Topical Review

Cell physiology of cAMP sensor Epac

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Epac is an acronym for the exchange proteins activated directly by cyclic AMP, a family of cAMP-regulated guanine nucleotide exchange factors (cAMPGEFs) that mediate protein kinase A (PKA)-independent signal transduction properties of the second messenger cAMP. Two variants of Epac exist (Epac1 and Epac2), both of which couple cAMP production to the activation of Rap, a small molecular weight GTPase of the Ras family. By activating Rap in an Epac-mediated manner, cAMP influences diverse cellular processes that include integrin-mediated cell adhesion, vascular endothelial cell barrier formation, and cardiac myocyte gap junction formation. Recently, the identification of previously unrecognized physiological processes regulated by Epac has been made possible by the development of Epac-selective cyclic AMP analogues (ESCAs). These cell-permeant analogues of cAMP activate both Epac1 and Epac2, whereas they fail to activate PKA when used at low concentrations. ESCAs such as 8-pCPT-2′-*O*-Me-cAMP and 8-pMeOPT-2′-*O*-Me-cAMP are reported to alter Na^+, K^+, Ca^{2+} and Cl^- channel function, intracellular $[Ca^{2+}]$, and Na^+–H^+ transporter activity in multiple cell types. Moreover, new studies examining the actions of ESCAs on neurons, pancreatic beta cells, pituitary cells and sperm demonstrate a major role for Epac in the stimulation of exocytosis by cAMP. This topical review provides an update concerning novel PKA-independent features of cAMP signal transduction that are likely to be Epac-mediated. Emphasized is the emerging role of Epac in the cAMP-dependent regulation of ion channel function, intracellular Ca^{2+} signalling, ion transporter activity and exocytosis.

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The discovery and characterization of a novel cAMP signal transduction mechanism that uses the Epac family of cAMP 'sensors' to regulate multiple cellular functions has dramatically reinvigorated interest in cyclic nucleotide research (Bos, 2003). Actions of cAMP, which at one time were thought to be mediated exclusively by protein kinase A (PKA), must now be re-evaluated in the light of an accumulating body of evidence that indicates a likely role for Epac in cell physiology (Holz, 2004; Seino & Shibasaki, 2005). By serving as a cAMP-binding protein with intrinsic guanine nucleotide exchange factor (GEF) activity, Epac couples cAMP production to the activation of Rap, a small molecular weight GTPase of the Ras family (Fig. 1). Cellular processes stimulated as a consequence of the Epac-mediated activation of Rap include integrin-mediated cell adhesion (Rangarajan *et al.* 2003), vascular endothelial cell barrier formation (Fukuhara *et al.* 2005; Kooistra *et al.* 2005), cardiac gap junction formation (Somekawa *et al.* 2005), mitogen-activated protein kinase (MAPK) signalling (Wang *et al.* 2006), hormone gene expression (Gerlo *et al.* 2006; Lotfi *et al.* 2006), and phospholipase C-epsilon (PLC-ε) activation (Schmidt *et al.* 2001). Thus, Epac is an exchange protein activated directly by cyclic AMP (de Rooij *et al.* 1998; Rehman *et al.* 2006), or in an alternative terminology, a cyclic AMP-regulated guanine nucleotide exchange factor (cAMPGEF) (Kawasaki *et al.* 1998; Ozaki *et al.* 2000).

The Rap GTPases are not the only interesting molecules with which Epac interacts (Fig. 1). Epac is also reported to interact with Ras GTPases (Li *et al.* 2006; De Jesus *et al.* 2006), microtubule-associated proteins (Yarwood, 2005), secretory granule-associated proteins such as Rim2 and Piccolo (Ozaki *et al.* 2000; Fujimoto *et al.* 2002; Shibasaki *et al.* 2004*a*,*b*), and the sulphonylurea receptor-1 (SUR1), a subunit of ATP-sensitive K^+ channels (Ozaki *et al.* 2000; Shibasaki *et al.* 2004*a*,*b*; Kang *et al.* 2006). Some of these interactions may underlie the

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recruitment of Epac to an intracellular compartment that is rich in Rap GTPase. Alternatively, Epac may act as a multifunctional protein, one in which cAMP exerts its effects not simply by promoting guanyl nucleotide exchange on Rap, but by allosterically regulating key molecules involved in cell physiology. Intriguingly, newly published findings demonstrate Epac-mediated actions of cAMP that influence Na^+, K^+, Ca^{2+}, and Cl^- channel function, $[Ca^{2+}]_i$, Na^+–H^+ and Na^+–K^+ transporter activity, and exocytosis in multiple cell types (see below).

cAMP-binding properties of Epac

Epac1 is also known as cAMPGEF-I, whereas Epac2 is referred to as cAMPGEF-II (Fig. 2). Epac1 is most prominent in the brain, heart, kidney, pancreas, spleen, ovary, thyroid and spinal cord, whereas Epac2 is less ubiquitous and is most prominent in discreet regions of the brain, as well as the adrenal glands, liver and pancreatic islets of Langerhans (de Rooij *et al.* 1998; Kawasaki *et al.* 1998; Ozaki *et al.* 2000; Ueno *et al.* 2001). Epac1 contains a single cAMP-binding domain, whereas Epac2 contains two – a lower-affinity cAMP-binding domain of uncertain significance designated as 'A', and a higher-affinity cAMP-binding domain that is physiologically relevant and which is designated as 'B'. The K_d for binding of cAMP to Epac1 is 2.8 μM, whereas for Epac2 the 'A' and 'B' binding sites exhibit a K_d of 87 and 1.2 μM, respectively (de Rooij *et al.* 2000; Christensen *et al.* 2003). Thus, both Epac1 and Epac2 bind cAMP *in vitro* with an affinity similar to that of the PKA holoenzyme (K_d 2.9 μM; Dao *et al.* 2006).

Given that Epac is activated *in vitro* by micromolar concentrations of cAMP, some uncertainty existed as to whether the intracellular concentration of cAMP would be high enough to activate Epac. To address this issue, Epac-based cAMP sensors exhibiting Förster resonance energy transfer (FRET) have been developed. These sensors bind cAMP with an affinity similar to endogenous Epac. When expressed in living cells, Epac-based FRET sensors are activated by agents that stimulate cAMP production (DiPilato *et al.* 2004; Nikolaev *et al.* 2004; Ponsioen *et al.* 2004; Landa *et al.* 2005). For example, one such sensor (Epac1-camps) detects oscillations of $[cAMP]_i$ that occur in MIN6 insulin-secreting cells (Fig. 3). Thus, there is good reason to believe that micromolar fluctuations of $[cAMP]_i$ do occur in living cells, and that such fluctuations are coupled to the activation of Epac.

Development of Epac-selective cAMP analogues

An important advance is the synthesis and characterization of cAMP analogues that are cell permeant and which activate Epac but not PKA when used at low concentrations (Enserink *et al.* 2002; Kang *et al.* 2003). Selective activation of Epac is conferred by the substitution of an -*O*-Me group for the -OH group normally present at the 2′ carbon of the ribose moiety of cAMP (cf. Fig. 4*A* and *B*). Although this 2′-*O*-Me substitution impairs the interaction of cAMP with PKA, it allows the 2′-*O*-Me cAMP analogue to act as an agonist at Epac. Epac-selective cAMP analogues (ESCAs) include 8-pCPT-2′-*O*-Me-cAMP (Fig. 4*B*), 8-pMeOPT-2′-*O*-Me-cAMP (Fig. 4*C*), and 8-pHPT-2′-*O*-Me-cAMP (not shown). These ESCAs provide unique pharmacological tools with which to assess potential PKA-independent actions of cAMP that may be Epac mediated.

Validation that an ESCA acts via Epac can be achieved by demonstrating a biological activity of 8-pCPT-2′-*O*-Me-cAMP that is not mimicked by the PKA-selective cAMP analogue 6-Bnz-cAMP (Fig. 4*D*). The action of 8-pCPT-2′-*O*-Me-cAMP should also be insensitive to inhibitors of PKA catalytic activity

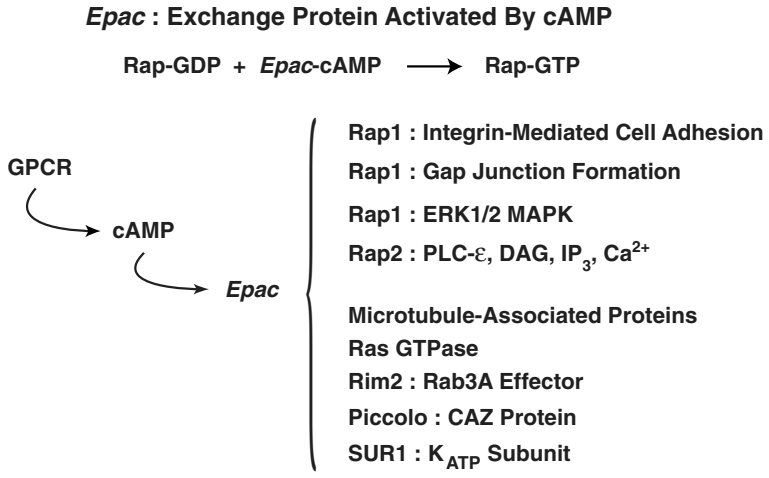

Figure 1. Signal transduction properties of Epac
When bound to cAMP, Epac catalyses the exchange of GDP for GTP on Rap GTPase. The activated form of Rap-GTP is then capable of promoting integrin-mediated cell adhesion, gap junction formation and ERK1/2 MAPK-mediated protein phosphorylation. Activated Rap also stimulates phospholipase C-epsilon (PLC-ε) which hydrolyses PIP_2 to generate diacylglycerol (DAG), and the Ca^{2+}-mobilizing second messenger IP_3. As illustrated, some actions of Epac may also be Rap independent. These actions of Epac may involve its interaction with microtubule-associated proteins, the Ras GTPases, secretory granule-associated proteins (Rim2, Piccolo), and the SUR1 subunit of K_{ATP} channels. Abbreviations: G protein coupled receptor, GPCR; cytoskeletal protein-associated with the active zone, CAZ; ATP-sensitive K^+ channel, K_{ATP}.

(H-89, KT5720, PKI), and moreover, the action of 8-pCPT-2′-*O*-Me-cAMP should be insensitive to Rp-cAMP, a cAMP analogue that blocks the activation of PKA by cAMP, but which does not prevent the activation of Epac in living cells (Dostmann *et al.* 1990; Eliasson *et al.* 2003; Kang *et al.* 2003, 2006; Rangarajan *et al.* 2003; Branham *et al.* 2006). Ruling out a role for PKA is necessitated by the fact that high concentrations ($> 100\ \mu$M) of 8-pCPT-2′-*O*-Me-cAMP can activate PKA, although with low efficacy (Christensen *et al.* 2003).

One impediment to the analysis of Epac signal transduction is that no specific pharmacological inhibitors exist with which to selectively block the binding of cAMP to Epac1 or Epac2. Furthermore, it is not yet possible to selectively inhibit the catalytic (GEF) function of Epac. To circumvent this problem, a molecular approach is available in which an Epac-mediated action of cAMP is inferred by demonstrating the failure of an ESCA to act in cells transfected with a dominant-negative Epac. These mutant forms of Epac fail to bind cAMP (Ozaki *et al.* 2000; Kang *et al.* 2001, 2005, 2006; Mei *et al.* 2002). Conversely, the action of an ESCA may be shown to be reproduced by a constitutively active Epac that is truncated to remove the cAMP-binding domain responsible for autoinhibition of the exchange factor's catalytic function (Morel *et al.* 2005). Although Epac knock-out mice are not yet reported to be available, it is possible to knock-down the expression of Epac using antisense oligodeoxynucleotides or small interfering RNA (siRNA). For the Epac2 expressed in pancreatic beta cells, the use of antisense oligodeoxynucleotides has revealed an important role for this exchange factor in the cAMP-dependent stimulation of insulin secretion (Kashima *et al.* 2001; Eliasson *et al.* 2003). For Epac1, the use of siRNA has revealed its role in the formation of endothelial cell tight junctions (Kooistra *et al.* 2005).

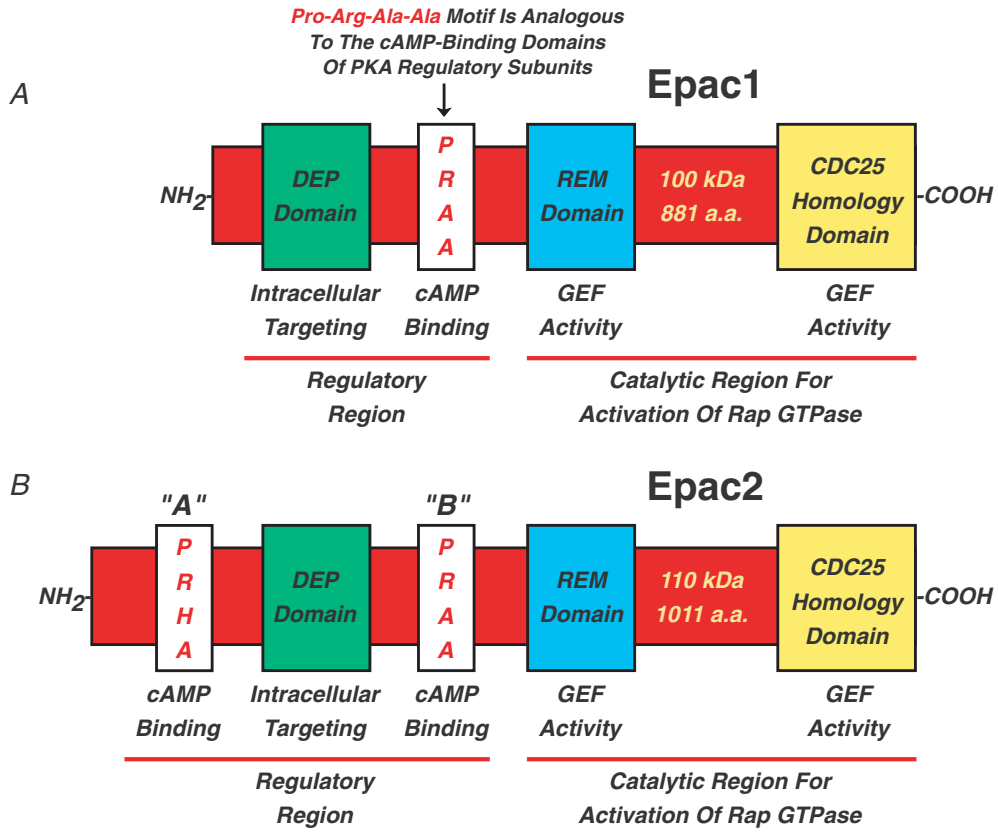

Figure 2. Molecular properties of the Epac family of cAMPGEFs
Epac1 is comprised of 881 amino acids (molecular mass 100 kDa), whereas Epac2 is comprised of 1011 amino acids (molecular mass 110 kDa). In the absence of cAMP, the regulatory region of Epac inhibits the guanine nucleotide exchange (GEF) function of the catalytic region. Binding of cAMP to Epac relieves this autoinhibition. The DEP domain of Epac located within the regulatory region contains sequence homologies to *d*isheveled, *E*gl I0 and *p*leckstrin. A Ras exchange motif (REM) and a CDC25 homology domain are found within the catalytic region. These two variants of Epac are coded for by two distinct genes, and evidence exists for both shorter and longer forms of the proteins (not shown).

Epac mediates the cAMP-dependent regulation of ion channel function

A previously unrecognized role for Epac in the cAMP-dependent regulation of ion channel function is now known to exist. One such example is the Epac-mediated inhibition of ATP-sensitive K^+ channels (K_{ATP} channels), as measured in pancreatic beta cells (Kang *et al.* 2006). Under conditions in which beta cells are dialysed with a low concentration of ATP, 8-pCPT-2′-*O*-Me-cAMP inhibits K_{ATP} channel activity, an effect not observed following transfection of cells with a dominant-negative Epac1. Interestingly, both Epac1 and Epac2 are shown to co-immunoprecipitate with SUR1, a subunit of the K_{ATP} channel (Kang *et al.* 2006). Thus, it has been proposed that Epac serves as an accessory subunit of K_{ATP} channels, possibly as a consequence of the binding of Epac to nucleotide binding fold-1 (NBF-1) of SUR1 (Ozaki *et al.* 2000; Shibasaki *et al.* 2004*a*,*b*; Kang *et al.* 2006). In one model proposed by Kang and co-workers (Fig. 5), SUR1 recruits Epac to the plasma membrane where Epac mediates the cAMP-dependent activation of Rap GTPase (Kang *et al.* 2006). Once activated, Rap stimulates PLC-ε (Schmidt *et al.* 2001), a phospholipase that catalyses hydrolysis of membrane-bound phosphatidylinositol 4,5-bisphosphate (PIP_2). As PIP_2 stimulates the activity of K_{ATP} channels by reducing the channel's sensitivity to ATP (Baukrowitz *et al.* 1998; Shyng & Nichols, 1998), an ability of Epac to promote PIP_2 hydrolysis may explain the inhibitory action of cAMP at beta-cell K_{ATP} channels.

In rat chromaffin cells there also exists Epac-mediated actions of cAMP to influence ion channel activity. A 2-day exposure of chromaffin cells to 8-pCPT-2′-*O*-Me-cAMP increases the low-voltage-activated T-type Ca^{2+} current, presumably by up-regulating the expression of $Ca_V3.1$ Ca^{2+} channel subunits (Novara *et al.* 2004). Increased T-type current lowers the threshold for action potential generation in this cell type, thereby facilitating exocytosis of adrenaline (epinephrine). Since secreted adrenaline is an

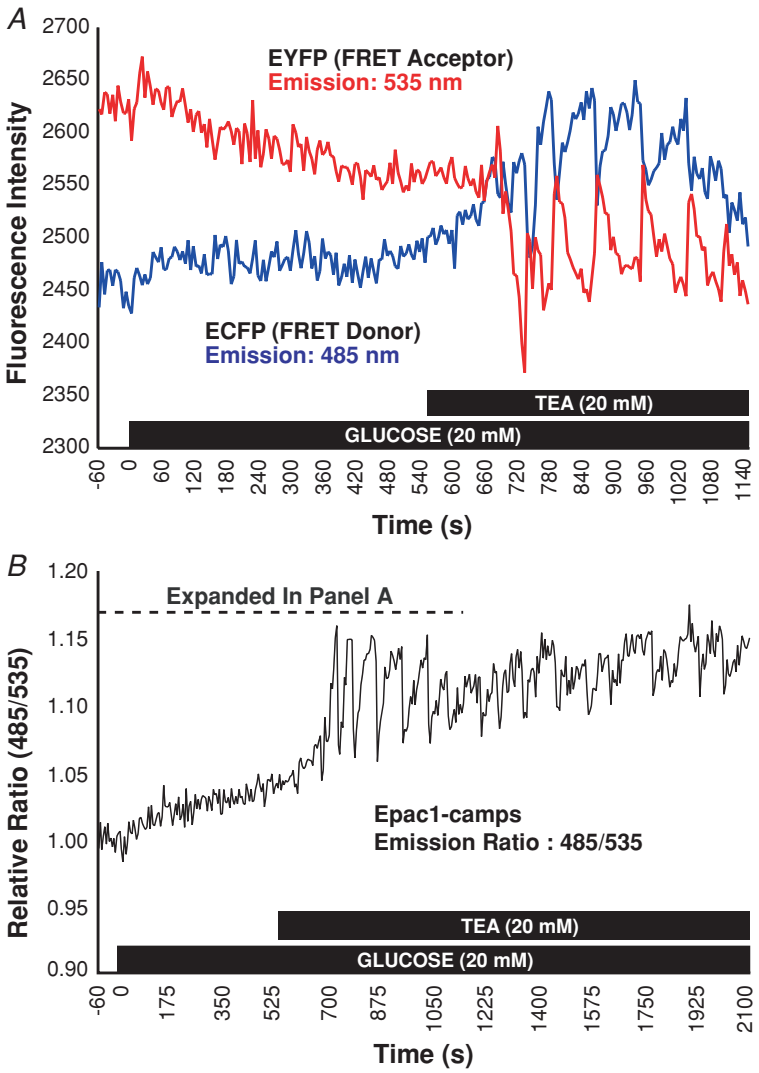

Figure 3. Detection of $[cAMP]_i$ using Epac1-camps
The $[cAMP]_i$ was measured in a single MIN6 insulin-secreting cell transfected with Epac1-camps, a cAMP sensor that incorporates the cyclic nucleotide-binding domain of Epac1 fused at its C-terminus with ECFP (FRET donor), and at its *N*-terminus with EYFP (FRET acceptor). Emitted light was measured at 485 and 535 nm in response to excitation at 440 nm (Landa *et al.* 2005). An increase of $[cAMP]_i$ produces a decrease of FRET. This action of cAMP is measured as a decrease of 535 nm emitted light accompanied by an increase of 485 nm emitted light. *A*, a MIN6 cell equilibrated in saline containing 2 mM glucose, and then challenged with a solution containing 20 mM glucose with or without 20 mM of the K^+ channel blocker tetraethylammonium ion (TEA). Application of 20 mM glucose alone produced a small increase of $[cAMP]_i$, whereas larger oscillations of $[cAMP]_i$ were observed upon introduction of TEA to the bath solution. TEA initiates oscillatory electrical activity in this cell type, an effect accompanied by oscillations of both $[Ca^{2+}]_i$ and $[cAMP]_i$ (Landa *et al.* 2005). *B*, data presented in panel *A* re-plotted as the relative ratio of 485/535 nm emitted light *versus* time. The complete experiment encapsulating 2160 s is illustrated.

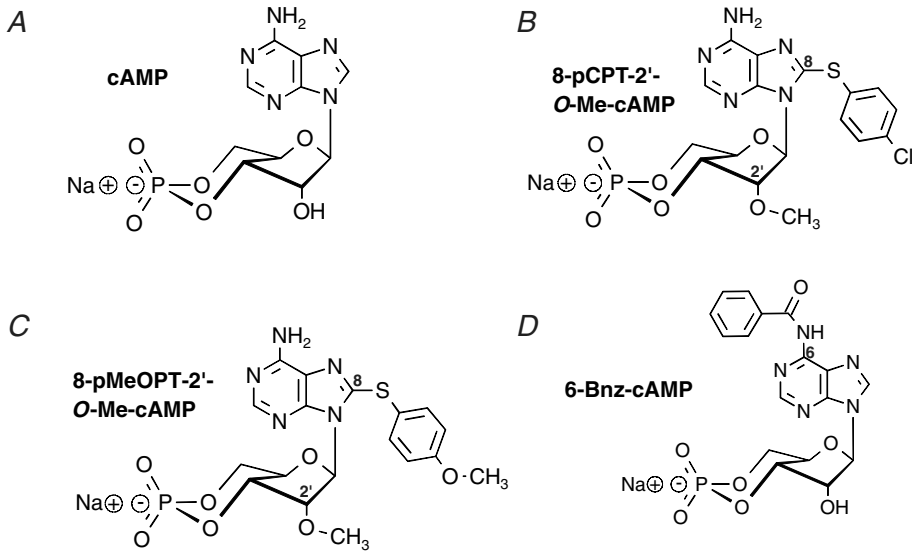

Figure 4. Chemical structures of cAMP analogues
Illustrated are the structures for cAMP (*A*), 8-pCPT-2′-*O*-Me-cAMP (*B*), 8-pMeOPT-2′-*O*-Me-cAMP (*C*), and 6-Bnz-cAMP (*D*). Both 8-pCPT-2′-*O*-Me-cAMP and 8-pMeOPT-2′-*O*-Me-cAMP are Epac selective, whereas 6-Bnz-cAMP is PKA selective. The naturally occurring second messenger cAMP activates both Epac and PKA. A chlorophenylthio substitution introduced at the 8′ position of cAMP (*B* and *C*) dramatically increase the lipophilicity of the cAMP analogues, thereby rendering them cell-permeant. Although not shown, an Sp- isomer of 8-pCPT-2′-*O*-Me-cAMP is also available. It activates Epac, whereas the Rp- isomer does not.

autocrine that stimulates cAMP production by activating chromaffin cell beta adrenergic receptors, there may exist Epac-mediated actions of cAMP that underlie 'chromaffin cell plasticity' and which dictate the level of expression of $Ca_V3.1$ in this cell type (Novara *et al.* 2004).

Does Epac influence the activity of ion channels in non-excitable cells? The answer seems to be yes. 8-pCPT-2′-*O*-Me-cAMP increases the channel open probability (P_o) of amiloride-sensitive Na^+ channels (ENaC) expressed in rat pulmonary epithelial cells

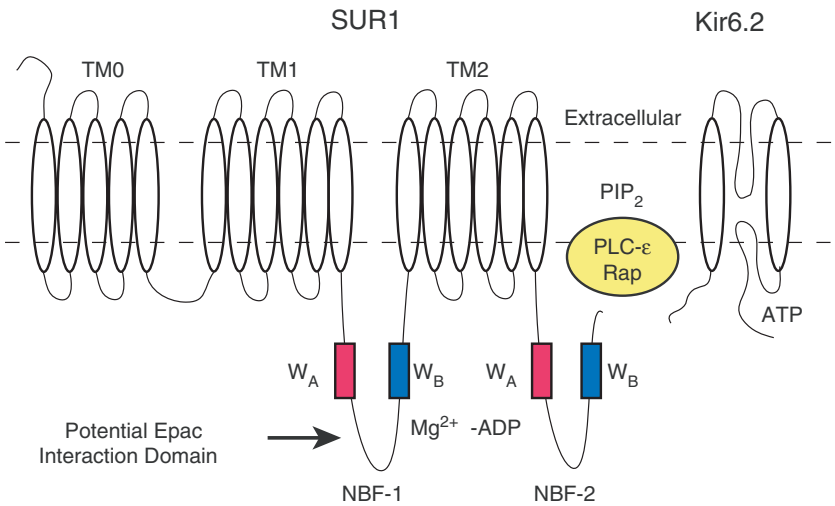

Figure 5. cAMP may inhibit K_{ATP} channel function in an Epac-mediated manner
Nucleotide-binding fold-1 (NBF-1) of the SUR1 subunit of K_{ATP} channels may recruit Epac to the plasma membrane. Binding of cAMP to Epac may then allow for the activation of plasma membrane-associated Rap GTPase. The activated form of Rap stimulates PLC-ε, and the PLC-ε-catalysed hydrolysis of PIP_2 results in the closure of K_{ATP} channels, possibly as a consequence of the increased sensitivity of these channels to ATP. Note that ATP inhibits K_{ATP} channel function by virtue of its interaction with the Kir6.2 subunit of the channel. In contrast, the activity of K_{ATP} channels is stimulated by Mg^{2+}-ADP, acting at the SUR1 subunit. Abbreviations: W_A and W_B, Walker A and Walker B motifs; TMO, TM1 and TM2, transmembrane clusters; NBF-2, nucleotide-binding fold-2.

(Helms *et al.* 2006). This effect is reproduced by the cAMP-elevating neurotransmitter dopamine acting via D_1 receptors. Interestingly, the stimulatory effect of cAMP on recombinant ENaC expressed in *Xenopus* oocytes is not abolished by mutagenesis of PKA phosphorylation sites in the cytosolic domain of ENaC (Yang *et al.* 2006). In contrast, the action of cAMP is reduced by mutagenesis of extracellular signal-regulated kinase (ERK) motifs. Since the Epac-mediated activation of Rap GTPase is reported to stimulate ERK MAPK (Wang *et al.* 2006), it appears that ERK-mediated phosphorylation of ENaC may explain how cAMP, acting via Epac, stimulates this channel's function.

One new study of rat hepatocytes demonstrates novel stimulatory effects of 8-pCPT-2′-*O*-Me-cAMP on Cl^- channel function (Aromataris *et al.* 2006). This action of the ESCA leads to the appearance of an outwardly rectifying Cl^- current with biophysical properties and Ca^{2+} dependence identical to that of the Cl^- current activated by cell swelling. Although this same Cl^- current is not activated by the PKA-selective cAMP analogue N^6-Bnz-cAMP, it is activated by the cAMP-elevating hormone glucagon, an effect not blocked by inhibitors of PKA. Since Epac2 is known to be expressed in hepatocytes (Ueno *et al.* 2001), it seems likely that it is Epac2 that mediates the stimulatory effect of glucagon on Cl^- channel function. Whether this effect of glucagon is secondary to cAMP-dependent activation of a membrane-associated phospholipase or a MAPK remains to be determined.

A role for Epac in the regulation of intracellular Ca^{2+} signalling

Although cAMP promotes Ca^{2+} influx and intracellular Ca^{2+} mobilization in multiple cell types, there is new evidence that these actions of cAMP are not exclusively PKA mediated. In pancreatic beta cells, there exists an Epac-mediated action of 8-pCPT-2′-*O*-Me-cAMP to mobilize Ca^{2+} from intracellular Ca^{2+} stores (Kang *et al.* 2003, 2005). This action of the ESCA promotes exocytosis (Kang *et al.* 2003), and it may also up-regulate mitochondrial ATP production (Tsuboi *et al.* 2003). Available information suggests three scenarios by which the action of 8-pCPT-2′-*O*-Me-cAMP might be achieved (Fig. 6). First, Epac might interact directly with intracellular Ca^{2+} release channels (IP_3 receptors, ryanodine receptors), thereby promoting their opening in response to Ca^{2+} or various Ca^{2+}-mobilizing second messengers (IP_3; cADP-ribose; NAADP). Second, Epac might act

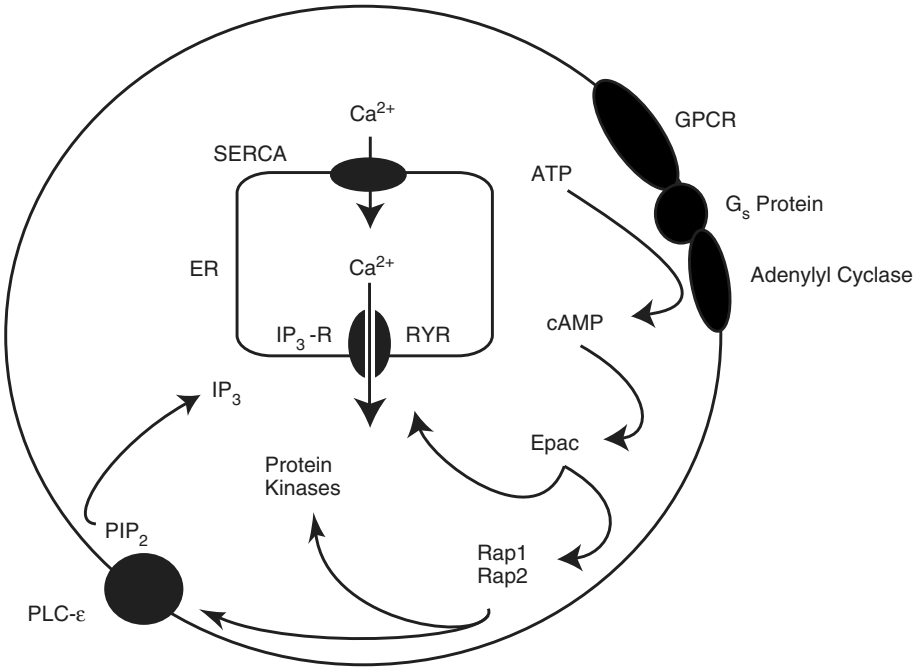

Figure 6. Epac mediates the Ca^{2+}-mobilizing action of cAMP
The mobilization of Ca^{2+} from endoplasmic reticulum (ER) Ca^{2+} stores may be facilitated as a consequence of the Epac-mediated action of cAMP to promote the opening of intracellular Ca^{2+} release channels corresponding to inositol trisphosphate receptors (IP_3-R) or ryanodine receptors (RYR). Such an effect of cAMP might be explained by the ability of Epac to interact directly with the channels. A second possibility is that Epac acts via Rap GTPases to stimulate protein kinases that phosphorylate and regulate the function of intracellular Ca^{2+} release channels. A third possibility is that the Epac-mediated activation of Rap GTPases leads to the stimulation of PLC-ε, which generates IP_3 by hydrolysing PIP_2. Abbreviations: GPCR, G protein-coupled receptor; SERCA, sarco-endoplasmic reticulum ATPase.

via Rap and ERK to promote the PKA-independent phosphorylation of these channels, thereby increasing their sensitivity to Ca^{2+} or Ca^{2+}-mobilizing second messengers. Third, Epac might act via Rap to stimulate PLC-ε, thereby hydrolysing PIP_2 and generating IP_3.

Modulatory actions of Epac at ryanodine receptor Ca^{2+} release channels seem likely because newly published findings demonstrate that in cardiac myocytes there exists a macromolecular complex consisting of Epac1, muscle-specific A-kinase anchoring protein (mAKAP), PKA, cAMP-phosphodiesterase (PDE), and the type-2 isoform (RYR-2) of the ryanodine receptor (Dodge-Kafka *et al.* 2005). Thus, it may be speculated that cAMP, acting via Epac, facilitates the release of Ca^{2+} from the cardiac sarcoplasmic reticulum, where RYR-2 is known to be expressed. In fact, 8-pCPT-2′-*O*-Me-cAMP increases the frequency of spontaneous oscillations of $[Ca^{2+}]_i$ in neonatal rat cardiac myocytes (Morel *et al.* 2005), and it also increases Ca^{2+} spark frequency in adult rat cardiac myocytes (Pereira *et al.* 2006). Furthermore, ryanodine-sensitive Ca^{2+}-mobilizing actions of 8-pCPT-2′-*O*-Me-cAMP exist in mouse pancreatic beta cells (Kang *et al.* 2001, 2005), mouse cerebellar granule cells (Ster *et al.* 2005), and rat renal inner medullary collecting duct (IMCD) cells (Yip, 2006), three cell types that express ryanodine receptors.

Ion transport processes regulated by Epac

Recently published findings provide evidence for a role of Epac in the acute inhibitory regulation of Na^+–H^+ exchanger 3 (NHE3) transporter activity in the brush border membrane (BBM) of rodent renal proximal tubules (Honegger *et al.* 2006). In this study, immunocytochemistry of mouse kidney slices demonstrates co-expression of Epac1 with NHE3 at the BBM, whereas treatment of these slices with 8-pCPT-2′-*O*-Me-cAMP (10–100 μM) produces a concentration-dependent inhibition of NHE3 activity. The effect of the ESCA is independent of major changes in the level of NHE3 transporter expression at the plasma membrane, and it is not associated with PKA-mediated phosphorylation of NHE3. Although the exact mechanism by which Epac regulates NHE3 remains to be determined, it is noteworthy that 8-pCPT-2′-*O*-Me-cAMP fails to inhibit NHE3 under conditions in which kidney slices are treated with PD98059, an inhibitor of MEK1/2 mitogen-activated protein kinases. This finding seems to indicate that MEK-mediated phosphorylation of an as-yet-to-be identified intermediary underlies Epac-mediated inhibition of NHE3.

Limited information also exists suggesting that Epac plays a role in the acute stimulation of ATP-dependent H^+–K^+ transporter activity in the intercalated Iα cells of rat renal collecting ducts (Laroche-Joubert *et al.* 2002). In this cell type, the cAMP-elevating hormone calcitonin stimulates a H^+,K^+-ATPase, an effect mimicked by cAMP, but which is insensitive to an inhibitor of PKA. Importantly, the intracellular administration of antibodies directed against Epac1, or its downstream effector Rap, blocks the action of calcitonin. Since the action of calcitonin is also reduced by U0126, an inhibitor of MEK1/2, and because calcitonin is shown to increase the phosphorylation status of the MEK substrate ERK, it is suggested that there exists in collecting duct cells a cAMP and Epac-mediated action of calcitonin to activate Rap, MEK and ERK in a sequential fashion. In this manner, calcitonin might recruit intracellular vesicles rich in H^+,K^+-ATPase to the plasma membrane (Laroche-Joubert *et al.* 2002).

Epac links cAMP production to the stimulation of exocytosis

Studies of cell types as distantly related as sperm, neurons and endocrine cells provide convincing evidence for a major role of Epac in the stimulation of exocytosis by cAMP (Renstrom *et al.* 1997; Ozaki *et al.* 2000; Kashima *et al.* 2001; Nakazaki *et al.* 2002; Eliasson *et al.* 2003; Kang & Holz, 2003; Shimomura *et al.* 2004; Chin & Abayasekara, 2004; Ma *et al.* 2005; Sedej *et al.* 2005; Branham *et al.* 2006; Hashiguchi *et al.* 2006; Liu *et al.* 2006; Yip, 2006). For example, 8-pCPT-2′-*O*-Me-cAMP potentiates the depolarization-induced exocytosis of large dense core secretory granules, an effect measured as an increase of membrane capacitance in voltage-clamped pancreatic beta cells (Eliasson *et al.* 2003) and pituitary melanotrophs (Sedej *et al.* 2005). These pro-secretagogue actions of 8-pCPT-2′-*O*-Me-cAMP are selective for exocytosis that is Ca^{2+} dependent, and which is initiated by the opening of voltage-dependent Ca^{2+} channels. Available evidence indicates that the action of 8-pCPT-2′-*O*-Me-cAMP results from its ability to activate a pool of intracellular Epac2 that is in close association with, or directly linked to, secretory granules (Fig. 7).

An Epac-mediated action of cAMP to potentiate Ca^{2+}-dependent exocytosis also occurs at presynaptic nerve endings located at the calyx of Held of the rodent central nervous system (Sakaba & Neher, 2001, 2003; Kaneko & Takahashi, 2004), and at the neuromuscular junctions of crayfish (Zhong & Zucker, 2005) and *Drosophila* (Cheung *et al*. 2006). For example, at the calyx of Held, the cAMP-elevating agent forskolin exerts a presynaptic action to facilitate evoked transmitter release, an effect mimicked by 8-Br-cAMP (Sakaba & Neher, 2001). This action of forskolin is most probably Epac mediated because it is reproduced by 8-pCPT-2′-*O*-Me-cAMP, whereas it is insensitive to inhibitors of PKA (Sakaba & Neher, 2001, 2003; Kaneko & Takahashi, 2004). More

detailed electrophysiological analyses provide evidence that cAMP acts via Epac to increase the probability that a readily releasable pool (RRP) of synaptic vesicles will undergo exocytosis in response to depolarization-induced Ca^{2+} influx (Sakaba & Neher, 2001, 2003; Kaneko & Takahashi, 2004). Simultaneously, cAMP may act via Epac to increase the number of synaptic vesicles available to undergo exocytosis. Surprisingly, this effect does not appear to be a generalized action of cAMP to increase the RRP size. Instead, it is a selective effect specific for a subpopulation of vesicles, those that exhibit a high probability of release (Sakaba & Neher, 2001, 2003; Kaneko & Takahashi, 2004).

What is the molecular basis for such stimulatory actions of cAMP? Seino and co-workers propose that cAMP exerts its effects via Epac2, which heterodimerizes with Rim2, a *R*ab3A GTPase-*i*nteracting *m*olecule previously reported to play a central role in the regulation of Ca^{2+}-dependent exocytosis (Fig. 7*A*). Through an as-yet-to-be defined mechanism, cAMP may act via Epac2 to enable Rim2 to promote the 'priming' of secretory granules, thereby rendering them release-competent (Ozaki *et al.* 2000; Fujimoto *et al.* 2002; Shibasaki *et al.* 2004*a*,*b*; Seino & Shibasaki, 2005). Since Rab3A is located on the cytoplasmic surface of secretory granules docked at the plasma membrane, its ability to recruit heterodimers of Rim2 and Epac2 might explain the action of cAMP to increase the size of the RRP of secretory granules available for exocytosis (Seino & Shibasaki, 2005).

In the special case of pancreatic beta cells, the action of cAMP to promote exocytosis may also be explained by the interaction of Epac2 with an intracellular pool of SUR1 located at or near the secretory granules (Fig. 7*B*). This conclusion is reached because the action of 8-pCPT-2′-*O*-Me-cAMP to potentiate Ca^{2+}-dependent exocytosis in beta cells is not observed in SUR1 knock-out mice (Eliasson *et al.* 2003). In the model proposed by Eliasson and co-workers, the binding of 8-pCPT-2′-*O*-Me-cAMP to Epac2 promotes the opening of ClC-3 chloride channels located in the secretory granule membrane. 8-pCPT-2′-*O*-Me-cAMP-induced influx of Cl^- into the secretory granule lumen creates an electromotive force that facilitates ATP-dependent H^+ uptake mediated by a v-type H^+-ATPase (Barg *et al.* 2001). Since SUR1 is expressed not only at the plasma membrane (pmSUR1), but also within the secretory granule membrane (sgSUR1) of beta cells (Geng *et al.* 2003), SUR1-mediated recruitment of Epac2 to the granules may allow for Epac2-mediated stimulation of ClC-3 channel function. Simultaneously, Epac2 might act in a more direct manner to up-regulate the activity of the v-type H^+-ATPase. In summary, these actions of Epac2 would allow for acidification and priming of the granules, thereby rendering them release-competent.

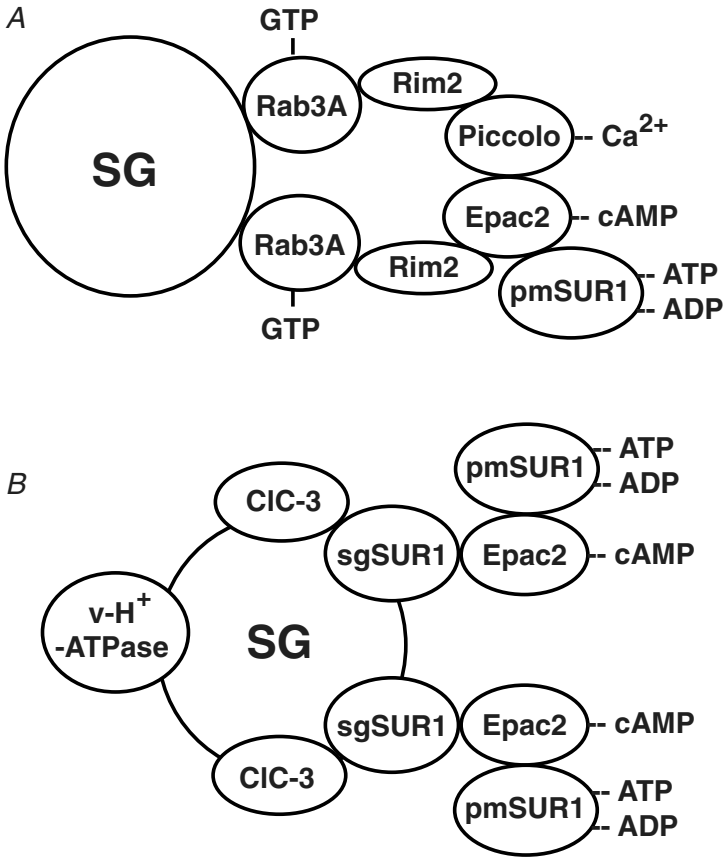

Figure 7. Interactions of Epac2 with secretory granule-associated proteins
A, in the model of Seino and co-workers, plasma membrane SUR1 (pmSUR1), Epac2, Rim2 and Piccolo form a macromolecular complex that interacts with the GTP-bound form of Rab3A to regulate the priming and exocytosis of secretory granules (SG). This model may also apply to presynaptic nerve endings in which synaptic vesicles are found in close association with Rim1. *B,* in the model of Eliasson and co-workers, Epac2 stimulates exocytosis by interacting with secretory granule-associated SUR1 (sgSUR1), and/or pmSUR1. Both sources of SUR1 may be necessary for the cAMP-dependent regulation of ClC-3 chloride channels. Uptake of Cl^- into the secretory granule facilitates granule acidification and priming mediated by the v-type H^+-ATPase.

Conclusion

As Epac is expressed in numerous cell types, and because Epac acts as an intermediary linking cAMP production to plasma membrane phospholipid hydrolysis, it may be predicted that the activated form of Epac will influence a broad array of physiological processes, most notably ion channel function, transporter activity and exocytosis. Of particular interest to cell physiologists are the high levels of expression of Epac1 and Epac2 in the heart and brain, respectively. What is the role of Epac in these excitable tissues, and which effector molecules in addition to Rap GTPase are regulated by Epac? Can new drugs be developed to target the G protein-coupled receptors that activate Epac, and if so which diseases might be treatable using these agents? A pharmacological approach of this sort seems reasonable in view of the demonstrated importance of Epac to cellular processes underlying immune system function (Aronoff *et al.* 2005), neuronal function (Maillet *et al.* 2003; Hucho *et al.* 2005; Robert *et al.* 2005), endocrine function (Holz, 2004), and cardiac function (Morel *et al.* 2005). Finally, with the advent of a molecular genetics approach taking advantage of Epac knock-out mice, a fuller apppreciation of the physiological importance of Epac to cell physiology should be attainable.

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