

# cAMP Sensor Epac and Gastrointestinal Function

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## 68.1 INTRODUCTION

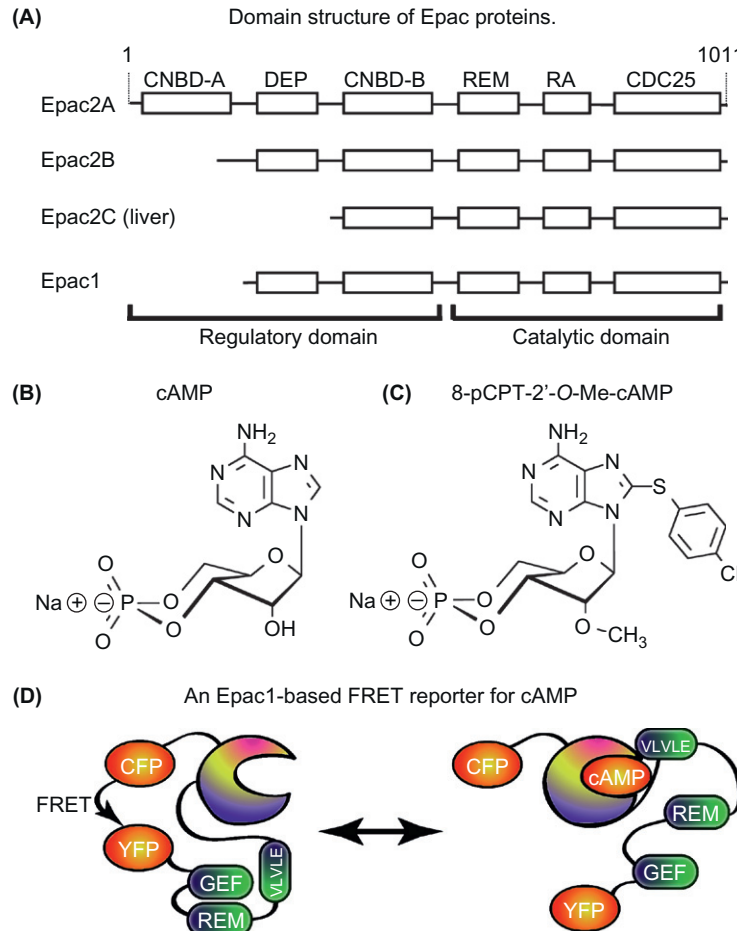
Adenosine-3',5'-cyclic monophosphate (cAMP) is a cytosolic second messenger derived from its precursor ATP by virtue of the activities of soluble adenylyl cyclases and transmembrane adenylyl cyclases that are under the control of G-protein-coupled receptors (GPCRs). Intracellular cAMP has the capacity to activate protein kinase A (PKA) and cyclic nucleotide-gated ion channels (CNGs), whereas these actions of cAMP are terminated by the cyclic nucleotide phosphodiesterase (PDE) catalyzed conversion of cAMP to 5'-AMP. In 1998, two groups independently reported the discovery of cAMP-regulated guanine nucleotide exchange factors (cAMP-GEFs) that are now designated as Epac1 and Epac2.<sup>1,2</sup> Concurrently, both teams of investigators established Epac1 and Epac2 to be cAMP-binding proteins that have the capacity to act catalytically via their CDC25 homology domains (CDC25-HD) to promote the exchange of GDP for GTP on GTPases of the Rap family (Figure 68.1A). This nucleotide exchange allows the GTP-bound and active form of Rap to signal through its downstream effectors,<sup>3</sup> one of which is a novel phosphoinositide-specific phospholipase C  $\epsilon$  (PLC $\epsilon$ ) that transduces stimulatory effects of cAMP on phosphatidylinositol bisphosphate (PIP<sub>2</sub>) hydrolysis.<sup>4</sup>

Subsequent to the discovery of Epac, crystallographic and structure–function analyses demonstrated that in the absence of cAMP, Epac exists in an autoinhibited state, and that the binding of cAMP to Epac promotes a conformational change that relieves this autoinhibition, allowing Epac to activate Rap.<sup>5–9</sup> Thus, Epac proteins were demonstrated to convey actions of cAMP that were independent of their binding to PKA or CNGs. Although it is now recognized that cAMP signals through Epac to influence numerous cellular processes in the endocrine pancreas, the cardiovascular system, and the brain,<sup>10–19</sup> it has only recently become apparent that these Epac-regulated processes also exist in various organs of the gastrointestinal

tract such as the exocrine glands, the hepatobiliary system, and the intestine. Thus, the main focus of the discussion presented in this chapter is to highlight what is known concerning this matter, and consequently pointing out future areas of investigation that might prove fruitful for gastrointestinal physiologists.

## 68.2 IDENTIFICATION OF Epac AS A TRANSDUCER OF INTRACELLULAR cAMP ACTION

The earliest investigations concerning cellular processes under the regulation of Epac revealed that this exchange factor has the capacity to regulate ion channel activity,<sup>20–23</sup> sodium/proton and urea transporter function,<sup>24,25</sup> intracellular Ca<sup>2+</sup> signaling,<sup>4,26–32</sup> and exocytosis<sup>27,32–39</sup> in a wide variety of cell types. Furthermore, it became apparent that these novel PKA and CNG-independent actions of Epac were complemented by its ability to mediate the cAMP-dependent control of cell adhesion,<sup>40</sup> endothelial barrier permeability,<sup>41–43</sup> gap junction formation,<sup>44</sup> gene expression,<sup>45–47</sup> growth,<sup>48–52</sup> differentiation,<sup>53,54</sup> and survival.<sup>55,56</sup> The role Epac plays in these processes was established by examining the action of a cAMP analog (8-pCPT-2'-O-Me-cAMP) that has the capacity to activate Epac1 and Epac2, but not PKA or CNGs (Figure 68.1B, C).<sup>57</sup> Thus, this Epac-selective cAMP analog (ESCA) was demonstrated to reproduce the Epac-mediated action of cAMP, or of cAMP-elevating agents such as hormones and neurotransmitters.<sup>15</sup> It was subsequently demonstrated that the biological activity of 8-pCPT-2'-O-Me-cAMP could be dramatically enhanced by conjugating it to an acetoxymethyl ester (AM-ester), improving its membrane permeability.<sup>58,59</sup> This modified analog is 8-pCPT-2'-O-Me-cAMP-AM (ESCA-AM), and it has recently been demonstrated to exert potent stimulatory effects on cytosolic Ca<sup>2+</sup> signaling and exocytosis, as evaluated

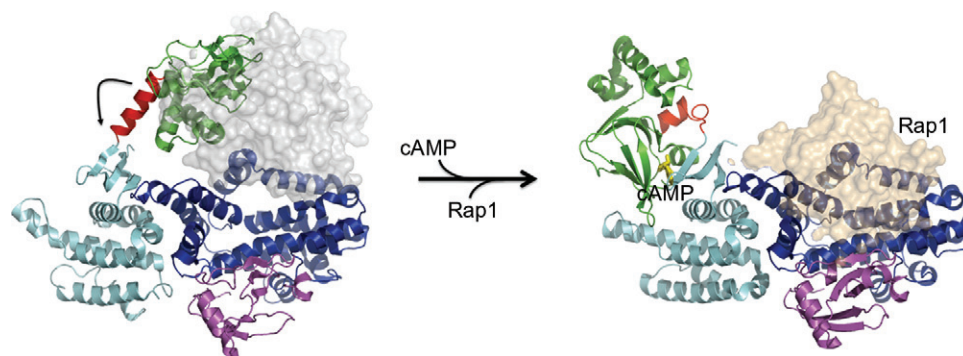


**FIGURE 68.1 Epac isoforms, Epac activators, and Epac-based biosensors.** (A) Domain structures of Epac1 and the three known isoforms of Epac2. Epac2A has two CNBDs, a CNBD-A, which is important for cellular localization, and a high-affinity site (CNBD-B), which is important for cAMP-dependent activation of GEF activity. The Dishevelled, Egl-10, Pleckstrin (DEP) domain is responsible for association of Epac2 with intracellular membranes, a REM domain stabilizes the tertiary structure of the catalytic region, and an RA domain allows the interaction of Epac2 with activated Ras. The CDC25 homology domain (CDC25) catalyzes guanine nucleotide exchange on Rap1 and activates it. Epac2B is specifically expressed in the adrenal cortex and lacks the CNBD-A.<sup>90</sup> Epac2C is found in the liver and lacks both the CNBD-A and DEP domains.<sup>105</sup> All three isoforms have GEF activity and activate Rap1. (B, C) Structures of cAMP and the Epac-selective cAMP analog 8-pCPT-2'-O-Me-cAMP. Note that the Epac selectivity of 8-pCPT-2'-O-Me-cAMP is conferred by a methoxy substitution at the 2' position on the ribose ring, a substitution that drastically reduces the ability of this analog to bind to and activate the regulatory subunits of PKA.<sup>57</sup> A chlorophenylthio (CPT) moiety at position 8 on the adenine ring also improves the selectivity of the analog for Epac, while causing it to be a membrane-permeant "super activator" of Epac.<sup>57</sup> (D) Epac1-based FRET biosensor that can be used to monitor changes in intracellular cAMP.<sup>67</sup> Binding of cAMP to the CNBD causes the hinge region to change conformation resulting in the spatial separation of the donor (CFP, excited at 440 nm with emission at 485 nm) and acceptor (YFP, 535 nm emission) fluorophores and the loss of energy transfer. This change results in an increase in the 485/535 nm emission ratio signifying an increase in cAMP levels. Note that after binding of cAMP, the LID region of Epac1 containing amino acid residues VLVLE becomes rearranged so that it does not interact with, and auto-inhibit, the catalytic domain of the molecule.

in assays of insulin secretion from pancreatic  $\beta$ -cells.<sup>60</sup> Interestingly, 8-pCPT-2'-O-Me-cAMP-AM is also reported to depolarize islet  $\beta$ -cells,<sup>61</sup> an effect attributable to its ability to act via Epac to inhibit ATP-sensitive  $K^+$  channels (K-ATP).<sup>22,27,61</sup> In fact, available evidence indicates that in  $\beta$ -cells, Epac2 interacts directly with the sulfonylurea receptor-1 (SUR1) subunit of K-ATP channels, controlling their function.<sup>21,36,62,63</sup> Thus, findings of this sort have established a foundation of knowledge applicable to studies of gastrointestinal physiology (see the following section).

### 68.2.1 Evidence that Intracellular Epac can be Activated by cAMP

It was initially thought that cAMP generated in response to GPCR activation might not be a very effective activator of Epac, since the *in vitro* cAMP-binding affinities of Epac proteins were found to be considerably less than that of PKA.<sup>64</sup> However, this concept has been challenged,<sup>65</sup> and it could be that Epac and PKA both bind cAMP with similar affinity when their interaction is studied under conditions that replicate what occurs in living cells. The ability



**FIGURE 68.2 Structural model of Epac2 activation by cAMP.** Diagram of X-ray crystal structures of the full-length apo-Epac2 (PDB ID: 2BYV; left panel) and Epac2 $\Delta$ 305:Sp-cAMPS:Rap1B complex (PDB ID: 3CF6; right panel) with the second CBD, REM, RA, and CDC25HD domains colored in green, cyan purple, and blue, respectively. The hinge helix in both structures is highlighted in red. In the cAMP-free, autoinhibitory Epac2 structure, the regulatory region is anchored by the hinge helix to sterically block the access of the catalytic core to Rap1. Binding of cAMP induces a major hinge motion (arrow) that reorients the regulatory lobe relative to catalytic lobe, and ultimately leads to an open Epac2 conformation and the exposure of the catalytic core of Epac2 for binding of Rap GTPase.

of cAMP to activate Epac can be inferred indirectly by monitoring Rap activation in cells that express both Epac and Rap.<sup>1,2</sup> However, a more direct approach is to monitor the ability of cAMP to activate genetically encoded biosensors that are fusion proteins of Epac and fluorescent proteins (Figure 68.1D). These Epac-based biosensors exhibit a change of fluorescence resonance energy transfer (FRET) when they bind cAMP, so it is possible to directly evaluate the cAMP-dependent activation of Epac by monitoring changes of FRET. Biosensors of this sort include one that contains the full-length Epac1 molecule fused to cyan (CFP) and yellow (YFP) fluorescent proteins,<sup>66,67</sup> or a shorter reporter that contains only the cAMP-binding domain (CNBD) of Epac1 fused to CFP and YFP.<sup>68</sup> By imaging single cells, or by monitoring FRET in monolayers of cells expressing these biosensors, it has been possible to confirm that intracellular Epac is activated not only by cAMP, but also by GPCR agonists that stimulate cAMP production.<sup>58,69,70</sup> Using this approach, a surprising finding was reported; Epac2 was shown to be activated by certain members (e.g., tolbutamide) of the sulfonyleurea class of blood glucose-lowering agents.<sup>71,72</sup> Although this finding is disputed,<sup>61,73</sup> it remains possible that Epac proteins might serve as intracellular targets of therapeutically relevant small molecules that are not necessarily related in structure to cAMP.

### 68.2.2 Mechanism of Epac Activation

At the molecular level, Epac1 and Epac2 are multidomain proteins with extensive sequence homology. Both share a C-terminal catalytic lobe that consists of a RAS exchange (REM) domain, a RAS association (RA) domain, and a CDC25-HD, while the N-terminal regulatory lobe contains a Dishevelled, Egl-10, Pleckstrin (DEP) homology domain and one or two CNBDs that exist in Epac1 or

Epac2, respectively (Figure 68.1A). Significant progress was made toward elucidating the molecular mechanism of Epac activation by cAMP. In particular, X-ray crystal structure determinations of the apo-full-length Epac2 protein,<sup>7</sup> or a ternary complex of a deletion Epac2 in complex with a cAMP analog and Rap1B (Epac2 $\Delta$ 305:Sp-cAMPS:Rap1B),<sup>6</sup> have provided a clear “before and after” snapshot of the cAMP-induced activation process in atomic detail (Figure 68.2).

In the apo-Epac2 structure, Epac2 exists in an autoinhibitory conformation with the access of the downstream effector to the C-terminal catalytic core sterically blocked by the N-terminal regulatory lobe. Binding of cAMP triggers a chain of structural reorganizations that is manifested by a localized “hinge” motion. During this process the last two turns of the hinge helix dissolve into an extended loop, which rotates the catalytic lobe about 90° sideway, frees the CDC25-HD domain from the autoinhibitory regulatory lobe, and allows the binding of downstream effectors, Rap1 or Rap2. This general model of Epac activation is based on X-ray crystallographic analyses and is further supported by studies using various solution structural and molecular biophysical techniques, including FRET,<sup>66</sup> computational hinge prediction,<sup>74</sup> peptide amide hydrogen/deuterium exchange mass spectrometry,<sup>75</sup> and small angle X-ray scattering.<sup>76</sup>

Despite these advances, our understanding of the mechanism of Epac activation remains incomplete. For example, Epac proteins are known to exist as a dynamic ensemble of multiple conformations in solution.<sup>9,76</sup> X-ray crystal structures capture only one of the many possible low-energy conformations: the one that is compatible with the crystal lattice. Furthermore, the truncated Epac2 protein that was crystallized in its active complex by Rehmann and co-workers lacked the first CNBD and also the DEP domain.<sup>6</sup>

Additional conformational changes and structural rearrangements within the N-terminal regulatory lobe in response to cAMP binding are expected to occur, and they may play critical roles for Epac functions *in vivo*.

### 68.2.3 Intracellular Targeting of Epac is Under Multiple Levels of Control

Epac1 and Epac2 each contain a DEP domain (Figure 68.1A) that plays an important role in the targeting of Epac to intracellular membranes.<sup>5,77</sup> It is also reported that the perinuclear targeting of Epac1 is conferred by a portion of the catalytic domain (Figure 68.1A) located at the exchange factor's C-terminus.<sup>78</sup> Thus, it may be speculated that the DEP and catalytic domains act in concert to determine the subcellular distribution of Epac1. Of additional interest is the finding that in cardiomyocytes a macromolecular complex exists in which Epac1 associates with the 4D3 isoform of cyclic nucleotide PDE, and muscle-specific A-kinase anchoring protein (AKAP), as well as PKA, ERK5 mitogen-activated protein kinase (MAPK), and the type 2 isoform of ryanodine receptor (RYR) intracellular Ca<sup>2+</sup> release channel.<sup>79</sup> Targeting of Epac1 to this complex is a consequence of the direct interaction of Epac1 with PDE4D3,<sup>79</sup> and the existence of this complex suggests a mechanism by which compartmentalized cAMP signal transduction is achieved.

Additional control over the subcellular targeting of Epac is achieved as a consequence of its interactions with proteins such as H-Ras GTPase,<sup>80,81</sup> Ran GTPase,<sup>82</sup> ezrin-radixin-moesin (ERM) proteins,<sup>83</sup> sulfonyleurea receptors,<sup>21,84</sup> RIM2,<sup>35,36</sup> Piccolo,<sup>62,85</sup> the TCL1 proto-oncogene product,<sup>86</sup> AKAP150,<sup>87</sup> and light-chain 2 of microtubule-associated protein 1A.<sup>88</sup> Many of these interactions are Epac isoform specific, and not all such interactions have been carefully assessed for both Epac1 and Epac2. However, it is possible that some of these interactions are under the control of cAMP since Epac1 translocates to the plasma membrane in response to cAMP-elevating agents.<sup>89</sup> One interesting finding concerning Epac2 is that its low-affinity CNBD (CNBD-A; Figure 68.1A) appears to target Epac2 to the plasma membrane, an effect independent of cAMP activation.<sup>90</sup> Since many of these regulatory processes may be cell type specific, their relative importance remains to be fully established.

### 68.2.4 Strategies for the Study of Epac Signal Transduction

Despite detailed structural information concerning the CNBDs of Epac proteins, no pharmacological antagonist of Epac activation has been identified.<sup>15</sup> Thus, many published studies have sought to validate a role for Epac in cellular processes by demonstrating that (1) the action

of cAMP or cAMP-elevating agents is reproduced by 8-pCPT-2'-O-Me-cAMP, (2) a selective activator of PKA such as 6-Bnz-cAMP fails to reproduce the action of 8-pCPT-2'-O-Me-cAMP, and (3) an inhibitor of PKA activation (Rp-cAMPS), or an inhibitor of PKA catalytic activity (H-89), fails to block the action of 8-pCPT-2'-O-Me-cAMP. Unfortunately, this sort of pharmacological approach does not achieve true target validation, and interpretation of the findings is complicated by the fact that some actions of Epac are conditional on permissive PKA activity.<sup>60,91</sup>

An alternative approach is to express recombinant Epac in transfected cells in order to evaluate whether a cellular process is altered by constitutively active or dominant-negative forms of Epac.<sup>26,36,46,61,76,77,92,93</sup> Methods of RNA interference have also been used successfully to achieve a "knockdown" of Epac protein expression.<sup>43,51,87,94-96</sup> Surprisingly, rather few mouse models exist in which a "knockout" of Epac gene expression has been achieved, particularly from the standpoint of knockouts that are cell type-specific. As of the date of this review, only three Epac knockout mouse models were reported. One is a targeted and global knockout of Epac<sup>2,97</sup> another is a gene trap global knockout of Epac2,<sup>98</sup> and a third is a targeted and global knockout of Epac1.<sup>99</sup> Although the existence of these knockout mice provides clear evidence that lethality is not a consequence of the knockout of a single Epac isoform, it has yet to be established what the consequences would be if both isoforms of Epac were knocked out simultaneously.

### 68.2.5 A Role for Epac in Exocrine Gland Function

Epac plays an active role in the control of exocrine parotid gland function, as demonstrated in prior studies of parotid acinar cells (Table 68.1). For example, amylase secretion from rat parotid acinar cells that express Epac1, Rap1, and the Epac-interacting protein RIM2, was found to be stimulated by 8-pCPT-2'-O-Me-cAMP, an effect not blocked by the PKA inhibitor H-89.<sup>100</sup> This secretagogue action could be measured in saponin-permeabilized acinar cells using a low concentration of 8-pCPT-2'-O-Me-cAMP (1  $\mu$ M), one that did not activate PKA, as validated through the use of a Kemptide-based PKA activation assay and acinar cell homogenates.<sup>100</sup> Similarly, the ESCA 8-pMeOPT-2'-O-Me-cAMP stimulated amylase secretion from mouse parotid acinar cells, and this effect was accompanied by Rap1 activation.<sup>101</sup> This secretagogue action of 8-pMeOPT-2'-O-Me-cAMP was mimicked by N<sup>6</sup>-phenyl-cAMP, a selective activator of PKA, as expected if amylase secretion is under the dual control of Epac1 and PKA.<sup>101</sup>

Epac1 also participates in the stimulation of amylase secretion from pancreatic acinar cells (Table 68.1).

**TABLE 68.1** Effects of Epac Activators in Parotid and Pancreatic Acini

Organ/Tissue	Effects of Epac Activator	Physiological Significance	References
Parotid acini	Stimulation of amylase secretion Rap activation	PKA-independent part of cAMP-stimulated enzyme secretion	101,100
Pancreatic acini	Enhancement of amylase secretion upon co-stimulation with carbachol	PKA-independent part of cAMP-stimulated enzyme secretion	102
Pancreatic acini	Synergistic effect on stimulation of amylase secretion by carbachol Rap activation	Mimics synergistic effect of the combination of cholecystokinin and VIP on enzyme secretion	103
Pancreatic acinar cells	Enhancement of carbachol-stimulated apical-to-basal Ca <sup>2+</sup> wave speed due to Ca <sup>2+</sup> release from intracellular store.	Not clear	104

Moreover, evidence exists that the activation of pancreatic acinar cell zymogens (trypsinogen, chymotrypsinogen) is also under the control of Epac1. Thus, 8-pCPT-2'-O-Me-cAMP was found to potentiate the action of a muscarinic cholinergic receptor agonist (carbachol) to stimulate Ca<sup>2+</sup>-dependent amylase release and zymogen activation in rat pancreatic acinar cells.<sup>102</sup> These actions of 8-pCPT-2'-O-Me-cAMP were mimicked by the ESCA 8-pHPT-2'-O-Me-cAMP, were not blocked by a PKA inhibitor, and were not accompanied by significant PKA activation, as validated in a CREB phosphorylation assay using pancreatic acinar cell homogenates.<sup>102</sup>

Available evidence indicates that Epac exerts its stimulatory effect on amylase secretion by activating Rap GTPase. Thus, in mouse pancreatic acinar cells, 8-pCPT-2'-O-Me-cAMP activated Rap1 and the action of 8-pCPT-2'-O-Me-cAMP to stimulate amylase release was reduced after treatment of acinar cells with adenovirus-directing expression of Rap1GAP. This GTPase-activating protein (GAP) converts Rap from its active to inactive state.<sup>103</sup> Once again, the secretagogue action of 8-pCPT-2'-O-Me-cAMP was not blocked by a PKA inhibitor.<sup>103</sup> Furthermore, the action of a cAMP-elevating neuropeptide (VIP) to stimulate amylase secretion was also unaffected by H-89, as expected if it is primarily Epac1 that serves to transduce stimulatory effects of cAMP on amylase secretion in this cell type.<sup>103</sup> This conclusion is consistent with the finding that Epac1 was localized to the zymogen granule membranes where Rap1 was also located.<sup>103</sup>

What remains uncertain is whether Epac1-dependent Rap1 activation promotes amylase secretion and zymogen activation by stimulating cytosolic Ca<sup>2+</sup> signaling within the acinar cells (Table 68.1). This might be the case because 8-pCPT-2'-O-Me-cAMP was reported to facilitate Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release (CICR) in rat pancreatic acinar cells.<sup>104</sup> This finding is in striking agreement with the

demonstrated ability of this same ESCA to facilitate CICR in pancreatic  $\beta$ -cells.<sup>27,93,98</sup> Thus, the Epac-dependent facilitation of CICR might provide a critical Ca<sup>2+</sup> signal that serves to control enzyme secretion, zymogen activation, and possibly fluid or electrolyte release from the acinar cells. Although it remains to be determined, this mechanism of cAMP-regulated CICR in pancreatic acinar cells may involve inositol trisphosphate receptor and ryanodine receptor intracellular Ca<sup>2+</sup> release channels that are under the control of Epac1 and Rap1.<sup>104</sup>

### 68.3 Epac PROTECTS HEPATOCYTES FROM APOPTOSIS

Human hepatocytes express a short form of Epac2 designated as Epac2C.<sup>105</sup> This variant of Epac2 is able to mediate the cAMP-dependent activation of Rap1, even though it lacks the CNBD-A and DEP domains (Figure 68.1A). Thus, it seems that activation of Epac2C might explain the finding that glucagon, cAMP, and 8-pCPT-2'-O-Me-cAMP exert a pro-survival effect on hepatocytes (Table 68.2).<sup>106-109</sup> For example, 8-pCPT-2'-O-Me-cAMP was reported to protect rat hepatocytes against apoptosis induced by bile acids, Fas ligand, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ).<sup>106</sup> These pro-survival actions of glucagon, cAMP, and 8-pCPT-2'-O-Me-cAMP in rat hepatocytes are proposed to involve the sequential activation of Epac, Rap, Src tyrosine kinase, PI3K, and protein kinase B (PKB, Akt).<sup>110</sup> This identified signal transduction pathway may not be the only mechanism by which an anti-apoptosis effect is achieved, since 8-pCPT-2'-O-Me-cAMP was also reported to act in rat hepatocytes to promote epidermal growth factor receptor (EGFR) activation with consequent PI3K and PKB activation.<sup>110</sup> Furthermore, 8-pCPT-2'-O-Me-cAMP was found to suppress bile acid-induced apoptosis of rat hepatocytes by promoting the PI3K-dependent

**TABLE 68.2** Effects of Epac Activators in the Hepatobiliary System

Organ/Tissue	Effects of Epac Activator	Physiological Significance	References
Hepatocytes	SOCS-3 gene induction, negative effect on PKA-dependent gene expression	Epac activation mimics effects of glucagon during fasting	113
Hepatocytes	Anti-apoptotic effects in bile acid-, Fas ligand-, and TNF- $\alpha$ -induced models of apoptosis	Apoptosis of hepatocytes is an important mechanism of many hepatic diseases	106, 108, 110, 111
Hepatocytes	Activation of Ca <sup>2+</sup> and Cl <sup>-</sup> currents	Epac activation mimics effect of the glucagon on Ca <sup>2+</sup> and Cl <sup>-</sup> currents	20
Kupffer cells	Inhibition of respiratory burst.	Suppression of the respiratory burst allows the malaria sporozoites to pass through phagocytes and to develop inside hepatocytes.	118
Stellate cells (Hepatic fibroblasts) Human hepatic stellate cell line	Profibrotic stimuli inhibit Epac1 expression Overexpression of Epac1 decreases TGF $\beta$ 1-induced collagen synthesis and promotes fibroblast migration	Hepatic fibrosis in the late stages of inflammation leads to organ damage and loss of function	120
Cholangiocytes	Increase of proliferation of the normal and PCK cholangiocytes.	Increased proliferation of cholangiocytes leads to formation of cholangiocyte-derived liver cysts	117

phosphorylation of focal adhesion kinase (FAK).<sup>111</sup> Just as intriguing, 8-pCPT-2'-*O*-Me-cAMP was demonstrated to inhibit lipopolysaccharide (LPS)-induced apoptosis in rat hepatocytes. This effect was attributed to its ability to reverse LPS induced activation of c-Jun N-terminal kinase (JNK), while also reversing the inhibitory effect of LPS on PKB.<sup>112</sup> Thus, good evidence exists that Epac mediates at least some of the pro-survival actions of cAMP in hepatocytes. However, it has yet to be definitively established that it is Epac2C that mediates these effects.

### 68.3.1 Evidence that Hepatic Gluconeogenesis is Epac Regulated

It was recently reported that the expression of key hepatic gluconeogenic enzymes is under the inhibitory control of Epac.<sup>113</sup> This finding is surprising in light of the fact that glucagon acts via cAMP and PKA to stimulate the expression of these same enzymes.<sup>114</sup> This seemingly paradoxical finding concerning the inhibitory action of Epac has been interpreted to indicate that under periods of long-term fasting, glucagon may downregulate hepatic gluconeogenesis and instead favor a switch to the mitochondrial  $\beta$ -oxidation of free fatty acids in order to generate ketone bodies as an alternative energy source.<sup>113</sup> Epac-mediated

inhibition of gluconeogenic enzyme expression is proposed to be a consequence of the action of Epac to induce the expression of SOCS3 (suppressor of cytokine signaling-3), and it has been proposed that SOCS3 counteracts PKA-dependent activation of gluconeogenic enzyme expression through a signal transduction mechanism that has yet to be identified.<sup>113</sup>

### 68.3.2 Additional Diverse Roles of Epac in Hepatobiliary Function

Cholangiocytes are epithelial cells that line the intrahepatic bile ducts, and this cell type is reported to express both Epac1 and Epac2.<sup>115</sup> Although it has yet to be established, the presence of Epac in cholangiocytes may explain the ability of GLP-1 receptor agonist exendin-4 to protect cholangiocytes from bile acid-induced apoptosis.<sup>116</sup> Similarly, cholangiocyte expression of Epac may explain the ability of 8-pCPT-2'-*O*-Me-cAMP to stimulate their proliferation, and in fact it was reported that this ESCA markedly stimulated cholangiocyte proliferation in the rat PCK model of autosomal recessive polycystic kidney disease (ARPKD).<sup>117</sup> Thus, it may be speculated that dysregulated Epac activity could be involved in hepatic cystogenesis, although this remains to be tested.

A particularly interesting story has emerged concerning a role for Epac in the suppression of Kupffer cell defense mechanisms in the liver. Kupffer cells are phagocytes, and they protect against invasion of hepatocytes by microbes and parasites such as the malaria sporozoite. However, the malaria sporozoite has developed the ability to act via a cell surface receptor (LRP-1) on Kupffer cells to raise levels of cAMP, suppressing the respiratory burst and intracellular reactive oxygen species (ROS) generation that normally allows Kupffer cells to kill off invading pathogens. Thus, it is remarkable that the Epac activator 8-pCPT-2'-O-Me-cAMP mimicked that action of cAMP to inhibit the respiratory burst in rat liver Kupffer cells, whereas the PKA activator *N*<sup>6</sup>-monobutyryl-adenosine-3',5'-cyclic monophosphate (6-MB-cAMP) did not.<sup>118</sup> This action of 8-pCPT-2'-O-Me-cAMP resembles its ability to suppress ROS production in human alveolar macrophages.<sup>119</sup> Since ROS production in alveolar macrophage results from the activity of NADPH oxidase,<sup>119</sup> an analogous mechanism might exist in Kupffer cells.

An additional notable role for Epac in liver physiology is the reported ability of Epac1 to suppress the hepatic stellate cell fibrogenesis that is induced by profibrogenic stimuli such as transforming growth factor  $\beta$  1 (TGF $\beta$ 1).<sup>120</sup> This is a significant finding because fibrogenesis stimulated by TGF $\beta$ 1 is mechanistically linked to reduced levels of Epac1 expression in multiple types of fibroblasts. Thus, there is the potential for a strategy to combat fibrosis, one in which activators of Epac1 expression and/or function could be considered as therapeutic agents in multiple pathophysiological disorders such as liver fibrosis, myocardial scar formation, pulmonary fibrosis, and scarring of the skin. This concept is consistent with the demonstrated expression of Epac1 in hepatic stellate cells, cardiac fibroblasts, lung fibroblasts, and skin fibroblasts.<sup>120</sup>

## 68.4 Epac PARTICIPATES IN THE REGULATION OF INTESTINAL EPITHELIAL CELL Cl<sup>-</sup> SECRETION

In addition to being implicated in the cAMP-dependent stimulation of hepatocyte Cl<sup>-</sup> channel function,<sup>20</sup> there is evidence that cAMP acts via Epac to stimulate the activity of a novel Cl<sup>-</sup> channel located in the apical membrane of intestinal epithelial cells.<sup>121</sup> Although the molecular identity of this Cl<sup>-</sup> channel is not yet known, it is hyperpolarization activated, exhibits inward rectification, displays a Cl<sup>-</sup> > Br<sup>-</sup> > I<sup>-</sup> ion selectivity, and mediates stimulatory effects of cAMP on Cl<sup>-</sup> secretion. Importantly, this novel Cl<sup>-</sup> channel does not correspond to the cystic fibrosis transmembrane conductance regulator (CFTR) Cl<sup>-</sup> channel, the activity of which is stimulated not by Epac, but by PKA.

In studies of human intestinal T84 cells and mouse ileal sheets, 8-pCPT-2'-O-Me-cAMP was demonstrated to stimulate Cl<sup>-</sup> secretion mediated by this novel Cl<sup>-</sup> channel, and this effect was blocked by intracellular administration of the Ca<sup>2+</sup> chelator BAPTA-AM.<sup>121</sup> Furthermore, it was demonstrated that Cl<sup>-</sup> secretion was also stimulated by forskolin, and that the PKA-independent action of forskolin was abolished by shRNA-mediated knockdown of Epac1. The action of forskolin to promote PKA-independent Cl<sup>-</sup> secretion in T84 cells correlated positively with its ability to activate Rap2, and forskolin also increased [Ca<sup>2+</sup>]<sub>i</sub>, an effect blocked by the PLC inhibitor U73122.<sup>121</sup> On the basis of these findings, it was proposed that a novel cAMP-regulated, and Ca<sup>2+</sup>-sensitive, Cl<sup>-</sup> conductance exists in the apical membrane of intestinal epithelial cells, one that might be activated as a consequence of Epac1 and Rap2 stimulated PLC $\epsilon$  activity.<sup>121</sup> Needless to say, it will be important to test this hypothesis by examining whether or not the knockout of PLC $\epsilon$  uncouples Epac activation from the stimulation of this novel Cl<sup>-</sup> channel.

### 68.4.1 Evidence that Epac2 Regulates Intestinal L-cell GLP-1 Biosynthesis

Intestinal L-cells are enteroendocrine cells that line the wall of the large intestine and that synthesize and secrete the blood glucose-lowering hormone glucagon-like peptide-1 (GLP-1).<sup>122</sup> Due to the fact that ingested nutrients stimulate the secretion of GLP-1 from L-cells, and since circulating GLP-1 stimulates insulin secretion by activating its GPCR located on pancreatic  $\beta$ -cells, it may be concluded that there exists an "entero-insular axis" that utilizes GLP-1 to couple intestinal nutrient sensing to insulin-dependent blood glucose control.<sup>123</sup> Within L-cells, GLP-1 is derived from its precursor proglucagon, and it is now established that proglucagon biosynthesis is under the control of cAMP due to this second messenger's stimulatory effect on glucagon gene transcription.<sup>124</sup> Since cAMP also stimulates GLP-1 secretion from L-cells,<sup>125</sup> there is currently great interest in identifying L-cell cAMP-elevating agents that might raise levels of circulating GLP-1. Indeed, such agents would be expected to act via GLP-1 to stimulate insulin secretion, and would be expected to lower levels of blood glucose in patients diagnosed with type 2 diabetes mellitus (T2DM).

Although it is not clear exactly what the natural stimulus is for cAMP production in the L-cells, available evidence indicates that a GPCR designated as GPR119 is expressed on this endocrine cell type, and that its activation by small molecule compounds such as AR231453 initiates GLP-1 secretion, and possibly GLP-1 biosynthesis.<sup>126</sup> Studies of this sort have been performed using GLUTag cells,<sup>127</sup>

an immortalized mouse L-cell line that not only expresses GPR119,<sup>126</sup> but also Epac2.<sup>45,125</sup> Using GLUTag cells, it was demonstrated that 8-pCPT-2'-*O*-Me-cAMP stimulated glucagon gene expression, as measured by increased levels of proglucagon mRNA.<sup>45</sup> Furthermore, the cAMP-elevating agent's forskolin and isobutylmethylxanthine (IBMX) acted in GLUTag cells to stimulate glucagon gene promoter activity, and this effect, was not blocked by an inhibitor of PKA.<sup>45</sup> Interestingly, these actions of 8-pCPT-2'-*O*-Me-cAMP, forskolin, and IBMX were accompanied by increased phosphorylation and activation of the ERK MAPKs.<sup>45</sup> It was concluded that there exists a cAMP-signaling mechanism in GLUTag cells, and possibly L-cells, which acts through Epac2, Rap1, and ERK to upregulate glucagon gene transcription. Expanding on this concept, it was recently proposed that the Epac-mediated activation of ERK results in the phosphorylation and nuclear exclusion of the transcriptional regulator Oct-1. This leads to a derepression of transcription factor *Cdx-2* expression, allowing *Cdx-2*, a positive regulator of glucagon gene expression, to transactivate the glucagon gene promoter.<sup>128</sup>

When evaluating these prior studies concerning effects of 8-pCPT-2'-*O*-Me-cAMP on GLUTag cell proglucagon mRNA expression, or glucagon gene promoter activity, it is important to note that 8-pCPT-2'-*O*-Me-cAMP can be metabolized intracellularly to generate metabolites of adenosine, and that these metabolites can act independently of Epac to control gene expression in other cell types.<sup>129,130</sup> Furthermore, 8-pCPT-2'-*O*-Me-cAMP has the capacity to directly inhibit cyclic nucleotide PDEs, raising levels of cAMP.<sup>131</sup> This PDE-mediated effect of 8-pCPT-2'-*O*-Me-cAMP can result in PKA activation with consequent alterations of cellular function that are independent of Epac activation.<sup>15</sup> Such Epac-independent actions of 8-pCPT-2'-*O*-Me-cAMP are particularly prominent when experiments are performed under conditions in which cells are treated with this ESCA for many hours.<sup>129,130</sup> Thus, it is reasonable to inquire as to whether Epac activation is really required in order for 8-pCPT-2'-*O*-Me-cAMP to upregulate glucagon gene transcription in the L-cells or L-cell lines. Although this issue has not been addressed in the published literature, it was established that overexpression of a dominant-negative Epac2 in L-cell lines such as GLUTag and STC-1 did in fact reduce the ability of forskolin to stimulate glucagon gene promoter activity.<sup>125</sup> Furthermore, the action of forskolin to increase levels of proglucagon mRNA in GLUTag and STC-1 cells was found to be unaffected by inhibitors of PKA.<sup>125</sup> What remains to be demonstrated is whether dominant-negative Epac2 also blocks the action of 8-pCPT-2'-*O*-Me-cAMP to stimulate glucagon gene expression. Perhaps the best test would be to determine whether Epac activators fail to upregulate proglucagon gene expression in L-cells of Epac2 knockout mice.

### 68.4.2 Does Epac2 Regulate Intestinal L-cell GLP-1 Secretion?

Studies of pancreatic  $\alpha$ -cells that synthesize and secrete glucagon have demonstrated that these cells express Epac2, and that 8-pCPT-2'-*O*-Me-cAMP enhances the  $\text{Ca}^{2+}$ -dependent exocytosis of glucagon.<sup>132,133</sup> Furthermore, studies of insulin-secreting  $\beta$ -cells have established that Epac2 activation also enhances exocytosis in this cell type.<sup>27,34,35,58,60,97,134,135</sup> Similarly, Epac2 activation in melanotrophs that secrete  $\alpha$ -MSH was shown to be associated with enhanced exocytosis,<sup>38</sup> and neurotensin secretion from endocrine cell line BON was also found to be stimulated in an Epac-mediated manner.<sup>91</sup> Thus, it is particularly surprising that 8-pCPT-2'-*O*-Me-cAMP was found not to stimulate the secretion of glucagon from the InR1-G9  $\alpha$ -cell line, while also failing to stimulate the secretion of GLP-1 from GLUTag cells.<sup>125</sup> It is important to note that 8-pCPT-2'-*O*-Me-cAMP has poor membrane permeability properties in some cells types. In fact, it is sometimes necessary to use the AM-ester of 8-pCPT-2'-*O*-Me-cAMP to be able to detect its stimulatory effect on exocytosis.<sup>58,60</sup> Since this AM-ester was not tested in prior studies of GLP-1 secretion, it remains an open question if Epac2 activation stimulates the secretion of GLP-1 from intestinal L-cells. It is also uncertain if the activation of Epac2 within L-cells treated with GPR119 agonists such as AR231453 underlies the therapeutically important action of these substances to raise levels of plasma GLP-1 in humans. Since GPR119 is also expressed on  $\beta$ -cells where its activation stimulates insulin secretion,<sup>136</sup> it could be that a common mechanism of Epac2 activation underlies GPR119 signal transduction and exocytosis in L-cells and  $\beta$ -cells.

## 68.5 CONCLUSION

Summarized here is a body of evidence that lends credibility to the claim that Epac proteins participate in the regulation of the gastrointestinal function. Ultimate validation of this claim will require a systematic analysis of what the consequences are of tissue- and cell-type-specific knockouts of Epac1 and Epac2 within the gastrointestinal tract. To what extent agonist stimulation of GPCRs results in preferential activation of PKA, Epac1, or Epac2 is also a question that has not yet been adequately addressed in the published literature concerning gastrointestinal physiology. One additional issue is whether some gastrointestinal diseases are initiated by genetic alterations of the *RAPGEF3* and *RAPGEF4* genes that code for Epac1 and Epac2, respectively. Furthermore, it remains to be determined whether the functionality of Epac1 and Epac2 can be modified by small molecule compounds that exert beneficial therapeutic actions *in vivo*. Given that there are substantial differences in the levels of expression of Epac1 and Epac2 in various cell types, it could be that Epac proteins



**TABLE 68.3** Effects of Epac Activators in the Intestine

Organ/Tissue	Effects of Epac Activator	Physiological Significance	References
Enteroendocrine cell lines	<i>Cdx-2</i> gene induction	<i>Cdx-2</i> stimulates proglucagon gene expression	128
Enteroendocrine cell lines	Proglucagon gene induction	Products of proglucagon gene expression in the enteroendocrine cells (GLP-1, GLP-2) are important in glucose homeostasis and gut function	45
Enteroendocrine cell lines	Stimulation of proglucagon gene expression and GLP-1 production	GLP-1 production in preparation for subsequent release without actually affecting glucose homeostasis	125
Human endocrine cell line BON	Stimulation of neurotensin secretion	Neurotensin plays an important role in gastrointestinal secretion, inflammation, and growth	91
Human intestinal cell line T14 Mouse small intestine	Stimulation of Cl <sup>-</sup> secretion	Intestinal Cl <sup>-</sup> secretion by a novel non-CFTR Cl <sup>-</sup> channel	121

constitute novel molecular targets for pharmacological intervention in the treatment of human diseases.

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## REFERENCES

- de Rooij J, Zwartkruis FJ, Verheijen MH, Cool RH, Nijman SM, Wittinghofer A, et al. Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature*. 1998;396:474–477.
- Kawasaki H, Springett GM, Mochizuki N, Toki S, Nakaya M, Matsuda M, et al. A family of cAMP-binding proteins that directly activate Rap1. *Science*. 1998;282:2275–2279.
- Raaijmakers JH, Bos JL. Specificity in Ras and Rap signaling. *J Biol Chem*. 2009;284:10995–10999.
- Schmidt M, Evellin S, Weernink PA, von Dorp F, Rehmann H, Lomasney JW, et al. A new phospholipase-C-calcium signalling pathway mediated by cyclic AMP and a Rap GTPase. *Nat Cell Biol*. 2001;3:1020–1024.
- de Rooij J, Rehmann H, van Triest M, Cool RH, Wittinghofer A, Bos JL. Mechanism of regulation of the Epac family of cAMP-dependent RapGEFs. *J Biol Chem*. 2000;275:20829–20836.
- Rehmann H, Arias-Palomo E, Hadders MA, Schwede F, Llorca O, Bos JL. Structure of Epac2 in complex with a cyclic AMP analogue and RAP1B. *Nature*. 2008;455:124–127.
- Rehmann H, Das J, Knipscheer P, Wittinghofer A, Bos JL. Structure of the cyclic-AMP-responsive exchange factor Epac2 in its auto-inhibited state. *Nature*. 2006;439:625–628.
- Rehmann H, Prakash B, Wolf E, Rueppel A, de Rooij J, Bos JL, et al. Structure and regulation of the cAMP-binding domains of Epac2. *Nat Struct Biol*. 2003;10:26–32.
- Rehmann H, Rueppel A, Bos JL, Wittinghofer A. Communication between the regulatory and the catalytic region of the cAMP-responsive guanine nucleotide exchange factor Epac. *J Biol Chem*. 2003;278:23508–23514.
- Bos JL. Epac: a new cAMP target and new avenues in cAMP research. *Nat Rev Mol Cell Biol*. 2003;4:733–738.
- Bos JL. Epac proteins: multi-purpose cAMP targets. *Trends Biochem Sci*. 2006;31:680–686.
- Cheng X, Ji Z, Tsalkova T, Mei F. Epac and PKA: a tale of two intracellular cAMP receptors. *Acta Biochim Biophys Sin (Shanghai)*. 2008;40:651–662.
- Gloerich M, Bos JL. Epac: defining a new mechanism for cAMP action. *Annu Rev Pharmacol Toxicol*. 2010;50:355–375.
- Holz GG. New insights concerning the glucose-dependent insulin secretagogue action of glucagon-like peptide-1 in pancreatic beta-cells. *Horm Metab Res*. 2004;36:787–794.
- Holz GG, Heart E, Leech CA. Synchronizing Ca<sup>2+</sup> and cAMP oscillations in pancreatic beta-cells: a role for glucose metabolism and GLP-1 receptors? Focus on “regulation of cAMP dynamics by Ca<sup>2+</sup> and G protein-coupled receptors in the pancreatic beta-cell: a computational approach”. *Am J Physiol Cell Physiol*. 2008;294:C4–6.
- Holz GG, Kang G, Harbeck M, Roe MW, Chepurny OG. Cell physiology of cAMP sensor Epac. *J Physiol*. 2006;577:5–15.
- Metrich M, Berthouze M, Morel E, Crozatier B, Gomez AM, Lezoualc’h F. Role of the cAMP-binding protein Epac in cardiovascular physiology and pathophysiology. *Pflugers Arch*. 2010;459:535–546.
- Roscioni SS, Elzinga CR, Schmidt M. Epac: effectors and biological functions. *Naunyn Schmiedeberg's Arch Pharmacol*. 2008;377:345–357.
- Seino S, Shibasaki T. PKA-dependent and PKA-independent pathways for cAMP-regulated exocytosis. *Physiol Rev*. 2005;85:1303–1342.
- Aromataris EC, Roberts ML, Barritt GJ, Rychkov GY. Glucagon activates Ca<sup>2+</sup> and Cl<sup>-</sup> channels in rat hepatocytes. *J Physiol*. 2006;573:611–625.
- Kang G, Chepurny OG, Malester B, Rindler MJ, Rehmann H, Bos JL, et al. cAMP sensor Epac as a determinant of ATP-sensitive

- potassium channel activity in human pancreatic beta cells and rat INS-1 cells. *J Physiol.* 2006;573:595–609.
22. Kang G, Leech CA, Chepurny OG, Coetzee WA, Holz GG. Role of the cAMP sensor Epac as a determinant of K-ATP channel ATP sensitivity in human pancreatic beta-cells and rat INS-1 cells. *J Physiol.* 2008;586:1307–1319.
  23. Ster J, De Bock F, Guerinou NC, Janossy A, Barrere-Lemaire S, Bos JL, et al. Exchange protein activated by cAMP (Epac) mediates cAMP activation of p38 MAPK and modulation of Ca<sup>2+</sup>-dependent K<sup>+</sup> channels in cerebellar neurons. *Proc Natl Acad Sci USA.* 2007;104:2519–2524.
  24. Honegger KJ, Capuano P, Winter C, Bacic D, Stange G, Wagner CA, et al. Regulation of sodium-proton exchanger isoform 3 (NHE3) by PKA and exchange protein directly activated by cAMP (EPAC). *Proc Natl Acad Sci USA.* 2006;103:803–808.
  25. Wang Y, Klein JD, Blount MA, Martin CF, Kent KJ, Pech V, et al. Epac regulates UT-A1 to increase urea transport in inner medullary collecting ducts. *J Am Soc Nephrol.* 2009;20:2018–2024.
  26. Kang G, Chepurny OG, Holz GG. cAMP-regulated guanine nucleotide exchange factor II (Epac2) mediates Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release in INS-1 pancreatic beta-cells. *J Physiol.* 2001;536:375–385.
  27. Kang G, Joseph JW, Chepurny OG, Monaco M, Wheeler MB, Bos JL, et al. Epac-selective cAMP analog 8-pCPT-2'-O-Me-cAMP as a stimulus for Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release and exocytosis in pancreatic beta-cells. *J Biol Chem.* 2003;278:8279–8285.
  28. Liu G, Jacobo SM, Hilliard N, Hockerman GH. Differential modulation of Cav1.2 and Cav1.3-mediated glucose-stimulated insulin secretion by cAMP in INS-1 cells: distinct roles for exchange protein directly activated by cAMP 2 (Epac2) and protein kinase A. *J Pharmacol Exp Ther.* 2006;318:152–160.
  29. Mangmool S, Shukla AK, Rockman HA. beta-Arrestin-dependent activation of Ca<sup>2+</sup>/calmodulin kinase II after beta(1)-adrenergic receptor stimulation. *J Cell Biol.* 2010;189:573–587.
  30. Oestreich EA, Wang H, Malik S, Kaproth-Joslin KA, Blaxall BC, Kelley GG, et al. Epac-mediated activation of phospholipase C(epsilon) plays a critical role in beta-adrenergic receptor-dependent enhancement of Ca<sup>2+</sup> mobilization in cardiac myocytes. *J Biol Chem.* 2007;282:5488–5495.
  31. Pereira L, Metrich M, Fernandez-Velasco M, Lucas A, Leroy J, Perrier R, et al. The cAMP binding protein Epac modulates Ca<sup>2+</sup> sparks by a Ca<sup>2+</sup>/calmodulin kinase signalling pathway in rat cardiac myocytes. *J Physiol.* 2007;583:685–694.
  32. Yip KP. Epac-mediated Ca<sup>2+</sup> mobilization and exocytosis in inner medullary collecting duct. *Am J Physiol Renal Physiol.* 2006;291:F882–890.
  33. Branham MT, Mayorga LS, Tomes CN. Calcium-induced acrosomal exocytosis requires cAMP acting through a protein kinase A-independent, Epac-mediated pathway. *J Biol Chem.* 2006;281:8656–8666.
  34. Eliasson L, Ma X, Renstrom E, Barg S, Berggren PO, Galvanovskis J, et al. SUR1 regulates PKA-independent cAMP-induced granule priming in mouse pancreatic B-cells. *J Gen Physiol.* 2003;121:181–197.
  35. Kashima Y, Miki T, Shibasaki T, Ozaki N, Miyazaki M, Yano H, et al. Critical role of cAMP-GEFII-Rim2 complex in incretin-potentiated insulin secretion. *J Biol Chem.* 2001;276:46046–46053.
  36. Ozaki N, Shibasaki T, Kashima Y, Miki T, Takahashi K, Ueno H, et al. cAMP-GEFII is a direct target of cAMP in regulated exocytosis. *Nat Cell Biol.* 2000;2:805–811.
  37. Sakaba T, Neher E. Direct modulation of synaptic vesicle priming by GABA(B) receptor activation at a glutamatergic synapse. *Nature.* 2003;424:775–778.
  38. Sedej S, Rose T, Rupnik M. cAMP increases Ca<sup>2+</sup>-dependent exocytosis through both PKA and Epac2 in mouse melanotrophs from pituitary tissue slices. *J Physiol.* 2005;567:799–813.
  39. Zhong N, Zucker RS. cAMP acts on exchange protein activated by cAMP/cAMP-regulated guanine nucleotide exchange protein to regulate transmitter release at the crayfish neuromuscular junction. *J Neurosci.* 2005;25:208–214.
  40. Rangarajan S, Enserink JM, Kuiperij HB, de Rooij J, Price LS, Schwede F, et al. Cyclic AMP induces integrin-mediated cell adhesion through Epac and Rap1 upon stimulation of the beta 2-adrenergic receptor. *J Cell Biol.* 2003;160:487–493.
  41. Cullere X, Shaw SK, Andersson L, Hirahashi J, Luscsinkas FW, Mayadas TN. Regulation of vascular endothelial barrier function by Epac, a cAMP-activated exchange factor for Rap GTPase. *Blood.* 2005;105:1950–1955.
  42. Fukuhara S, Sakurai A, Sano H, Yamagishi A, Somekawa S, Takakura N, et al. Cyclic AMP potentiates vascular endothelial cadherin-mediated cell-cell contact to enhance endothelial barrier function through an Epac-Rap1 signaling pathway. *Mol Cell Biol.* 2005;25:136–146.
  43. Kooistra MR, Corada M, Dejana E, Bos JL. Epac1 regulates integrity of endothelial cell junctions through VE-cadherin. *FEBS Lett.* 2005;579:4966–4972.
  44. Somekawa S, Fukuhara S, Nakaoka Y, Fujita H, Saito Y, Mochizuki N. Enhanced functional gap junction neofunction by protein kinase A-dependent and Epac-dependent signals downstream of cAMP in cardiac myocytes. *Circ Res.* 2005;97:655–662.
  45. Lotfi S, Li Z, Sun J, Zuo Y, Lam PP, Kang Y, et al. Role of the exchange protein directly activated by cyclic adenosine 5'-monophosphate (Epac) pathway in regulating proglucagon gene expression in intestinal endocrine L cells. *Endocrinology.* 2006;147:3727–3736.
  46. Morel E, Marcantoni A, Gastineau M, Birkedal R, Rochais F, Garnier A, et al. cAMP-binding protein Epac induces cardiomyocyte hypertrophy. *Circ Res.* 2005;97:1296–1304.
  47. O'Neil JS, Maywood ES, Chesham JE, Takahashi JS, Hastings MH. cAMP-dependent signaling as a core component of the mammalian circadian pacemaker. *Science.* 2008;320:949–953.
  48. Metrich M, Laurent AC, Breckler M, Duquesnes N, Hmitou I, Courillau D, et al. Epac activation induces histone deacetylase nuclear export via a Ras-dependent signalling pathway. *Cell Signal.* 2010;22:1459–1468.
  49. Metrich M, Lucas A, Gastineau M, Samuel JL, Heymes C, Morel E, et al. Epac mediates beta-adrenergic receptor-induced cardiomyocyte hypertrophy. *Circ Res.* 2008;102:959–965.
  50. Misra UK, Pizzo SV. Epac1-induced cellular proliferation in prostate cancer cells is mediated by B-Raf/ERK and mTOR signaling cascades. *J Cell Biochem.* 2009;108:998–1011.
  51. Murray AJ, Shewan DA. Epac mediates cyclic AMP-dependent axon growth, guidance and regeneration. *Mol Cell Neurosci.* 2008;38:578–588.
  52. Van Kolen K, Dautzenberg FM, Verstraeten K, Royaux I, De Hoogt R, Gutknecht E, et al. Corticotropin releasing factor-induced ERK phosphorylation in AtT20 cells occurs via a cAMP-dependent mechanism requiring EPAC2. *Neuropharmacology.* 2010;58:135–144.

53. Kiermayer S, Biondi RM, Imig J, Plotz G, Hauptenthal J, Zeuzem S, et al. Epac activation converts cAMP from a proliferative into a differentiation signal in PC12 cells. *Mol Biol Cell*. 2005;16:5639–5648.
54. Woolfrey KM, Srivastava DP, Photowala H, Yamashita M, Barbolina MV, Cahill ME, et al. Epac2 induces synapse remodeling and depression and its disease-associated forms alter spines. *Nat Neurosci*. 2009;12:1275–1284.
55. Kwon G, Pappan KL, Marshall CA, Schaffer JE, McDaniel ML. cAMP Dose-dependently prevents palmitate-induced apoptosis by both protein kinase A- and cAMP-guanine nucleotide exchange factor-dependent pathways in beta-cells. *J Biol Chem*. 2004;279:8938–8945.
56. Shao W, Yu Z, Fantus IG, Jin T. Cyclic AMP signaling stimulates proteasome degradation of thioredoxin interacting protein (TxNIP) in pancreatic beta-cells. *Cell Signal*. 2010;22:1240–1246.
57. Enserink JM, Christensen AE, de Rooij J, van Triest M, Schwede F, Genieser HG, et al. A novel Epac-specific cAMP analogue demonstrates independent regulation of Rap1 and ERK. *Nat Cell Biol*. 2002;4:901–906.
58. Chepurny OG, Leech CA, Kelley GG, Dzhura I, Dzhura E, Li X, et al. Enhanced Rap1 activation and insulin secretagogue properties of an acetoxymethyl ester of an Epac-selective cyclic AMP analog in rat INS-1 cells: studies with 8-pCPT-2'-O-Me-cAMP-AM. *J Biol Chem*. 2009;284:10728–10736.
59. Vliem MJ, Ponsioen B, Schwede F, Pannekoek WJ, Riedl J, Kooistra MR, et al. 8-pCPT-2'-O-Me-cAMP-AM: an improved Epac-selective cAMP analogue. *Chembiochem*. 2008;9:2052–2054.
60. Chepurny OG, Kelley GG, Dzhura I, Leech CA, Roe MW, Dzhura E, et al. PKA-dependent potentiation of glucose-stimulated insulin secretion by Epac activator 8-pCPT-2'-O-Me-cAMP-AM in human islets of Langerhans. *Am J Physiol Endocrinol Metab*. 2010;298:E622–633.
61. Leech CA, Dzhura I, Chepurny OG, Schwede F, Genieser HG, Holz GG. Facilitation of beta-cell K-ATP channel sulfonylurea sensitivity by a cAMP analog selective for the cAMP-regulated guanine nucleotide exchange factor Epac. *Islets*. 2010;2:72–81.
62. Shibasaki T, Sunaga Y, Fujimoto K, Kashima Y, Seino S. Interaction of ATP sensor, cAMP sensor, Ca<sup>2+</sup> sensor, and voltage-dependent Ca<sup>2+</sup> channel in insulin granule exocytosis. *J Biol Chem*. 2004;279:7956–7961.
63. Shibasaki T, Sunaga Y, Seino S. Integration of ATP, cAMP, and Ca<sup>2+</sup> signals in insulin granule exocytosis. *Diabetes*. 2004;53(suppl 3):S59–62.
64. Christensen AE, Selheim F, de Rooij J, Dremier S, Schwede F, Dao KK, et al. cAMP analog mapping of Epac1 and cAMP kinase. Discriminating analogs demonstrate that Epac and cAMP kinase act synergistically to promote PC-12 cell neurite extension. *J Biol Chem*. 2003;278:35394–35402.
65. Dao KK, Teigen K, Kopperud R, Hodneland E, Schwede F, Christensen AE, et al. Epac1 and cAMP-dependent protein kinase holoenzyme have similar cAMP affinity, but their cAMP domains have distinct structural features and cyclic nucleotide recognition. *J Biol Chem*. 2006;281:21500–21511.
66. DiPilato LM, Cheng X, Zhang J. Fluorescent indicators of cAMP and Epac activation reveal differential dynamics of cAMP signaling within discrete subcellular compartments. *Proc Natl Acad Sci USA*. 2004;101:16513–16518.
67. Ponsioen B, Zhao J, Riedl J, Zwartkruis F, van der Krogt G, Zaccolo M, et al. Detecting cAMP-induced Epac activation by fluorescence resonance energy transfer: Epac as a novel cAMP indicator. *EMBO Rep*. 2004;5:1176–1180.
68. Nikolaev VO, Bunemann M, Hein L, Hannawacker A, Lohse MJ. Novel single chain cAMP sensors for receptor-induced signal propagation. *J Biol Chem*. 2004;279:37215–37218.
69. Harbeck MC, Chepurny O, Nikolaev VO, Lohse MJ, Holz GG, Roe MW. Simultaneous optical measurements of cytosolic Ca<sup>2+</sup> and cAMP in single cells. *Sci STKE*. 2006;2006:16.
70. Landa Jr. LR, Harbeck M, Kaihara K, Chepurny O, Kitiphongspattana K, Graf O, et al. Interplay of Ca<sup>2+</sup> and cAMP signaling in the insulin-secreting MIN6 beta-cell line. *J Biol Chem*. 2005;280:31294–31302.
71. Hinke SA. Epac2: a molecular target for sulfonylurea-induced insulin release. *Sci Signal*. 2009;4.
72. Zhang CL, Katoh M, Shibasaki T, Minami K, Sunaga Y, Takahashi H, et al. The cAMP sensor Epac2 is a direct target of antidiabetic sulfonylurea drugs. *Science*. 2009;325:607–610.
73. Tsalkova T, Gribenko AV, Cheng X. Exchange protein directly activated by cyclic AMP isoform 2 is not a direct target of sulfonylurea drugs. *Assay Drug Dev Technol*. 2011;9:88–91.
74. Yu S, Fan F, Flores SC, Mei F, Cheng X. Dissecting the mechanism of Epac activation via hydrogen-deuterium exchange FT-IR and structural modeling. *Biochemistry*. 2006;45:15318–15326.
75. Brock M, Fan F, Mei FC, Li S, Gessner C, Woods Jr. VL, et al. Conformational analysis of Epac activation using amide hydrogen/deuterium exchange mass spectrometry. *J Biol Chem*. 2007;282:32256–32263.
76. Tsalkova T, Blumenthal DK, Mei FC, White MA, Cheng X. Mechanism of Epac activation: structural and functional analyses of Epac2 hinge mutants with constitutive and reduced activities. *J Biol Chem*. 2009;284:23644–23651.
77. Qiao J, Mei FC, Popov VL, Vergara LA, Cheng X. Cell cycle-dependent subcellular localization of exchange factor directly activated by cAMP. *J Biol Chem*. 2002;277:26581–26586.
78. Borland G, Gupta M, Magiera MM, Rundell CJ, Fuld S, Yarwood SJ. Microtubule-associated protein 1B-light chain 1 enhances activation of Rap1 by exchange protein activated by cyclic AMP but not intracellular targeting. *Mol Pharmacol*. 2006;69:374–384.
79. Dodge-Kafka KL, Soughayer J, Pare GC, Carlisle Michel JJ, Langeberg LK, Kapiloff MS, et al. The protein kinase A anchoring protein mAKAP coordinates two integrated cAMP effector pathways. *Nature*. 2005;437:574–578.
80. Li Y, Asuri S, Rebhun JF, Castro AF, Paranavitana NC, Quilliam LA. The RAP1 guanine nucleotide exchange factor Epac2 couples cyclic AMP and Ras signals at the plasma membrane. *J Biol Chem*. 2006;281:2506–2514.
81. Liu C, Takahashi M, Li Y, Song S, Dillon TJ, Shinde U, et al. Ras is required for the cyclic AMP-dependent activation of Rap1 via Epac2. *Mol Cell Biol*. 2008;28:7109–7125.
82. Liu C, Takahashi M, Li Y, Dillon TJ, Kaeck S, Stork PJ. The interaction of Epac1 and Ran promotes Rap1 activation at the nuclear envelope. *Mol Cell Biol*. 2010;30:3956–3969.
83. Gloerich M, Ponsioen B, Vliem MJ, Zhang J, Zhao J, Kooistra MR, et al. Spatial regulation of cyclic AMP-Epac1 signaling in cell adhesion by ERM proteins. *Mol Cell Biol*. 2010;30:5421–5431.
84. Purves GI, Kamishima T, Davies LM, Quayle JM, Dart C. Exchange protein activated by cAMP (Epac) mediates cAMP-dependent but

- protein kinase A-insensitive modulation of vascular ATP-sensitive potassium channels. *J Physiol.* 2009;587:3639–3650.
85. Fujimoto K, Shibasaki T, Yokoi N, Kashima Y, Matsumoto M, Sasaki T, et al. Piccolo, a  $\text{Ca}^{2+}$  sensor in pancreatic beta-cells. Involvement of cAMP-GEFII/Rim2. Piccolo complex in cAMP-dependent exocytosis. *J Biol Chem.* 2002;277:50497–50502.
  86. Misra UK, Kaczowka SJ, Pizzo SV. Interaction between TCL1 and Epac1 in the activation of Akt kinases in plasma membranes and nuclei of 8-CPT-2-O-Me-cAMP-stimulated macrophages. *Cell Signal.* 2008;20:130–138.
  87. Nijholt IM, Dolga AM, Ostroveanu A, Luiten PG, Schmidt M, Eisel UL. Neuronal AKAP150 coordinates PKA and Epac-mediated PKB/Akt phosphorylation. *Cell Signal.* 2008;20:1715–1724.
  88. Magiera MM, Gupta M, Rundell CJ, Satish N, Ernens I, Yarwood SJ. Exchange protein directly activated by cAMP (EPAC) interacts with the light chain (LC) 2 of MAP1A. *Biochem J.* 2004;382:803–810.
  89. Ponsioen B, Gloerich M, Ritsma L, Rehmann H, Bos JL, Jalink K. Direct spatial control of Epac1 by cyclic AMP. *Mol Cell Biol.* 2009;29:2521–2531.
  90. Niimura M, Miki T, Shibasaki T, Fujimoto W, Iwanaga T, Seino S. Critical role of the N-terminal cyclic AMP-binding domain of Epac2 in its subcellular localization and function. *J Cell Physiol.* 2009;219:652–658.
  91. Li J, O'Connor KL, Cheng X, Mei FC, Uchida T, Townsend Jr. CM, et al. Cyclic adenosine 5'-monophosphate-stimulated neurotensin secretion is mediated through Rap1 downstream of both Epac and protein kinase A signaling pathways. *Mol Endocrinol.* 2007;21:159–171.
  92. Hochbaum D, Hong K, Barila G, Ribeiro-Neto F, Altschuler DL. Epac, in synergy with cAMP-dependent protein kinase (PKA), is required for cAMP-mediated mitogenesis. *J Biol Chem.* 2008;283:4464–4468.
  93. Kang G, Chepurny OG, Rindler MJ, Collis L, Chepurny Z, Li WH, et al. A cAMP and  $\text{Ca}^{2+}$  coincidence detector in support of  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release in mouse pancreatic beta cells. *J Physiol.* 2005;566:173–188.
  94. Borland G, Bird RJ, Palmer TM, Yarwood SJ. Activation of protein kinase Calpha by EPAC1 is required for the ERK- and CCAAT/enhancer-binding protein beta-dependent induction of the SOCS-3 gene by cyclic AMP in COS1 cells. *J Biol Chem.* 2009;284:17391–17403.
  95. Idevall-Hagren O, Barg S, Gylfe E, Tengholm A. cAMP mediators of pulsatile insulin secretion from glucose-stimulated single beta-cells. *J Biol Chem.* 2010;285:23007–23018.
  96. Ostroveanu A, van der Zee EA, Eisel UL, Schmidt M, Nijholt IM. Exchange protein activated by cyclic AMP 2 (Epac2) plays a specific and time-limited role in memory retrieval. *Hippocampus.* 2010;20:1018–1026.
  97. Shibasaki T, Takahashi H, Miki T, Sunaga Y, Matsumura K, Yamanaka M, et al. Essential role of Epac2/Rap1 signaling in regulation of insulin granule dynamics by cAMP. *Proc Natl Acad Sci USA.* 2007;104:19333–19338.
  98. Dzhura I, Chepurny OG, Kelley GG, Leech CA, Roe MW, Dzhura E, et al. Epac2-dependent mobilization of intracellular  $\text{Ca}^{2+}$  by glucagon-like peptide-1 receptor agonist exendin-4 is disrupted in  $\beta$ -cells of phospholipase C- $\epsilon$  knockout mice. *J Physiol.* 2010;588:4871–4889.
  99. Kai AK, Lam AK, Zhang X, Lai AKW, Xu A, Vanhoutte PM, et al. Targeted disruption of exchange protein directly activated by cyclic-AMP1 in mice leads to altered islet architecture and reduced b-cell distribution of GLUT-2. *Diabetes.* 2009;58:LB20–LB21.
  100. Shimomura H, Imai A, Nashida T. Evidence for the involvement of cAMP-GEF (Epac) pathway in amylase release from the rat parotid gland. *Arch Biochem Biophys.* 2004;431:124–128.
  101. Wu CY, DiJulio DH, Jacobson KL, McKnight GS, Watson EL. The contribution of AKAP5 in amylase secretion from mouse parotid acini. *Am J Physiol Cell Physiol.* 2010;298:C1151–1158.
  102. Chaudhuri A, Husain SZ, Kolodecik TR, Grant WM, Gorelick FS. Cyclic AMP-dependent protein kinase and Epac mediate cyclic AMP responses in pancreatic acini. *Am J Physiol Gastrointest Liver Physiol.* 2007;292:G1403–1410.
  103. Sabbatini ME, Chen X, Ernst SA, Williams JA. Rap1 activation plays a regulatory role in pancreatic amylase secretion. *J Biol Chem.* 2008;283:23884–23894.
  104. Shah AU, Grant WM, Latif SU, Mannan ZM, Park AJ, Husain SZ. Cyclic AMP accelerates calcium waves in pancreatic acinar cells. *Am J Physiol Gastrointest Liver Physiol.* 2008;294:G1328–1334.
  105. Ueno H, Shibasaki T, Iwanaga T, Takahashi K, Yokoyama Y, Liu LM, et al. Characterization of the gene EPAC2: structure, chromosomal localization, tissue expression, and identification of the liver-specific isoform. *Genomics.* 2001;78:91–98.
  106. Cullen KA, McCool J, Anwer MS, Webster CR. Activation of cAMP-guanine exchange factor confers PKA-independent protection from hepatocyte apoptosis. *Am J Physiol Gastrointest Liver Physiol.* 2004;287:G334–343.
  107. Graf D, Reinehr R, Kurz AK, Fischer R, Haussinger D. Inhibition of tauroolithocholate 3-sulfate-induced apoptosis by cyclic AMP in rat hepatocytes involves protein kinase A-dependent and -independent mechanisms. *Arch Biochem Biophys.* 2003;415:34–42.
  108. Sinclair EM, Yusta B, Streutker C, Baggio LL, Koehler J, Charron MJ, et al. Glucagon receptor signaling is essential for control of murine hepatocyte survival. *Gastroenterology.* 2008;135:2096–2106.
  109. Webster CR, Usechak P, Anwer MS. cAMP inhibits bile acid-induced apoptosis by blocking caspase activation and cytochrome c release. *Am J Physiol Gastrointest Liver Physiol.* 2002;283:G727–738.
  110. Gates A, Hohenester S, Anwer MS, Webster CR. cAMP-GEF cytoprotection by Src tyrosine kinase activation of phosphoinositide-3-kinase p110 beta/alpha in rat hepatocytes. *Am J Physiol Gastrointest Liver Physiol.* 2009;296:G764–774.
  111. Usechak P, Gates A, Webster CR. Activation of focal adhesion kinase and JNK contributes to the extracellular matrix and cAMP-GEF mediated survival from bile acid induced apoptosis in rat hepatocytes. *J Hepatol.* 2008;49:251–261.
  112. Ponzetti K, King M, Gates A, Sawkat Anwer M, Webster CLR. Cyclic AMP-guanine exchange factor activation inhibits JNK-dependent lipopolysaccharide-induced apoptosis in rat hepatocytes. *Hepatic Medicine.* 2010;2:1–11.
  113. Gaudy AM, Clementi AH, Campbell JS, Smrcka AV, Mooney RA. Suppressor of cytokine signaling-3 is a glucagon-inducible inhibitor of PKA activity and gluconeogenic gene expression in hepatocytes. *J Biol Chem.* 2010;285:41356–41365.
  114. Jiang G, Zhang BB. Glucagon and regulation of glucose metabolism. *Am J Physiol Endocrinol Metab.* 2003;284:E671–678.
  115. Masyuk AI, Gradilone SA, Banales JM, Huang BQ, Masyuk TV, Lee SO, et al. Cholangiocyte primary cilia are chemosensory organelles that detect biliary nucleotides via P2Y12 purinergic receptors. *Am J Physiol Gastrointest Liver Physiol.* 2008;295:G725–734.

116. Marzioni M, Alpini G, Saccomanno S, Candelaresi C, Venter J, Rychlicki C, et al. Exendin-4, a glucagon-like peptide 1 receptor agonist, protects cholangiocytes from apoptosis. *Gut*. 2009;58:990–997.
117. Banales JM, Masyuk TV, Gradilone SA, Masyuk AI, Medina JF, LaRusso NF. The cAMP effectors Epac and protein kinase a (PKA) are involved in the hepatic cystogenesis of an animal model of autosomal recessive polycystic kidney disease (ARPKD). *Hepatology*. 2009;49:160–174.
118. Uсынin I, Klotz C, Frevert U. Malaria circumsporozoite protein inhibits the respiratory burst in Kupffer cells. *Cell Microbiol*. 2007;9:2610–2628.
119. Aronoff DM, Canetti C, Serezani CH, Luo M, Peters-Golden M. Cutting edge: macrophage inhibition by cyclic AMP (cAMP): differential roles of protein kinase A and exchange protein directly activated by cAMP-1. *J Immunol*. 2005;174:595–599.
120. Yokoyama U, Patel HH, Lai NC, Aroonsakool N, Roth DM, Insel PA. The cyclic AMP effector Epac integrates pro- and anti-fibrotic signals. *Proc Natl Acad Sci USA*. 2008;105:6386–6391.
121. Hoque KM, Woodward OM, van Rossum DB, Zachos NC, Chen L, Leung GP, et al. Epac1 mediates protein kinase A-independent mechanism of forskolin-activated intestinal chloride secretion. *J Gen Physiol*. 2010;135:43–58.
122. Holz GG, Chepurny OG. Glucagon-like peptide-1 synthetic analogs: new therapeutic agents for use in the treatment of diabetes mellitus. *Curr Med Chem*. 2003;10:2471–2483.
123. Kieffer TJ, Habener JF. The glucagon-like peptides. *Endocr Rev*. 1999;20:876–913.
124. Jin T. Mechanisms underlying proglucagon gene expression. *J Endocrinol*. 2008;198:17–28.
125. Islam D, Zhang N, Wang P, Li H, Brubaker PL, Gaisano HY, et al. Epac is involved in cAMP-stimulated proglucagon expression and hormone production but not hormone secretion in pancreatic alpha- and intestinal L-cell lines. *Am J Physiol Endocrinol Metab*. 2009;296:E174–181.
126. Chu ZL, Carroll C, Alfonso J, Gutierrez V, He H, Lucman A, et al. A role for intestinal endocrine cell-expressed g protein-coupled receptor 119 in glycemic control by enhancing glucagon-like peptide-1 and glucose-dependent insulinotropic peptide release. *Endocrinology*. 2008;149:2038–2047.
127. Drucker DJ, Jin T, Asa SL, Young TA, Brubaker PL. Activation of proglucagon gene transcription by protein kinase-A in a novel mouse enteroendocrine cell line. *Mol Endocrinol*. 1994;8:1646–1655.
128. Wang P, Wang Q, Sun J, Wu J, Li H, Zhang N, et al. POU homeodomain protein Oct-1 functions as a sensor for cyclic AMP. *J Biol Chem*. 2009;284:26456–26465.
129. Enyeart JA, Enyeart JJ. Metabolites of an Epac-selective cAMP analog induce cortisol synthesis by adrenocortical cells through a cAMP-independent pathway. *PLoS One*. 2009;4:e6088.
130. Enyeart JA, Liu H, Enyeart JJ. cAMP analogs and their metabolites enhance TREK-1 mRNA and K<sup>+</sup> current expression in adrenocortical cells. *Mol Pharmacol*. 2010;77:469–482.
131. Laxman S, Riechers A, Sadilek M, Schwede F, Beavo JA. Hydrolysis products of cAMP analogs cause transformation of *Trypanosoma brucei* from slender to stumpy-like forms. *Proc Natl Acad Sci USA*. 2006;103:19194–19199.
132. De Marinis YZ, Salehi A, Ward CE, Zhang Q, Abdulkader F, Bengtsson M, et al. GLP-1 inhibits and adrenaline stimulates glucagon release by differential modulation of N- and L-type Ca<sup>2+</sup> channel-dependent exocytosis. *Cell Metab*. 2010;11:543–553.
133. Ma X, Zhang Y, Gromada J, Sewing S, Berggren PO, Buschard K, et al. Glucagon stimulates exocytosis in mouse and rat pancreatic alpha-cells by binding to glucagon receptors. *Mol Endocrinol*. 2005;19:198–212.
134. Hatakeyama H, Takahashi N, Kishimoto T, Nemoto T, Kasai H. Two cAMP-dependent pathways differentially regulate exocytosis of large dense-core and small vesicles in mouse beta-cells. *J Physiol*. 2007;582:1087–1098.
135. Kelley GG, Chepurny OG, Schwede F, Genieser HG, Leech CA, Roe MW, et al. Glucose-dependent potentiation of mouse islet insulin secretion by Epac activator 8-pCPT-2'-O-Me-cAMP-AM. *Islets*. 2009;1:260–265.
136. Chu ZL, Jones RM, He H, Carroll C, Gutierrez V, Lucman A, et al. A role for beta-cell-expressed G protein-coupled receptor 119 in glycemic control by enhancing glucose-dependent insulin release. *Endocrinology*. 2007;148:2601–2609.

## NOTE ADDED IN PROOF

After submission of this manuscript, it was reported that Epac participates in bile acid-stimulated hepatocyte polarization. The reference is: Fu D, Wakabayashi Y, Lippercolt Schwartz J, Arias IM. Bile acid stimulates hepatocyte polarization through a cAMP-Epac-MEK-LKB1-AMPK pathway. *Proc Natl Acad Sci USA*. 2011;108:1403–1408.