envelope (Zhang et al., 2011; Fig. 4B). Thus, studies that potentially link Epac to cardiac hypertrophy are still under debate.

To add even more complexity, earlier studies report that the PLD product phosphatidic acid activates PDE4D3 (Grange et al., 2000) and that PLD expression is upregulated in response to ventricular pressure-overload hypertrophy (Peivandi et al., 2005). These findings implicate that alterations in activity and/or expression of factors that are part of cAMP-sensing multiprotein complexes, such as PDE4D3 (Dodge-Kafka et al., 2005), or factors that determine the subcellular localization of Epac, such as phosphatidic acid (Consonni et al., 2012), exhibit different signaling properties and therefore alter the ability of Epac to act either anti- and/or pro-hypertrophic in the heart. In vascular smooth muscle Epac1 enhances the expression of the α_{2C}-adrenergic receptor through a Rap1-RhoA signaling pathway (Jeyaraj et al., 2012), suggesting that Epac may even alter the expression profile of β_1- and/or β_2-adrenergic receptors known to be linked to cardiac functions. In addition, a β-arrestin-CaMKII-Epac1 complex is formed in response to the stimulation of the β_1-adrenergic receptor, but not of the β_2-adrenergic receptor (Mangmool et al., 2010). Ligand-directed or -biased agonism through β-arrestin has been reviewed in the context of heart failure (Whalen et al., 2011; Reiter et al., 2012), suggesting that β-arrestin-Epac1 complex formation may contribute to cardiac hypertrophy. As outlined above cardiac hypertrophy may lead to heart failure (Lohse et al., 2003; El-Armouche and Eschenhagen, 2009), a process predominantly relying on cardiac signaling in response to the β_1-adrenergic receptor. Indeed, recent studies show that heart failure changes primarily compartmentalized cAMP signaling in response to the β_2-adrenergic receptor, but not in response to the β_1-adrenergic receptor (Nikolaev et al., 2010). By use of nanoscale live scanning ion conductance combined

with fluorescence resonance energy transfer techniques, it is shown that the β_1-adrenergic receptor is distributed throughout the entire cardiomyocyte cell surface encompassing the transverse T-tubules and the cell crest, whereas the β_2-adrenergic receptors exclusively localized in transverse T-tubules (Nikolaev et al., 2010). These are novel findings that perfectly reflect their functional diversity in the heart. Nevertheless, the relative contribution of the adrenergic receptor subtypes to the apparent link between Epac and cardiac hypertrophy remains to be studied.

B. Epac and the Brain

1. Role of Epac in Neuronal Differentiation and Regeneration. Until very recently, most biologic effects of cAMP known to regulate molecular mechanisms of a wide variety of neuronal processes, including development, growth, differentiation, neurotransmitter release, excitability, remodeling, plasticity, learning, and memory, have been assigned to PKA (Tronson and Taylor, 2007; Abel and Nguyen, 2008; Lee and Silva, 2009). Several studies, however, clearly show that Epac, acting either alone or in concert with PKA, regulates neuronal physiology through its signaling properties to a plethora of distinct effectors and contributes to the pathophysiology of several diseases (Grandoch et al., 2010). Epac regulates neuronal differentiation, neurite outgrowth, and axon regeneration, suggesting that Epac plays a key role in the development and the maintenance of the nervous system. In PC12 cells, initial findings demonstrated that the Epac activator 8-pCPT-2′-*O*-Me-cAMP induces neurite outgrowth (Christensen et al., 2003). Subsequent studies show that, depending on the duration of signaling, Epac converts cAMP from a proliferative into a differentiation signal (Kiermayer et al., 2005). In retinal ganglion cells, Epac links, both in vitro and in vivo, ganglion cell survival and axon growth to PKA and soluble AC (Corredor et al., 2012). As Epac regulates the phosphorylation of PKB/Akt in primary cortical neurons (Nijholt et al., 2008), this Epac signaling route probably supports neuronal survival. In human SH-SY5Y neuroblastoma cells, Epac regulates neuronal differentiation in response to the pituitary adenylate cyclase-activation polypeptide (PACAP)-38 (Monaghan et al., 2008). Of note, neuronal differentiation in response to PACAP-38 involves activation of Rit by Epac independent of Rap (Shi et al., 2006; Fig. 5). As Rit is implicated in neurotrophin signaling (Shi and Andres, 2005), these findings indicate that Epac uses a non-classic signaling property to create a novel molecular link to neuronal development and regeneration.

Although few studies indicate that developmental and/or regenerative neuronal responses do not rely on Epac (Emery and Eiden, 2012; Xu et al., 2012), a growing list of recent studies point to a central function of Epac. Together with PKA, Epac regulates polarized responses of neuronal growth cone attraction and repulsion. The key axon guidance cues, however, from attraction to repulsion require a switch from Epac to PKA activation (Murray et al., 2009a). Next to Epac-induced neurite outgrowth in rat dorsal root ganglia, Epac enhances neurite regeneration on adult spinal cord in vitro (Murray and Shewan, 2008; Fig. 5), suggesting that Epac represents a target to induce axon regeneration in vivo. In neurons within the pre-Bötzinger complex, Epac induces neurite outgrowth and reinforces neuronal bursting activity (Mironov et al., 2011; Mironov and Skorova, 2011), suggesting that Epac signaling properties compensate for neurodevelopmental deficits characteristic for the Rett syndrome.

2. Regulation of Neuronal Signaling by Epac. In glutamatergic synapses, Epac enhances neurotransmitter release from the rat brain calyx of Held (Sakaba and Neher, 2003) and in the crayfish neuromuscular junction (Zhong and Zucker, 2005). In dorsal root ganglion, Epac activates PKCε translocation and contributes to perception of inflammatory pain in response to PGE_2-induced P_2X_3 receptor responses (Hucho et al., 2005; Fig. 5; Table 1); the upregulated Epac1 expression in inflamed neurons is expected to sensitize pain perception (Wang et al., 2007). Intriguingly, recent studies provide new insights into the role of Epac in inflammatory hyperalgesia. It is shown that the low nociceptor G protein-coupled receptor kinase 2 (GRK2) leads to prolonged inflammatory hyperalgesia through biased signaling from PKA to Epac-Rap1, ERK/PLCε (Eijkelkamp et al., 2010). In cultured mouse cerebral neurons, Epac activates neuronal excitability upon modulation of Ca^{2+}-dependent K^+-channels through Rap and p38 mitogen activated protein kinase (Ster et al., 2007, 2009; Fig. 5). In the suprachiasmatic nuclei of the hypothalamus, Epac is part of the core component of the mammalian circadian pacemaker (O'Neill et al., 2008). In spine synapses, Epac2 promotes dynamic remodeling and depression of spiny synapses through the postsynaptic adhesion molecule neuroligin 3, the scaffold protein PSD-95, and Rap (Woolfrey et al., 2009; Penzes et al., 2011).

3. Learning and Memory Retrieval. Several recent studies indicate that Epac regulates distinct steps in processes required for synaptic plasticity, learning, and memory. In the CA1 area of mouse hippocampal slices, Epac enhances long-term potentiation known to be one characteristic of synaptic plasticity through ERK1/2 signaling (Gelinas et al., 2008). In the hippocampus, Epac mediates PACAP-dependent long-term depression through Rap, p38 mitogen-activated protein kinase, and PSD-95/Disc-large/ZO-1 (PDZ) homology domains (Ster et al., 2009). Independent of PKA, Epac enhances memory consolidation in the hippocampus

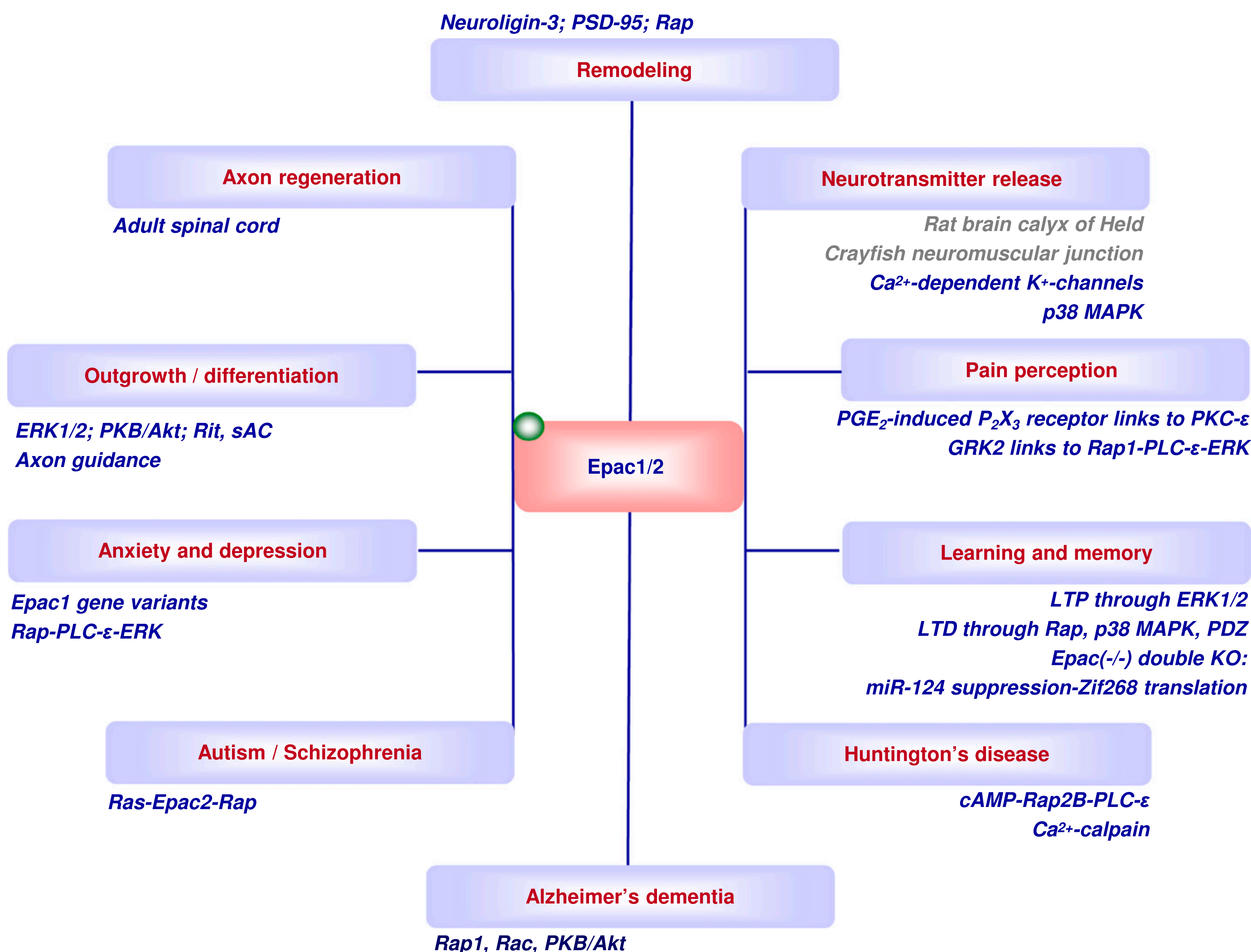


Fig. 5. Epac and the brain. Epac is linked to diverse neuronal processes including neuronal differentiation, neurite outgrowth, axon regeneration, remodeling, neurotransmitter release, pain perception, as well as learning and memory. Next to physiologic neuronal processes linked to Epac, Epac is implicated in brain disorders, such as anxiety and depression, autism, schizophrenia, Alzheimer's disease, and Huntington's disease. Identified signaling routes are indicated. LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; miR-124, microRNA-124; PKB/Akt, phosphoinositide 3-kinase-dependent protein kinase B/Akt; sAC, soluble adenylyl cyclase.

(Ma et al., 2009). In dopamine-β-hydroxylase-deficient mice, coapplication of selective activators for both PKA and Epac rescues memory impairments in contextual fear conditioning (Ouyang et al., 2008). Subsequent studies indicate that β_2-adrenergic receptors through coupling to $G_{i/o}$ and β_1-adrenergic receptors through coupling to G_s have opposing effects on hippocampus-dependent emotional memory (Schutsky et al., 2011), suggesting that the beneficial effects of Epac on memory retrieval are also controlled by the relative input of adrenergic receptor subtypes. Intrahippocampal injection of the Epac activator 8-pCPT-2′-*O*-Me-cAMP demonstrates that Epac2 specifically enhances time-limited memory retrieval and contextual fear conditioning (Ostroveanu et al., 2010). Recent studies in Epac(−/−) double knockout mice confirmed these initial findings. The double knockout mice of Epac1 and Epac2 exhibit severe defects in long-term potentiation, spatial learning, and social interactions through a Rap1-dependent suppression of miR-124 and subsequent Zif268 translation (Yang et al., 2012; Fig. 5). These research findings offer several novel concepts to the scientific community. First, as both Epac1(−/−) and Epac2(−/−) are phenotypically normal (Yang et al., 2012), the concept that both Epac1 and Epac2 bear functional redundancy is further supported. Second, it is the first report that links Epac to the mechanisms of post-transcriptional regulation by microRNAs. Currently, individual microRNAs are believed to act as pivotal modulators of mammalian functions during both development and disease (Filipowicz et al., 2008). Additional studies in Epac2-deficient mice indicate that Epac2 depletion is sufficient to induce impairments in social interactions, ultrasonic vocalization, and cortical structures (Srivastava et al., 2012a), suggesting that both Epac1 and Epac2 control

a distinct subset of neuronal functions with potential alterations under diseased conditions.

4. The Epac Signalosome in Brain Disorders. Indeed, recent studies link specific defects in neuronal Epac1 and/or Epac2 signaling to brain disorders (Table 1). In human subjects diagnosed for autism, an Epac2 rare coding variant controls basal dendritic morphology through a Ras-Eapc2-Rap pathway (Srivastava et al., 2012b). In addition, reduced signaling of $G\alpha_s$ is associated with schizophrenia symptoms through defects in Epac signals (Kelly et al., 2009). Initial studies associate Epac-1 gene variants with anxiety and depression (Middeldorp et al., 2010). Additional studies demonstrate that the corticotropin releasing factor receptor modulates anxiety and depression through a wide range of Epac-associated signals including Rap-PLCε-ERK (Hauger et al., 2009; Fig. 5). In human brain regions associated with Alzheimer's disease, upregulation of Epac1 mRNA correlates with a downregulation of Epac2 mRNA (McPhee et al., 2005). Initial studies in primary neurons show that Epac increases the activity of α-secretase and subsequently the secretion of the neuroprotective and memory-enhancing soluble amyloid precursor protein through both Rap1 and Rac (Maillet et al., 2003; Robert et al., 2005; Fig. 5). Studies in primary cortical neurons indicate that Epac, primarily Epac2, induces phosphorylation of PKB/Akt known to support neuronal survival and memory processes (Nijholt et al., 2008), suggesting that reduced Epac2 functioning in patients with Alzheimer's disease promotes loss of neuroprotective PKB/Akt signaling. Next to the neurodegenerative disorder Alzheimer's disease, Epac is linked to polyglutamine disorders, including Huntington's disease (Sarkar et al., 2009). It is shown that Epac regulates autophagy independent of mTOR through cAMP-Rap2B-PLCε- and thereby supports Ca^{2+}-calpain signaling (Williams et al., 2008; Fig. 5).

These findings indicate that the link of Epac, acting either alone and/or in concert PKA, to several brain disorders requires its localization to specific brain regions and signaling to distinct effectors in a time- and space-limited manner. In murine primary cortical neurons and HT-4 cells, Epac2 enhances PKB/Akt phosphorylation through Rap1 complexed to AKAP79/150 (Nijholt et al., 2008). In addition, Epac promotes neuronal survival and neurite growth through a nuclear complex composed of PKA and sAC (Corredor et al., 2012), suggesting that distinct cAMP microdomains maintained by sAC profoundly alter the signaling properties of Epac. The ability of Epac2 to promote remodeling of spiny synapses requires a distinct complex composed of neuroligin-3/PSD95/Rap complex (Woolfrey et al., 2009). Earlier reports indicate that the scaffold PSD-95 also interacts with the Ras-GAP p135 SynGAP (Chen et al., 1998), indicating that PSD-95 might represent the molecular link between Epac2 and Ras in neurons. Finally, GRK2 binds to Epac1 and thereby promotes biased signaling through Rap1-PLC-ε-ERK (Eijkelkamp et al., 2010), indicating that new G protein-coupled receptor signaling paradigms modulate the neuronal signaling properties of Epac. In conclusion, these findings indicate that next to complex formation of Epac with members of the AKAP family scaffolding through PSD-95 and GRK2 may alter its impact on neuronal processes both under physiological and pathophysiological settings.

C. Epac and the Pancreas

1. Regulation of Insulin Secretion by Epac. The metabolic disease diabetes is expected to be a leading cause of disability, morbidity, and premature mortality by the year 2030, which will severely affect both developed and developing countries (Rydén et al., 2007; Chalmers and Cooper, 2008; Danaei et al., 2011). The complications of diabetes primarily affect the eyes, kidneys, nerves, and the cardiovascular system, causing several microvascular-associated complications, including kidney failure. Current treatment of diabetes, particularly type 2 diabetes, focuses on reducing glucose levels by elevating the plasma levels of insulin through several distinct molecular mechanisms, including inhibition of ATP-sensitive K^+-channels by sulfonylureas on pancreatic β-cells (Doyle and Egan, 2007; Verspohl, 2012). Insulin is produced in the islet of Langerhans in the pancreatic β-cells. Glucose induces a pulsatile insulin secretion, and it has been proposed that the observed initial and late secretion of insulin point to functionally distinct granule pools (Tengholm and Gylfe, 2009). The incretin hormone glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide are produced in the gut upon duodenum entry of food containing fat, peptides, and/or glucose. In pancreatic β-cells, glucose-induced insulin secretion is by the cAMP signaling pathway following GLP-1 receptor stimulation (Holz et al., 2006; Doyle and Egan, 2007; Fig. 6). By use of evanescent-wave fluorescence imaging as a new technique for single-cell measurements of cAMP, it is reported that glucose-induced cAMP oscillations are linked to the pulsatile insulin secretion in the pancreatic MIN6 and in primary mouse β-cells (Dyachok et al., 2008). Several studies indicate that the effect of cAMP acts via activation of Epac—partly in cooperation with PKA. Thus, silencing studies have assigned Epac2 as the key player in glucose-induced insulin secretion through pulsatile cAMP oscillations (Idevall-Hagren et al., 2010). As described in detail in section I.D, Epac2 is allosterically activated through sulfonylureas, which are widely used as antidiabetic drugs. These findings further link Epac to insulin secretion as sulfonylureas potentiate glucose-induced insulin secretion upon binding to the SUR1 subunit of ATP-sensitive

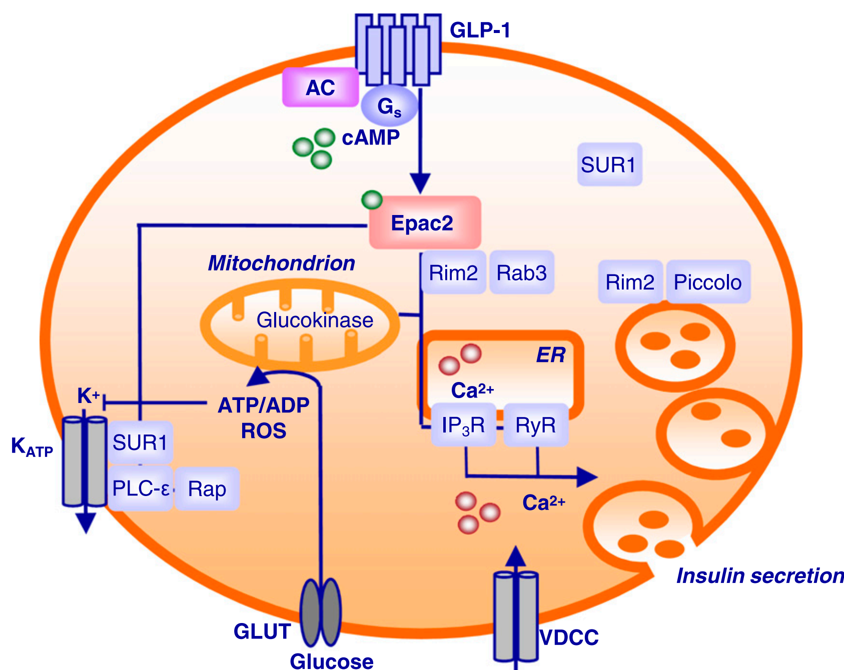


Fig. 6. Epac and the pancreas. In pancreatic β-cells, glucose-induced insulin secretion is potentiated through Epac2-Rim2-Piccolo signaling in response to stimulation of the GLP-1 receptor. Closure of ATP-sensitive K^+-channels (K_{ATP}) is promoted through Epac2-SUR1 interaction and Epac2-Rap-dependent stimulation of PLC-ε, the latter known to increase channel ATP sensitivity through hydrolysis of PIP_2. Subsequent membrane depolarization induces opening of voltage-dependent Ca^{2+}-channels (VDCC) and facilitates insulin secretion through fusion of insulin granules with the plasma membrane. Interaction of Epac2 with Rim2 and Piccolo promotes insulin granule fusion and thereby insulin secretion, such as the activation of calcium channels on the endoplasmic reticulum (ER). Epac2 interacts in a Rim2-Rab3-dependent manner with glucokinase and thereby enhances mitochondrial metabolism of glucose. IP_3R, inositol-1,4,5-trisphosphate receptor; ROS, reactive oxygen species.

K^+-channels, upon direct interaction of sulfonylurea with arginine 447 in Epac2 (Zhang et al., 2009; Herbst et al., 2011). The initial findings, however, ignite a controversial debate among experts in the field (Tsalkova et al., 2011; Parnell et al., 2012; Rehmann, 2012; Fig. 6). Nevertheless, the studies directly link Epac2 to both sulfonylureas and SUR1, implicating that Epac2 captures a rather central function to fine tune the SUR1-dependent effects of the antidiabetic sulfonylureas (Doyle and Egan, 2007; Verspohl, 2012).

Epac2, which is the predominantly expressed Epac isoform in pancreatic β-cells, is required for the potentiation of insulin secretion by incretins (Kashima et al., 2001). Additional studies show that Epac2 binds to Rim (Rab3-interacting molecule) and to Rim2, a novel regulator of fusion of vesicles to the plasma membrane (Ozaki et al., 2000; Shibasaki et al., 2004). Potentiation of Ca^{2+}-dependent insulin granule exocytosis in response to GLP-1 relies, at least in part, on Epac2 (Kang et al., 2001, 2003). In pancreatic β-cells, the Ca^{2+} sensor Piccolo stabilizes the Epac2-Rim2 complex through heterodimerization (Fujimoto et al., 2002). Likewise, Epac2 directly interacts with the SUR1 subunit of ATP-sensitive K^+ channels and thereby regulates priming of pancreatic β-cells (Eliasson et al., 2003; Fig. 6). Recent findings indicate that GLP-1 potentiates glucose-induced insulin secretion by enhancing glucokinase activity in pancreatic β-cells through Epac2-Rim2-Rab3 (Park et al., 2012). Interestingly, GLP-1 potentiates both Ca^{2+}-dependent insulin granule excocytosis and glucose-induced insulin secretion through Epac2-Rim2-Rab3 signaling. In primary pancreatic β-cells from Epac2 knockout mice, the initial phase of insulin secretion is severely reduced (Shibasaki et al., 2007), further substantiating that Epac2 is of central importance for the release of insulin from pancreatic β-cells and thus for the regulation of blood glucose. Interestingly, GLP-1 enhances glucokinase activity through Epac2-Rim2-Rab3 (Park et al., 2012; Fig. 6). Although the studies link glucokinase to Epac2, these findings indicate that Epac not only regulates insulin secretion but also profoundly alters the metabolism of glucose because glucokinase controls mitochondrial consumption of glucose. In conclusion, several molecular mechanisms that regulate glucose-mediated insulin secretion upon engagement of a sophisticated network of scaffold proteins and ion channels have identified Epac2 as a key player.

GLP-1-induced potentiation of Ca^{2+}-dependent insulin granule excocytosis is mediated by Epac2 (Kang et al., 2001, 2003). Likewise, the Epac activator 8-pCPT-2′-*O*-Me-cAMP induces a ryanodine-sensitive Ca^{2+}-mobilization in pancreatic β-cells (Kang et al., 2001, 2003; Holz et al., 2006), suggesting that Epac2 also contributes to calcium-induced calcium release from the endoplasmic reticulum. Earlier studies indicate that Epac induces inositol-1,4,5-trisphosphate-sensitive Ca^{2+}-mobilization through Rap2B-dependent

stimulation of PIP_2-hydrolyzing PLC-ε (Schmidt et al., 2001), a mechanism being expected to contribute to Ca^{2+}-dependent insulin granule excocytosis. In addition, the hydrolysis of PIP_2 through Epac2 is expected to increase ATP-sensitive K^+-channels responsiveness to ATP and subsequently to promote their closure (Holz et al., 2006), leading to insulin secretion. Indeed, recent studies show that PLC-ε links Epac2 to the potentiation of insulin secretion in mouse islets of Langerhans (Dzhura et al., 2011; Fig. 6). In transgenic mice that lack the Epac effector PLC-ε, the Epac activator 8-pCPT-2′-*O*-Me-cAMP loses the ability to potentiate glucose-induced insulin secretion. Importantly, however, these mice also lose their ability to increase calcium in pancreatic β-cells (Dzhura et al., 2011), indicating that a Rap1-regulated PLC-ε also links Epac2 to Ca^{2+}-dependent insulin granule excocytosis. Since a recent study in cardiomyocytes reported on scaffolding of PLC-ε to mAKAP-β (Zhang et al., 2011), it is tempting to speculate that cellular PLC-ε scaffolding contributes to its ability to potentiate glucose-induced insulin secretion as well.

2. The Epac Signalosome in Diabetes-Related Diseases. Recent studies link signaling properties of Epac1 and/or Epac2 to diabetes-related diseases, particularly kidney failure. Activation of Epac by 8-pCPT-2′-*O*-Me-cAMP induces protection of tubular epithelial cells from cisplatin-induced apoptosis (Qin et al., 2012). In a mouse model of ischemia-induced kidney failure, the Epac activator 8-pCPT-2′-*O*-Me-cAMP preserves the epithelial barrier function during hypoxia in vitro (Stokman et al., 2011). Importantly, intrarenal administration of 8-pCPT-2′-*O*-Me-cAMP reduces renal failure in vivo through a reduced expression of the tubular stress marker clusterin-α and lateral expression of β-catenin, which is indicative for sustained tubular function (Stokman et al., 2011). In accordance with the expression profile of Epac1 and Epac2 in the kidney (Ulucan et al., 2007; Li et al., 2008), silencing studies indicate that the renal protective effects require Epac1 (Stokman et al., 2011; Qin et al., 2012). Recent studies indicate that the GLP-1 receptor agonist exendin-4 suppresses the production of detrimental reactive oxygen species through Epac (Mukai et al., 2011), which may contribute to the protection against renal ischemia-reperfusion injury through Epac1. Reactive oxygen species (Fig. 6) also intermingle with the mitochondrial glucose consumption controlled by glucokinase, in particular under disease conditions such as diabetic nephropathy (Singh et al., 2008; Kanwar et al., 2011). Complex mechanisms obviously contribute to glucose homeostasis through the signaling properties of Epac. Recent studies indicate that, in contrast to the positive effects of Epac2, Epac1 mediates high glucose-induced renal proximal tubular cell hypertrophy through Akt and the cyclin-dependent kinase inhibitor p21 (Sun et al., 2011). In addition, Epac induces the resistance to leptin through an impairment of leptin-induced Rap1-SOCS-3 signaling and subsequent elevation of JAK-STAT responses (Fukuda et al., 2011). Thus, Epac bears, in addition to its beneficial effects, rather deleterious signaling properties under diabetes-related disorders. In this context it is unclear whether the finding that Epac1 is increased in the cortical tubules of the kidney in a mouse model of streptozotocin-induced diabetes (Sun et al., 2011; Table 1) is a cause or a consequence. Interestingly, the level of induction was correlated with the level of glucose in the blood of these animals (Sun et al., 2011). A causal relation between increased glucose levels and increased expression of Epac2 was demonstrated by the finding that D-glucose, but not L-glucose, induces Epac1 expression in human kidney HK-2 cells (Sun et al., 2011; Table 1). Future studies on the relative contributions of Epac1 versus Epac2 in regulating glucose homeostasis are warranted. Studies in Epac1 and Epac2 transgenic mice under disease conditions would help to clarify whether Epac2 indeed is beneficial, whereas Epac1 is detrimental. Clearly, this research will also greatly benefit from subtype selective pharmacological activators and inhibitors (see section I.C).

D. Epac and the Vasculature

1. Epac and the Endothelial Barrier Function. The vascular endothelium covers the inner lining of blood vessels and serves as an interface between circulating blood and surrounding tissue. The semi-permeable endothelial barrier dynamically regulates exchange of ions, solutes, and cells both through and between cells or the endothelium (Mehta and Malik, 2006; Vestweber, 2007; Giepmans and van Ijzendoorn, 2009; Pannekoek et al., 2009). During acute inflammation, leukocytes transmigrate through the permeable endothelial barrier; however, chronic inflammation also leads to excessive extravasation of blood components and fluid accumulation into the extravascular space, causing edema and vascular dysfunction (Hirase and Node, 2012; Vestweber, 2012). The barrier is maintained by tight junctions and adherens junctions, creating strong cell-cell contacts. Tight junctions act as size-selective barriers and maintain cell polarity due to their apical-lateral cell surface localization (Chiba et al., 2008; Anderson and Van Itallie, 2009). Adherens junctions are located at the more basal cell side and express (V)E-cadherin and catenin (Giepmans and van Ijzendoorn, 2009; Pannekoek et al., 2009; Vestweber et al., 2009). (V)E-cadherin interacts with α-catenin and/or β-catenin and thereby connects the adherens junctions to the circumferential belt of actin filaments to strengthen cell-cell contacts. Barrier disrupting agents, including tumor necrosis factor-α, thrombin, and the bacterial endotoxin lipopolysaccharide, alter the dynamic actin-microtubule network architecture of tight

and adherens junctions (Niessen, 2007; Vestweber, 2007; Vestweber et al., 2009, 2010; Sayner, 2011; Fig. 7).

Reduction of the barrier leakage is achieved by cAMP elevation in response to β_2-agonists, prostanoids, and forskolin and has been linked to the inhibition of actin-microtubule dynamics linked to tight and adherens junctions under tight control of members of the Ras family, in particular Rho GTPases and Rap. Several studies indicate that Epac, acting either alone and/or in concert with PKA, reduces barrier leakage predominantly through its signaling properties to Rho via Rap on the actin-microtubule network. Notably, Epac1 modulates the endothelial barrier independent from the Rho GTPases and Rap (see below). As Rap1 promotes cell-cell junction formation through signaling to E-cadherin-catenin and integrin-extracellular matrix complexes (Retta et al., 2006; Pannekoek et al., 2009; Noda et al., 2010; Suh and Han, 2010; Fig. 7), reduction of endothelial permeability through Rap1 activation by Epac1 most likely reflects its signaling property to the actin-microtubule network. Several studies indicate that Epac promotes microtubule elongation and thereby enhances the endothelial barrier through VE-cadherin and integrins (Sehrawat et al., 2008, 2011). As earlier studies indicate that microtubulin enhances the exchange activity of Epac toward Rap1 (Magiera et al., 2004; Gupta and Yarwood, 2005; Borland et al., 2006, 2009), the interaction of Epac with the actin-microtubule network seems to be bidirectional in nature. In addition, interaction of Epac with ERM proteins seems to serve as an additional molecular link to promote cell adhesion (Gloerich et al., 2010; Ross et al., 2011), a process known to rely on the communication between integrins and extracellular matrix components (Pannekoek et al., 2009; Fehon et al., 2010). ERM proteins require for their full activation PIP_2, which is produced by phosphatidyl-4-phosphate

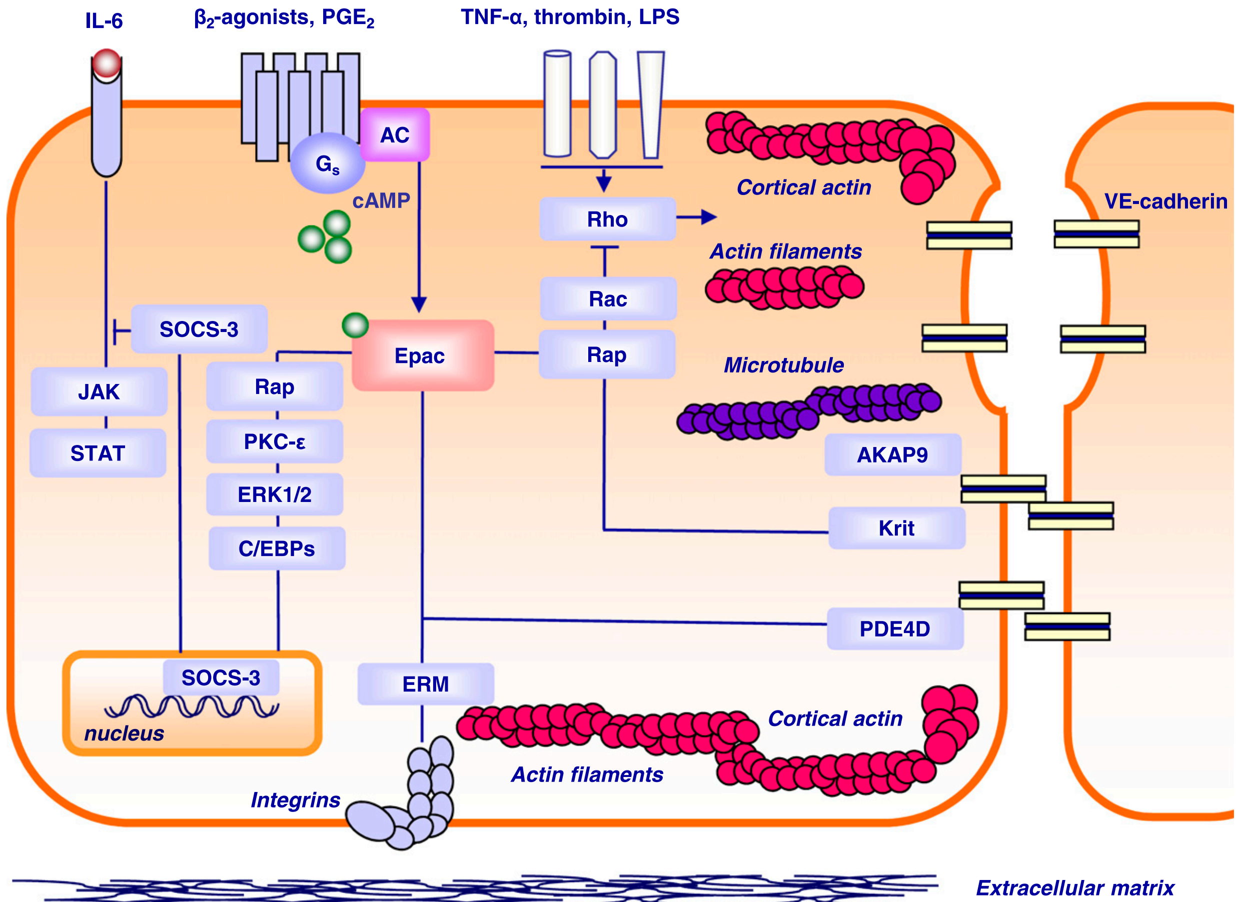


Fig. 7. Epac and the vasculature. Barrier disrupting agents, such as TNF-α, thrombin, and LPS, signal to Rho and subsequently reduce the VE-cadherin-dependent endothelial barrier through a reduced stability of cortical actin and microtubules as well as an increased formation of actin stress fibers. Epac reduces endothelial barrier leakage through skewing the balance of RhoA/Rac1 activation toward Rac1, thereby leading to a reduction of the phosphorylation of the Rho-Rho-kinase target myosin light chain and subsequent reduction of actin stress fiber formation. In addition, Epac promotes microtuble growth. Epac directly interacts with PDE4D at VE-cadherin-bearing cell-cell contacts, a process further supporting its endothelial barrier stabilizing function. Interaction of Epac with AKAP9 represents a direct link between VE-cadherin and integrins. The Epac effector Rap1 stabilizes β-catenin-bearing cell-cell contacts through Krit. Targeting of Epac to integrins at junctional sites seems to be supported through ERM-Epac interactions. Epac limits proinflammatory IL-6 actions through induction of the suppressor of IL-6 receptor signaling SOCS-3 and subsequent inhibits JAK-STAT signaling through Rap-PKC-ε-ERK1/2-C/EBPs. C/EBP, CCAAT/enhancer-binding protein; JAK, Janus kinase; Krit, Krev1 interaction trapped gene; LPS, lipopolysaccharide; PDE4D, phosphodiesterase 4D; STAT, signal transducer and activator of transcription; TNF-α, tumor necrosis factor-α.

5-kinases in a phosphatidic acid-sensitive manner (Oude Weernink et al., 2000, 2007; Weernink et al., 2004). Recent findings indicate that subcellular localization of Epac1 is determined by its interaction with phosphatidic acid (Consonni et al., 2012; Gloerich et al., 2012). The phosphatidic acid-producing PLD1 acts as an Epac effector and represents a direct molecular link to the actin cytoskeleton through phosho-cofilin (López de Jesús et al., 2006; Han et al., 2007). Thus, interaction of Epac with the actin-microtubule network might be rather diverse in nature and therefore contribute to the sophisticated mechanisms leading to proper endothelial barrier function and cell adhesion.

2. Other Endothelial Functions Regulated by Epac. In vascular endothelial cells, Epac1 induces the transcriptional activity of CCAAT/enhancer-binding protein through protein kinase Cα and ERK1/2 (Yarwood et al., 2008; Borland et al., 2009; Woolson et al., 2009) and thereby promotes the expression of SOCS-3. Epac1-dependent induction of SOCS-3 inhibits the IL-6 receptor trans-signaling complex and subsequently limits proinflammatory actions of IL-6 in vascular endothelial cells (Parnell et al., 2012; Wiejak et al., 2012; Fig. 7). It is noteworthy that Epac1 thereby protects the vascular endothelium from IL-6-induced dysfunction, at least in part through inhibition of the JAK-STAT pathway. In the human promonocytic cell line U937, fibrinogenic TGF-β reduced the expression of Epac1 transcript and thereby reduced the transmigratory capacity of monocytes (Basoni et al., 2005), suggesting that Epac achieves limitations of proinflammatory signals through elaborated effects on both leukocytes and endothelial cells.

In addition, modulation of actin-myosin contractility by Epac can also be envisioned. Myosin IIA and IIB are primarily expressed at cell-cell contacts (De La Cruz and Ostap, 2004), and myosin, as part of the circumferential belt and bounded to actin, can control the shape of the cell via this belt. In MCF7 breast epithelial cells (Smutny et al., 2010; Fig. 8), junctional localization of myosin IIA requires next to E-cadherin adhesion, Rho-Rho-kinase, and myosin light chain-kinase activation, and thereby subsequently increase the contractile force of circumferential belt and tight junction integrity. Myosin IIB, via Rap1A, supports myosin IIA-Rho-Rho-kinase signaling to E-cadherin and to myosin light chain kinase and thereby also subsequently induces the stabilization of the apical ring structure and enhancement of the junctional integrity (Smutny et al., 2010; Fig. 8). In support of the findings in endothelial cells, transformation of the human pancreatic carcinoma epithelial-like cell line PANC-1 with constitutively active Rac1 (V12) redistributes E-cadherin-β-catenin complexes to cell-cell contacts through IQ motif containing (IQ)GAP1, whereas cell transformation with dominant negative Rac1 (N17) exerts opposing effects (Hage et al., 2009). Together with the finding that IQGAP1 binds the Epac effector Rap1 (Jeong et al., 2007), Epac-dependent compartmentalized cAMP signaling might require scaffolding proteins including IQGAPs. Thus, Epac, by activation of Rap, may regulate barrier function of endothelial as well as epithelial cells. Several recent findings point also to a proper RhoA versus Rac1 signal balance (Lawrence et al., 2002; Lorenowicz et al., 2007a,b; Baumer et al., 2008a,b; Sayner, 2011). Thus, Rap1 and Rho GTPases RhoA and Rac1 capture key functions to preserve structural and functional properties of tight and adherens junctions (Fig. 7). Confusingly, Rac1 promotes both endothelial barrier stabilization and destabilization (Pullar et al., 2004; Netherton et al., 2007; Raymond et al., 2007; Baumer et al., 2008b; Rampersad et al., 2010; Spindler et al., 2011; Spindler and Waschke, 2011). Distinct barrier protection properties of Rac1, however, in microvascular versus macrovascular endothelial cells seem to be reasonable (Spindler et al., 2011).

3. Epac and Vascular Smooth Muscle Functioning. Epac seems to be to able alleviate symptoms associated with vascular dysfunctions, including atherosclerosis and (pulmonary) hypertension (Yokoyama et al., 2008a; Frumkin, 2012; Hirase and Node, 2012), through targeting vascular smooth muscle functions. In rat aortic smooth muscle cells, Epac inhibits ATP-sensitive K^{+}-channels through Ca^{2+}-dependent calcineurin (Purves et al., 2009), suggesting that Epac, in particular Epac1 being primarily expressed in vascular smooth muscle, controls vascular tone and blood flow. Indeed, specific activation of Epac induces relaxation of rat aorta (Sukhanova et al., 2006) and rabbit and rat pulmonary aorta (Murray et al., 2009b; Zieba et al., 2011) through downregulation of RhoA-dependent myosin light chain phosphatase phosphorylation (Zieba et al., 2011; Fig. 8B). Thus, Epac alters the Rho/Rac balance and thereby regulates biologic responses that rely on actin-myosin signals. Interestingly, the mRNA and protein expression of both Epac1 and Epac2 are downregulated in patients with pulmonary hypertension as well as in rats with monocrotalin-induced pulmonary hypertension (Murray et al., 2009; Table 1). The functional consequence of the reduced Epac expression is clear from the reduced ability of the Epac activator 8-pCPT-2′-*O*-Me-cAMP to relax pulmonary arteries from monocrotalin-treated mice (Murray et al., 2009). Intriguingly, Epac-mediated arterial smooth muscle relaxation also involves activation of nitric oxide synthase in the endothelium (Garcia-Morales et al., 2012). Loss of Epac1 expression in the endothelium due to hypoxia (Garcia-Morales et al., 2012), which may induce pulmonary hypertension by inducing vasoconstriction, could contribute to the development of pulmonary hypertension.

In addition to regulating vascular tone, Epac induces adhesion of microvascular smooth muscle cells and promotes vascular smooth muscle migration (Yokoyama

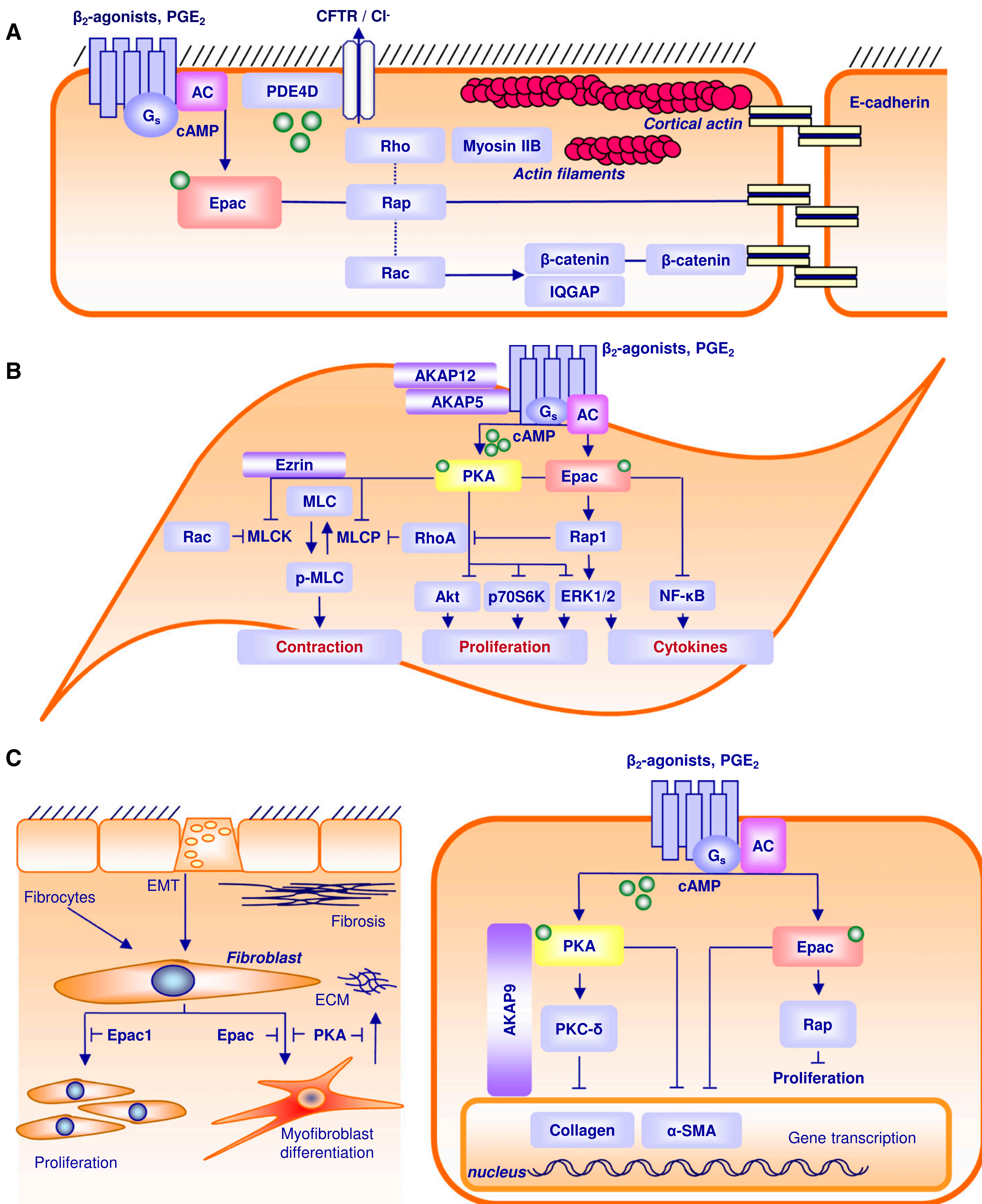


Fig. 8. Epac and the lung. In airway epithelium, elevation of cAMP in response to β_2-agonists and prostanoids activates Rap through Epac and thereby promotes signaling through Rho and Rac via yet unidentified mechanisms. Rac destabilizes the IQGAP-β-catenin complex and releases β-catenin to E-cadherin-bearing cell-cell contacts, a process known to act in concert with Rap-dependent E-cadherin recruitment and subsequently to stabilize the epithelial barrier. Rho, in concert with myosin IIB, enhances the stability of actin filaments and cortical actin through phosphorylation of myosin light

et al., 2008a; Eid, 2012). Thus, specific activation of Epac as well as overexpression of Epac1 induces migration of rat aortic smooth muscle cells via Epac1-mediated activation of Rap1 (Yokoyama et al., 2008a). By contrast, specific activation of PKA inhibits cell migration. Interestingly, the AC activator forskolin induces migration at lower concentrations and inhibits migration at higher doses (Yokoyama et al., 2008a), indicating cAMP effector selectivity at different cAMP concentrations. The importance of Epac1 in cell migration is supported by the finding that Epac1, but not Epac2, is upregulated during neointima formation following vascular injury in mice (Yokoyama et al., 2008a; Table 1). Furthermore, Epac inhibits vascular smooth muscle proliferation either alone and/or concert with PKA through JNK-ERK (Hewer et al., 2011; Mayer et al., 2011). Together with the finding that Epac induces the early gene nuclear receptor subfamily 4, which seems to act anti-atherogenic (Mayer et al., 2011), these findings indicate that Epac may modulate phenotypical modulation of vascular smooth muscle cells. Conversely, Epac also bears the capacity to mediate procontractile functions in the vasculature, as Epac1 enhances the expression of the α_{2C}-adrenergic receptor through a Rap1-RhoA signaling pathway (Jeyaraj et al., 2012). In conclusion, these findings indicate that Epac exhibits several distinct biologic responses in vascular smooth muscle cells, the relative net outcome may even differ from the vessel type.

4. Regulatory Role of the Epac Signalosome in the Vasculature. In human dermal microvascular endothelial cells, cAMP-dependent Rac1 activation requires both vasodilator-stimulated phosphoprotein and AKAP-anchored PKA (Baumer et al., 2008b; Spindler et al., 2010, 2011; Spindler and Waschke, 2011). Rac1 activation through AKAP-anchored PKA is sensitive to the PKA-binding blocking peptide st-Ht31 (Schlegel and Waschke, 2009). PKA-dependent phosphorylation of vasodilator-stimulated phosphoprotein contributes to the barrier protection (Bogatcheva et al., 2009). In HUVECs, PDE4D-bearing VE-cadherin-based multiprotein complexes control the vascular permeability, a process requiring Epac1 (Raymond et al., 2007; Rampersad et al., 2010; Fig. 7). In human microvascular endothelial cells, Epac induces activation of Rac1, enhancement of the barrier function, and redistribution of VE-cadherin to cell-cell contacts (Baumer et al., 2008b; Spindler et al., 2011). On the molecular level, mechanisms leading to Rac1 activation through Epac1 are solved in human pulmonary artery endothelial cells in vitro and in ventilator-induced lung injury in vivo (Birukova et al., 2007, 2008, 2009, 2010; Xing and Birukova, 2010). The cAMP elevating compounds PGE_2, prostacyclin I_2, and the atrial natriuretic peptide activate Rac1 through Epac1, Rap1, the Rac-specific exchange factors Tiam1-Vav2 signals, and p115 Rho-GEF-dependent RhoA inhibition, processes acting in concert with PKA (Fig. 7). Thus, protection of the endothelial barrier by Epac1 resembles mechanisms known to induce smooth muscle relaxation (Roscioni et al., 2011b; Zieba et al., 2011; Fig. 8). A reduction of the phosphorylation of the Rho-Rho-kinase target myosin light chain skews the balance of RhoA/Rac1 activation toward Rac1.

Earlier studies reported on the first molecular link of Epac and the actin-microtubule and its impact on the regulation of the barrier function in HUVECs and human pulmonary aortic endothelial cells (Cullere et al., 2005; Fukuhara et al., 2005; Sehrawat et al., 2008; Noda et al., 2010). Recent studies demonstrate that Krit1 (Krev1 interaction trapped gene) is required for the stabilization of β-catenin-bearing cell-cell contacts by the Epac effector Rap1 and that the Epac-Rap1 effector Krit1 is required for the maintenance of the endothelial barrier (Glading et al., 2007; Stockton et al., 2010). Loss of Krit1, known to account for the endothelial junction disease cerebral cavernous malformations (Stockton et al., 2010), induced destabilization of the endothelial barrier by increasing the phosphorylation of the Rho-Rho-kinase target myosin light chain (Fig. 7). Although the molecular link to Epac has still to be studied, these findings implicate that Epac-bearing multiprotein complexes are of utmost importance for endothelial barrier maintenance.

Recent studies in HUVECs and human dermal microvascular endothelial cells, indeed, indicate that AKAP9 (also known as AKAP450) complexes with Epac1, promotes microtubule growth to coordinate integrin signaling at lateral cell borders, and thereby

chain. PDE4D creates a functional cAMP diffusion domain and thereby promotes cystic fibrosis transmembrane conductance regulator (CFTR) channel function, a process supported by the cortical actin (A). Elevation of cAMP in response to β_2-agonists and prostanoids activates both PKA and Epac and thereby induces airway smooth muscle relaxation, inhibits airway smooth muscle proliferation, and modulates cytokine secretion. Epac induces airway smooth muscle relaxation through inhibition of RhoA and activation of Rac1, a process that may involve the dual-specific AKAP ezrin known to modulate Rho-Rho-kinase. PKA induces airway smooth muscle relaxation through signaling to MLCK and MLCP. Epac and PKA inhibit airway smooth muscle proliferation and cytokine secretion through distinct signaling to PKB/Akt, p70S6K, ERK1/2, and NF-κB (B). Activation of PKA and Epac differentially inhibits specific fibroblast functions; activation of PKA inhibits airway fibroblast collagen synthesis, whereas activation of Epac inhibits fibroblast proliferation. Both PKA and Epac contribute to the inhibition of fibroblast transdifferentiation to myofibroblasts in response to β_2-agonists and prostanoids. PKA inhibits collagen synthesis by inhibiting the profibrotic kinase PKC-δ, a process supported by AKAP9. Epac activates Rap1, which subsequently inhibits fibroblast proliferation. Both PKA and Epac inhibit transdifferentiation and thus α-smooth muscle actin (α-SMA) expression. The molecular mechanisms involved, however, remain to be determined. C/EBP, CCAAT/enhancer-binding protein; CFTR, cystic fibrosis transmembrane conductance regulator; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; Krit, Krev1 interaction trapped gene; MLC, myosin light chain; MLCK, myosin light chain kinase; MLCP, myosin light chain phosphatase; PDE4D, phosphodiesterase 4D; PKB/Akt, protein kinase B/Akt

enhances endothelial barrier properties of Epac1 (Sehrawat et al., 2011; Fig. 7). Surprisingly, AKAP9 does not alter Epac1-dependent GTP-loading of Rap1, reorganization of cortical actin, and de novo VE-cadherin adhesion (Sehrawat et al., 2011), suggesting that AKAP9 integrates functions of VE-cadherin and integrins at junctional sites to enhance the endothelial barrier in response to activation of Epac (Fig. 7). Thus, several recent studies indicate that compartmentalization of cAMP signaling in endothelial cells (as reviewed by Feinstein et al., 2012) might turn out to be of key importance for the maintenance of central endothelium-associated signaling properties including a proper and tight control of the endothelial barrier.

E. Epac and the Lung

Asthma and COPD are both chronic inflammatory airways diseases characterized by airway obstruction and airway remodeling, albeit with different etiology and specific pathologic features (Barnes, 2008; Hogg and Timens, 2009). Next to inflammatory cells, structural cells, including epithelial cells, airway smooth muscle cells and (myo)fibroblasts, interactively regulate key airway responses (Hogg and Timens, 2009; Hurst et al., 2010; van den Berge et al., 2011). Next to anti-inflammatory drugs, compounds that elevate intracellular levels of cAMP, particularly β_2-agonists and PDE4 inhibitors, represent current disease treatment. Recent studies report on signaling of Epac in the lung and strengthen the notion that compartmentalized cAMP is controlled by a distinct subset of cAMP-sensing multiprotein complexes that permit cell-type specific functions.

1. Epac and the Airway Epithelium. The airway epithelium constitutes protection through its barrier, mucus production, ciliary beating, antimicrobial production, and immune responses to deleterious agents (Knight and Holgate, 2003; Lai and Rogers, 2010). Airway epithelial barrier disruption leads to epithelium remodeling, goblet cell metaplasia, and mucus gland hypertrophy in asthma and COPD (Fahy and Dickey, 2010). The cAMP signaling pathway is thought to regulate the barrier function (see also section II.D), although the molecular mechanisms that regulate the epithelial barrier are less characterized. In a mouse model of allergic asthma, chronic application of β_2-adrenoceptor inverse agonists reduces airway epithelial barrier dysfunctions (Callaerts-Vegh et al., 2004; Lin et al., 2008; Nguyen et al., 2008, 2009; Dickey et al., 2010; Walker et al., 2011), suggesting that ligand-directed signaling or biased agonism is operational in the airway epithelium. In polarized human Calu-3 airway epithelial cells, a functional cAMP diffusion barrier maintained by PDE4D determines the function of cystic fibrosis transmembrane conductance regulator chloride channel (Barnes et al., 2005), indicating that functional responses to cAMP in airway epithelial cells rely on cAMP microdomains (Fig. 8A). Indeed, in human airway epithelial cells, regulation of the cystic fibrosis transmembrane conductance regulator requires next to the subcortical cytoskeleton compartmentalized cAMP signaling (Monterisi et al., 2012). Different ACs and PDEs are expressed in airway epithelium (Defer et al., 2000; Hanoune and Defer, 2001; Small et al., 2003; Tantisira et al., 2005; Pierre et al., 2009), which may have the potential to generate cAMP-sensing multiprotein complexes exhibiting a distinct composition to maintain specific airway epithelium functions. In human bronchial epithelial cells, the COPD inducer cigarette smoke disrupts the epithelial barrier and specifically downregulates AKAP9 (Oldenburger et al., 2011). As recent studies report on the maintenance of the endothelial barrier through AKAP9 (Sehrawat et al., 2011; Fig. 7), these findings indicate that AKAP9 is a key regulator of both the endothelial and epithelial barrier. The role of Epac in these processes is currently unknown. However, Epac1 and Epac2 as well as the Epac effectors Rap1 and Rap2, which regulate epithelial integrity in *Caenorhabditis elegans* (Pellis-van Berkel et al., 2005), are expressed in human bronchial epithelial cells (S.S. Roscioni, C.R.S. Elzinga, I.S.T. Bos, M.H. Menzen, F.J. Warnders, H. Maarsingh, A.J. Halayko, H. Meurs, M. Schmidt, unpublished observations) and may therefore be involved in the cAMP-mediated effects on the epithelial barrier. Taken together, compartmentalized cAMP signaling seems also to account for the proper functioning of the epithelial barrier.

2. Regulation of Airway Smooth Muscle Tone and Secretory Function by the Epac Signalosome. Airway smooth muscle cells control airway caliber and contribute to bronchoconstriction, local inflammation, wound healing, and remodeling (Hirst, 2003; Halayko et al., 2008; Damera and Panettieri, 2011; Billington et al., 2012). Classically, the bronchodilating effect of β_2-agonists was assumed to be mediated by PKA, which reduces contractile force through inhibition of myosin light chain phosphorylation (Pfitzer, 2001; Giembycz and Newton, 2006; Penn, 2008; Billington et al., 2012). However, it has been shown that additional PKA-independent mechanisms contribute to airway smooth muscle relaxation (Spicuzza et al., 2001). Recent studies in airway smooth muscle preparations show that Epac, presumably by downstream activation of Rap1B, inhibits myosin light chain phosphorylation through RhoA inhibition and Rac1 activation causing relaxation (Roscioni et al., 2011a; Zieba et al., 2011; Fig. 8B). Regulation of smooth muscle contraction requires a tightly controlled network of the actin-microtubule cytoskeleton (Olson and Nordheim, 2010), most likely acting in concert with members of the ERM family known to regulate cytoskeleton driven processes, including migration, motility, and contraction (Niggli and Rossy, 2008; Fehon et al., 2010). In airway

smooth muscle, a recent study report on the expression of the dual-specific AKAP protein ezrin and its impact on compartmentalized cAMP signaling (Horvat et al., 2012). Ezrin interacts with Rho-Rho kinase signaling (Bretscher et al., 2002; Niggli and Rossy, 2008; Gloerich et al., 2010), suggesting that ezrin contributes to the regulation of airway smooth muscle contraction by regulating calcium sensitivity. Future studies should address the potential role of ezrin in mediating cAMP-induced airway smooth muscle relaxation and its relation with Epac.

Airway smooth muscle cells synthesize and release multiple mediators, including extracellular matrix proteins and cytokines-chemokines (Hirst, 2003; Damera and Panettieri, 2011). In airway smooth muscle, the cAMP-elevating compounds PGE_2 and β_2-agonists increase the release of the chemokines IL-6 and IL-8 in response to TNF-α and bradykinin (Huang et al., 1998; Pang and Knox, 1998; Ammit et al., 2002; Roscioni et al., 2009). Recent studies indicate that both Epac1 and Epac2 as well as PKA enhance bradykinin-induced IL-8 release through Rap1 and ERK1/2 (Roscioni et al., 2009). Thus, Epac bears the potential to potentiate inflammation in airway smooth muscle cells. Conversely, Epac, acting in concert with PKA, inhibits IL-1β-induced release of GM-CSF (granulocyte-macrophage colony-stimulating factor), RANTES (regulated on activation, normal T cell expressed, and secreted) and eotaxin, and reduced IL-1β-induced ICAM1 expression (Hallsworth et al., 2001; Ammit et al., 2002; Clarke et al., 2004; Kaur et al., 2008). Recent studies in human airway smooth muscle show that both Epac1 and Epac2 limit the release of IL-8 in response to cigarette smoke extract through inhibition of NF-κB, whereas the inhibitory effect of PKA is mediated via inhibition of ERK1/2 (Oldenburger et al., 2012; Fig. 8B). Importantly, the expression of Epac1, but not Epac2, is reduced in cigarette smoke extract-exposed airway smooth muscle cells and lung tissue from COPD patients (Oldenburger et al., 2012; Table 1), suggesting that cigarette smoke may reduce the anti-inflammatory effects of cAMP by downregulation of Epac1. The cigarette smoke-induced downregulation of Epac1 resembles the effect of TGF-β on the Epac1 expression level (Yokoyama et al., 2008c); however, the underlying molecular mechanisms still have to be defined. The notion that, depending on the stimulus, Epac may act both proinflammatory and anti-inflammatory is rather exciting. The recent finding that airway smooth muscle cells also express AKAP family members, including ezrin, AKAP79 (AKAP5), and AKAP250 (AKAP12) (Horvat et al., 2012; Poppinga et al., 2012) suggests that cAMP compartmentalization through distinct AKAPs determines inflammatory properties of Epac (Fig. 8B).

3. Epac as a Target to Treat Airway Remodeling. Airway wall remodeling in asthma is characterized by increased airway smooth muscle mass through both hyperplasia and hypertrophy and airway fibrosis (Ebina et al., 1993; Kuwano et al., 1993; Jeffery, 2001, 2004; Benayoun et al., 2003; Woodruff et al., 2004; Dekkers et al., 2009) and leads to lung function decline and airway hyperresponsiveness (Lambert et al., 1993; Saetta et al., 1998; Hogg et al., 2004; Oliver et al., 2007). Exposure to mitogenic stimuli switches the airway smooth muscle phenotype from a (normo)contractile to a proliferative, hypocontractile state characterized by increased expression of proliferative markers and decreased contractile responses associated with decreased expression of contractile proteins, a process reversible in nature (Halayko and Amrani, 2003, Dekkers et al., 2009). Mitogen-induced airway smooth muscle cells proliferation is reduced in response to β_2-agonists and even more pronounced in response to PGE_2 (Tomlinson et al., 1994; Stewart et al., 1999; Lee et al., 2001; Kassel et al., 2008; Roscioni et al., 2011a; Yan et al., 2011). PGE_2 may act antimitogenic or promitogenic via distinct EP receptor subtypes (Yan et al., 2011). Inhibition of airway smooth muscle proliferation does not correlate with the generation of cAMP in response to β_2-agonists and PGE_2 (Kassel et al., 2008; Yan et al., 2011), pointing to the contribution of compartmentalized cAMP signaling. In primary human lung fibroblasts, PGE_2 and the PDE inhibitors roflumilast and piclamilast diminish fibroblast proliferation and myofibroblast differentiation (Selige et al., 2010, 2011). The PDE4 inhibitor rolipram also reduced epithelial-mesenchymal transition (Kolosionek et al., 2009). PGE_2-induced cAMP elevation exerts antifibrotic properties in human and mouse lung fibroblasts, and defects in the synthesis of PGE_2 correlate with the degree of airway remodeling in mice (Bauman et al., 2010; Okunishi et al., 2011; Stumm et al., 2011). PGE_2 also inhibits collagen deposition in fibrotic lung fibroblasts through a PGE_2-sensing AKAP450 (AKAP9)-PKA-protein phosphatase 2A multiprotein complex (Okunishi et al., 2011; Fig. 8C). The extracellular mediator plasmin profoundly alters the AKAP complex signaling properties, restoring PGE_2 sensitivity and subsequent inhibition of activated fibroblasts (Okunishi et al., 2011). Interestingly, a distinct subset of AKAPs family members directs PGE_2 toward the enhancement of toll-like receptor signaling (Kim et al., 2011) and may therefore be important for asthma and COPD pathophysiology. As AKAP9 has been reported to complex with PDE4 (Tasken et al., 2001), the presence of PDE4 in the PGE_2-sensing AKAP9 complex in lung fibroblasts awaits additional investigations. The distinct composition of the cAMP-responsive multiprotein complexes is expected to generate and maintain local cAMP gradients in a cell-type specific fashion.

Several recent studies indicate that next to PKA (Misior et al., 2008; Yan et al., 2011), Epac contributes to the inhibition of cell proliferation (Kassel et al., 2008;

Roscioni et al., 2011a). Epac-induced antiproliferative effects associated with the normalization of mitogen-induced hypocontractility and maintenance of normal expression of contractile protein (Roscioni et al., 2011a,c). Intriguingly, Epac inhibits phenotype switching by inhibition of ERK1/2, whereas PKA inhibits ERK1/2 and phosphoinositide 3-kinase (Roscioni et al., 2011a, Fig. 8B). As mentioned above, expression of a distinct subset of AKAPs in human airway smooth muscle (Horvat et al., 2012; Poppinga et al., 2012) and bronchial epithelial cells (Oldenburger et al., 2011; Schmidt et al., 2011) maintains compartmentalized cAMP signaling of PKA and Epac and modifies functional responses. Collectively, Epac inhibits mitogen-induced phenotypic switching of airway smooth muscle cells, which may potentially be regulated AKAP-containing complexes. Alterations in spatiotemporal dynamics of cAMP may therefore profoundly alter biologic responses of airway smooth muscle. Future studies should define the precise impact of AKAP-Epac-PKA interactions on airway smooth muscle function and responses to pharmacological interventions.

Airway fibrosis encompasses increased deposition of extracellular matrix proteins in response to abnormal wound repair through excessive recruitment and activation of (myo)fibroblasts from resident mesenchymal cells, circulating fibrocytes, and/or epithelial-to-mesenchymal transition (Postma and Timens, 2006; Salazar and Herrera, 2011; Wynn, 2011). Resident fibroblasts are transformed upon "activation" into the more contractile, proliferative, and secretory active myofibroblasts, a process importantly driven by TGF-β (Bartram and Speer, 2004; Leask and Abraham, 2004; Meneghin and Hogaboam, 2007). In human lung fibroblasts, cAMP-elevating compounds, such as PGE_2, EP_2 receptor agonists, β-adrenoceptor agonists, the direct AC activator forskolin, and PDE inhibitors, suppress the transdifferentiation into myofibroblasts as indicated by a reduced expression of α-smooth muscle actin (Kolodsick et al., 2003; Liu et al., 2004; Baouz et al., 2005; Dunkern et al., 2007; Thomas et al., 2007; Lamyel et al., 2011). The PKA agonist 6-Bnz-cAMP and the Epac agonist 8-CPT-2′-*O*-Me-cAMP also reduce the expression of α-smooth muscle actin (Lamyel et al., 2011), suggesting that fibroblast transformation is regulated by PKA and Epac (Fig. 8C).

In MRC-5 lung fibroblasts, expression of both Epac1 and Epac2 mRNA is shown (Haag et al., 2008), although protein expression was only observed for Epac1 (Huang et al., 2007, 2008; Haag et al., 2008). Unfortunately, our current knowledge about the fibroblast-specific expression profile of Epacs is predominantly based on studies in human fetal lung fibroblasts, and only a rather limited amount of studies point to a (functional) role of Epacs in primary adult human lung fibroblasts (Huang et al., 2007, 2008). In (primary) human lung fibroblasts PKA and Epac differentially regulate fibroblast proliferation and collagen synthesis. Epac agonists only inhibit fibroblast proliferation, presumably through Rap1, to a similar extent as β_2-agonists and prostanoids, whereas PKA activation only inhibits collagen synthesis, presumably via inhibition of PKC-δ (Zhang et al., 2004; Huang et al., 2007, 2008; Haag et al., 2008; Fig. 8C). The effects of Epac are most likely mediated via Epac1, as downregulation of Epac1, but not Epac2, largely prevented the inhibitory effects of butaprost and 8-pCPT-2′-*O*-Me-cAMP on fibroblast proliferation (Huang et al., 2007, 2008; Haag et al., 2008). Interestingly, relative to Epac2, expression of Epac1 becomes dominant in the adult lung compared with fetal tissue (Ulucan et al., 2007), suggesting that Epac's function in the lung may alter during development. On the basis of our current knowledge of the expression profile of Epac1 and Epac2 in lung fibroblasts, it is tempting to assign Epac-dependent functional responses to Epac1. In this respect it is important to note that TGF-β reduces the expression of Epac1 in rat lung fibroblasts (Yokoyama et al., 2008c; Table 1). Because a direct interaction between Epac1 and TGF-β1 has been observed with subsequent inhibition of Smad-dependent TGF-β signaling (Conrotto et al., 2007), it is tempting to speculate that loss of Epac1 expression by TGF-β promotes the transformation of resident fibroblasts into active myofibroblasts due to a dysfunction in the dynamic signaling features of the endogenous suppressor cAMP.

In conclusion, Epac plays an important role in regulating airway functions, such as inflammatory cytokine release, cell proliferation, airway smooth muscle contraction, and the production of extracellular matrix proteins. In vivo studies using transgenic mice and/or pharmacological agents targeting the Epac pathway are needed to further clarify the role of Epac in the pathophysiology and treatment of airways diseases, such as asthma and COPD.

III. Concluding Remarks and Future Directions

The discovery of Epac has ignited a new epoch of research designated to further decipher formerly unexpected signaling properties of the "old" second messenger cAMP and adds another layer of complexity that permit a tightly controlled fine tuning of pivotal biologic processes. X-ray crystallography and NMR spectroscopy provide the first molecular clues about a sophisticated dynamic equilibrium model of Epacs' closed (autoinhibited or inactive) and open (active) states (Rehmann et al., 2006; Das et al., 2008). Surely, currently developed novel fluorescence imaging techniques are a solid fundament for translational studies to visualize the complex temporal dynamics of Epac's activation in vivo.

Since the discovery of Epac in 1998, research into its signaling properties denotes two scientific milestones.

First, the development of the specific Epac activators 8-pCPT-2′-*O*-Me-cAMP (Enserink et al., 2002; Rehmann et al., 2006, 2007) and its prodrug 8-pCPT-2′-*O*-Me-cAMP-AM (Vliem et al., 2008), which exhibits improved cell permeability. Both compounds act as superagonists for both Epac1 and Epac2 (Enserink et al., 2002) and thereby deliver the first reliable insights into the biologic properties of Epac. The second milestone is the development of the first pharmacological Epac-selective antagonists (Chen et al., 2012a; Tsalkova et al., 2012a,b). The 5-cyano-6-oxo-1,6-dihydro-pyrimidines derivatives 6g (HJCO198) and 6h (HJCO197) act specifically on Epac but not on PKA, with even some preference of 6g (HJCO198) for Epac2 and 6h (HJCO197) for Epac1. Initial cell-based studies together with molecular docking data offer promising findings that these novel compounds will serve as a lead to further develop antagonists with improved subtype selectivity for Epac and thereby to substantiate biologic properties of Epac to unravel the role of the Epac signalosome.

Epac is linked to the progression of several devastating diseases, including cardiac hypertrophy, which may eventually lead to the development of heart failure. Epigenetic regulation of gene expression through selective nuclear export of HDAC isoenzymes (Pereira et al., 2012; Ruiz-Hurtado et al., 2012) may turn out to be an important mechanism for disease development, including heart failure. Post-translational regulation by microRNAs is a novel mechanism in the context of learning and memory impairments linked to Epac (Yang et al., 2012). Such mechanisms presumably offer clues into the highly dynamic developmental and disease-related expression profile of Epac. Future studies that unravel the molecular mechanisms underlying the loss of Epac1 in fibrosis and COPD are warranted (Yokoyama et al., 2008c; Oldenburger et al., 2012). Knockout mice of either Epac1 or Epac2 as well as Epacs' double knockout mice (Shibasaki et al., 2007; Suzuki et al., 2010; Srivastava et al., 2012a; Yang et al., 2012) substantiate the notion that Epac is indispensable for proper functioning of pancreatic β-cells and neuronal functions to prevent the development of diabetes and Alzheimer's disease. Such studies support a link between the release of insulin from pancreatic β -cells and Epac2 (Shibasaki et al., 2007). However, it is still unclear whether Epac2, acting either alone or in concert with Epac1, is responsible for synaptic plasticity, memory, and learning (Srivastava et al., 2012; Yang et al., 2012). The development of additional conditional and temporal transgenic mouse models as well as agonists and antagonists with improved subtype selectivity for Epac1 and Epac2 will support a more detailed assignment of biologic functions to either Epac1 or Epac2 or even unravel their concerted action. Importantly, successful in vivo application of the Epac activator 8-pCPT-2′-*O*-Me-cAMP in animal models of ischemia-induced kidney failure (intrarenal injection; Stokman et al., 2011) and memory retrieval in contextual fear conditioning (intrahippocampal infection; Ostroveanu et al., 2010) has been described. This demonstrates that this Epac activator maintains its biologic activity under physiologic conditions and thus can be used as pharmacological treatment.

The majority of biologic responses currently linked to Epac are dependent on its exchange activity toward Rap1 and Rap2; however, Epac also exerts signaling properties independent of its canonical GEF activity. The multidomain structure of Epac supports its interaction with newly identified interactions partners, including all members of the ERM family (Gloerich et al., 2010a; Ross et al., 2011) and the nuclear-pore associated small GTPase Ran and RanBP2 (Liu et al., 2010a; Gloerich et al., 2011). Such mechanisms underpin the highly dynamic nature of Epac-related biologic responses that require a tightly controlled network of the actin-microtubule cytoskeleton, processes nowadays linked to ERM proteins known to connect transmembrane events to the cytoskeleton. The link between Epac and phosphatidic acid (Consonni et al., 2012; Gloerich et al., 2012) further strengthens the interactions between ERM proteins and Epac by creating a positive feedforward loop. The complex protein-protein/protein-lipid interactions of Epac are linked to the existence of cAMP-sensing multiprotein complexes maintained by the AKAP family members, mAKAP, AKAP5, and AKAP9 (Dodge-Kafka et al., 2005; Nijholt et al., 2008; Sehrawat et al., 2011). Together with the potential link to cAMP microdomains maintained by sAC (Corredor et al., 2012), such complexes generate compartmentalized cAMP signaling and lead to cell-type specific functions of Epac. As AKAP are also the molecular interaction partner of PKA, AKAP-bearing complexes are the most logical explanation for biologic responses driven by both PKA and Epac. Future challenges are defined by the development of small molecules that specifically inhibit these protein-protein and/or protein-lipid interactions. Newly developed AKAP complex disruptors (Patel et al., 2010; Christian et al., 2011), small molecules inhibitors that disrupt membrane localization of the Epac signalosome (Dekker et al., 2010), and inhibitors of the epigenetic machinery are rather promising (Dekker and Haisma, 2009; Ghizzoni et al., 2011). Improvement of our knowledge on the cellular functions, (post)transcriptional modifications, and expression patterns of Epac proteins and the Epac signalosome will greatly facilitate the development of improved pharmacotherapy.

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Wrote or contributed to the writing of the manuscript: Schmidt, Dekker, Maarsingh.

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Correction to "Exchange Protein Directly Activated by cAMP (epac): A Multidomain cAMP Mediator in the Regulation of Diverse Biological Functions"

In the above article [Schmidt M, Dekker FJ, and Maarsingh H (2013) *Pharmacol Rev* **65:**670-709], the wording "to reduce" is incorrect on page 692, legend to Fig. 6, and on page 693, left column, line 5. The correct wording is "to increase."

The corrections are as follows:

Page 692, Fig. 6. Closure of ATP-sensitive K^+-channels (K_{ATP}) is promoted through Epac2-SUR1 interaction and Epac2-Rap-dependent stimulation of PLC-ε, the latter known to increase channel ATP sensitivity through the hydrolysis of PIP_2.

Page 693, left column, line 5. In addition, the hydrolysis of PIP_2 through Epac2 is expected to increase ATP-sensitive K^+-channels responsiveness to ATP and subsequently to promote their closure (Holz et al., 2006), leading to insulin secretion.

The online version of this article has been corrected.

The authors regret this error and any inconvenience it may have caused.