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Review Article

cAMP/PKA signaling compartmentalization in cardiomyocytes: Lessons from FRET-based biosensors

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ABSTRACT

3′,5′-cyclic adenosine monophosphate (cAMP) is a ubiquitous second messenger produced in response to the stimulation of G protein-coupled receptors (GPCRs). It regulates a plethora of pathophysiological processes in different organs, including the cardiovascular system. It is now clear that cAMP is not uniformly distributed within cardiac myocytes but confined in specific subcellular compartments where it modulates key players of the excitation–contraction coupling as well as other processes including gene transcription, mitochondrial homeostasis and cell death. This review will cover the major cAMP microdomains in cardiac myocytes. We will describe recent work using pioneering tools developed for investigating the organization and the function of the major cAMP microdomains in cardiomyocytes, including the plasma membrane, the sarcoplasmic reticulum, the myofilaments, the nucleus and the mitochondria.

1. Introduction

3′,5′-Cyclic adenosine monophosphate (cAMP) is an intracellular second messenger that is generated in response to activation of a wide range of membrane receptors, belonging to the G protein-coupled receptor (GPCR) superfamily. Upon ligand binding, an active G protein is released and triggers a membrane bound adenylyl cyclase (AC) that generates cAMP from ATP. cAMP in turn regulates a plethora of physiological and pathological processes in different organs, including the cardiovascular system, and this is achieved by activation of the so-called cAMP effectors, including cAMP-dependent protein kinase (protein kinase A, PKA), exchange protein directly activated by cAMP (EPAC), cyclic-nucleotide-gated ion channels (CNG) and the recently discovered Popeye domain containing proteins (POPDC) [1,2].

The master function of cAMP in the heart is undoubtedly the regulation of the fight-or-flight response, a physiological reaction that occurs in response to a perceived harmful event, attack, or threat to survival. This reaction involves the activation of β-adrenergic receptors (β-ARs) and ensures positive chronotropic (increased heart rate), inotropic (increased force of contraction) and lusitropic (increased rate of relaxation) effects. This is primarily achieved by PKA-mediated phosphorylation of the proteins that participate in the coupling of cell excitation to myocyte contraction, also known as calcium-handling proteins, including L-type Ca^{2+} channels (LTCC) at the plasmalemma, phospholamban (PLB) and ryanodine receptors (RyR) on the sarcoplasmic reticulum (SR); and troponin I (TPNI) and myosin binding protein C (MyBP-C) on the myofilaments [3,4].

Beside the control of cardiac excitation-contraction coupling, cAMP directs a wide array of other processes which are diverse and at times opposing, including for instance gene transcription, mitochondrial homeostasis and cell death. As a consequence of the plethora of functions that progressively emerged to be under the control of cAMP, for long time the main question in the field has remained how different signals relying on the same intracellular second messenger could be translated into the appropriate cellular response. Seminal work by independent laboratories led to the nowadays well-established view that, within the cell, cAMP is made available in spatially and temporally restricted microdomains, and that different stimuli impact on well-confined cAMP pools while leaving others untouched [2,5]. This mechanism eventually ensures that only selective subsets of PKA, and, in turn, of its substrates, are engaged in response to activation of a specific receptor subtype, eventually translating into an unambiguous cellular response.

Compartmentalized cAMP/PKA signaling is now recognized as

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important for physiology and pathophysiology. Evidence indicate that the spatial heterogeneity of the cAMP signal is altered in pathological stress condition, and multiple drugs currently in use for the treatment of cardiac disease target the cAMP/PKA pathway. Unfortunately, the details of how cAMP/PKA compartmentalization is compromised in cardiac disease are still not completely understood. In the last years, this gap in knowledge has prompted the development of adequate molecular tools for the investigation of cAMP microdomains. Intense research by independent groups has led to the development of biosensors for the detection of changes in both cAMP and PKA activity in real time in intact living cells, with very high spatial and temporal resolution. These sensors are based on the principle of Fluorescence Resonance Energy Transfer (FRET) and, in the case of cAMP detection, take advantage of the cAMP binding domains present in PKA [2] or EPAC [6,7]. The binding of cAMP to these modules results in a conformational change of the probe and the ensuing reduction or increase (depending on the probe) of the FRET signal, which can be easily quantified. Since the description of the first cAMP biosensor by Zaccolo & Pozzan in 2002, different variants have been conceived and it has also turned out that the development of targeted, microdomain-specific biosensors is more challenging than expected. The major limitation of such probes is that targeted probes usually carry different dynamic ranges and cAMP affinities than their untargeted counterpart, thus rendering the direct comparison of FRET responses produced by the two different biosensors quite complex. A new generation of targeted probes allowing detection of compartmentalized cAMP with high fidelity and accuracy have been just recently discovered and may help in expanding our knowledge on how cAMP compartmentalization is altered in cardiac disease and how can be manipulated for therapeutic purposes [8,9].

This review aims at providing an overview of the major cAMP microdomains in cardiac myocytes and their regulation in cardiac physiopathology. A comprehensive description of the vast literature relative to the role of cardiac cAMP compartmentalization is beyond the scope of this work. We will focus our attention on and describe recent studies exploiting new generation untargeted and domain-targeted biosensors for investigating the organization and the function of the major cAMP microdomains in cardiomyocytes, including the plasma membrane, the sarcoplasmic reticulum, the myofilaments, the nucleus and the mitochondria. For more information on previous studies and/or other aspects of cAMP compartmentalization, we will refer the reader to dedicated reviews.

2. Basic principles of cAMP compartmentalization

cAMP compartmentalization is guaranteed by the formation in different subcellular compartments of cAMP signalosomes, i.e. multi-protein complexes combining together key players of the cAMP signaling. The prototypical cAMP signalosome includes at least (i) a cAMP effector, primarily PKA, (ii) one phosphodiesterase (PDE), the enzyme responsible for the degradation of cAMP, and (iii) a scaffold protein namely A-kinase anchoring protein (AKAP) which anchors the whole complex to a specific subcellular location. If under basal conditions the localized PDE maintains cAMP concentrations below an activation threshold for the PKA which remains dormant, upon hormonal stimulation the increased production of cAMP overcomes the rate of PDE-mediated degradation and stimulates PKA activity. Finally, PKA phosphorylation of the PDE stimulates its cAMP hydrolytic activity, thereby driving cAMP levels back to basal [10]. This peculiar organization of cAMP enzymes eventually facilitates the regulation of the levels of cAMP and of PKA activation in certain subcellular locations.

More than 50 genes encoding distinct AKAPs have been identified so far, and among the most relevant for the cardiovascular system are the short AKAP7, which anchors PKA to the LTCC at the plasma membrane, mAKAP (also known as AKAP6) which tethers PKA to RyRs at the SR, long AKAP7 which links PKA to Sarco-endoplasmic reticulum calcium ATPase (SERCA2A) complex and Yotiao that binds PKA to the plasma membrane channel KCNQ1 (for extensive reviews on cardiac AKAPs see [11,12]).

Similarly, the other key players of cAMP compartmentalization, PDEs, can be grouped in at least 11 families, including 20 genes and about 50 different isoforms. Nevertheless, the established view is that PDE3 and PDE4 are predominant in the heart and their cAMP hydrolytic activity is essential for shaping cAMP microdomains within cardiomyocytes (for extended reviews on cardiac PDEs see [13]). Extensive work has established that PDE4 primarily localizes close to plasmalemmal LTCC, while PDE3 is enriched at the SR in close proximity of the PLB/SERCA2 complex, although, as discussed below, the recent development of improved FRET-based biosensors suggests a multifaceted role of the different PDE isoforms.

Finally, besides the concerted action of AKAPs and PDEs, the spatial and temporal definition of cAMP domains may also rely on the different subcellular localization of PKA holoenzymes, and in particular of PKA regulatory subunits. If PKA regulatory subunit type I (PKA-RI) is mainly cytosolic, PKA regulatory subunit type II (PKA-RII) associates with the particulate fraction [14,15]. This implies that when the two catalytic subunits (PKA-C) are released from the PKA holoenzyme, depending on the type of regulatory adaptor they are bound to, they may not be free to diffuse within the cell. Rather, they could be limited by spatial boundaries that thus lead to activation of local, but not distant PKA substrates. Nevertheless, the textbook view that PKA-C subunits are released upon cAMP elevation has been recently overcome by the observation that catalytically active PKA holoenzymes remain intact within the cytoplasm [16], leaving the way open to new possibilities of cAMP signaling compartmentalization.

3. Different generations of FRET biosensors for monitoring cAMP and PKA activity

The first genetically encoded FRET biosensor for cAMP was described by the pioneering work of Zaccolo and colleagues, who exploited the ability of the R subunits of PKA to dissociate from the C subunits of the kinase upon binding to cAMP [2]. The probe was generated by fusing R and C subunits of PKA to CFP and YFP, respectively, implying that in conditions of low cAMP, the probe is maintained in its inactive holotetrameric conformation leading to maximal FRET signal, while a rise in cAMP induces a conformational change that releases active PKA-C, with a consequent reduction in FRET. Despite these first-generation biosensors allowed to significantly progress our understanding of cAMP compartmentalization in cardiac myocytes and other cells [2,17,18], they had major limitations. First, they were rapidly saturated, as a direct consequence of the high intracellular cAMP levels (which is usually in the micromolar range), leading to a significant loss of resolution at physiological concentrations of cAMP. Second, multimeric biosensors, like the RII-CFP/C-YPF, required equal expression of both subunits to form the PKA tetramer, a condition that was not always easily attainable. These problems were at least partially overcome by the design by three independent laboratories of unimolecular probes exploiting the cyclic nucleotide binding domain of EPAC1/2 (Table 1; for extensive reviews on the technical features of the different probes, see [19,20]). Nikolaev and colleagues first sandwiched the cAMP-binding module of EPAC1 and EPAC2 between CFP and YFP, generating EPAC1-camps and EPAC2-camps biosensors, respectively [6]. Zhang group instead produced a family of cAMP indicators named ICUE (Indicator of cAMP Using EPAC), by fusing the full-length EPAC1 to ECFP and YFP (or improved versions of these fluorophores, like the circularly permuted Venus at lysine 194; cpV-L194) [21–23]. Finally, the Jalink lab developed a similar probe carrying a full-length catalytically inactive EPAC1 (with a T781A/F782A double mutation) [7]. In parallel, Zhang lab conceived a family of FRET reporters of PKA activity, namely AKAR, consisting of a phosphoamino acid binding domain, linked to a PKA-specific substrate sequence, flanked by CFP and YFP [24]

Table 1
Characteristics of non-targeted and targeted FRET-based biosensors for cAMP.

Name	cAMP binding motif	Fluorophores	Targeting sequence	Notes	References
Non-targeted					
RII-CFP/C-YFP	PKA-RII	YFP/CFP	–		[2]
EPAC1-camps	EPAC1 cAMP-binding site	YFP/CFP	–		[6]
EPAC2-camps	EPAC2 cAMP-binding site	YFP/CFP	–		[6]
ICUE1	Full-length EPAC1	ECFP- Citrine	–		[21]
ICUE2	N-terminally truncated EPAC1 ($EPAC^{1149–881}$)	ECFP- Citrine	–		[23]
ICUE3	N-terminally truncated EPAC1 ($EPAC^{1149–881}$)	ECFP- cpV-L194 (circularly permuted Venus at lysine 194)	–	Increased dynamic range over ICUE1 and ICUE2	[22]
EPAC H30 (EPAC (δDEP-CD)	full-length EPAC1 (catalytically-inactive, T781A/F782A double mutation)	YFP/CFP	–		[7]
$EPACS^{H187}$	full-length EPAC1 (glutamine to glutamic acid mutation at position 270)	Turquoise/Venus	–	"Fourth generation" EPAC sensors (enhanced photostability, dynamic range and signal-to-noise ratio)	[61]
CUTie	Second cyclic nucleotide binding domain (CNBD) from PKA-RIIB	YFP/CFP (YFP was inserted within loop 4–5 of CNBD)	–	Allows subcellular targeting without altering the kinetics or the maximal amplitude of FRET responses	[9]
Plasma membrane					
pm-EPAC2-camps	From EPAC2-camps	EYFP/ECFP	SH4 motif of Lyn kinase	Effectively targeted to sarcolemma	[35]
β_1-AR-EPAC2	From EPAC2-camps	YFP/CFP	Full-length β_1-AR	Targeted to the plasma membrane	[36]
pm-ICUE1	From ICUE1	ECFP/Citrine	From small guanosine triphosphatase K-ras4B		[21]
EPAC2-MyrPalm	From EPAC2-camps	YFP/CFP	N-terminal acylation sequence (GCINSKRKD) from Lyn kinase	lipid raft/caveolar membrane fractions	[46]
EPAC2-CAAX	EPAC2 cAMP-binding site	YFP/CFP	CAAX box sequence (KKKKSKTKCVIM) from Rho GTPase	non-raft membrane microdomains	[46]
PLM-EPAC1	From EPAC2-camps	EYFP/ECFP	Full-length PLM	Targeted to Sodium-potassium ATPase	[47]
AKAP79-CUTie	From CUTie	EYFP/ECFP	AKAP79	Targeted to plasma membrane	[9]
Nucleus					
NLS-ICUE1	From ICUE1	ECFP/Citrine	nuclear localization signal (PKKKRKVEDA)	appropriately targeted to the nucleus	[21]
$EPACS^{H187}$-3NLS	From $EPACS^{H187}$	Turquoise/Venus	nuclear localization signal (PKKKRKVEDA)	appropriately targeted to the nucleus	[62]
Mitochondria					
MitoCOX-ICUE1	From ICUE1	ECFP/Citrine	From subunit IV of cytochrome *c* oxidase (COX)	Only partially targeted to mitochondria	[21]
Mito-DAKAP1-ICUE1	From ICUE1	ECFP/Citrine	From DAKAP1a	Effectively targeted to mitochondria	[21]
4mtH30	From H30	EYFP/ECFP	From human COXVIII (4 copies)	Good targeting (very small missorting in the cytosol)	[65]
Other compartments					
RI_EPAC and RII_EPAC	From EPAC1-camps	YFP/CFP	dimerization/docking domain from either RIα (amino acids 1 to 64) or RIIβ (amino acids 1 to 49)	RI_EPAC is cytosolic, RII_EPAC localizes at the membrane	[15]
EPAC1-PLB	From EPAC1-camps	YFP/CFP	N-terminus of PLB	Targeted to sarcoplasmic reticulum	[8]
TPNI-CUTie	From CUTie	YFP/CFP	TPNI	Targeted to myofilaments	[9]

Table 2
Characteristics of non-targeted and targeted FRET-based biosensors for PKA activity.

Name	cAMP binding motif	Fluorophores	Targeting sequence	Notes	References
Non-targeted					
AKAR3-NES	FHA1, forkhead associated domain 1 from Rad53p (residues 22–162)	ECFP/Venus	C-terminal nuclear export signal (NES) sequence	Excluded from the nucleus	[31]
Plasma membrane					
PM-AKAR3	From AKAR3	ECFP/Venus	Plasma membrane targeted sequence KKKKKSKTKCVIM	Plasma membrane targeting	[31]
Nucleus					
AKAR3-NLS	From AKAR3	ECFP/Venus	Nuclear localization signal (NLS) sequence PKKKRKVEDA	Appropriately targeted to the nucleus	[31]

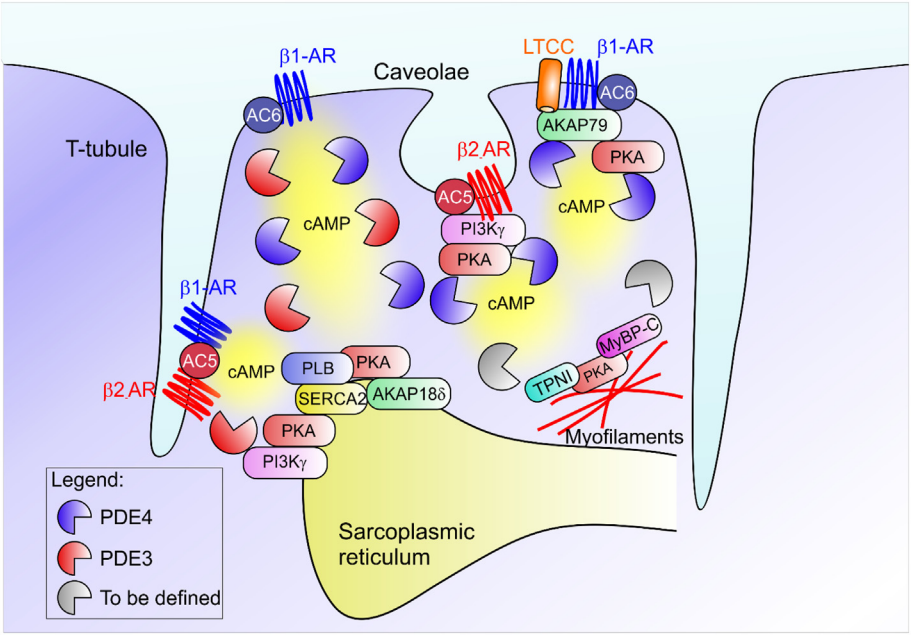

Fig. 1. Key players of cAMP compartmentalization at the plasma membrane, sarcoplasmic reticulum and myofilaments.
cAMP compartmentalization is guaranteed by distinct localization within the cell of the different cAMP/PKA pathway actors, including membrane receptors, adenylyl cyclase (ACs) and cAMP-phosphodiesterases (cAMP-PDEs). B1-adrenergic receptors (β1-ARs), which are among the major membrane receptors expressed by cardiomyocytes localize at the T-tubule and non-T-tubule regions of the plasma membrane, while the cAMP responses evoked by the other β-AR subtype, β2-AR, arise from the T-tubules only. The two major cAMP-producing enzymes of cardiomyocytes, AC5 and AC6, are also differentially localized, with AC5 being coupled to both β1-ARs and β2-ARs, and AC6 being present only in β1-AR compartments. Furthermore, different A-kinase anchoring proteins (AKAPs) localize cAMP-dependent kinase PKA to distinct subcellular locations in close proximity of its targets. For instance, AKAP79 anchors PKA to β-AR and L-type calcium channel (LTCC) at the plasma membrane, allowing cAMP-mediated regulation of the calcium (Ca2+) channel. AKAP18δ assembles a macromolecular complex at the sarcoplasmic reticulum (SR) which includes phospholamban (PLB), controlling Ca2+ reuptake in the SR. Finally, PI3K functions as an atypical AKAP, restraining β2-AR-evoked cAMP responses, both at the SR and at the plasma membrane. Barriers of cAMP-PDEs further contributes to shape cAMP microdomains by forming channels of preferential communication between distinct sites. While PDE3 and PDE4 primarily control the communication between plasma membrane and SR, the PDEs involved in the attenuation of cAMP signaling at myofilaments, specifically at troponin I (TPNI) sites, are still to be determined. For additional details, refers to Sections 4.1, 4.2 and 4.3.

(Table 2). The rationale underlying the construction of this sensor was that PKA phosphorylation of its substrate induces binding of the phosphorylated substrate to the phosphoamino acid binding domain, leading to an increase in FRET.

In the attempt to monitor cAMP changes and PKA activation in spatially restricted microdomains, most of the probes described above were subsequently targeted to distinct subcellular compartments by either introducing a signal sequence or linking the sensor to specific proteins, including for example AKAPs, AC, β-AR and others (Table 1 and 2). Nevertheless, in many cases these strategies led to a significant reduction in the maximal amplitude of the FRET signal, likely stemming from the interference of the subcellular targeting sequence/domain with the proper folding of the probe. This inconvenient was recently solved by Surdo and colleagues who designed a new FRET sensor based on a different topology and consisting in moving YFP from the amino terminus of the sensor to the loop 4–5 within the cyclic nucleotide binding domain (CNBD) of the PKA-RIIβ [9]. This approach allowed minimalizing the risk of perturbing the folding of either the CNBD or the YFP. Of note, the fusion of this sensor, named CUTie (cAMP Universal Tag for imaging experiments), to a number of targeting proteins does not significantly alter the kinetics or the maximal amplitude of the FRET change [9].

Nevertheless, despite the important advance made by the introduction of FRET-based techniques, most studies require transfection of genetically encoded fluorescent reporters into isolated cells. This approach has important limitations, as terminally differentiated cells, such adult cardiomyocytes, are generally refractory to transfection/infection procedures. Furthermore, isolated myocytes do not faithfully recapitulate the complex biology of the whole heart. In order to be able to monitor cAMP dynamics in a more (patho)physiological setting, Calebiro et al. generated reporter mice with ubiquitous expression of the EPAC1-based biosensor [25]. Interestingly, this mouse model was used in combination with a stereomicroscope system to measure cAMP changes in whole perfused heart [9,26] and this in vivo technology allowed the Authors to assess the effect of atrial neural network modulation on left ventricular cAMP levels in an integrated model of mouse heart [26]. Similarly, Nikolaev lab generated a transgenic mouse model expressing a targeted cAMP sensor, allowing the analysis of microdomain-specific second messenger dynamics in the vicinity of SERCA2, not only in healthy hearts, but also in disease conditions [8].

4. cAMP compartments in cardiac myocytes

4.1. Plasma membrane

At the plasma membrane, the ability of cAMP to elicit receptor-

specific responses is based on its compartmentalization. The first suggestion of cellular cAMP compartmentation came from observations that stimulation of membrane β-ARs leads to increased cAMP levels and PKA activity and enhances force of contraction, while PGE1 application does not lead to contractile activity in spite of the increase in cAMP and PKA activation [14]. The generation of delineated cAMP compartments can be in part explained by distinct localization of signaling pathway actors, such as membrane receptors and ACs, as well as cAMP-PDEs (Fig. 1). This section will describe membrane micro/nanodomains involving these actors.

Among the 9 AC isoforms, AC5 and AC6 are the main enzymes expressed in the heart and have divergent roles in ventricular myocytes [27]. AC5 is reported to be present in caveolin 3-enriched domains of T-tubules and is coupled to both β_1-ARs and β_2-ARs, the two main isoforms of cardiac β-ARs, whereas AC6 is present at the plasma membrane outside T-Tubules and is coupled to β_1-AR [28]. It has been demonstrated that genetic inactivation of AC5 activity (AC5KO) may be protective in response to chronic β-AR stimulation and to chronic pressure overload since it reduces the decline in cardiac function and protects against apoptosis [29]. On the other hand, AC6 deletion (AC6KO) increases mortality during sustained β-AR stimulation [30].

The assessment of cAMP levels produced by β-ARs and other GPCRs was made possible by the generation of the first FRET biosensors based on EPAC, PKA and PKA substrates [2,31,32]. The development of such probes led to the conclusive demonstration that cAMP pools activated upon isoprenaline (ISO, β-AR agonist) or prostacyclin (PGE2, prostaglandin receptor agonist) are discrete. By exploiting EPAC-based FRET biosensors for cAMP selectively targeted to PKA-RI (cytosolic) and PKA-RII (membrane) compartments, Di Benedetto and coworkers elegantly showed that ISO generates a rise in cAMP more pronounced in the PKA II domain than in the PKA I domain, while PGE2 has the opposite effect [15]. Plasma membrane cAMP dynamics were further investigated by linking EPAC-based biosensors to different targeting signals or membrane proteins, including for example a targeting sequence from Lyn kinase [33,34], a catalytically inactive version of AC [35] or a β-AR [36], allowing to better decipher the compartmentalization of β-AR/cAMP signaling [33,36–38]. By exploiting a plasma membrane-targeted version of EPAC2-camps sensor (pm-EPAC2), Ghigo et al. revealed an abnormal cAMP accumulation after β_2-AR activation in cardiomyocytes lacking the non-canonical AKAP, PI3Kγ [33]. This study also reported that downstream of β_2-ARs, PI3Kγ participates in multiprotein complexes linking PKA to the activation of major cardiac PDE isoforms, including PDE3A, PDE4A and PDE4B (Fig. 1). The study by Mika et al. further confirmed a role for PDE4B in the control of membrane cAMP, showing that PDE4B mediates a crucial PKA-dependent feedback that controls β_1AR-dependent cAMP signals in a restricted subsarcolemmal domain [37]. In agreement with these studies, PDE4B was found to be an integral component of the LTCC complex at the sarcolemma, where it controls cAMP levels and PKA-phosphorylation, hence the activity, of the channel under β-AR stimulation [39]. By using an EPAC2-camps sensor fused to the C-terminus of β_1-AR, Richter and colleagues conclusively demonstrated the critical role of PDE in the regulation of β-AR/cAMP signaling in subsarcolemmal domains. This study showed that antagonists of β_1-AR increase cAMP levels locally without stimulating cAMP production [36]. This effect is explained by the finding that binding of β_1-antagonists causes the dissociation of a preformed complex between β_1ARs and PDE4s, thereby reducing the local cAMP hydrolysis and increasing submembrane cAMP and PKA activity [36].

Later on, a combination of Scanning Ion Conductance Microscopy (SICM) and FRET allowed to further identify the different cAMP pools produced by different β-AR subtypes [40–42]. SICM is able to resolve complex membrane topography and the nanopipette permits application of picoliters of solution to cells; FRET microscopy can then offer a read-out on the relative presence of cAMP response. The resolution of SICM using this hybrid technique has been around 200 nm, allowing T-tubule openings to be observed in adult cardiomyocytes. This means that the openings of individual T-tubular regions can be targeted for agonist application. The presence of cAMP responses can then be investigated at points both near and far from the region of application to assess the relative diffusion of the cAMP response upon agonist application. Using this high-resolution technique, Nikolaev et al. demonstrated that β_1-AR-induced cAMP responses occur both at the T-tubule and non-T-tubule regions of the plasma membrane. In contrast, β_2-AR-dependent cAMP signals arise from the T-tubules only [41] (Fig. 1). Further work using a modified high-resolution SICM setup enabling to resolve structures on the scale of 50 nm, demonstrated that caveolae, membrane structures found in T-tubules and in the crest membranes of ventricular myocytes, are critically involved in the assembly of receptors and signaling components in this compartment [43,44]. A recent study provides evidence that sarcolemmal membrane organization, i.e. the regularity of T-tubules and the number of caveolae, varies between the different regions of the heart. Wright and colleagues report a smaller degree of signaling microdomain organization in cells at the apex of the myocardium, whereas this organization is increased in cells from the base of the heart. In cells from the apex, PDE4 activity is lower allowing β_2-AR-induced cAMP to influence myocyte contractility [45]. Of note, these findings were replicated in the basal and apical regions of hearts isolated from pm-EPAC2 transgenic mice [45]. Overall, these findings provide varying levels of nanostructural control for cAMP-mediated effects responsible for regional differences in the myocardium.

Recently, cAMP measurement within nanodomains of the plasma membrane has been achieved by targeting biosensors in lipid rafts or non-rafts domains [46]. Two different versions of the EPAC2-camps probe, including EPAC2-MyrPalm, which contains an acylation sequence that targets the probe to lipid raft domains of the plasma membrane, and EPAC2-CAAX, which contains a prenylation sequence that targets the probe to non-raft domains of the plasma membrane were generated. The authors then validated that different membrane domains contribute to the formation of distinct pools of cAMP under basal conditions as well as following receptor stimulation in adult ventricular myocytes [46]. β-ARs are expressed in both lipid raft and non-raft domains and produce cAMP responses that are extremely different from those elicited by E-type prostaglandin receptors, which are instead expressed exclusively in non-raft domains. Intriguingly, basal cAMP levels associated with lipid raft and non-raft domains have been shown to be different, and this could be explained by differences in basal AC activity. In addition, PDE2, PDE3, and PDE4 work together in regulating cAMP levels associated with both lipid raft and non-raft domains, while PDE3 plays a more prominent role in the bulk cytoplasm [46].

Very recently, Nikolaev's group developed a new FRET-based biosensor, PLM-EPAC1, by fusing the EPAC1 sensor to the C-terminus of phospholemman (PLM), to monitor real time cAMP dynamics in the vicinity of the sodium-potassium ATPase/PLM complex. This study revealed a tight regulation of this microdomain by the β_2-AR/cAMP/PKA axis and local pools of PDE3, which was lost in heart failure [47]. With the same strategy of fusing FRET sensors to protein components of multiprotein complexes involved in cAMP compartmentalization, Surdo et al. produced a cAMP probe fused to the plasma membrane AKAP79 (AKAP79-CUTie) which allowed the authors to precisely assess cAMP signals within the AKAP79/β-AR/adenylyl cyclase/LTCC complex at the plasmalemma, providing tools and fundamental mechanistic insights into subcellular adrenergic signaling in normal and pathological cardiac function (see section 4.3 for extensive discussion) [9] (Fig. 1).

4.2. Sarcoplasmic reticulum

A key function under the control of cAMP at the SR is the reuptake of diastolic Ca^{2+} via the SR-Ca^{2+} ATPase (SERCA2). Cyclic AMP exerts its control on SERCA2 indirectly, by promoting PKA-mediated

phosphorylation of its associated protein, PLB. In resting conditions, SERCA2 is inhibited by unphosphorylated PLB, while upon catecholamine stimulation, PLB is phosphorylated by PKA and its inhibitory effect on SERCA2 is released, allowing more efficient reuptake of cytosolic Ca^{2+} [4]. Lygren and colleagues first uncovered the cAMP signalosome responsible for such compartmentalized regulation. This is directed by AKAP18δ that acts as a scaffold to tether PKA to the SR and in close proximity to PLB and SERCA2. Disrupting AKAP18δ–PLB interaction with specific molecular disruptors, e.g. a short peptide from PLB covering the AKAP18δ-binding domain, results in a significant decrease of both PLB phosphorylation and Ca^{2+} re-uptake in the SR [48]. The nature of the PDE involved in this process has also been uncovered. This is likely to be PDE3A, which was recently demonstrated to be part of a multiprotein complex including not only PLB, AKAP18δ and PKA, but also the phosphatase type 2A [49] (Fig. 1).

Two independent laboratories also suggested the involvement of another AKAP to the regulation of such microdomain, phosphoinositide 3-kinase γ (PI3Kγ) [33,50,51]. This was first described as a non-canonical AKAP in virtue of the fact that it directly binds to the regulatory subunit of PKA, though its PKA-binding sequence is unique and not conserved among other AKAPs [12]. In support of a key role of PI3Kγ-anchored PKA in the control of SR-cAMP, PI3Kγ knock-out cardiomyocytes display enhanced PLB phosphorylation as well as increased Ca^{2+} spark occurrence and amplitude upon adrenergic stimulation. Of note, PKA-mediated phosphorylation of RyRs, the other main target of PKA at the SR, was not affected by the absence of PI3Kγ, demonstrating that this AKAP is implicated in a well-spatially restricted cAMP microdomain. FRET experiments with a cytosolic and a plasma membrane-tethered cAMP biosensor, EPAC2-camps and pm-EPAC2-camps, respectively, revealed that this regulation relies on modulation of different PDE isoforms at this site, primarily PDE3 [33], although a contribution of PDE4 cannot be excluded [50] (Fig. 1).

Further insights into how this microdomain is regulated came from the development of transgenic mice expressing a PLB-targeted, FRET-based, biosensor for cAMP (EPAC1-PLB). This approach was the first to allow the direct detection of subcellular cAMP dynamics in the SERCA2 complex in adult ventricular cardiomyocytes, isolated from healthy and diseased hearts [8]. By taking advantage of this sensor, Sprenger et al. uncovered the existence of a privileged communication between the β_1-AR and the PLB/SERCA2 microdomain, as demonstrated by the finding that β_1-AR agonists elicit greater cAMP responses at the SR than in the bulk cytosol. Interestingly, combined inhibition of PDE3 and PDE4 abolishes the difference between the cAMP responses detected with the cytosolic sensor and EPAC1-PLB, suggesting that PDEs facilitate the communication between the two compartments, likely by “channeling” cAMP from the membrane to the SR microdomain. More specifically, this study proposes a model where “barriers” of PDE4 and PDE3 form a channel connecting these two compartments and whereby cAMP formed after stimulation of a particular membrane receptor can reach a compartment deep inside the cell, like the PLB/SERCA2, in a privileged manner (Fig. 1). This communication appears to be impaired in conditions of cardiac hypertrophy, as diseased cardiomyocytes no longer show a difference in the cAMP response elicited by β-AR agonists in the two compartments. This is explained by changes in local PDE activities, and in particular by a reduction of PDE4, but not PDE3 activity in the cytosol which no longer guarantees the proper channeling of cAMP.

Finally, the other major target of cAMP at the SR is the ryanodine receptor (RyR) that, upon phosphorylation by PKA, is activated and responsible for a massive release of Ca^{2+} into the cytosol, ultimately driving contraction [4]. The description of the cAMP signaling domain centered on RyRs dates back to the work of Lehnart and colleagues, who first showed that the core of this signalosome is mAKAP, which tethers together PDE4D3, protein phosphatase 1, protein phosphatase 2A and PKA type II. This work also demonstrated the pathophysiological relevance of such regulation, showing that this multiprotein complex is disturbed in human failing hearts and that genetic ablation of PDE4D3 in mice aggravates post-infarction remodeling [52]. Unfortunately, RyR-targeted FRET probes for cAMP and PKA detection have not been developed so far. Thus, the full comprehension of the regulation of this domain in health and disease awaits the development of such adequate tools.

4.3. Myofilaments

Cyclic AMP is the major determinant of myofilament Ca^{2+} sensitivity. Upon β-AR stimulation, PKA-mediated phosphorylation of troponin I (TPNI) and myosin-binding protein C (MyBP-C), results in a decrease in Ca^{2+} sensitivity, which has been associated with increased cardiac contractility and output [3,4,53]. Our understanding of the organization of the cAMP signaling at the myofilament site has long been limited by the availability of adequate tools, i.e. cAMP biosensors with nanoscale resolution. This gap in knowledge was recently filled by the development of CUTie that, different from the previous generations of biosensors, allows the direct comparison of cAMP levels in different subcellular compartments with high accuracy and without loss in sensitivity [9] (see discussion in section 3). Surdo et al. exploited this new-generation FRET probe to interrogate cAMP dynamics in the environment immediately surrounding macromolecular complexes, including TPNI and MyBP-C at the myofilaments [9]. By using three different versions of CUTie, fused to AKAP18δ (SR-targeted), TPNI (myofilament-targeted) and AKAP79 (plasma membrane-targeted), they found that, the β-adrenergic agonist isoproterenol (ISO) induces significant smaller and delayed cAMP responses at TPNI than at AKAP18δ and AKAP79 sites. Pan-PDE inhibition with IBMX totally abolishes the differences of the cAMP responses induced by ISO in the three compartments, indicating that PDE activity is most strongly limiting cAMP levels at TPNI versus SR or plasmalemmal sites.

Intriguingly, even within the myofilaments, there is differential regulation of PKA-dependent phosphorylation at TPNI and MyBP-C sites, since ISO was found to increase the phosphorylation of MyBP-C, but not that of TPNI. This finding suggests that local regulation of cAMP may occur within tens of nanometers within the myofilament structure, between TPNI and MyBPC sites that are in very close physical proximity [9] (Fig. 1). How this regulation is achieved is still mysterious and further investigation will shed light on the molecular nature of the signalosomes involved.

Of note, Surdo and colleagues further demonstrated that the subcellular compartment of myofilaments is particularly vulnerable to the changes of cAMP signaling that occur in conditions such as cardiac hypertrophy and heart failure (HF). In neonatal rat ventricular myocytes exposed to norepinephrine, a well-established in vitro model of cardiac hypertrophy, ISO-induced cAMP responses are almost undetectable at TPNI sites compared to control, non-hypertrophied cells, while cAMP signals at AKAP79 and AKAP18δ sites do not differ significantly in control versus diseased cardiomyocytes. These findings may have profound clinical implications as the identification of the PDE isoforms involved in this attenuation of the cAMP signal at TPNI may provide a novel therapeutic target for HF.

4.4. Nucleus

The nucleus is also directly involved in cAMP compartmentation in cardiac myocytes. DiPilato et al. first developed two targeted versions of the cAMP reporter ICUE1, namely a plasma membrane-targeted (pm-ICUE1) and a nuclear ICUE1 (NLS-ICUE1), for direct comparison of the cAMP dynamics at these two compartments [21]. When fused to a nuclear localization sequence (NLS), NLS-ICUE1 response to β-AR stimulation was smaller than the response of the untargeted counterpart ICUE1. Interestingly, the β-AR-stimulated cAMP response in the nucleus is not delayed compared with that from untargeted ICUE1. This finding indicated that the pool of cAMP available in the nucleus, although possibly smaller than the cytosolic one, is not kinetically limited, likely

because of the fast diffusion of cAMP from cytosol to nucleus. Moreover, this study showed that β-AR stimulation induces an acute cAMP response at the plasma membrane, followed by a response in the nucleus, which reaches the plateau in 2 to 3 min. Furthermore, co-expression of pm-ICUE1 and of a nuclear version of the AKAR probe for PKA activity (AKAR-NLS) revealed that the acute nuclear β-AR-cAMP response contrasts with the delayed PKA phosphorylation events detected in the nucleus, which require 20 to 30 min to reach the maximum. Thus, the presence of this nuclear pool of cAMP is not sufficient to generate a detectable phosphorylation of AKAR by PKA within the nucleus on the same timescale [21].

It has been suggested that factors may limit the access of cAMP to the nucleus, leading to a reduced pool of the second messenger in this compartment. In muscle cells, a perinuclear pool of PDE (PDE4D3) and PKA, targeted through mAKAP, is reported to play a role in restricting nuclear cAMP signaling [10]. mAKAPb organizes a cAMP signaling complex including PKA and PDE4D3 at the nuclear envelope. Since PKA activates PDE4D3, local cAMP levels are modulated by this negative feedback loop [10]. This complex including mAKAPb was then shown to modulate pathological hypertrophy [54,55].

By measuring cytoplasmic and nuclear cAMP and PKA activity with AKAR3 and ICUE1 probes in adult rat ventricular myocytes, Haj Slimane et al. showed that β-AR stimulation of nuclear PKA activity is temporally dissociated from that of nuclear cAMP elevation and from that of cytoplasmic PKA activation. This is consistent with activation of the PKA holoenzyme in the cytoplasm and translocation of the C subunits to the nucleus. They showed that PDE4 is an upstream negative regulator of this process, with PDE4D playing an important role in controlling nuclear PKA activity, whereas the phosphatases PP1 and PP2A are downstream negative regulators. Importantly, the contribution of PP1 and PP2A to the termination of the β-AR PKA response differs between these two compartments, with PP1 being predominant in the nucleus [56]. This finding may have important functional consequences for the control of nuclear PKA targets such as the CREB family of transcription factors [57,58] and class II histone deacetylases 4 and 5 (HDAC4 and 5) [55–58], which are involved in pathological cardiac remodeling and in the adverse effects of chronic β-AR stimulation. On these grounds, it has been proposed that PKA favors nuclear accumulation of HDAC5, thereby inhibiting cardiomyocyte hypertrophy [59,60]. Altogether these results illustrate the dynamic and versatile nature of nuclear cAMP signaling, which may exert both beneficial and detrimental influences on the adult heart depending on the respective balance between the different target effectors.

Finally, using cytoplasmic (EPACS^{H187} [61] and AKAR3-NES [31]) and nuclear (EPACS^{H187}-3NLS and AKAR3-NLS) reporters to examine cAMP signals and PKA activity, Bedioune et al recently reported that β_1- and β_2-ARs differentially regulate nuclear PKA activity and ICER expression in adult cardiomyocytes. The Authors showed that PDE4 prevents activation by β_2-AR of a mAKAP-targeted PKA pool at the nuclear envelope which is required for PKA activation inside the nucleus and contributes to ICER induction [62].

4.5. Mitochondria

It is now well accepted that mitochondria also represent sites of compartmentalized cAMP signaling. All the components of the cAMP/PKA axis can be found in these organelles, including the catalytic and the regulatory subunit of PKA, AKAPs, PDEs and several PKA substrates, although the exact location and the molecular composition of the different mitochondrial PKA signalosomes are still incompletely understood [63].

A major matter of debate in the field has long been the source of cAMP responsible for the activation of the mitochondrial pool of PKA. This controversy primarily stemmed from the unavailability of adequate tools for the monitoring of cAMP accumulation at mitochondria. The first FRET sensors allowing the study of cAMP dynamics at this subcellular location were developed by DiPilato and colleagues. They generated two different sensors, by fusing ICUE1 [21] to the targeting sequence of subunit IV of cytochrome *c* oxidase (MitoCOX-ICUE1) and to a mitochondria-targeting motif taken from the N-terminal sequence of DAKAP1a (Mito-DAKAP1-ICUE1). In cells expressing either constructs, activation of β-ARs by isoprenaline resulted in a FRET response similar to that observed in cells with the cytosolic probe ICUE1, leading the authors to conclude that cAMP produced in the cytoplasm diffuses rapidly into the mitochondrial matrix through a yet undefined mechanism [21]. This view was challenged by three independent studies arguing that the inner mitochondrial membrane is impermeable to cAMP [64–66]. Acin-Perez and colleagues suggested that cAMP is generated inside the matrix by a soluble form of adenylate cyclase (sAC) in response to bicarbonate and that cAMP, in turn, activates a mitochondrial isoform of protein kinase A, PKA, leading to the phosphorylation and activation of the respiratory chain [64,67]. This model was conclusively proved by the elegant work of Di Benedetto and colleagues who demonstrated that stimulation of transmembrane AC (tmACs) with forskolin fails to increase mitochondrial cAMP, while activation of a calcium and bicarbonate-regulated soluble adenylyl cyclase (sAC) localized inside the matrix produces a significant cAMP elevation with mitochondria [65] (Fig. 2). The discrepancies between these studies likely lies in the different localization signals used to target the sensors to mitochondria (Table 1). While the probes described by DiPilato and colleagues show only a marginal accumulation in mitochondria and are missorted in the cytosol, Di Benedetto and colleagues exploited a new sensor with improved mitochondrial targeting, obtained by fusing repetitive targeting sequences from subunit VIII of the human cytochrome oxidase (COX) at the N terminus of the EPAC1-based sensor H30 [7] (4mtH30).

The presence of PKA itself in the mitochondria is also controversial. Di Benedetto and colleagues provided hints on the existence of a mitochondrial pool of PKA, as they show that PKA inhibition with a mitochondrial targeted version of the endogenous protein kinase inhibitor peptide (PKI) reduces ATP production, considered to be the major outcome of PKA activity in mitochondria [65]. This view was questioned by the work of Lefkimmiatis and coworkers who targeted the FRET-based sensors for PKA activity, AKAR3-4 [31], to the matrix

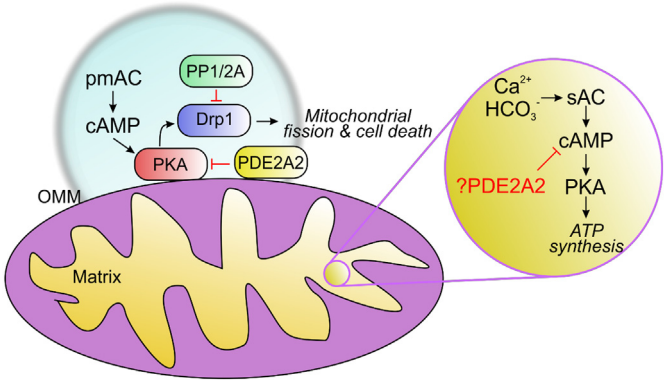

Fig. 2. Key players of cAMP compartmentalization at mitochondria.
Mitochondria represent sites of compartmentalized cAMP signaling. A pool of cAMP can be produced inside the matrix by a calcium and bicarbonate-regulated soluble adenylyl cyclase (sAC) and is primarily responsible of the regulation of ATP synthesis. While in other tissues, like brain and liver, PDE2A critically contributes to the regulation of this pool of cAMP within mitochondria, in cardiomyocytes PDE2A2 appears to be bound to the outer mitochondrial membrane (OMM) and to control a pool of cAMP produced at the plasma membrane by the hormone-responsive plasma membrane adenylyl cyclase (pmAC). This pmAC/cAMP/PKA/PDE2A signaling eventually converges on the regulation of Drp1, a well-known PKA substrate implicated in the regulation of mitochondrial morphology. In addition to PDE2A, a pool of soluble phosphatases, mainly PP1 and PP2A, further contributes to signal termination.

(mito-AKAR3/4) and the outer mitochondrial membrane (OMM-AKAR3/4). They found that mito-AKAR3 does not respond to cytosolic cAMP generated by forskolin plus the non-selective PDE inhibitor IBMX, whereas OMM-AKAR3 did, leading to the conclusion that PKA activity is not present within mitochondria at least in HeLa cells [66]. However, a recent study by Wang et al. suggests the existence in cardiomyocytes of a mitochondrial signaling pathway localized inside the matrix that involves the other effector of cAMP, EPAC1 [68], supporting the concept that all actors of cAMP signaling may be present within cardiac mitochondria. Future studies are awaited to clarify the scenario.

On the other hand, there is now clear consensus on the presence of PDEs within mitochondria. The observation that cAMP can be produced inside the matrix by sAC [65] implies that a PDE must be present in loco to degrade the second messenger locally. Early studies based on cell fractionation experiments and PDE activity assays revealed two different active enzymes in mitochondria, one associated with the OMM and the other one with the inner mitochondrial membrane (IMM) [69]. Further work revealed that the major PDE isoform expressed in mitochondria is PDE2A2, which contains an amphipathic helix of 19 amino acids at the N-terminus that is believed to be responsible of the targeting of the enzyme to the matrix [67]. Although a mitochondrial PDE2A2 was initially described in neurons and hepatocytes, more recent studies confirm the presence of this enzyme also in the cardiac organelle, though localized in a different mitochondrial subdomain [67,70]. In brain and in liver, PDE2A is tethered to the mitochondrial matrix and controls a pool of cAMP generated at this site by sAC, eventually impacting on ATP synthesis [67]. In contrast, cardiomyocyte PDE2A2 appears to be bound to mitochondrial membranes and to control a pool of cAMP produced at the plasma membrane by the hormone-responsive plasma membrane adenylyl cyclase (pmAC) [70]. This pmAC/cAMP/PKA/PDE2A signaling eventually converges on the regulation of Drp1, a well-known PKA substrate implicated in the regulation of mitochondrial morphology (Fig. 2). Inhibition of this specific subset of PDE2A2 results in elongated mitochondria and protection from mitochondrial-dependent cell death. Of note, these effects are unchanged in cells lacking sAC, further corroborating the view that this regulation does not rely on a sAC/cAMP/PKA/PDE2A located in the matrix [70]. In addition, many reports agree that PDE2 activity is altered in heart disease, such as in hypertrophy, HF and myocardial infarction (MI) [71–73], but the contribution of mitochondrial PDE2 has not yet been investigated. However, PDE2 is not the only PDE localized in mitochondria. Evidence indicates that one of the two PDE8 isoforms, PDE8A, colocalizes with known mitochondrial markers. This observation is indicative of the enrichment of PDE8A to or very near to mitochondria. Unfortunately, this has been reported only in testicular Leydig cells [74], and whether PDE8 isoforms are present at these organelles in other cell types, such as cardiomyocytes, is still unexplored.

The view that PDEs are the solely responsible of the generation of sharp microdomains of PKA activity, including those at mitochondria, has been recently challenged. Burdyga et al. propose that cAMP degradation and PKA inactivation do not *per se* terminate the cascade and that this ultimately depends on the dephosphorylation of PKA targets by phosphatases [75]. By using cytosolic as well as OMM-targeted sensors for cAMP (EPACS^{H187}) [61] and PKA activity (AKAR3–4) [31], they uncovered that the same cAMP trigger produces different patterns of PKA activation in the two compartments, with PKA activity being significantly higher at the OMM than in the cytosol. Intriguingly, the two compartments attain comparable levels of PKA-mediated phosphorylation of the targets when phosphatases are inhibited, highlighting the critical importance of these enzymes in generating functional cAMP/PKA microdomains. This study proposes a model where dephosphorylation of both cytosolic and OMM-bound PKA targets relies on pools of soluble phosphatases, mainly PP1 and PP2A, but the limited access of phosphatases to the PKA targets present at the OMM generates persistent PKA-dependent signals at this site [75] (Fig. 2). Overall, this study thus identifies another regulatory layer of cAMP/PKA compartmentalization that is downstream of, and may occur in parallel with, the PDE-mediated compartmentalization of the messenger itself, as parts of the mechanisms that define functionally distinct cAMP signaling microdomains.

5. Conclusion

Since its discovery by Sutherland in 1957, numerous studies have allowed to better understand the critical role of cAMP in cardiovascular pathophysiology. The use of β-blockers as an efficient therapy for the treatment of chronic heart failure (HF), the administration of PDE3 inhibitor in acute HF and the more recent development of LCZ696 (combination of neprilysin inhibitor and angiotensin receptor blocker) highlight the interest of modulating cyclic nucleotide signaling in HF. More than 50 years of basic research have revealed details on the subcellular organization of cAMP signaling in cardiac myocytes, leading to the concept that this compartmentation is essential for adequate processing and targeting of the information generated at the membrane. The development of more and more sophisticated tools for detecting cAMP signals at micro-, and more recently, nano- scale in vivo, have led to clear evidence of an alteration of cAMP compartmentation in HF. However, there are still many aspects of the regulation of cardiac function by cAMP that remain elusive. For instance, the respective role of PKA isoforms in the heart is undefined and little is known about the specific contribution of POPDC proteins to heart function regulation. In a therapeutic perspective, it seems crucial to determine if EPAC inhibition, PKA inhibition or specific PDE-subtype activation could be beneficial for the treatment of HF.

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Conflict of interest

None.

References

[1] T. Brand, R. Schindler, New kids on the block: the Popeye domain containing (POPDC) protein family acting as a novel class of cAMP effector proteins in striated muscle, Cell. Signal. 40 (2017) 156–165.
[2] M. Zaccolo, T. Pozzan, Discrete microdomains with high concentration of cAMP in stimulated rat neonatal cardiac myocytes, Science. 295 (2002) 1711–1715.
[3] D.M. Bers, Cardiac excitation-contraction coupling, Nature 415 (2002) 198–205.
[4] D.A. Eisner, J.L. Caldwell, K. Kistamas, A.W. Trafford, Calcium and excitation-contraction coupling in the heart, Circ. Res. 121 (2017) 181–195.
[5] J. Jurevicius, V.A. Skeberdis, R. Fischmeister, Role of cyclic nucleotide phosphodiesterase isoforms in cAMP compartmentation following beta2-adrenergic stimulation of ICa,L in frog ventricular myocytes, J. Physiol. 551 (2003) 239–252.
[6] V.O. Nikolaev, M. Bunemann, L. Hein, A. Hannawacker, M.J. Lohse, Novel single chain cAMP sensors for receptor-induced signal propagation, J. Biol. Chem. 279 (2004) 37215–37218.
[7] B. Ponsioen, J. Zhao, J. Riedl, F. Zwartkruis, G. van der Krogt, M. Zaccolo, et al., Detecting cAMP-induced Epac activation by fluorescence resonance energy transfer: Epac as a novel cAMP indicator, EMBO Rep. 5 (2004) 1176–1180.
[8] J.U. Sprenger, R.K. Perera, J.H. Steinbrecher, S.E. Lehnart, L.S. Maier, G. Hasenfuss, et al., In vivo model with targeted cAMP biosensor reveals changes in receptor-microdomain communication in cardiac disease, Nat. Commun. 6 (2015) 6965.
[9] N.C. Surdo, M. Berrera, A. Koschinski, M. Brescia, M.R. Machado, C. Carr, et al., FRET biosensor uncovers cAMP nano-domains at beta-adrenergic targets that dictate precise tuning of cardiac contractility, Nat. Commun. 8 (2017) 15031.
[10] K.L. Dodge, S. Khouangsathiene, M.S. Kapiloff, R. Mouton, E.V. Hill, M.D. Houslay, et al., mAKAP assembles a protein kinase A/PDE4 phosphodiesterase cAMP signaling module, EMBO J. 20 (2001) 1921–1930.
[11] G.K. Carnegie, C.K. Means, J.D. Scott, A-kinase anchoring proteins: from protein

complexes to physiology and disease, IUBMB Life 61 (2009) 394–406.
[12] A. Perino, A. Ghigo, E. Ferrero, F. Morello, G. Santulli, G.S. Baillie, et al., Integrating cardiac PIP3 and cAMP signaling through a PKA anchoring function of p110gamma, Mol. Cell 42 (2011) 84–95.
[13] D. Mika, J. Leroy, G. Vandecasteele, R. Fischmeister, PDEs create local domains of cAMP signaling, J. Mol. Cell. Cardiol. 52 (2012) 323–329.
[14] I.L. Buxton, L.L. Brunton, Compartments of cyclic AMP and protein kinase in mammalian cardiomyocytes, J. Biol. Chem. 258 (1983) 10233–10239.
[15] G. Di Benedetto, A. Zoccarato, V. Lissandron, A. Terrin, X. Li, M.D. Houslay, et al., Protein kinase A type I and type II define distinct intracellular signaling compartments, Circ. Res. 103 (2008) 836–844.
[16] F.D. Smith, J.L. Esseltine, P.J. Nygren, D. Veesler, D.P. Byrne, M. Vonderach, et al., Local protein kinase A action proceeds through intact holoenzymes, Science. 356 (2017) 1288–1293.
[17] M. Mongillo, T. McSorley, S. Evellin, A. Sood, V. Lissandron, A. Terrin, et al., Fluorescence resonance energy transfer-based analysis of cAMP dynamics in live neonatal rat cardiac myocytes reveals distinct functions of compartmentalized phosphodiesterases, Circ. Res. 95 (2004) 67–75.
[18] A. Terrin, S. Monterisi, A. Stangherlin, A. Zoccarato, A. Koschinski, N.C. Surdo, et al., PKA and PDE4D3 anchoring to AKAP9 provides distinct regulation of cAMP signals at the centrosome, J. Cell Biol. 198 (2012) 607–621.
[19] K. Gorshkov, J. Zhang, Visualization of cyclic nucleotide dynamics in neurons, Front. Cell. Neurosci. 8 (2014) 395.
[20] J.U. Sprenger, V.O. Nikolaev, Biophysical techniques for detection of cAMP and cGMP in living cells, Int. J. Mol. Sci. 14 (2013) 8025–8046.
[21] L.M. DiPilato, X. Cheng, J. Zhang, Fluorescent indicators of cAMP and Epac activation reveal differential dynamics of cAMP signaling within discrete subcellular compartments, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 16513–16518.
[22] L.M. DiPilato, J. Zhang, The role of membrane microdomains in shaping beta2-adrenergic receptor-mediated cAMP dynamics, Mol. BioSyst. 5 (2009) 832–837.
[23] J.D. Violin, L.M. DiPilato, N. Yildirim, T.C. Elston, J. Zhang, R.J. Lefkowitz, beta2-adrenergic receptor signaling and desensitization elucidated by quantitative modeling of real time cAMP dynamics, J. Biol. Chem. 283 (2008) 2949–2961.
[24] J. Zhang, Y. Ma, S.S. Taylor, R.Y. Tsien, Genetically encoded reporters of protein kinase A activity reveal impact of substrate tethering, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 14997–15002.
[25] D. Calebiro, V.O. Nikolaev, M.C. Gagliani, T. de Filippis, C. Dees, C. Tacchetti, et al., Persistent cAMP-signals triggered by internalized G-protein-coupled receptors, PLoS Biol. 7 (2009) e1000172.
[26] C. Jungen, K. Scherschel, C. Eickholt, P. Kuklik, N. Klatt, N. Bork, et al., Disruption of cardiac cholinergic neurons enhances susceptibility to ventricular arrhythmias, Nat. Commun. 8 (2017) 14155.
[27] C. Boularan, C. Gales, Cardiac cAMP: production, hydrolysis, modulation and detection, Front. Pharmacol. 6 (2015) 203.
[28] V. Timofeyev, R.E. Myers, H.J. Kim, R.L. Woltz, P. Sirish, J.P. Heiserman, et al., Adenylyl cyclase subtype-specific compartmentalization: differential regulation of L-type Ca2+ current in ventricular myocytes, Circ. Res. 112 (2013) 1567–1576.
[29] S. Okumura, D.E. Vatner, R. Kurotani, Y. Bai, S. Gao, Z. Yuan, et al., Disruption of type 5 adenylyl cyclase enhances desensitization of cyclic adenosine monophosphate signal and increases Akt signal with chronic catecholamine stress, Circulation. 116 (2007) 1776–1783.
[30] T. Tang, N.C. Lai, A.T. Wright, M.H. Gao, P. Lee, T. Guo, et al., Adenylyl cyclase 6 deletion increases mortality during sustained beta-adrenergic receptor stimulation, J. Mol. Cell. Cardiol. 60 (2013) 60–67.
[31] M.D. Allen, J. Zhang, Subcellular dynamics of protein kinase A activity visualized by FRET-based reporters, Biochem. Biophys. Res. Commun. 348 (2006) 716–721.
[32] M. Raspe, J. Klarenbeek, K. Jalink, Recording intracellular cAMP levels with EPAC-based FRET sensors by fluorescence lifetime imaging, Methods Mol. Biol. 1294 (2015) 13–24.
[33] A. Ghigo, A. Perino, H. Mehel, A. Zahradnikova Jr., F. Morello, J. Leroy, et al., Phosphoinositide 3-kinase gamma protects against catecholamine-induced ventricular arrhythmia through protein kinase A-mediated regulation of distinct phosphodiesterases, Circulation. 126 (2012) 2073–2083.
[34] T.M. Mohamed, D. Oceandy, M. Zi, S. Prehar, N. Alatwi, Y. Wang, et al., Plasma membrane calcium pump (PMCA4)-neuronal nitric-oxide synthase complex regulates cardiac contractility through modulation of a compartmentalized cyclic nucleotide microdomain, J. Biol. Chem. 286 (2011) 41520–41529.
[35] S. Wachten, N. Masada, L.J. Ayling, A. Ciruela, V.O. Nikolaev, M.J. Lohse, et al., Distinct pools of cAMP centre on different isoforms of adenylyl cyclase in pituitary-derived GH3B6 cells, J. Cell Sci. 123 (2010) 95–106.
[36] W. Richter, D. Mika, E. Blanchard, P. Day, M. Conti, beta1-adrenergic receptor antagonists signal via PDE4 translocation, EMBO Rep. 14 (2013) 276–283.
[37] D. Mika, W. Richter, R.E. Westenbroek, W.A. Catterall, M. Conti, PDE4B mediates local feedback regulation of beta(1)-adrenergic cAMP signaling in a sarcolemmal compartment of cardiac myocytes, J. Cell Sci. 127 (2014) 1033–1042.
[38] V.O. Nikolaev, M. Bunemann, E. Schmitteckert, M.J. Lohse, S. Engelhardt, Cyclic AMP imaging in adult cardiac myocytes reveals far-reaching beta1-adrenergic but locally confined beta2-adrenergic receptor-mediated signaling, Circ. Res. 99 (2006) 1084–1091.
[39] J. Leroy, W. Richter, D. Mika, L.R. Castro, A. Abi-Gerges, M. Xie, et al., Phosphodiesterase 4B in the cardiac L-type Ca(2)(+) channel complex regulates Ca(2)(+) current and protects against ventricular arrhythmias in mice, J. Clin. Invest. 121 (2011) 2651–2661.
[40] A.V. Glukhov, M. Balycheva, J.L. Sanchez-Alonso, Z. Ilkan, A. Alvarez-Laviada, N. Bhogal, et al., Direct evidence for microdomain-specific localization and remodeling of functional L-type calcium channels in rat and human atrial Myocytes, Circulation. 132 (2015) 2372–2384.
[41] V.O. Nikolaev, A. Moshkov, A.R. Lyon, M. Miragoli, P. Novak, H. Paur, et al., Beta2-adrenergic receptor redistribution in heart failure changes cAMP compartmentation, Science. 327 (2010) 1653–1657.
[42] S. Schobesberger, P. Wright, S. Tokar, A. Bhargava, C. Mansfield, A.V. Glukhov, et al., T-tubule remodelling disturbs localized beta2-adrenergic signalling in rat ventricular myocytes during the progression of heart failure, Cardiovasc. Res. 113 (2017) 770–782.
[43] N.K. Bhogal, A. Hasan, J. Gorelik, The development of Compartmentation of cAMP signaling in cardiomyocytes: the role of T-tubules and Caveolae microdomains, Journal of Cardiovascular Development and Disease. 5 (2018).
[44] P.T. Wright, V.O. Nikolaev, T. O'Hara, I. Diakonov, A. Bhargava, S. Tokar, et al., Caveolin-3 regulates compartmentation of cardiomyocyte beta2-adrenergic receptor-mediated cAMP signaling, J. Mol. Cell. Cardiol. 67 (2014) 38–48.
[45] P.T. Wright, N.K. Bhogal, I. Diakonov, L.M.K. Pannell, R.K. Perera, N.I. Bork, et al., Cardiomyocyte membrane structure and cAMP compartmentation produce anatomical variation in beta2AR-cAMP responsiveness in murine hearts, Cell Rep. 23 (2018) 459–469.
[46] S.R. Agarwal, J. Gratwohl, M. Cozad, P.C. Yang, C.E. Clancy, R.D. Harvey, Compartmentalized cAMP signaling associated with lipid raft and non-raft membrane domains in adult ventricular Myocytes, Front. Pharmacol. 9 (2018) 332.
[47] Z. Bastug-Ozel, P.T. Wright, A.E. Kraft, D. Pavlovic, J. Howie, A. Froese, et al., Heart failure leads to altered beta2-adrenoceptor/cyclic adenosine monophosphate dynamics in the sarcolemmal phospholemman/Na,K ATPase microdomain, Cardiovasc. Res. 115 (2019) 546–555.
[48] B. Lygren, C.R. Carlson, K. Santamaria, V. Lissandron, T. McSorley, J. Litzenberg, et al., AKAP complex regulates Ca2+ re-uptake into heart sarcoplasmic reticulum, EMBO Rep. 8 (2007) 1061–1067.
[49] S. Beca, F. Ahmad, W. Shen, J. Liu, S. Makary, N. Polidovitch, et al., Phosphodiesterase type 3A regulates basal myocardial contractility through interacting with sarcoplasmic reticulum calcium ATPase type 2a signaling complexes in mouse heart, Circ. Res. 112 (2013) 289–297.
[50] B.G. Kerfant, D. Zhao, I. Lorenzen-Schmidt, L.S. Wilson, S. Cai, S.R. Chen, et al., PI3Kgamma is required for PDE4, not PDE3, activity in subcellular microdomains containing the sarcoplasmic reticular calcium ATPase in cardiomyocytes, Circ. Res. 101 (2007) 400–408.
[51] A. Ghigo, M. Laffargue, M. Li, E. Hirsch, PI3K and calcium signaling in cardiovascular disease, Circ. Res. 121 (2017) 282–292.
[52] S.E. Lehnart, X.H. Wehrens, S. Reiken, S. Warrier, A.E. Belevych, R.D. Harvey, et al., Phosphodiesterase 4D deficiency in the ryanodine-receptor complex promotes heart failure and arrhythmias, Cell. 123 (2005) 25–35.
[53] R.J. Solaro, M. Henze, T. Kobayashi, Integration of troponin I phosphorylation with cardiac regulatory networks, Circ. Res. 112 (2013) 355–366.
[54] K.L. Dodge-Kafka, J. Soughayer, G.C. Pare, J.J. Carlisle Michel, L.K. Langeberg, M.S. Kapiloff, et al., The protein kinase A anchoring protein mAKAP coordinates two integrated cAMP effector pathways, Nature. 437 (2005) 574–578.
[55] C.L. Passariello, J. Li, K. Dodge-Kafka, M.S. Kapiloff, mAKAP-a master scaffold for cardiac remodeling, J. Cardiovasc. Pharmacol. 65 (2015) 218–225.
[56] Z. Haj Slimane, I. Bedioune, P. Lechene, A. Varin, F. Lefebvre, P. Mateo, et al., Control of cytoplasmic and nuclear protein kinase A by phosphodiesterases and phosphatases in cardiac myocytes, Cardiovasc. Res. 102 (2014) 97–106.
[57] B. Ding, J. Abe, H. Wei, H. Xu, W. Che, T. Aizawa, et al., A positive feedback loop of phosphodiesterase 3 (PDE3) and inducible cAMP early repressor (ICER) leads to cardiomyocyte apoptosis, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 14771–14776.
[58] H. Tomita, M. Nazmy, K. Kajimoto, G. Yehia, C.A. Molina, J. Sadoshima, Inducible cAMP early repressor (ICER) is a negative-feedback regulator of cardiac hypertrophy and an important mediator of cardiac myocyte apoptosis in response to beta-adrenergic receptor stimulation, Circ. Res. 93 (2003) 12–22.
[59] C.W. Chang, L. Lee, D. Yu, K. Dao, J. Bossuyt, D.M. Bers, Acute beta-adrenergic activation triggers nuclear import of histone deacetylase 5 and delays G(q)-induced transcriptional activation, J. Biol. Chem. 288 (2013) 192–204.
[60] C.H. Ha, J.Y. Kim, J. Zhao, W. Wang, B.S. Jhun, C. Wong, et al., PKA phosphorylates histone deacetylase 5 and prevents its nuclear export, leading to the inhibition of gene transcription and cardiomyocyte hypertrophy, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 15467–15472.
[61] J. Klarenbeek, J. Goedhart, A. van Batenburg, D. Groenewald, K. Jalink, Fourth-generation epac-based FRET sensors for cAMP feature exceptional brightness, photostability and dynamic range: characterization of dedicated sensors for FLIM, for ratiometry and with high affinity, PLoS One 10 (2015) e0122513.
[62] I. Bedioune, F. Lefebvre, P. Lechene, A. Varin, V. Domergue, M.S. Kapiloff, et al., PDE4 and mAKAPbeta are nodal organizers of beta2-ARs nuclear PKA signalling in cardiac myocytes, Cardiovasc. Res. 114 (2018) 1499–1511.
[63] S. Monterisi, M. Zaccolo, Components of the mitochondrial cAMP signalosome, Biochem. Soc. Trans. 45 (2017) 269–274.
[64] R. Acin-Perez, E. Salazar, M. Kamenetsky, J. Buck, L.R. Levin, G. Manfredi, Cyclic AMP produced inside mitochondria regulates oxidative phosphorylation, Cell Metab. 9 (2009) 265–276.
[65] G. Di Benedetto, E. Scalzotto, M. Mongillo, T. Pozzan, Mitochondrial Ca(2)(+) uptake induces cyclic AMP generation in the matrix and modulates organelle ATP levels, Cell Metab. 17 (2013) 965–975.
[66] K. Lefkimmiatis, D. Leronni, A.M. Hofer, The inner and outer compartments of mitochondria are sites of distinct cAMP/PKA signaling dynamics, J. Cell Biol. 202 (2013) 453–462.
[67] R. Acin-Perez, M. Russwurm, K. Gunnewig, M. Gertz, G. Zoidl, L. Ramos, et al., A phosphodiesterase 2A isoform localized to mitochondria regulates respiration, J. Biol. Chem. 286 (2011) 30423–30432.

[68] Z. Wang, D. Liu, A. Varin, V. Nicolas, D. Courilleau, P. Mateo, et al., A cardiac mitochondrial cAMP signaling pathway regulates calcium accumulation, permeability transition and cell death, Cell Death Dis. 7 (2016) e2198.
[69] B. Cercek, M.D. Houslay, Submitochondrial localization and asymmetric disposition of two peripheral cyclic nucleotide phosphodiesterases, The Biochemical Journal. 207 (1982) 123–132.
[70] S. Monterisi, M.J. Lobo, C. Livie, J.C. Castle, M. Weinberger, G. Baillie, et al., PDE2A2 regulates mitochondria morphology and apoptotic cell death via local modulation of cAMP/PKA signalling, eLife. 6 (2017).
[71] H. Mehel, J. Emons, C. Vettel, K. Wittkopper, D. Seppelt, M. Dewenter, et al., Phosphodiesterase-2 is up-regulated in human failing hearts and blunts beta-adrenergic responses in cardiomyocytes, J. Am. Coll. Cardiol. 62 (2013) 1596–1606.
[72] C. Vettel, M. Lindner, M. Dewenter, K. Lorenz, C. Schanbacher, M. Riedel, et al., Phosphodiesterase 2 protects against catecholamine-induced arrhythmia and preserves contractile function after myocardial infarction, Circ. Res. 120 (2017) 120–132.
[73] A. Zoccarato, N.C. Surdo, J.M. Aronsen, L.A. Fields, L. Mancuso, G. Dodoni, et al., Cardiac hypertrophy is inhibited by a local pool of cAMP regulated by phosphodiesterase 2, Circ. Res. 117 (2015) 707–719.
[74] M. Shimizu-Albergine, L.C. Tsai, E. Patrucco, J.A. Beavo, cAMP-specific phosphodiesterases 8A and 8B, essential regulators of Leydig cell steroidogenesis, Mol. Pharmacol. 81 (2012) 556–566.
[75] A. Burdyga, N.C. Surdo, S. Monterisi, G. Di Benedetto, F. Grisan, E. Penna, et al., Phosphatases control PKA-dependent functional microdomains at the outer mitochondrial membrane, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) (E6497-E506).