HHS Public Access

Author manuscript

Gene. Author manuscript; available in PMC 2019 July 17.

Published in final edited form as:

Gene. 2016 January 10; 575(2 Pt 3): 577–583. doi:10.1016/j.gene.2015.09.029.

Structure and functional roles of Epac2 (RAPGEF4)

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Abstract

Epac (exchange protein activated by cyclic-AMP) 2 is a direct target of 3'−5'-cyclic adenosine monophosphate (cAMP) and is involved in cAMP-mediated signal transduction through activation of the Ras-like small GTPase Rap. Crystallographic analyses revealed that activation of Epac2 by cAMP is accompanied by dynamic structural changes. Epac2 is expressed mainly in brain, neuroendocrine and endocrine tissues, and is involved in diverse cellular functions in the tissues. In this review, we summarize the structure and function of Epac2. We also discuss the physiological and pathophysiological roles of Epac2, and the possibility of Epac2 as a therapeutic target.

Keywords

Epac2; cAMP; Rap

1. Introduction

3'−5'-cyclic adenosine monophosphate (cAMP), which is one of the major second messengers derived from adenosine triphosphate (ATP) by adenylate cyclase following various extracellular stimulations, regulates many biological processes. Initially, it was considered that cAMP exerts its actions through activation of cAMP-dependent protein kinase A (PKA) or interaction with cyclic nucleotide binding ion channels (Beavo and Brunton, 2002).

In 1998, a novel cAMP target protein cAMP-GEF, also called Epac (hereafter referred to as Epac) was identified by two independent groups using computational database search (de Rooij et al., 1998) or differential display screen (Kawasaki et al., 1998). While PKA transduces cAMP signaling by direct phosphorylation of target proteins, Epac-mediated signaling depends mainly on its activating effect on the small GTPases Rap, Rap1 and Rap2.

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(de Rooij et al., 1998; Kawasaki et al., 1998; de Rooij et al., 2000; Gloerich and Bos, 2011). The active forms of Rap interact specifically with their effector proteins and activate downstream targets to control divergent biological processes (Frische and Zwartkruis, 2010).

This review briefly summarizes the structure, function, intracellular signaling, and physiological and pathophysiological roles of Epac, in particular of the Epac2 isoform, and also mentions the potential of Epac2 as a drug target for treatment of diseases.

2. Structure and function of Epac2

There are two isoforms of Epac, Epac1 and Epac2, which are coded by *Rapgef3* and *Rapgef4* genes, respectively. The coding region of *Rapgef4* gene comprises 31 exons and 30 introns located on chromosome 2q31. *Rapgef3* mRNA is ubiquitously expressed, with high levels in thyroid, kidney, ovary, skeletal muscle, and heart, and with relatively low levels in brain. The expression of *Rapgef4* mRNA is more restricted and is predominant in brain and neuroendocrine and endocrine tissues (de Rooij et al., 1998; Kawasaki et al., 1998; Ozaki et al., 2000).

Epac2 is a multi-domain protein with molecular weight of ~116 kDa consisting of two parts, the regulatory and catalytic regions. The amino-terminal regulatory region contains two cyclic nucleotide-binding domains (cNBD-A and cNBD-B) and a DEP (Dishevelled, Egl-10, and Pleckstrin) domain (Bos, 2006). Epac1 has a similar domain organization to Epac2, but has only one cNBD in the regulatory region. cNBD-A of Epac2 has lower binding affinity to cAMP compared with cNBD-B (de Rooij et al., 2000). It is proposed that cNBD-A determines the subcellular localization (Niimura et al., 2009) and that the DEP domain is responsible for its membrane association and altering the localization of Epac1 to the plasma membrane (Qiao et al., 2002; Consonni et al., 2012). The carboxy-terminal catalytic region consists of a CDC25 homology domain (CDC25-HD), a Ras exchange motif (REM), and a Ras association (RA) domain (Bos, 2006). The REM is thought to be involved in both stabilization of CDC25-HD and intramolecular interaction with the second cNBD. Interaction of a RA domain of Epac2 with an active GTP-bound form of Ras may control the localization of Epac2 and regulate the spatio-temporal activation of Rap (Li et al., 2006).

Three isoforms of Epac2, Epac2A, Epac2B, and Epac2C, produced by alternative promoter usage and differential splicing, have been identified. Epac2A, named Epac2 originally, is expressed mainly in brain and neuroendocrine and endocrine tissues such as pituitary and pancreatic islets as mentioned above. Epac2B, which lacks the cNBD-A domain, is similar to Epac1 in domain structure, and is expressed mainly in adrenal gland (Niimura et al., 2009). Epac2C, which lacks both a cNBD-A and a DEP domain, is expressed predominantly in liver (Ueno et al., 2001) (Fig. 1A). The tissue-specific expression of the Epac2 isoforms is regulated by DNA methylation of alternative promoters (Hoivik et al., 2013). Physiological roles of Epac2B in adrenal grand and Epac2C in liver have not been fully elucidated. However, Epac2C is likely to control bile acid-stimulated canalicular formation in the liver (Fu et al., 2011).

The major function of Epac2 is guanine-nucleotide exchange for Rap1 and Rap2. Small GTPases cycle between an inactive GDP-bound form and an active GTP-bound form. They are tightly regulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs), which are responsible for stimulation of GTP loading and catalysis of GTP hydrolysis, respectively. CDC25-HD of Epac2 interacts with GDP-bound Rap1, and its subsequent activation by exchange of GDP for GTP stimulates downstream signaling through interaction with its specific effector proteins. Well-known functions of Rap are regulation of actin cytoskeletal dynamics such as integrin-mediated cell adhesion, cadherin-mediated formation of cell junctions and potentiation of insulin secretion in pancreatic β-cells (Shibasaki et al., 2007; Roscioni et al., 2008; Frische and Zwartkruis, 2010).

Crystallographic analyses have advanced our understanding of the mechanism underlying cAMP-induced activation of Epac2 at the molecular level (Rehmann et al., 2006; Rehmann et al., 2008). In the crystal structure of the full length of Epac2 in the absence of cAMP, a catalytic region is masked by a regulatory region forming auto-inhibited conformation (Fig. 1B). cNBD-B interacts with this catalytic region both by ion bonds with REM and by forming beta strand-like secondary structures with REM and CDC25-HD, which are called ionic latch and switchboard, respectively. Thus, in auto-inhibited form, a regulatory region of Epac2 prevents Rap1 from accessing the catalytic region and therefore sterically inhibits the activation of Rap1 (Rehmann et al., 2006). Analysis of the crystal structure of the ternary complex of Epac2, cAMP analog, and Rap1B implies a mechanism of Epac2 activation as follows. The binding of cAMP to cNBD-B induces a dynamic conformational change that forms an open-conformation in which the catalytic region is rotated about 90° to the side and thus is translated closer to cNBD-B. Interaction of the cAMP analog with both cNBD-B and a REM ensures stabilization of the open conformation. This dynamic conformational change allows the Rap1-interacting region in the GEF domain to be exposed at its surface, thereby inducing GEF activity toward Rap1 (Rehmann et al., 2008) (Fig. 1B).

3. Roles of Epac2 in pancreatic islets

3.1. Insulin secretion from pancreatic β-cells

Type 2 diabetes is becoming a greater global health problem; the number of patients is rapidly increasing in both developed and developing countries. Type 2 diabetes is associated with impaired insulin secretion from pancreatic β-cells and impaired insulin action in peripheral target tissues. Insulin secretion from pancreatic β-cells is regulated by various factors. Although glucose is physiologically the most important regulator of insulin secretion (Henquin, 2000), hormones, neurotransmitters, and fatty acids also are required for normal regulation of insulin secretion mainly through G-protein-coupled receptors (Ahren, 2009; Seino et al., 2011). Among them, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), gut hormones called incretins, which are secreted from enteroendocrine cells following meal ingestion, are known to stimulate insulin secretion in a glucose-dependent manner (Drucker, 2006; Nauck, 2009; Seino et al., 2010). GLP-1 and GIP bind to their specific receptors, the GLP-1 receptor and GIP receptor, respectively (Thorens, 1992; Usdin et al., 1993; Yamada et al., 1995). As these incretin receptors are coupled to Gs-protein, their activation increases the intracellular cAMP concentration. Thus,

cAMP-signaling plays a crucial role in insulin secretion from pancreatic β-cells to maintain glucose homeostasis. The action of cAMP in pancreatic β-cells had long been thought to be mediated through activation of PKA (Montague and Howell, 1972). However, cAMP stimulates exocytosis of insulin granules from a readily releasable pool (RRP), and it was found that this effect is unaffected by PKA inhibition, suggesting an alternative pathway by which cAMP enables insulin potentiation distinct from that of the PKA-dependent pathway (Renstrom et al., 1997). In yeast two-hybrid screening for a novel target protein for cAMP using the sulfonylurea receptor SUR1, the regulatory subunit of the pancreatic β-cell ATP-sensitive potassium (K_{ATP}) channel as bait, the cAMP-binding protein cAMP-GEFII (Epac2) was identified in mouse insulin-secreting MIN6 cells (Ozaki et al., 2000).

Glucose-induced insulin secretion (GIIS) occurs in a biphasic manner, a first phase of prompt, marked, and transient increase followed by a second phase of moderate and sustained increase (Curry et al., 1968). The first and second phases are associated with exocytosis of insulin granules from the RRP and a reserve pool (RP), respectively (Henquin, 2000; Barg et al., 2002; Bratanova-Tochkova et al., 2002). Although the precise mechanisms of biphasic secretion are not completely unraveled, the first phase is most likely evoked primarily by entry of Ca^{2+} into the β-cells; the second phase is sustained by Ca^{2+} and various metabolic signals generated by glucose-metabolism (Seino et al., 2011). In addition, F-actin remodeling is involved in the second phase of insulin secretion (Wang et al., 2007; Uenishi et al., 2013).

To determine the role of Epac2 in insulin secretion, global *Rapgef4*$^{-/-}$mice were generated (Shibasaki et al., 2007). *Rapgef4*$^{-/-}$mice are fertile, and there are no apparent abnormalities in general appearance or behavior (the mice are available on request to the corresponding author). TIRFM analysis of isolated β-cells from *Rapgef4*$^{-/-}$mice indicated that the Epac2/Rap1signal is involved mainly in augmentation of the first phase of insulin secretion (Shibasaki et al., 2007). A biosimulation study suggested that the Epac2/Rap1signaling augments insulin secretion by increasing the size of the RRP and recruiting insulin granules from the RRP to the plasma membrane (Shibasaki et al., 2007). In Epac2-mediated exocytosis of insulin granules, Epac2 forms a complex with Rim2 (Ozaki et al., 2000; Kashima et al., 2001) that functions as scaffold for a variety of proteins involved in exocytosis. In pancreatic β-cells, Rim2 is localized in both plasma membrane and insulin granules, and determines the docking and priming states of exocytosis (Shibasaki et al., 2004; Yasuda et al., 2010). Epac2 also interacts with Piccolo, a possible Ca^{2+} sensor (Fujimoto et al., 2002), which increases the stability of the Epac2-Rim2 complex. Intricate interactions among Epac2, Rim2, Piccolo, and Rab3 play many important roles in cAMP-regulated insulin granule exocytosis (Shibasaki et al., 2004). On the other hand, a recent study of *Rapgef3*$^{-/-}$mice has shown that Epac1 also is involved in insulin secretion, but primarily in GIIS rather than in cAMP-potentiated insulin secretion (Kai et al., 2013). However, since Epac1 is expressed at a low level in pancreatic islets compared to Epac2, the physiological role of Epac1 remains to be clarified.

It is well accepted that changes in the intracellular Ca^{2+} concentration play a pivotal role in the regulation of insulin granule exocytosis. In this regard, Epac2 was shown to regulate cytosolic Ca^{2+} dynamics in its potentiating effects on insulin secretion. This might involve

phospholipase C-ε (PLC-ε), an isoform of the PLC family, which links Epac2 and various channel activities involved in cytosolic Ca^{2+} levels including those of the K_{ATP} channel, ryanodine receptor (RyR), and inositol 1,4,5-triphosphate (IP_3) receptor. Indeed in PLC-ε knockout (*Plce*$^{-/-}$) mice, Epac2-activated insulin potentiation was severely inhibited (Dzhura et al., 2011). PLC-ε has a domain structure containing two RA domains at its carboxy-terminus (Kelley et al., 2001), and PLC-ε is activated by the association of the active form of Rap to a RA domain (Schmidt et al., 2001; Song et al., 2002; Dzhura et al., 2010). It was shown that Epac2 modulates the activity of the K_{ATP} channels in pancreatic β-cells (Kang et al., 2006). The mechanism of this phenomenon was inferred from the finding that phosphatidylinositol 4,5-bisphosphate (PIP_2) activates the K_{ATP} channel and decreases its sensitivity toward ATP (Baukrowitz et al., 1998). Thus, Epac/Rap-mediated hydrolysis of PIP_2 localized around the K_{ATP} channel by PLC-ε, which hydrolyzes PIP_2 to generate IP_3, might increase the sensitivity of the channel to ATP, thereby inhibiting the channel (Holz et al., 2006).

GLP-1 is known to mobilize Ca^{2+} from endoplasmic reticulum (ER) to cytosol in pancreatic β-cells (Gromada et al., 1995; Holz et al., 1999). In this process, Epac2 is involved in RyR-mediated Ca^{2+} mobilization (Kang et al., 2001; Kang et al., 2003). Although the precise mechanism of RyR-mediated Ca^{2+} mobilization remains to be elucidated, these findings suggest that Epac2 is involved in Ca^{2+}-induced Ca^{2+} release from ER. In addition, it has been suggested that activation of PLC-ε by Epac2 stimulates IP_3 receptor-mediated Ca^{2+} mobilization (Schmidt et al., 2001).

Epac2 was also reported to regulate glucose metabolism. GLP-1 may increase the activity of glucokinase, the rate-limiting enzyme in glycolysis, through Epac2-Rim2-Rab3 signaling (Park et al., 2012). Ca^{2+} mobilization by GLP-1 has been reported to stimulate mitochondrial ATP synthesis in MIN6 cells (Tsuboi et al., 2003).

3.2. Glucagon secretion from pancreatic α-cells

Glucagon, which is secreted from pancreatic α-cells, also has an essential role in glucose-homeostasis: it stimulates glycogenolysis and gluconeogenesis in liver. Glucagon secretion from α-cells is regulated by a variety of factors including nutrients such as glucose and amino acids, hormones, and neurotransmitters (Quesada et al., 2008; Sandoval and D'Alessio, 2015). Adrenaline stimulates glucagon secretion by L-type Ca^{2+} channel-dependent exocytosis through activation of Epac2 as well as PKA via a large increase in intracellular cAMP levels by binding to the Gs-coupled β-adrenergic receptors on α-cells (De Marinis et al., 2010). On the other hand, GLP-1 was reported to exert an inhibitory effect on glucagon secretion (De Marinis et al., 2010). However, whether or not this is a direct effect on α-cells or an indirect effect through somatostatin secretion from δ-cells is inconclusive (de Heer et al., 2008).

4. Roles of Epac2 in CNS

It is well known that cAMP regulates the molecular mechanisms of a wide variety of neuronal processes through an Epac-dependent pathway as well as a PKA-dependent pathway. In brain, both Epac1 and Epac2 are expressed (Kawasaki et al., 1998). Consistent

with the observation that Epac2 is expressed abundantly in neuronal cells (Kawasaki et al., 1998), diverse roles of Epac2 in brain have been pointed out regarding neurotransmitter release, neuronal differentiation, neurite growth, memory, learning and pathophysiological roles in some brain disorders such as Alzheimer's disease and autism (Schmidt et al., 2013).

4.1. Neurotransmitter release

Epacs were shown to be involved in the enhancement of neurotransmitter release in glutamatergic synapses from calyx of Held and in the crayfish neuromuscular junction. (Sakaba and Neher, 2001; Zhong and Zucker, 2005; Gekel and Neher, 2008). Interaction of Epac2 with Rim1, which is an isoform of the Rim family and is expressed predominantly in brain, may participate in regulated exocytosis of synaptic vesicles in neuronal cells as well as in large dense core granules in pancreatic β-cells (Ozaki et al., 2000). A recent study of *Rapgef4*$^{-/-}$mice demonstrated the involvement of Epac2 in cAMP-dependent potentiation of neurotransmission through maintenance of the RRP at mossy fiber synapses in the hippocampus during sustained transmission or after tetanus-induced mossy fiber-long-term potentiation (LTP) (Fernandes et al., 2015).

4.2. Neural development and remodeling

Epac has roles in development of brain including neurite growth and neuronal differentiation. Initial observations showed that Epac-specific agonist induces neurite outgrowth in rat pheochromocytoma PC12 cells (Christensen et al., 2003). In an additional study using PC12 cells, it was shown that the Epac signal extended the duration of PKA-dependent extracellular signal-regulated kinase (ERK) 1/2 activation, and thereby converted cAMP from a proliferative into a neurite outgrowth-promoting signal (Kiermayer et al., 2005). Epac also has a role in mechanisms of cAMP-regulated axon growth and guidance, and in mediating axon regeneration in adult mammalian CNS tissue. (Murray and Shewan, 2008).

Growing evidence supports isoform-specific roles of Epac2 in these processes. It was suggested recently that while the cAMP sensor Rapgef2 is involved in signaling to ERK to mediate neuritogenesis, Epac2 can induce the growth arrest involved in the process of differentiation through signaling to the p38 mitogen activated protein kinase (MAPK) (Emery et al., 2014). In spine synapses, Epac2 induces synapse remodeling by altering synapse shrinkage and turnover, and also causes functional depression of spiny synapses through forming a molecular complex with postsynaptic adhesion molecule neuroligin 3, which is responsible for membrane translocation of Epac2 and consequent activation of Rap (Woolfrey et al., 2009; Penzes et al., 2011).

4.3. Higher brain function

The Epac-specific activator 8-pCPT-2'-*O*-Me-cAMP (8-pCPT) enhances maintenance of LTP in CA1 of mouse hippocampal slices through ERK signaling (Gelinas et al., 2008). In hippocampal CA1 excitatory synapses, Epac mediates pituitary adenylate cyclase-activating peptide-dependent long-term depression (LTD) through activation of p38-MAPK, mobilization of Ca^{2+}, and protein synthesis (Ster et al., 2009). These findings suggest that Epac is likely to have important roles in synaptic plasticity, thus controlling higher brain

functions such as memory and learning. Intrahippocampal injection of 8-pCPT has been reported to improve fear memory retrieval in contextual fear conditioning but not its acquisition and consolidation (Ostroveanu et al., 2010). This study also suggested that Epac2 is involved in the time-limited memory retrieval (Ostroveanu et al., 2010). In murine primary cortical neurons and HT-4 cells, Epac2 enhanced phosphorylation of PKB/Akt, which is known to support neuronal survival and memory processes through Rap1 activation. In these processes, AKAP150 regulates phosphorylation of PKB/Akt by controlling two cAMP pathways, the Epac2-dependent and PKA-dependent pathways (Nijholt et al., 2008). The double knockout of *Rapgef3* and *Rapgef4* in forebrain of mice impaired LTP, spatial learning, and social interaction. These impairments were mediated by microRNA miR-124 transcription and zinc finger protein Zif268 translation (Yang et al., 2012). In addition, Epac2 depletion was found to induce impairments in social interaction, ultrasonic vocalization and cortical structure. In these mice, abnormal columnar organization occurred in the anterior cingulate cortex, the region related to socially-driven interactions, and dendric spine motility and density on cortical neurons were reduced (Srivastava et al., 2012a).

Recent genetic studies suggested a role of Epac2 in the pathophysiological mechanism of autism. In 2003, rare coding mutations in Epac2 were identified in patients with autism (Bacchelli et al., 2003). Overexpression of this mutated form of Epac2 reduced the basal dendrite complexity in cortical pyramidal neurons and disrupted the interaction between Epac2 and Ras, suggesting that Epac2 enables crosstalk between Ras and Rap signaling and takes part in the regulation of basal dendrite complexity in cortical neurons (Srivastava et al., 2012b).

5. Functions of Epac2 in heart

In heart, stimulation of β-adrenergic receptors, which is coupled with Gs-protein, increases the intracellular cAMP levels. cAMP has physiological roles in cardiac functions including cardiac contractility, relaxation, heart rate and automaticity (Bers, 2008). Although these stimulations are necessary for part of normal adaptation to increase in physiologic demand, chronic elevation of cAMP due to sustained β-adrenergic stimulation has been associated with hypertrophy, arrhythmia and eventually development of heart failure (El-Armouche and Eschenhagen, 2009). These phenotypes might be due to over-activation of Epac as well as PKA.

In heart, Epac1 is predominantly expressed compared with Epac2 (Kawasaki et al., 1998; Metrich et al., 2008). Epac1 but not Epac2 is increased in pressure overload-induced hypertrophy (Ulucan et al., 2007) and knockdown of Epac1 inhibits β-adrenergic receptor-induced hypertrophy (Metrich et al., 2008). Epac1-mediated hypertrophy involves activation of hypertrophic transcription regulators such as NFAT (nuclear factor of activated T-cells) (Morel et al., 2005; Metrich et al., 2008) and MEF2 (Metrich et al., 2010; Pereira et al., 2012) through activation of several kinds of small GTPases such as Rac, Rap2B and H-Ras with PLC, phosphatase calcineurin and CaMKII. In contrast, Epac2 is more likely to be involved in enhancing susceptibility to arrhythmia in mice. It was reported that the Epac activator, 8-pCPT, caused ventricular tachycardia through the Ca^{2+}/CaMKII pathway (Hothi et al., 2008). Using *Rapgef3*$^{-/-}$mice, *Rapgef4*$^{-/-}$mice, and *Rapgef3*$^{-/-}$; *Rapgef4*$^{-/-}$mice, it

was shown that Epac2 but not Epac1 participates in the arrhythmogenic effect through CaMKII-dependent diastolic sarcoplasmic reticulum (SR) Ca^{2+} release; this process involves the β1-adrenargic receptor, Epac2, CaMKIIδ, and phosphorylation of the S2814 residue of RyR2 (Pereira et al., 2013). Furthermore, a study using fluorescent Epac2 ligand (Φ-*O*-Me-cAMP) showed distinct distributions between Epac1 and Epac2 in mice myocytes. Epac2 is localized along T-tubules while Epac1 is localized around the nucleus, supporting a role for Epac2 in arrhythmogenic SR Ca^{2+} leak in mice (Pereira et al., 2015). Whether or not Epac2 contributes to the progression of arrhythmia in human is unknown.

Epac2 also participates in atrial natriuretic peptide (ANP) secretion by heart in mice. The GLP-1 receptor was found to be expressed at cardiac atria, and activation of the receptor increased the plasma ANP concentration, which contributes to the antihypertensive effect through vascular smooth muscle relaxation and natriuresis in kidney. In this process, Epac2 is shown to link activation of the GLP-1 receptor and ANP secretion through PLC-dependent signals in cardiomyocytes (Kim et al., 2013).

6. Epac2 as a potential therapeutic target

Epac is considered to be a promising drug target for various diseases (Parnell et al., 2015). Epac2 as well as PKA has an important role in cAMP-mediated insulin potentiation. Epac2 agonist is expected to stimulate insulin secretion in a glucose-dependent manner, so it would have low risk of hypoglycemia clinically, as is key for incretin-based anti-diabetic therapies. Epac2 agonists might therefore have benefits for treatment of type 2 diabetes with impaired insulin secretion from pancreatic β-cells.

So far, Epac-specific agonists have been developed based on modification of cAMP. The important discovery of the Epac-specific cAMP analog 8-pCPT (Enserink et al., 2002) and its membrane-permeable derivative 8-pCPT-AM (Vliem et al., 2008) deepened our understanding of Epac-dependent and PKA-independent actions of cAMP signaling. However, because of its non-selectivity toward Epac isoforms, application to clinical use of these analogs is not possible due to side effects, on cardiac function for example. Indeed, sustained activation of cAMP signaling caused Epac1-dependent hypertrophy and fibrosis in heart in mice, as described above. A novel cAMP-analog Sp-8-BnT-cAMPS, which displays a great selectivity to Epac2 at cell level, has recently been developed (Schwede et al., 2015). It will be useful in further studies of Epac2.

It was shown that sulfonylurea drugs, widely used for the treatment of type 2 diabetes, are potent selective activators for Epac2 as assessed by a FRET (fluorescence resonance energy transfer) system, by which activation and subsequent dynamic structural change of Epac2 can be monitored (Zhang et al., 2009). However, other groups failed to detect the direct activation of Epac2 by sulfonylureas using cell free system (Tsalkova et al., 2011; Rehmann, 2012). Using molecular docking simulation, site-directed mutagenesis, Epac2A-FRET biosensor, and direct sulfonylurea-binding experiments, the amino acid residues Cys105, Gly114, Ser116 and His124 in the cNBD-A of Epac2A that interact with sulfonylureas have recently been identified (Takahashi et al., 2013). Binding of sulfonylureas to Epac2A depends on the concentration of cAMP and the structures of the drugs. Sulfonylureas and

cAMP cooperatively activate Epac2A through binding to cNBD-A and cNBD-B, respectively. As the plasma membrane is a crucial factor in Epac2A's activation of Rap1 (Liu et al., 2008), activation of Epac2A by sulfonylureas may require the cellular environment. Thus, it is considered that sulfonylureas except for gliclazide activate Epac2/Rap1 signaling in concert with cAMP signaling in its augmenting effect on insulin secretion (Takahashi et al., 2015). Since sulfonylureas have no effects on activation of Epac1 or PKA, these findings may provide a clue to the development of Epac2-specific agonists independent of the structure of cAMP.

7. Conclusion

Since the discovery of Epac proteins in 1998, accumulating studies have shed light on PKA-independent cAMP action in various cell types and tissues. Other than in the pancreatic islets, brain, and heart summarized in this review, Epac has been revealed to function in a wide range of organs and cell types including vasculature, lung, kidney, endometrium, osteoclasts, and inflammatory cells. Despite such advances, a great number of issues remain to be elucidated, including the isoform-specific molecular and physiological functions and spatio-temporal regulation of Epacs in each organ. Generation of tissue-specific genetically-modified animals and development of isoform-selective agonist or antagonist will lead not only to the further understanding of isoform-specific functions but also the discovery of novel drugs for treating various diseases.

Acknowledgments

This review and the corresponding Gene Wiki article are written as part of the Cardiac Gene Wiki Review series--a series resulting from a collaboration between the journal GENE, the Gene Wiki Initiative, and the BD2K initiative. The Cardiac Gene Wiki Initiative is supported by National Institutes of Health (GM089820 and GM114833). Additional support for Gene Wiki Reviews is provided by Elsevier, the publisher of GENE. We would like to thank members of our laboratory for their contributions to our studies cited in this review. The studies in our laboratory were supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sport, Science and Technology, Japan.

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Highlights

- Exchange protein directly activated by cAMP 2 (EPAC2) is encoded by *Rapgef4* gene.
- Epac2 functions as a guanine-exchange factor regulated by cAMP.
- Epac2 has diverse roles in cellular functions of pancreatic islets, central nervous system and heart.
- Epac2 is a potential therapeutic target for treatment of type 2 diabetes.

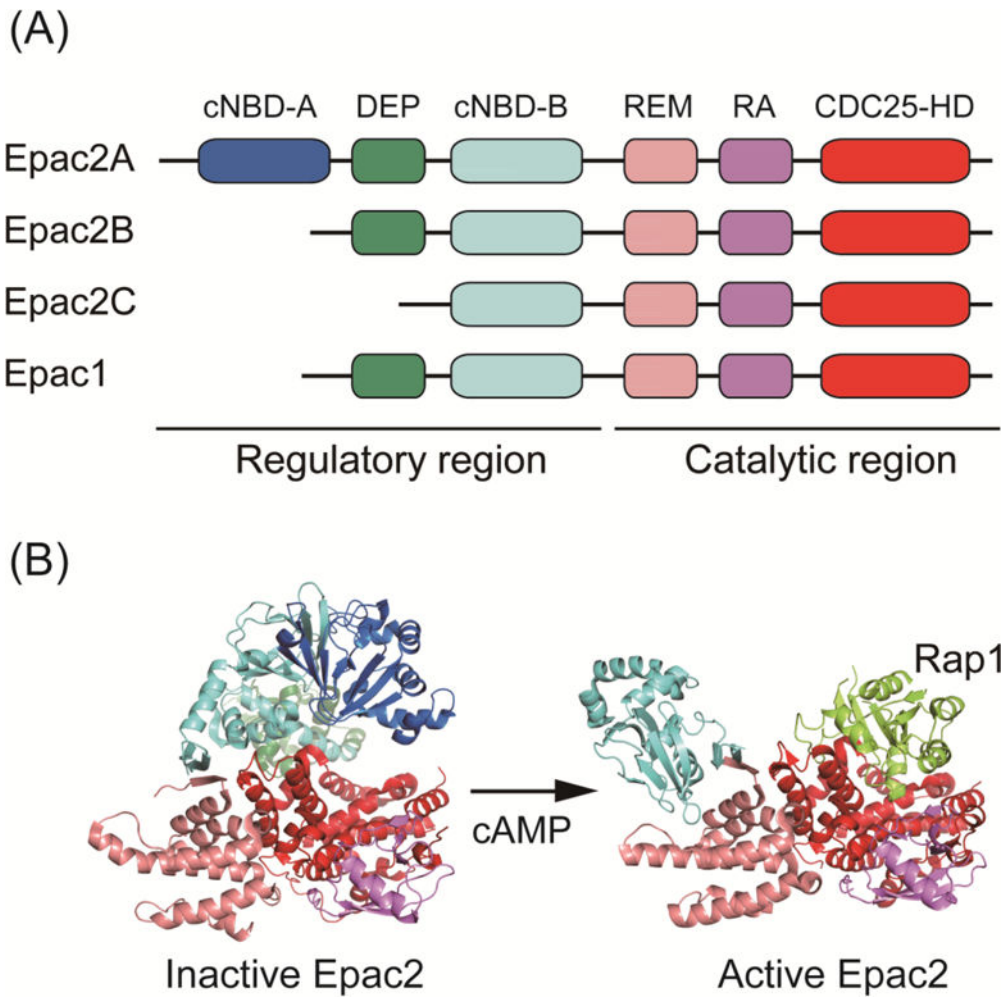

Figure 1. Domain structure of Epacs and mechanism of activation of Epac2.

(A) Domain structure of Epac1 and Epac2. Epacs have an amino-terminal regulatory region and a carboxy-terminal catalytic region. The regulatory region contains one or two cyclic nucleotide binding domains (cNBD) and a DEP (Dishevelled, Egl-10, and Pleckstrin) domain. The catalytic region contains a REM (Ras exchange motif), a RA (Ras association) domain and a CDC25-HD (CDC25 homology domain).

(B) Crystal structures of inactive-form (PDB code 2BYV) and active-form (in complex with Rap1B and cAMP analog; PDB code 3CF6) of Epac2. In the inactive-form of Epac2, the Ras-binding region in CDC25-HD is masked by the regulatory region. The binding of cAMP to cNBD-B induces a conformational change, enabling access by Rap to the catalytic region.

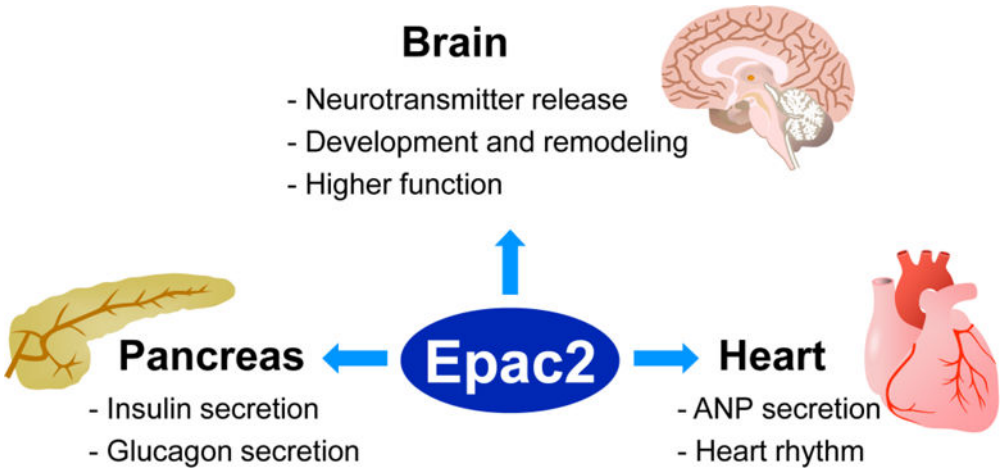

Figure 2.
Functional roles of Epac2 in pancreas, brain, and heart.
See text for details.