Cyclic AMP signaling in cardiac myocytes

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Abstract

The cyclic nucleotide 3', 5'-cyclic adenosine monophosphate (cAMP) is a ubiquitous second messenger of paramount importance in the regulation of the cardiac pump. It is now well established that cAMP is confined in specific subcellular compartments where it modulates various targets associated in signalosomes to control e.g. excitation-contraction coupling or gene transcription. In this review, we summarize notable breakthroughs on how cAMP is synthesized, degraded and compartmentalized. We describe its effectors, including newly described targets with emerging roles in heart. We also briefly discuss innovative methods recently developed to bring new insights on the role of these cAMP microdomains to control cardiac function.

Introduction

Since the discovery 60 years ago of the cyclic nucleotide cyclic adenosine 3′,5′-monophosphate (cAMP) many questions arouse and many answers were given on how this second messenger controls cardiac function. The sympathetic nerve/β-adrenergic receptor (β-AR)/adenylyl cyclase (AC)/cAMP/protein kinase A (PKA) axis is the canonical route to stimulate cardiac rhythm (chronotropy), contractile force (inotropy) and relaxation (lusitropy) [1]. PKA phosphorylates many of the proteins critically involved in the excitation contraction coupling (ECC). These include the L-type Ca^{2+} channels (LTCCs) [2] to increase the amplitude of $I_{Ca,L}$, the ryanodine receptor 2 (RyR2) [3] to increase its opening, phospholamban (PLB) to relieve its inhibitory effect on the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2) [4] and contractile proteins, such as troponin I (TnI) and myosin binding protein C, to accelerate cardiac relaxation [1]. Additionally, PKA phosphorylates the phosphatase 1 inhibitor [5], β-AR receptors and phosphodiesterases (PDEs) [6] to modulate intracellular signaling pathways that control not only ECC but also gene transcription, apoptosis and cell survival, and thus cardiac remodeling. While PKA is a major target for cAMP, the cyclic nucleotide also acts independently of the kinase binding directly to various proteins. The aim of the present review is not to be exhaustive, but to give an overview of the current knowledge on cAMP pathways in cardiac cells and discuss new perspectives in the field.

cAMP synthesis

All seven transmembrane receptors coupled to heterotrimeric G proteins (GPCRs) driving cAMP production do not lead to similar functional effects, but activate specifically localized effectors comforting the hypothesis of a compartmentation of cAMP signals [6]. The localization of the enzymes (ACs) which catalyze the conversion of ATP to cAMP and pyrophosphate is also determinant for the spatial organization of these signals. The main sources of cAMP in the cardiomyocytes are transarcolemmal ACs which are activated by $G_{\alpha s}$ freed upon GPCR activation by catecholamines and various hormones. Among the nine known AC isoforms, AC5 and AC6 are the main enzymes expressed in heart [7]. While both enzymes share 65% homology and are activated by $G_{\alpha s}$, but

inhibited by $G_{\alpha i}$, PKA and Ca^{2+}, extensive investigations led to the general conclusion that both enzymes have diametrically opposite roles in heart [8]. This could be due to their differential localization: AC5 is present in the nuclear envelop [9,10] and in caveolin-3 domains of the T-tubule membrane and is coupled to β_1- and β_2-ARs [11]; AC6 is expressed in the plasma membrane outside the T-tubular system and is coupled to β_1-ARs only [11]. However, the expression of β_2-ARs in myocytes was recently challenged in mouse heart [12]. The difference in the intrinsic enzymatic activities of AC5 and AC6 or their different modulation by PKC-dependent phosphorylation could also explain some of their functional differences [8]. AC5 deletion resulted in either enhanced or unaltered contractile performances and decreased sensitivity or blunted maximal response to β-AR stimulation [13-15]. AC6 KO animals exhibit reduced left ventricular contractile function and relaxation [13]. Interestingly, AC5 deletion increases longevity due to increased antioxidant capacities [16], improves exercise performance [17] and protects the heart against catecholamine infusion and pressure overload [15,18]. Similarly, AC6 KO mice are also protected against pressure overload [19]. However, the major difference between the two isoforms is that cardiac overexpression of AC5 leads to an impaired heart function [20] and exacerbates the cardiomyopathy induced by chronic catecholamine stress [21], whereas overexpression of the AC6 isoform improves heart function in murine cardiomyopathy due to $G_{\alpha q}$ overexpression [22]. Furthermore, AC5 inhibition prevents heart failure (HF) induced by myocardial infarction or by chronic catecholamine stress in mice, and protects against myocardial apoptosis [23] and myocardial ischemia in mice and swine [24]. Gene transfer of AC6 confirmed its beneficial effects since it increased left ventricular (LV) function and attenuated deleterious LV remodeling in a pig model of HF [25]. This paved the way to gene therapy with AC6 in HF patients, and a recent randomized clinical trial demonstrated improved LV function after intracoronary delivery of an adenovirus encoding AC6 in HF, with most benefits in patients with nonischemic etiology [26●]. However, larger trials are warranted to confirm these positive outcomes.

A cardiac role for soluble AC (sAC), which is activated by bicarbonate and Ca^{2+} and is insensitive to G proteins, has recently began to emerge. Its presence within the mitochondria has been suggested to regulate oxidative phosphorylation. In neonatal cardiomyocytes, mitochondrial cAMP production measured using a fluorescence resonance energy transfer (FRET) biosensor follows Ca^{2+} oscillation in the cytosol suggesting that these organelles can integrate an oscillatory Ca^{2+} signal to increase cAMP in their matrix [27]. This was confirmed by our group which demonstrated a protective role for sAC against cell death, apoptosis as well as necrosis [28●]. Upon stimulation with bicarbonate and Ca^{2+} of isolated mitochondria, sAC produces cAMP, which in turn stimulates oxygen consumption, increases the mitochondrial membrane potential and ATP production giving further evidences of sAC in the organelles [28●]. However, cAMP analogues failed to alter mitochondrial respiration in mitochondria from pig hearts in another study [29]. Thus, cAMP signaling within the matrix remains controversial [30] and further studies are required to confirm the role of sAC in the mitochondria.

cAMP targets in heart

Among cAMP receptors, PKA is a major player in the cAMP signaling pathways in heart. Two general classes of PKA are expressed, named PKA-I and PKA-II, due to differences in their regulatory (R) subunits, respectively RI and RII. Two R subunits dimerize and each associates with two catalytic (C) subunits to form holoenzymes. PKA-RI is mainly cytosolic while PKA-RII associates with the particulate fraction [31,32] and with key proteins of the ECC, and is considered as the main isoform responsible for the inotropic effects of a β-AR stimulation. PKA also phosphorylates the endogenous inhibitor of phosphatase 1 (I-1), causing inhibition of the enzyme which acts as a distal amplifier of β-AR signaling [5]. The role of PKA-RI in heart remains elusive. However, overexpression of a microARN, mir-208a, decreased RI expression and the β-AR inotropic effects, suggesting a potential role for this isoform to control ECC [33●]. PKA activation occurs when two cAMP molecules bind to each R subunit. This allows a conformational change leading to the dissociation of the C subunit. However, this dogma has been recently challenged by the discovery of disordered and flexible regions within RII bound to A kinase anchoring proteins (AKAPs) that determine how far from the AKAP the C region can operate and define the efficiency of the binding of the kinase to its target [34●●]. Further evidences confirmed that AKAPs constrain C subunits that operate within a radius of 200 Å but dissociate when cAMP reaches supraphysiological levels allowing it to reach nuclear targets [35]. This model of static C subunits orchestrating the phosphorylation of peculiar substrates nicely fits the rapid and specific PKA-RII phosphorylation of the key proteins of the ECC. It also explains the slow kinetics of PKA activation in the nucleus despite equivalently rapid elevations of cytosolic and nuclear cAMP levels [36,37]. It is well known that high catecholamine concentrations and prolonged β-AR stimulation, unlike brief β-AR agonist applications, can lead to PKA phosphorylation of transcription factors of the cAMP response element (CRE)-Binding protein (CREB) family [36,37] to induce cardiac hypertrophy. Increased phosphorylation/activation of CREB is an important factor in the up-regulation of ICER (inducible cAMP early repressor) expression which inhibits the antiapoptotic BCL-2 expression [38]. BCL-2 and the dynamin related protein 1 (Drp1) are both PKA substrates suggesting that PKA may regulate not only apoptosis but also mitochondria dynamics [39●]. Indeed, mitochondrial cAMP-PKA signaling cascade was found to control mitochondria elongation and mitochondrial cell-death [30]. However, the presence of PKA within the matrix [28●] or at the membrane of the organelle [30] is under debate. Additionally, PKA may control gene transcription via other nuclear proteins such as Class II histone deacetylases (HDACs) [40-42]. β-ARs can also activate the signal transducer and activator of transcription 3 (STAT3), which is critical for the transcription of β-AR signaling components including receptors, PKA and Ca^{2+} channels [43]. PKA can also exert cardioprotective effects via the phosphorylation of Hsp20 [44].

Besides activating PKA, cAMP binds to, and modulates the gating of, the hyperpolarization-activated, cyclic nucleotide-gated (HCN) channel HCN4, the main isoform expressed in the sinus node, the atrioventricular node and Purkinje fibers, leading to positive chronotropic and dromotropic effects [45].

Epac (Exchange Protein directly Activated by cAMP) proteins are other important cAMP effectors in heart [46]. Initially, Epac was found to affect Ca^{2+} homeostasis in neonatal cardiomyocytes [47] and this was then confirmed in adult cells, where Epac, downstream of the β-AR, leads to SR Ca^{2+} leak by activating Ca^{2+}/Calmodulin kinase II (CaMKII) which phosphorylates RyR2 [48] and PLB [49] via a sequential activation of Rap, PLCε, and PKCε [50,51]. To do so, one Epac isoform (Epac2) is localized in the Z striation of the cardiomyocyte [52,53]. However, an alternative pathway has been recently proposed which involves a β_1-AR/Epac/PI3K/Akt/NOSynthase1/CaMKII cascade [54]. Epac also exerts pro-arrhythmic effects by its ability to decrease potassium currents [55]. Moreover, inotropic effects of Epac activation by increasing myofilament Ca^{2+} sensitivity were reported [56]. Epac1, the second isoform expressed in heart, is localized around the nucleus [52], suggesting a role in controlling nuclear function. Accordingly, chronic Epac activation leads to cardiac hypertrophy [47,57] by initiating HDAC5 nuclear export and subsequent activation of MEF2 [58]. Epac downregulates the subunit of the potassium voltage-gated channel subfamily E member 1 (KCNE1) [59] and increases TRPC3/4 channel expression [60]. Furthermore, Epac1 activation also leads to autophagy activity in response to chronic activation of β-AR [61]. Epac1 might also be expressed within mitochondria where its activation by cAMP generated by sAC would protect mitochondria from Ca^{2+} overload and cell death [28●]. However, another recent study demonstrated that invalidation of Epac1 reduces infarct size and cardiomyocyte apoptosis induced by myocardial ischemia/reperfusion injury [62●]. Thus, like for the mitochondrial cAMP/PKA signaling pathway, future studies are warranted to fully understand the functional role of cAMP/Epac1 in this organelle.

In the early 2000's, a fourth class of cAMP effector encoded by Popeye domain containing (*Popdc*) genes with domains resembling the secondary structure of the cAMP-binding domain of the R subunit of PKA-II was discovered [63●●]. Of the three known *Popdc* genes (*1-3*) [64], *Popdc1* and *2* are highly expressed in cardiac tissue, especially in the conduction system including the sinus and atrioventricular nodes [63●●]. While no cardiac hypertrophy nor ECG modification was noticed in KO animals for the two genes, these mice exhibited bradycardia under mental stress or β-AR stimulation in an age dependent manner [63●●]. Interestingly, patients with a *Popdc1* mutation display early onset AV-block arrhythmias [65]. To influence pacemaker activity and electric conduction, Popdc interacts with the potassium channel TREK-1 to increase its expression at the plasma membrane, thus enhances K^+ current density, both phenomena being negatively modulated by cAMP [63●●]. Popdc interacts with caveolin 3 within caveolae in the T-tubules [66]. The number of binding partners of Popdc is increasing and the presence of the protein at the nuclear envelope suggests a role in transcriptional activity [65]. Indubitably, new functions for Popdc proteins in heart will be discovered in the near future.

cAMP elimination from cardiomyocytes

cAMP synthesis is counterbalanced by elimination processes to terminate the activation of cAMP effectors. This is mostly achieved by phosphodiesterases (PDEs) which specifically cleave the 3′,5′-cyclic phosphate moiety of cAMP to produce 5′-AMP. PDEs share conserved catalytic domains but differ markedly in their N-domains which contain diverse elements allowing their dimerization, binding of regulatory small molecules, phosphorylation and localization. Among the eleven known PDE families, five are expressed in heart to degrade cAMP: PDE1, which is activated by Ca^{2+}-calmodulin, PDE2, which is activated by cGMP, and PDE3, which is inhibited by cGMP, degrade both cGMP and cAMP while PDE4 and PDE8 are specific for cAMP. Each family can be encoded by several genes which together generate a multitude of PDE isoforms by the use of different translation initiation sites and alternative mRNA splicing. Extensive work to delineate the role in cardiac cells of these various enzymes has been performed, that is described in details in various reviews [6,67-69].

Despite its high level of expression in human and rodent cardiomyocytes [70,71], the functional role of PDE1 in myocytes is poorly documented. However, simultaneous measurements of Ca^{2+} signals and cAMP levels in paced cardiomyocytes demonstrated a role for PDE1 in shaping cAMP signals in a Ca^{2+} dependent manner to control ECC [72●]. Two isoforms of PDE1, PDE1A and C are expressed in heart [71]. Inhibition or genetic downregulation of PDE1A was found to prevent hypertrophy induced by various neurohumoral stimuli via a cGMP/PKG rather than cAMP mechanism [71]. PDE1C deficiency or inhibition attenuated apoptosis, in a cAMP/PKA and PI3K/AKT dependent manner *in vitro* and its genetic deletion attenuated cardiac remodeling and dysfunction induced by transverse aortic constriction in mice [73]. Accordingly, vinpocetine, a PDE1 inhibitor, prevented myocyte hypertrophy, fibroblast activation by TGF-β and fibrotic gene expression thus pathological remodeling induced by chronic stimulation with angiotensin II [74], confirming that PDE1 activation contributes to pathological cardiac remodeling.

Recently, PDE2 gained a lot of attention. PDE2 represents a minor part of total cAMP hydrolytic activity at basal, but its cAMP hydrolytic activity is stimulated up to 30-fold by cGMP. PDE2 was shown to inhibit cardiac LTCC in various species, including humans [6] and measurements with FRET-based sensors showed that PDE2 counteracts the effects of β-AR stimulation downstream of β_3-ARs [75]. In contrast to PDE3 and PDE4 which expression and activity are generally decreased in pathological hypertrophy and HF [76-78], PDE2 is increased in animal models as well as in human HF and this blunts β-AR/cAMP signals suggesting a protective mechanism against increased circulating catecholamines [79]. Accordingly, PDE2 overexpression improves cardiac function after myocardial infarction and protects against catecholamine-induced arrhythmias [80●●]. However, conflicting results were reported showing that PDE2 exerts prohypertrophic effects by blunting PKA-mediated phosphorylation of nuclear factor of activated T cells (NFAT) [81●●]. Furthermore, PDE2 inhibition was found to exert cardioprotective effects, resulting in elongated mitochondria and protecting against apoptotic cell death [30] and

improving mitochondrial respiration in sepsis induced myocardial dysfunction [82]. To do so, PDE2 would be part of a cAMP/PKA signaling domain at the surface of the organelle [30]. Whether PDE2 activation or inhibition produces cardioprotective effects is therefore a matter of debate [83,84] that will be probably arbitrated by ongoing studies.

PDE3 and PDE4 are the main PDE families controlling cAMP and ECC in heart. While PDE3 prevails in human and large mammal cardiac tissue [85], PDE4 dominates to control cAMP produced upon β-AR stimulation to control ECC in rodents [86,87], but also regulates cAMP levels and Ca^{2+} homeostasis in human atria and in dog cardiomyocytes [88,89]. PDE3 is encoded by two genes, *pde3A* and *pde3B*. PDE3A is responsible for the inotropic and chronotropic effects of PDE3 inhibitors. Because it associates with PLB in a phosphorylation-dependent manner [90], PDE3A controls SERCA2 activity and Ca^{2+} reuptake in the SR [91]. Furthermore, PDE3A is associated with myocyte apoptosis, likely through sustained induction of ICER [92], leading to the hypothesis that its overexpression may limit cardiac damages. Accordingly, overexpression of this isoform prevents ischemia/reperfusion (I/R)-induced myocardial infarction [93].

The PDE4 family is encoded by four genes (*pde4a–d*). Most of our knowledge of the roles of individual PDE4 subtypes in the heart is limited to PDE4D. It was found to regulate the PKA phosphorylation of RyR2 and its invalidation promotes sensitivity to ventricular arrhythmias and late-onset dilated cardiomyopathy [77]. PDE4D is crucial for the control of Ca^{2+} homeostasis in atria and its expression is decreased in atrial fibrillation [88], a cause of stroke. Interestingly, single nucleotide polymorphism (SNPs) in the *pde4d* gene were shown to be associated with cardiogenic shock [94] and cardioembolism [95] further suggesting its importance in the control of sinus rhythm. Similarly to PDE3A, PDE4D also associates with the PLB/SERCA2 complex and regulates SR Ca^{2+} load in the mouse heart [96,97]. It also controls the slowly activating delayed rectifier potassium channels [98], the desensitization of β_1- and β_2-ARs, associating either directly or indirectly through β-arrestin with the receptors [99-101]. PDE4D also degrades cAMP signals at the perinuclear region to regulate cardiac hypertrophy [102]. Lately, PDE4B was identified as an integral component of the LTCC complex to control $I_{Ca,L}$ during β-AR stimulation [97]. PDE4B KO mice exhibit an increased susceptibility to ventricular arrhythmias upon catecholamine stimulation, probably linked to enhanced $I_{Ca,L}$ [97]. Although RyR2 phosphorylation by PKA did not seem to be affected in adult PDE4B KO mice [97], a recent study indicated that it is increased in neonatal PDE4B KO myocytes [103], suggesting that altered RyR2 regulation may also contribute to these arrhythmias. Recently, we showed that under β-AR stimulation, inhibition of PDE4 (as well as inhibition of PDE3) exerted inotropic effects via PKA but led to spontaneous diastolic Ca^{2+} waves via both PKA and CaMKII, suggesting the potential use of CaMKII inhibitors as adjuncts to PDE inhibitors to limit their proarrhythmic effects [104].

PDE8A is also expressed in cardiomyocytes and its invalidation leads to exacerbated β-AR stimulation of the ECC and perturbation of the Ca^{2+} homeostasis [105]. However, further studies are required to fully understand the role of PDE8A in heart.

While hydrolysis by PDEs represents the main process to eliminate cAMP produced upon neurohormonal stimulations, there is also a contribution of nucleotide efflux from the cardiomyocyte. This is achieved by multidrug-resistance proteins (MRPs), of which the MRP4 isoform is crucial for cAMP homeostasis, since *Mrp4* KO mice exhibit enhanced cAMP and contractility and develop cardiac hypertrophy with age [106].

cAMP compartmentation in cardiomyocytes

The first evidence for a compartmentation of cAMP signaling in heart came from experiments showing that upon β-AR stimulation, cAMP and force of contraction were enhanced and soluble and particulate PKA activated, while upon PGE1 application, no change in contractile activity was observed despite an increase in cAMP and activation of soluble PKA [32,107]. Since then, a tremendous amount of work allowed to demonstrate that cAMP signaling is compartmentalized within the cardiomyocyte [6,8,69]. Different localizations of receptors and ACs are partly responsible for this compartmentation. Differentially localized β_1- and β_2-AR, AC5 and AC6 to caveolar T-tubular domains *vs.* non caveolar domains [11,108] and sAC in the mitochondria [28●] constitute various production sites in discrete membrane domains leading to the activation of cAMP effectors in close vicinity. Furthermore, it has been recently suggested that mitochondria act as physical barriers to limit cAMP diffusion in cardiomyocytes [109,110] and that association of PKA-RII at the surface of the organelle may play a crucial role in the limitation of cAMP diffusion [110]. Local specificity in cAMP functions is also achieved by the specific localization of its effectors. AKAPs, by localizing PKA in close proximity to its substrates play an essential role [111]. For example, PKA-RII was found associated with LTCC via the short AKAP7 (AKAP18α) [112], with RyR2 via mAKAP (AKAP6) [113], with SERCA2A complex via the long AKAP7 (AKAP18δ) [114], and with KCNQ1 [115]. By coordinating signaling pathways, AKAPs are crucial to achieved localized temporal regulation of β-AR stimulation. This is further attested by arrhythmias associated with mutations or SNPs in the yotiao gene associated with long QT [116,117]. PKA is not the sole cAMP effector found in macromecular complexes; for example, Epac1 was also found together with PKA, ERK5 and PDE4D tethered by the AKAP6 at the nuclear envelop to control cardiac hypertrophy [102]. Over the past 30 years, PDEs have been described as essential players in the compartmentation of cAMP [6,67,69]. Associated in macromolecular complexes, PDEs control cAMP levels at the vicinity of cAMP effectors such as the key ECC proteins as discussed earlier. Their role in limiting cAMP emanation from β-AR-stimulated AC was first demonstrated in cardiac cells studying the impact of PDE inhibition on $I_{Ca,L}$ regulation by local application of isoprenaline, suggesting their role as enzymatic barrier limiting cAMP diffusion [118]. Until then, only biochemical methods

involving cell fractionation gave a rough idea of cAMP signaling compartmentation [32,107]. Later, overexpression of exogenous mutant CNG channels allowed indirect cAMP measurements but limited at the subsarcolemmal compartment [87,119]. The advent of FRET bisosensors based on Epac [120], PKA [121], or PKA substrates [122] allowed to directly visualize cAMP concentration changes [123]. New technologies and the development of new biosensors permits now cAMP measurements in microdomains. Epac1-based sensors fused with the regulatory subunits of PKA-RI or PKA-RII allowed to visualize the differential cAMP increase induced by either β-AR stimulation or PGE1 in RII and RI compartments, respectively [124]. More recently, a sophisticated approach combining SCIM and FRET-imaging demonstrated the functional distribution of β-ARs subtypes in crests and T-tubules, taking advantage of the glass pipette used for cell surface topography measurements to locally and selectively activate β_1- or β_2-ARs [108]. A move towards cAMP measurements within “nanodomains” has been made by fusing sensors based on Epac1 or on the cAMP binding domain of the RII subunit of PKA with specific peptide sequences allowing their targeting at the membrane [125], in lipid rafts or non-rafts domains [126], to compartments including key multiprotein complexes involved in ECC such as SERCA2 protein [127,128●], TnI [128●], and the LTCC complex [125,128●]. Furthermore, combining genetic animal models overexpressing an Epac1-based biosensor with a stereomicroscope system recently allowed to measure cAMP changes directly in perfused heart in more physiological conditions [129●●]. Undoubtedly, in the near future, all these new approaches will provide exciting new insights concerning the organization of cAMP nanodomains within the cardiomyocyte and their functional roles.

Acknowledgements

Research from our laboratory was supported by the Investment for the Future program ANR-11-IDEX-0003-01 within the LABEX ANR-10-LABX-0033, a grant from ANR (ANR-16-ECVD-0007-01) and the 2016 Lamonica prize in cardiology (to RF).

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Figure Legend

Figure 1: The cartoon shows the differential cAMP signaling pathways and the localization of the various signalosomes in the cardiomyocyte including newly described transduction cAMP signals in the mitochondria. sAC, soluble adenylate cyclase; AC, adenylyl cyclase; AKAP, A-kinase anchoring protein; CaMKII, Ca^{2+}-Calmodulin kinase II; β-AR, β-adrenergic receptors.

Table 1: Physiological role of key players in the cAMP signalling pathways in heart and known implications in cardiac diseases.

Actors of cAMP signaling in cardiomyocytes	isoforms	Physiological roles	Known implications in cardiac diseases
AC5		cAMP synthesis upon β_2-AR stimulation [11]	Increased expression in HF [130]
AC6		cAMP synthesis upon β_1-AR stimulation [11]	Decreased expression in HF [130]
sAC		Regulation of oxidative stress, increase in mitochondrial respiration [27,28●] (but see [29])	Cardioprotective against cell death, apoptosis and necrosis [28●]
Short AKAP7 (AKAP18α)		Association of PKA-RII with LTCC [112]	
Long AKAP7 (AKAP18δ)		Association of PKA-RII with SERCA2A [114]	
AKAP6 (mAKAP)		- Association of PKA-RII with RyR2 and PDE4D [113] - Association of PKA-RII, Epac1, ERK5 and PDE4D at the nuclear envelope [102]	Coordinates signalling pathways leading to hypertrophy and cardiac remodelling [111]
AKAP9 (Yotiao)		Association of PKA-RII with KCNQ1 [115]	- Decreased interaction with PKA-RII in dilated cardiomyopathy [131] - SNPs associated with long QT [117]
PKA	PKA-RI	Control of ECC [33●]	- Antihypertrophic effects via NFAT phosphorylation [81●●] - Apoptosis via BCL-2 phosphorylation [39●] - Cardioprotective effects via Hsp20 [44] - Gene transcription/hypertrophic signals via phosphorylation of CREB [36,37], STAT3 [43], HDAC [40-42], BCL-2 [38] - Ca^{2+} homeostasis perturbations/arrhythmias [104]
	PKA-RII	- Phosphorylation of key proteins of ECC [32] - Phosphorylation of PDEs to increase cAMP hydrolysis [87,119] - Phosphorylation of the phosphatase inhibitor-1 [5]	
EPAC	Epac1	- Excitation-transcription coupling [58]	- Increased expression in HF [57] - Hypertrophy [47,57]

	Epac2	- Activation of CaMKII leading to RyR2 and PLB phosphorylation [48-51,55] - Regulation of Ca^{2+} sensitivity of myofilaments [56]	- Autophagy [61] - Apoptosis [28●,62●] - Diminishes KCNE1 expression [59] - Increases TRPC3/4 expression [60] - Ca^{2+} homeostasis perturbations/arrhythmias [53]
Popdc1 and 2		Pacemaker/electrical conductivity by enhancing TREK1 functional expression [63●●]	AV block associated with popdc1 mutation [65]
PDE1		Control of ECC [70]	- Hypertrophy [71] - Apoptosis [73] - Fibrotic gene expression [74]
PDE2		Control of ECC [6,79]	- Increased expression in heart failure [79] - Pro-hypertrophic [81●●] or antihypertrophic [79] - Apoptosis [39●] - Mitochondrial respiration in sepsis [82]
PDE3		- Control of ECC [87,90] - PLB phosphorylation [90,91]	- Decreased expression in hypertrophy [76] - Prevents Ca^{2+} homeostasis perturbations/arrhythmias [91,104] - Apoptosis [92,93]
PDE4		- Control of ECC - PKA phosphorylation of PLB, RyR2, LTCC and KCNQ1 [77,96,97,103,104] - β-AR desensitization [99,100]	- Decreased expression in hypertrophy [76], heart failure [77] and atrial fibrillation [88] - Prevents Ca^{2+} homeostasis perturbations/arrhythmias [77,88,104] - Hypertrophy [101,102] - SNPs in *Pde4d* gene associated with cardiogenic stroke [94] and cardioembolism [95]
PDE8		Control of ECC [105]	
MRP4		cAMP extrusion [106]	

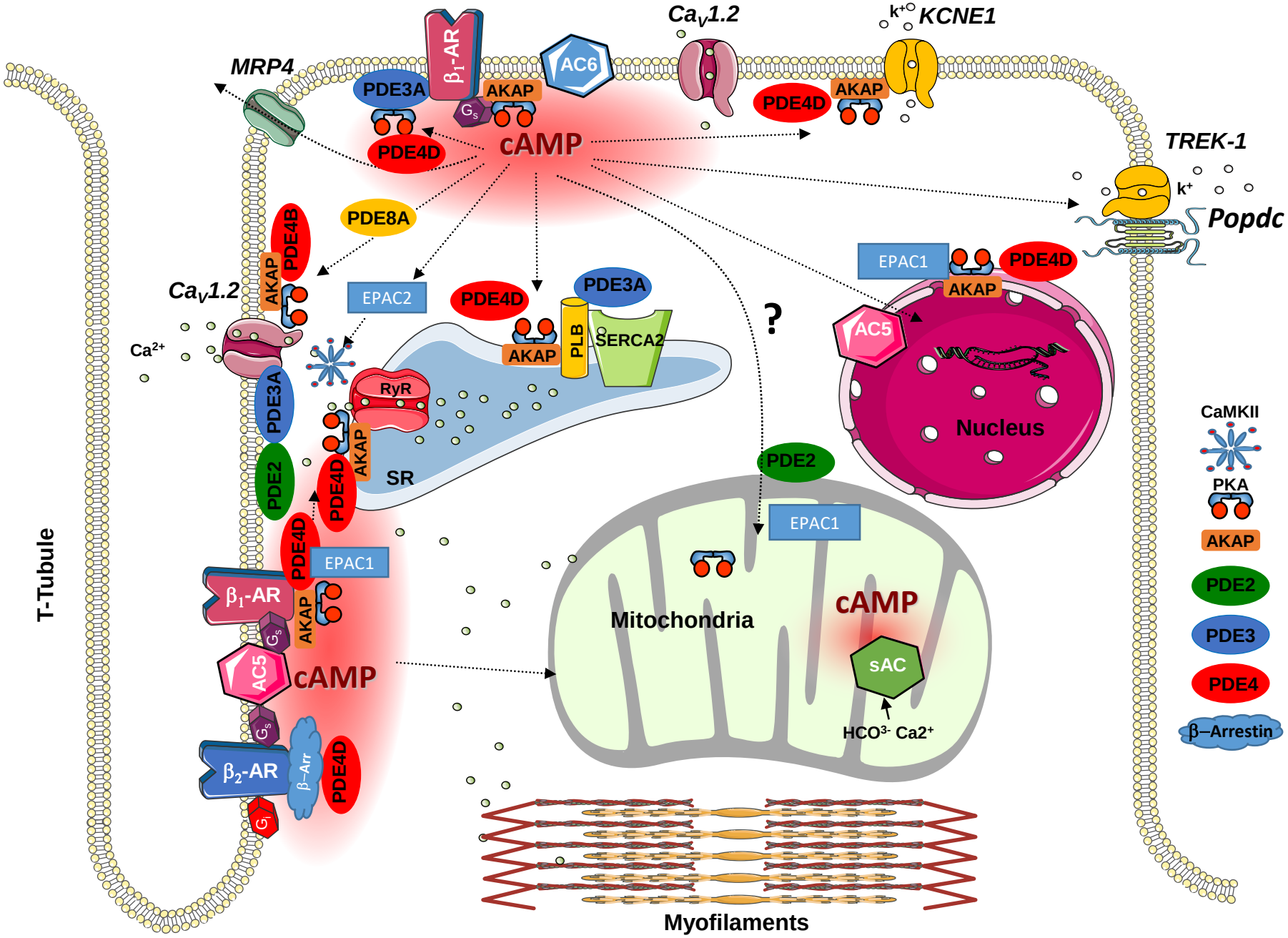
MRP4
β1-AR
AC6
PDE3A
AKAP
Gs
PDE4D
cAMP
CaV1.2
k+ KCNE1
PDE4D
AKAP
TREK-1
k+
Popdc
PDE8A
PDE4B
AKAP
CaV1.2
Ca2+
EPAC2
PDE4D
PDE3A
PLB
SERCA2
AKAP
RyR
SR
PDE3A
PDE2
PDE4D
AKAP
?
EPAC1
PDE4D
AKAP
AC5
Nucleus
PDE2
EPAC1
PDE4D
EPAC1
β1-AR
Gs
AKAP
AC5
cAMP
Gs
β2-AR
β–Arr
Gi
PDE4D
Mitochondria
cAMP
sAC
HCO3- Ca2+
T-Tubule
Myofilaments
CaMKII
PKA
AKAP
PDE2
PDE3
PDE4
β–Arrestin

Figure 1