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Subcellular Organization of the cAMP Signaling Pathway

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Abstract——The field of cAMP signaling is witnessing exciting developments with the recognition that cAMP is compartmentalized and that spatial regulation of cAMP is critical for faithful signal coding. This realization has changed our understanding of cAMP signaling from a model in which cAMP connects a receptor at the plasma membrane to an intracellular effector in a linear pathway to a model in which cAMP signals propagate within a complex network of alternative branches and the specific functional outcome strictly depends on local regulation of cAMP levels and on selective activation of a limited number of branches within the network. In this review, we cover some of the early studies and summarize more recent evidence supporting the model of compartmentalized cAMP signaling, and we discuss how this knowledge is starting to provide original mechanistic insight into cell physiology and a novel framework for the identification of disease mechanisms that potentially opens new avenues for therapeutic interventions.

Significance Statement——cAMP mediates the intracellular response to multiple hormones and neurotransmitters. Signal fidelity and accurate coordination of a plethora of different cellular functions is achieved via organization of multiprotein signalosomes and cAMP compartmentalization in subcellular nanodomains. Defining the organization and regulation of subcellular cAMP nanocompartments is necessary if we want to understand the complex functional ramifications of pharmacological treatments that target G protein–coupled receptors and for generating a blueprint that can be used to develop precision medicine interventions.

I. Introduction

Over 60 years ago, the discovery by Earl Sutherland that cAMP is responsible for hormone-induced glycogen breakdown in the liver led to the formulation of the fundamental concept of the intracellular second messenger: a small molecule that relays into the cell the information carried by a hormone, a neurotransmitter, or another extracellular cue and triggers the cascade of biochemical reactions required to achieve the appropriate cellular response to that specific stimulus (Sutherland and Rall, 1958). In follow-up studies, cAMP was found to be synthetized at the cell membrane by adenylyl cyclases (ACs) after activation of seven transmembrane receptors that couple with GTP-binding proteins [G protein–coupled receptors (GPCRs)] (Levitzki, 1988; Mons et al., 1998; Selbie and Hill, 1998). G proteins are trimeric $\alpha\beta\gamma$ complexes, in which the α subunit can either stimulate AC enzymatic activity (Gαs) or inhibit it (Gαi), resulting in either an increase or reduction of intracellular cAMP levels (Rodbell et al., 1971; Bloom et al., 1978; Ross et al., 1978). Although Gs and Go were thought for a long time to be the only heterotrimeric G proteins, several additional variants have since been identified (Hepler and Gilman, 1992; Ross and Wilkie, 2000; Wettschureck and Offermanns, 2005). Mammalian cells express an assortment of ACs from a set of nine membrane-integral isoforms (AC1–9) participating in the Gs protein-coupled

receptor (GsPCR) signaling (Dessauer et al., 2017) and one soluble AC (AC10) that is not directly linked to the GsPCR signaling but responds to bicarbonate (Wiggins et al., 2018). cAMP signals are translated into action by several effector proteins, including protein kinase A (PKA) (Walsh et al., 1968; Krebs and Beavo, 1979), the exchange protein directly activated by cAMP (EPAC) (de Rooij et al., 1998), cyclic nucleotide–gated ion channels (Fesenko et al., 1985), and the more recently identified Popeye domain containing proteins (Brand, 2005). Phosphodiesterases (PDEs) are a superfamily of enzymes that comprises eight different families with cAMP-degrading activity (PDE1, 2, 3, 4, 7, 8, 10, 11). These enzymes are transcribed from multiple genes that in turn can generate several isoforms (Beavo et al., 1971; Cheung, 1971; Marchmont and Houslay, 1980; Conti et al., 1984; Lugnier, 2006; Francis et al., 2011a). Phosphatases counteract PKA-mediated phosphorylation, terminating the cAMP signal (Khandelwal et al., 1976; Ingebritsen and Cohen, 1983). Evidence generated over the years shows that this pathway triggers a plethora of cellular effects, at times with opposing functional outcomes, including cell proliferation and differentiation (Stork and Schmitt, 2002); gene expression (Yamamoto et al., 1988); apoptosis (Weissinger et al., 1997; Suen et al., 2008); several metabolic processes, such as insulin secretion (Brisson et al., 1972), glycogen synthesis (Rous, 1970), and lipogenesis (Geelen and Vaartjes, 1977); and many others.

When Mayer and colleagues observed that in cardiac myocytes catecholamines and prostaglandins raise cellular cAMP to similar levels but have distinct functional effects (Hayes et al., 1980; Beavo and Brunton, 2002), it became clear that the idea that the cellular response to hormones depends on a linear transduction of the message from the receptor to the intracellular effector via cAMP was too simplistic. Those early studies showed that hearts challenged with the β-adrenergic receptor (AR) agonist isoproterenol contract more forcefully (positive inotropy), whereas prostaglandin has no effect on cardiac contractility, even when both stimuli produce similar amounts of cAMP and comparable levels of PKA activation. Clearly, a "linear cascade" model linking hormone binding to its receptor with generation of cAMP, activation of PKA, and substrate phosphorylation could not explain those observations. Although the pleiotropic role of cAMP in the regulation of cellular function became increasingly apparent (Kuo and Greengard, 1969), the question of how cAMP mediates hormone-specific effects remained unanswered for several decades. The last 20 years, however, have seen exciting developments demonstrating that the components of the cAMP signaling pathway are compartmentalized within the cell and that spatial confinement and local regulation are critical for hormonal specificity (Houslay and Milligan, 1997; Steinberg and Brunton, 2001; Beavo and Brunton, 2002; Zaccolo and Pozzan, 2002; Nikolaev et al., 2006; Scott and Pawson,

2009; Houslay, 2010). Here, we review some of the most recent literature to illustrate our current understanding of cAMP subcellular compartments, with a focus on those studies that link local regulation of cAMP with function.

II. Imaging cAMP Nanodomains

The development of genetically encoded molecular tools for monitoring cAMP in real time provided a key methodological breakthrough, as it allowed direct imaging of compartmentalized cAMP signaling in living cells (Adams et al., 1991; Zaccolo et al., 2000; Nikolaev et al., 2004; Ponsioen et al., 2004). This technique exploits the phenomenon of fluorescence resonance energy transfer (FRET) and is based on optical imaging of intact cells expressing genetically encoded probes that provide a readout of cAMP dynamics as they happen in the complex intracellular environment. The biosensors typically include a cAMP binding domain from PKA (Zaccolo et al., 2000; Zaccolo and Pozzan, 2002) or EPAC (DiPilato et al., 2004; Nikolaev et al., 2004; Ponsioen et al., 2004) sandwiched between two spectral variants of the GFP. On cAMP binding to the cAMP binding domain, a conformational change occurs, and the distance between the two fluorophores is modified, affecting the efficiency of energy transfer. The change in FRET can be detected with a conventional optical microscope, making the methodology easy to apply (Stangherlin et al., 2014). The subcellular resolution afforded by these sensors and the possibility to quantitatively monitor the second messenger in real time provided information on cAMP dynamics with unprecedented level of detail (Koschinski and Zaccolo, 2015). With these sensors, it was possible to demonstrate that the cAMP response to activation of GPCRs is not homogeneous within the cell, but the concentration of cAMP is higher within confined subcellular sites (Zaccolo and Pozzan, 2002). The heterogeneous change in cAMP results in only a limited subset of effector proteins being activated (Di Benedetto et al., 2008). Other studies showed that different GPCRs couple with different ACs (Bundey and Insel, 2006) and generate distinct local pools of cAMP (Houslay and Milligan, 1997). The spatial confinement of these pools is disrupted on inhibition of PDEs, indicating the key role of these enzymes in cAMP compartmentalization (Jurevicius and Fischmeister, 1996; Terrin et al., 2006; Iancu et al., 2007; Shcherbakova et al., 2007; Chen et al., 2008, 2009; Leroy et al., 2008; Feinstein et al., 2012; Mika et al., 2012; Stangherlin and Zaccolo, 2012; Cooper and Tabbasum, 2014; Guellich et al., 2014; Froese and Nikolaev, 2015).

In a recent advancement of the FRET-based imaging technique, the cAMP sensors have been genetically modified to target them to different subcellular compartments and monitor cAMP signaling events at specific

sites (DiPilato et al., 2004; Allen and Zhang, 2006; Liu et al., 2011; Lefkimmiatis et al., 2013; Sprenger et al., 2015; Agarwal et al., 2017; Pendin et al., 2017; Surdo et al., 2017). This methodological refinement further enhanced the spatial resolution of the approach, providing a more detailed map of the organization of the cAMP signaling network. The new targeted tools have contributed additional strong evidence for compartmentalized cAMP signaling, have uncovered novel complexity and interconnectivity in the mechanisms that regulate cAMP subcellular domains, and are starting to reveal the functional relevance of individual cAMP pools. One aspect that has emerged from studies using targeted reporters is that the radius of individual cAMP domains can be as small as a few tens of nanometers. In cardiac myocytes, for examples, these sensors revealed that the plasmalemma, sarcoplasmic reticulum, and the myofilaments, structures that are at most 300 nm apart, experience distinct amplitude and kinetics of the cAMP signal in response to catecholamine stimulation (Surdo et al., 2017). Another study in neurons showed that application of the guidance molecule ephrin-A5 generates a cAMP signal in the sub–plasma membrane space adjacent to lipid rafts but not in the space proximal to nonraft regions of the membrane (Averaimo et al., 2016), indicating that the size of the functional cAMP pools generated must be in the range of 20–200 nm, which is the estimated size of lipid rafts. These are important observations, as they imply that individual functionally relevant cAMP pools can be below the resolution limit of an optical microscope and that cAMP compartments can go undetected unless analyzed using a targeted reporter.

III. Contribution to Compartmentalization of Individual Pathway Components

A. Gs Protein Coupled Receptors

An individual cell detects the message from a huge number of extracellular cues via a multitude of specific GsPCRs at the plasma membrane, and the unique informational content of each extracellular stimulus is retained even though all these signals converge toward raising intracellular cAMP. Clear evidence shows that the different localization of GsPCRs at the plasma membrane contributes to triggering a stimulus-specific cellular response (Averaimo et al., 2016; Halls, 2019). The localization of GsPCRs within different plasma membrane domains (e.g., rafts versus nonraft domains) can physically couple the receptor with diverse components of the signaling cascade, e.g., specific G proteins and AC isoforms (Insel et al., 2005; Pontier et al., 2008; Magalhaes et al., 2012; Agarwal et al., 2018). For example, in the neuroblastoma cell line PC12, in which AC6 and AC7 are the two predominant cyclase isoforms, the cAMP response to activation of the pituitary adenylate

cyclase–activating polypeptide type I receptor by its selective endogenous neuropeptide agonist pituitary adenylate cyclase–activating polypeptide 38 was unaffected by knockdown of AC7 but abolished by knockdown of AC6, indicating that coupling of pituitary adenylate cyclase–activating polypeptide type I receptor and AC6 is an exclusive requirement for pituitary adenylate cyclase–activating polypeptide signaling (Emery et al., 2015). In adult mouse cardiac myocytes, caveolin-3 was found to direct the localization of AC5 predominantly to T-tubular invaginations of the plasmalemma, whereas AC6 was found to reside outside the T-tubules, where it is responsible for β_1-AR–mediated enhancement of the L-type Ca^{2+} current (Timofeyev et al., 2013). Another study found that the cAMP signal generated by activation of β_2-AR is mainly confined to T-tubules, where its localization requires caveolin-3 (Wright et al., 2014), whereas β_1-AR–induced cAMP signals are more diffuse across the entire cell (Nikolaev et al., 2010). The arrangement of receptors at the plasma membrane and their preferential coupling with ACs is not static, and this can significantly contribute to fine-tuning the cellular response to a given stimulus. That activation can affect the localization of GPCRs at specific plasma membrane domains has long been recognized, as studies have shown, for example, that treatment with agonist leads to a marked decline in the abundance of β_2-AR (but not β_1-AR) in caveolae (Rybin et al., 2000). In addition, GPCRs can switch their coupling to ACs in a regulated manner. For example, in uterine smooth muscle cells, α_2-AR stimulation is inhibitory in early pregnancy via predominant Gαi-mediated inhibition of ACs but becomes stimulatory after midterm pregnancy via selective G$\beta\gamma$ activation of AC2 (Zhou et al., 2000, 2007). Evidence also suggests that the spatial arrangement of GPCRs can be disrupted in disease. For example, in chronic heart failure, β_2-ARs redistribute from the T-tubules to nontubular portions of the plasmalemma, leading to loss of compartmentalization of the β_2-AR–dependent cAMP signal (Nikolaev et al., 2010). Selective stimulation of β_1-AR generates a more global cAMP signal and activates proapoptotic pathways and hypertrophy (Engelhardt, 2007), whereas selective stimulation of β_2-AR results in a more confined cAMP signal, is antiapoptotic, and has protective effects on the myocardium (Yano et al., 2008). It is possible that the redistribution of β_2-AR observed in heart failure may contribute to the failing phenotype as a consequence of disrupted cAMP compartmentation, although direct experimental evidence for a causative role is still missing.

Evidence supports confinement of GsPCRs to membrane hot spots defined by actin fibers, microtubules, and clathrin-coated pits where both receptors and G proteins are concentrated and where G protein coupling and signaling preferentially occur (Magalhaes et al., 2012). Activation of the relaxin family peptide receptor 1 (RXFP1) is an example of how differential coupling of

GsPCR with distinct signaling pathway components can result in subcellular context-specific responses. Studies have shown that picomolar relaxin can generate cAMP by binding to a preformed complex involving the relaxin receptor RXFP1 and $G\alpha s$/AC2. A slower second wave of cAMP is then generated upon binding of nanomolar relaxin to RXFP1; activation of $G\alpha13$ leads to cAMP synthesis via a pathway involving $G\beta\gamma$-phosphoinositide 3-kinase/protein kinase C (PKC) and AC5 (Nguyen and Dessauer, 2005; Halls et al., 2006, 2007; Dschietzig et al., 2011). However, evidence supports distinct functional outcomes downstream of these different RXFP1-activated pathways, with the $G\alpha s$-coupled pathway promoting cAMP response element–controlled gene transcription and the $G\alpha13$ pathway regulating nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB)-mediated gene transcription (Halls et al., 2007). Interestingly, the expression level of different $G\alpha13$ subunits can vary significantly—for example, in disease conditions (Dschietzig et al., 2011)—providing further mechanisms whereby the functional outcome of receptor activation and cAMP signaling depends on the contingent cellular state.

A recent study connects differential expression levels of $G\alpha$ subunits and sub–plasma membrane location of GPCR as factors that contribute to the response output downstream of a certain receptor. The data show that the level of expression of different $G\alpha$ subunits affects efficacy and potency of β-AR ligands, a feature that the authors attribute to $G\alpha$ subunit–directed partitioning of the receptors in different sub–plasma membrane domains (Onfroy et al., 2017). The hypothesis is that the impact of $G\alpha$ subunits on receptor membrane distribution could account for receptor-biased pharmacology such that a specific ligand could promote different conformations of the GPCR/G protein complexes, with different outputs depending on their compartmentalization at the cell surface. How biased signaling intersects with compartmentalized cAMP signaling is an important question that remains largely unexplored (Shen et al., 2019).

In addition to differential expression and localization at the plasma membrane, the ability of at least some $G\alpha s$-coupled receptors to trigger cAMP production after internalization provides additional mechanisms for diversification of the cAMP message at a temporal and spatial level (Irannejad et al., 2013; Gidon et al., 2014). The conventional paradigm is that, upon activation, receptors are internalized as part of the GPCR desensitization process, which involves receptor phosphorylation and recruitment of β-arrestin followed by GPCR endocytosis (Freedman and Lefkowitz, 1996). Receptor internalization and its degradation in the lysosome provides a mechanism that protects the cell from excessive stimulation, and receptor recycling back to the plasma membrane induces resensitization and protects cells from prolonged hormone resistance (Zhang and Eggert, 2013).

However, studies combining imaging of real-time cAMP signaling and GPCR trafficking events in the intact cell have challenged the dogma that internalized GPCRs are inactive by showing that sustained cAMP signaling can occur from GsPCR embedded in internalized vesicles (Freedman and Lefkowitz, 1996; Ferrandon et al., 2009; Calebiro et al., 2010; Lan et al., 2012; Irannejad et al., 2013; Stoddart et al., 2015; Namkung et al., 2016; Pavlos and Friedman, 2017), where they couple with ACs that are internalized with the receptor (Lazar et al., 2020).

The first direct evidence that internalized ligand/GPCR complexes can stimulate synthesis of cAMP in mammalian cells came from two independent studies showing that thyroid stimulating hormone receptor (TSHR) and parathyroid hormone receptor, respectively, are able to elicit a "second wave" of Gs protein activation after ligand-induced internalization (Calebiro et al., 2009; Ferrandon et al., 2009). Conformation-specific single-domain antibodies that can directly probe the activated state of β_2-AR were used to demonstrate that the adrenergic agonist isoproterenol promotes an immediate activation of β_2-AR and Gs protein at the plasma membrane and, within several minutes after agonist application, a delayed activation at the membrane of early endosomes (Irannejad et al., 2013). The temporal shift of these two waves of cAMP signals provides opportunities for encoding complex biologic information that the cell can translate into specific cellular effects (Grundmann and Kostenis, 2017). Evidence indicates that signaling by internalized GPCR is functionally relevant and is responsible, for example, for meiosis resumption in the oocyte within the follicle (Lyga et al., 2016) or regulation of transcription of certain cAMP-dependent genes (Tsvetanova and von Zastrow, 2014; Godbole et al., 2017). One study used a bacteria-derived adenylyl cyclase that allows cAMP production to be acutely induced by light and that was selectively targeted either to the plasma membrane or to the membrane of endosomes. cAMP generated on light stimulation at the endosomes, but not at the plasma membrane, triggered expression of the phosphoenolpyruvate carboxykinase 1 gene, a cAMP-regulated enzyme, confirming that information can be encoded in the spatial pattern of cAMP signals and supporting the notion that cAMP synthesis at the endosomes can effectively uncouple transcriptional control from cAMP generated at the plasma membrane (Tsvetanova and von Zastrow, 2014). Different Gs-coupled receptors can adopt distinct modalities of signaling after internalization. For example, whereas internalized parathyrod hormone receptors or β_2-adrenergic receptors appear to signal from the early endosome compartment (Ferrandon et al., 2009; Irannejad et al., 2013) and their retrograde trafficking to the trans Golgi coincides with signal termination (Feinstein et al., 2011), signaling from internalized TSHR occurs only at the Golgi/trans Golgi network (TGN) and not at early endosome

membranes (Godbole et al., 2017). For the latter modality, the model is that thyroid stimulating hormone (TSH)/TSHR complexes traffic to the TGN and there activate Gs proteins to induce cAMP synthesis. This leads to activation of a local PKA subset and regulation of gene transcription. Importantly, inhibition of TSHR internalization shows no effect on the overall amplitude of the cAMP response but prevents cAMP/PKA signaling at the Golgi as well as phosphorylation of the cAMP response element binding protein (CREB) and gene transcription. In line with this model, subcellular anchoring of PKA to A-kinase anchoring proteins (AKAPs) appears to be required for TSH-dependent regulation of gene transcription (Calebiro et al., 2006), although the detailed architecture of this Golgi/TGN signalosome and its regulation remain largely to be defined. Thus, receptor-specific signaling after internalization can potentially occur at distinct intracellular sites, providing additional opportunities to achieve response specificity via compartmentalization of cAMP.

A number of studies have also documented cAMP signaling from intracellular GPCRs that are located at intracellular membranes independently from receptor internalization (Kumar et al., 2008; Suofu et al., 2017; Dahl et al., 2018; Nash et al., 2019). For example, studies show that a pre-existing β_1-AR receptor pool that is not delivered from the cell surface resides at the Golgi, where it can be activated by endogenous ligands that enter the cell by facilitated transport via the organic cation transporter 3 (Irannejad et al., 2017). Activation of this internal pool of receptors induces Epac-phospholipase Cε (PLCε)–dependent phosphatidylinositol-4-phosphate (PI4P) hydrolysis in a process that has been linked to catecholamine-dependent cardiomyocyte hypertrophy (Nash et al., 2019).

B. Adenylyl Cyclases

Two classes of mammalian ACs, the nine transmembrane (AC1 to AC9) and the soluble (AC10) enzyme, generate cAMP from ATP either in response to GsPCR activation or bicarbonate, respectively, or via cross talk with other signaling pathways (Hindson, 2003). All transmembrane ACs share the same general architecture, yet they vary considerably outside their catalytic and transmembrane domains and are subject to complex regulation by a number of different modulators, including G proteins, PKA, protein kinase C, and calcium (Sunahara et al., 1996; Dessauer et al., 2017). The specific regulation and subcellular localization of different transmembrane AC isoforms provide an ideal mechanism for compartmentalization of cAMP signaling (Cooper and Tabbasum, 2014; Halls and Cooper, 2017; Johnstone et al., 2018). For example, AC1, AC3, and AC8 are Ca^{2+} activated, whereas AC5 and AC6 are inhibited by Ca^{2+} (Wachten et al., 2010; Halls and Cooper, 2017). Ca^{2+} regulation, however, can be complex, as AC3, for example, is activated by Ca^{2+}/calmodulin and inhibited by the Ca^{2+}/calmodulin protein kinase CaMKII (Wei et al., 1998). Although both AC5 and AC6 are Ca^{2+}-inhibited, disruption of AC5 increases longevity and protects against aging-induced cardiomyopathy (Yan et al., 2007), whereas deletion of AC6 has opposite effects, leading to increased mortality upon β-AR stimulation–induced cardiomyopathy (Tang et al., 2013). These opposite effects have been ascribed to the different localization of these two enzymes at the plasma membrane. In arterial smooth muscle cells, AC6 and, to some extent, AC3 have been associated with vasodilatory pathways via regulation of K^+ channels (Ostrom et al., 2002; Nelson et al., 2011), whereas AC5 was shown to mediate vasoconstriction in response to high glucose. Super-resolution analysis of arterial myocytes revealed a nanometer proximity between subpopulations of AC5 and the L-type Ca^{2+} subunit Cav1.2. Glucose-induced AC5 activity was found to mediate the activation of an anchored PKA pool, phosphorylation of Cav1.2, potentiation of L-type Ca^{2+} channel activity, increased $[Ca^{2+}]_i$, and vasoconstriction, a chain of events that the authors suggest underpins increased myogenic tone during diabetic hyperglycemia (Syed et al., 2019).

In other studies, targeted FRET-based sensors were used to detect cAMP in different subcellular domains in GH3B6 cells, a pituitary cell line that secretes prolactin in response to thyrotropin-releasing hormone (TRH) and vasoactive intestinal peptide (VIP). These two hypothalamic hormones act via different signaling pathways: TRH activates a Gq-coupled receptor and signals via inositol 1,4,5-trisphosphate (IP_3)-dependent Ca^{2+} release from the endoplasmic reticulum (ER), whereas VIP activates a G_s-coupled receptor that signals via cAMP. The authors could measure an increase in cAMP at the plasma membrane in response to VIP when using a sensor targeted via a myristylation and palmitoylation motif, but no cAMP signal was detected when using a sensor targeted to the plasma membrane via fusion to AC8, suggesting a coupling of the VIP receptor with ACs other than AC8. In contrast, when the same cells were stimulated with TRH, a dramatic increase in cAMP was registered by an AC8-targeted sensor, whereas the cAMP response at regions of the plasma membrane away from AC8 was significantly smaller (Wachten et al., 2010). The authors found that the different local cAMP response was due to Ca^{2+}-dependent activation of AC8 and to Ca^{2+}-dependent inhibition of AC5 and AC6 (as well as to concomitant stimulation of cytosolic PDE1 by Ca^{2+}) (Wachten et al., 2010). These findings illustrate how different stimuli can generate locally disparate cAMP responses via selective engagement of individual transmembrane ACs, which respond according to their contingent regulatory status.

The structural basis for the ability of different transmembrane ACs to participate in distinct signaling pathways appears to be their unique coupling with signaling elements at the plasma membrane (Fig. 1).
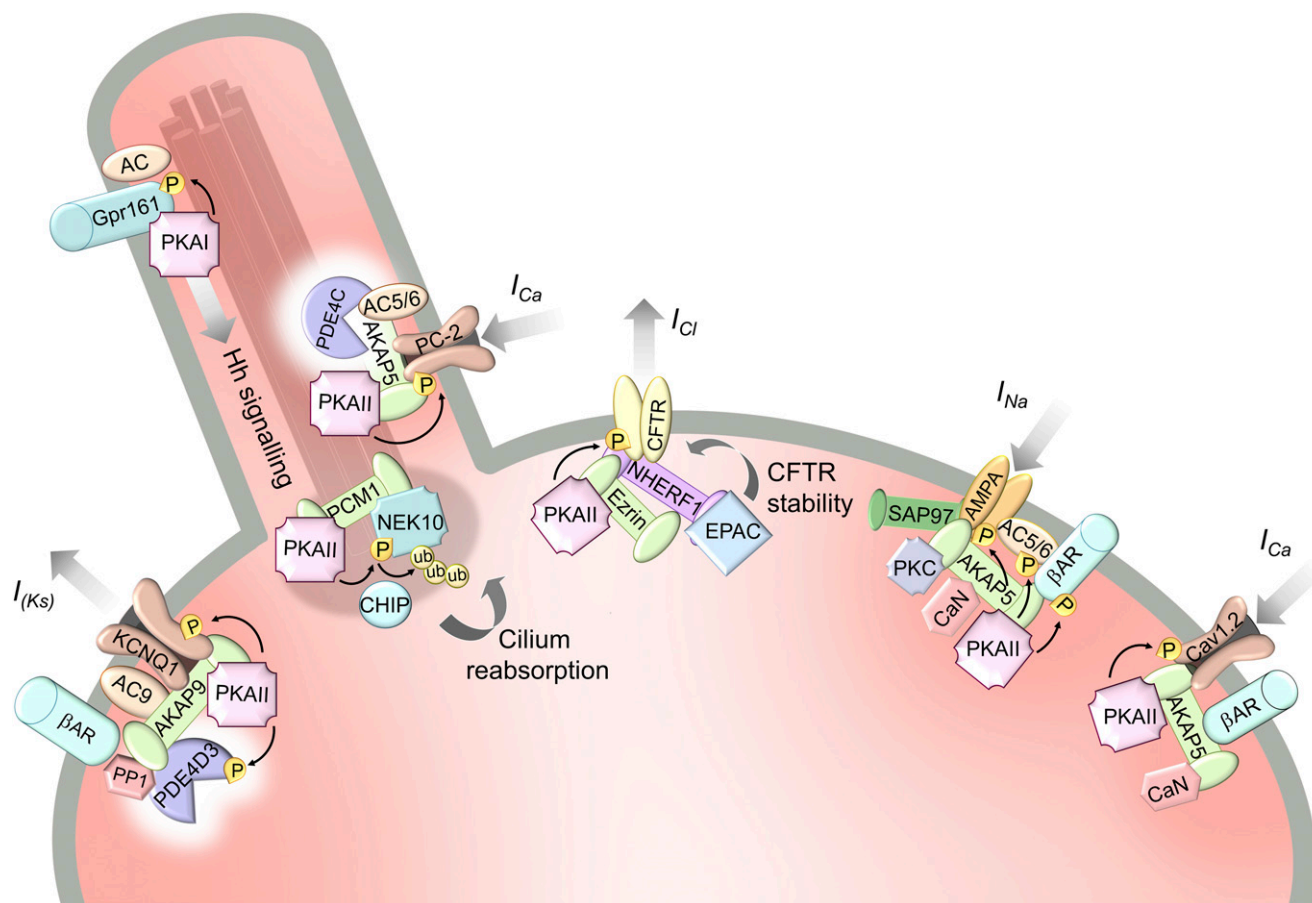
Fig. 1. Plasma membrane and primary cilium cAMP signalosomes. A selection of the signalosomes that localize at the plasma membrane and primary cilium are illustrated. PKA-dependent phosphorylation is indicated in yellow. The cellular function that each signalosome controls is indicated by the gray arrows. Ub indicates ubiquitination. The red gradient indicates cAMP. The white halo surrounding the phosphodiesterases indicates reduced local concentration of cAMP. For details of the different protein components, see the main text. I_{Ca}, calcium current; I_{Cl}, cloride current; I_{Na}, sodium current; Hh, hedgehog protein; NHERF1, Na^+/H^+ exchanger regulatory factor 1; P, phosphate group; PCM1, pericentriolar material 1; SAP-97, synapse-associated protein 97.

A number of transmembrane ACs have been found to be part of multiprotein complexes organized by AKAP (see below) (Bauman et al., 2006; Dessauer, 2009; Li et al., 2012) and including GPCRs/G proteins (Dupré et al., 2007), PDEs, and phosphatases (Crossthwaite et al., 2006) (see below). These complexes provide a local platform for a compartmentalized source of cAMP at the plasma membrane, where ACs can undergo feedback inhibition via PKA (e.g., AC5, 6, and 8) or feed-forward regulation via PKC (e.g., AC2) (Bauman et al., 2006; Willoughby et al., 2012; Efendiev et al., 2013; Shen and Cooper, 2013). In addition, interaction of an AC to an AKAP complex can lead to greater local increases in cAMP. For example, anchoring of an AC to AKAP5 was shown to sensitize the PKA phosphorylation of the anchored transient receptor potential cation channel subfamily V member 1 (TRPV1) channel to the effects of cAMP by shifting the forskolin dose-response curve by ~100-fold (Efendiev et al., 2013). Similarly, coexpression of AC9 and the AKAP Yotiao was found to sensitize phosphorylation of the α subunit [potassium voltage-gated channel subfamily Q member 1 (KCNQ1)] of the slowly activating delayed rectifier potassium channel I(Ks) to lower concentrations of β-AR stimulation compared with cells expressing only AC9 or Yotiao (Li et al., 2012).

In contrast to AC1–9, AC10 is not regulated by G proteins and is activated by bicarbonate (Chen et al., 2000; Bitterman et al., 2013) and ATP (Zippin et al., 2013) and is stimulated by Mn^{2+} and Ca^{2+} (Braun, 1975; Braun and Dods, 1975). Although originally identified as a soluble enzyme, soluble AC has been subsequently reported to be present at specific subcellular sites, including mitochondria, nucleus, centrioles, mitotic spindles, and midbodies (Zippin et al., 2003; Valsecchi et al., 2014; Wiggins et al., 2018). At these sites, AC10 is thought to provide a local source of cAMP that is triggered in response to changes in cellular metabolic state. A number of cellular functions have been associated to AC10-generated cAMP, including sperm capacitation and fertility (Esposito et al., 2004), ATP production (Acin-Perez et al., 2009; Di Benedetto et al., 2013), regulation of lysosomal acidification (Rahman et al., 2016), apoptosis (Ladilov and Appukuttan, 2014), and the downstream

effects from the trafficking corticotropin-releasing hormone receptor (Inda et al., 2016).

C. Protein Kinase A

PKA is the most extensively studied effector of cAMP (Walsh et al., 1968; Taskén and Aandahl, 2004). In its inactive form, PKA is a heterotetramer composed of two regulatory (R) and two catalytic (C) subunits (Walsh et al., 1968; Corbin and Keely, 1977; Sowadski et al., 1985). Four types of R subunits (RIα, RIβ, RIIα, and RIIβ) and three C subunits (Cα, Cβ, and Cγ) have been described (Diskar et al., 2010). In the absence of cAMP, a dimer of R subunits binds to and blocks the activity of two C subunits. Binding of cAMP to the R subunits causes a conformational change that removes their inhibitory action on C (Taylor et al., 2005, 2013), allowing phosphorylation of protein targets preferentially at a [R/K][R/K/X]X[pS/pT] consensus motif (Taylor et al., 1990). The most significant difference between RI and RII isoforms is the autophosphorylation potential of the inhibitor site in RII subunits at S114, which affects the interaction between RII and C subunits and which, when phosphorylated, results in a reduced activation threshold for PKA (Taylor et al., 1990). PKA tetramers containing RI-type subunits are activated at lower concentrations of cAMP compared with those including RII, providing a mechanism for concentration-dependent selective activation of different PKA subsets. (Dostmann et al., 1990; Moll et al., 2008). Genetic studies demonstrate that PKA subunits are nonredundant (Brandon et al., 1995; Cummings et al., 1996; Hensch et al., 1998), and the different sensitivity to cAMP can contribute to signal compartmentalization, as it provides a means for differential activation of PKA isoforms based on the local cAMP concentration. This is achieved via anchoring of PKA to the multiscaffolding proteins AKAPs, which tether PKA at defined subcellular locations in proximity to specific PKA substrates (see below).

Other mechanisms involving PKA can further contribute to compartmentalize the action of this enzyme. For example, the E3 ubiquitin ligase really interesting new gene-H2 (RING-H2) protein praja2, a component of the ubiquitin proteasome system (UPS) (Yu et al., 2002), has been reported to form a stable complex with, and to be phosphorylated by, PKA. cAMP elevation followed by PKA activation leads to praja2 phosphorylation and activation of UPS-mediated degradation of R subunits. By reducing the R-to-C ratio, this positive-feedback mechanism can promote sustained PKA substrate phosphorylation in response to cAMP (Lignitto et al., 2011). Praja2 targets PKA to distinct cell compartments, including plasma membrane, cellular organelles, and perinuclear membrane, thus potentially providing a means for local regulation of PKA activity. The Praja2-dependent effect on the amplitude and duration of PKA signaling was shown to impact the phosphorylation level of the transcription factor CREB and nuclear gene

transcription, significantly contributing to synaptic plasticity and long-term memory (Lignitto et al., 2011; Rinaldi et al., 2015). An additional mechanism involving ubiquitination of PKA C subunits has been reported (Eccles et al., 2016). ARHGAP36, a member of the Rho GTPase-activating protein family, was shown to directly bind PKA C via a pseudosubstrate motif and to potently inhibit PKA activity. The interaction of ARHGAP36 with PKA C quickly promotes their ubiquitination and targets them for endolysosomal degradation, leading to effective suppression of PKA signaling. ARHGAP36-mediated endolysosomal degradation of C subunits can therefore provide a mechanism for timely inactivation and selective degradation of a restricted pool of C subunits and, as described for Paja2-dependent ubiquitination of R subunits, may contribute to the compartmentalization of cAMP/PKA signaling (Eccles et al., 2016).

D. A-Kinase Anchoring Protein

The AKAPs play a fundamental role in signal compartmentalization, as they are responsible for tethering PKA holoenzyme to specific subcellular locations in physical proximity to PKA targets, increasing the specificity and efficiency of signaling (Lees-Miller and Anderson, 1989; Scott et al., 1990; Hulme et al., 2003; Lygren et al., 2007; Skroblin et al., 2010). All AKAPs identified so far (>30) share a short characteristic motif that serves to anchor PKA R subunits. The interaction between AKAPs and PKA occurs via binding of the dimerization-docking domain, located at the N terminus of the PKA R subunit, to an amphipathic α-helix, generally encompassing 14–18 residues, that is conserved among the AKAP family members. All four R subunit isoforms contain the conserved dimerization-docking domain (Banky et al., 1998; Gold et al., 2006). AKAPs include additional interaction sites that are responsible for their subcellular targeting and mediate interaction with other components of the cAMP pathway, including GPCRs (Malbon et al., 2004; Malbon, 2007), ACs (Piggott et al., 2008; Dessauer, 2009), phosphatases (Coghlan et al., 1995; Dodge-Kafka et al., 2010), PDEs (Dodge et al., 2001; Scott et al., 2013), as well as other kinases, including PKC, mitogen-activated protein kinase kinase (MEK)1/2, extracellular signal-regulated kinases (ERK)1/2, polycystic kidney disease (PKD), and others (Klauck et al., 1996; Tasken et al., 2001; Scott et al., 2013). The diverse subcellular targeting domains present in AKAPs recruit kinase activities to specific subcellular compartments, such as the plasma membrane, nucleus, mitochondria, endo/sarcoplasmic reticulum, the primary cilium, and other organelles (Langeberg and Scott, 2015; Torres-Quesada et al., 2017) (see below; illustrated in Figs. 1–3). Given the ability of each AKAP to bind and compartmentalize a different assortment of kinases, their substrates, and a variety of regulatory enzymes, a large diversity of PKA-focused signaling
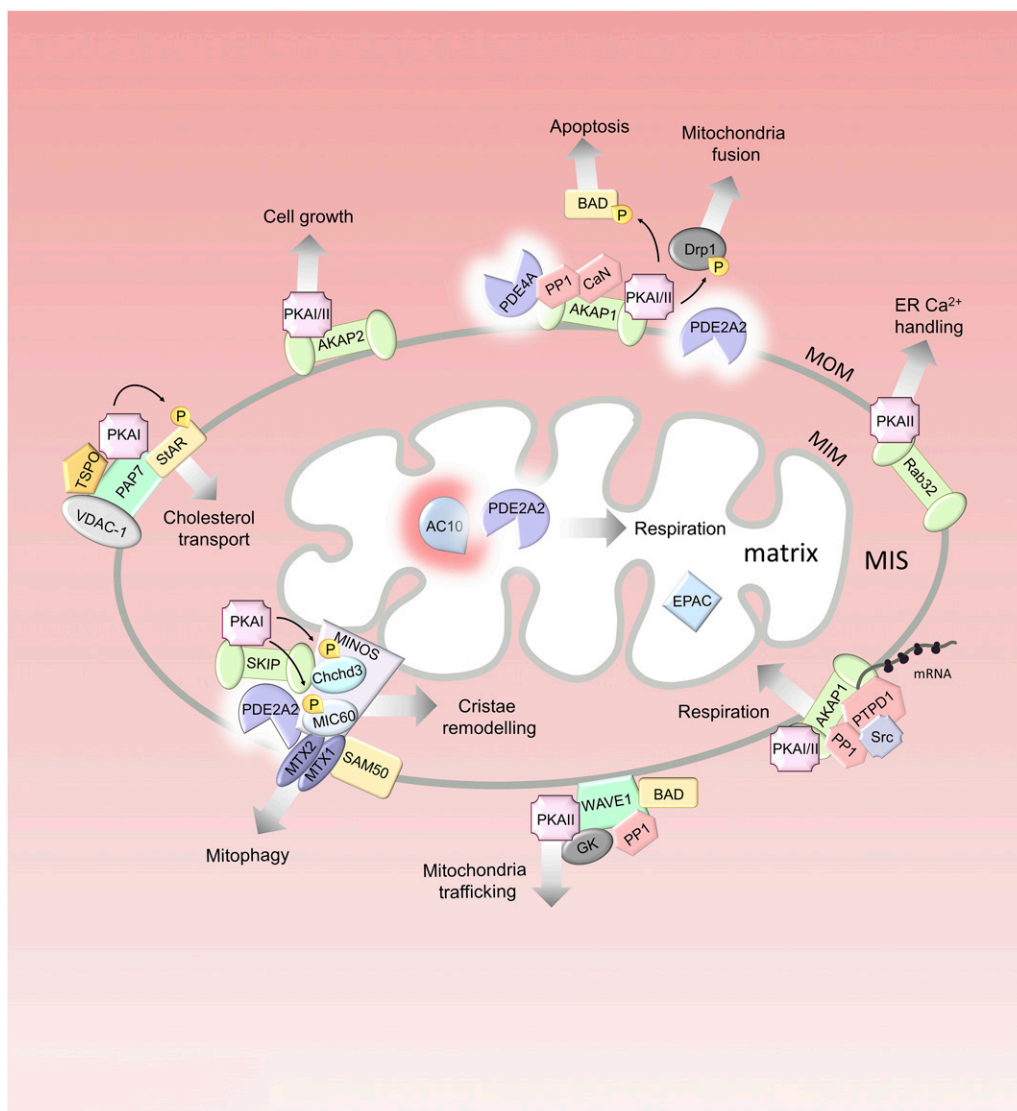
Fig. 2. Mitochondrial cAMP signalosomes. Multiple cAMP signalosomes localize to the mitochondria. Red gradients illustrate cAMP. The MOM is permeable to cytosolic cAMP, whereas the MIM is not. In the matrix, AC10 generates the second messenger locally. For the individual protein components of the signalosomes, see the main text. GK, glucokinase; P, phosphate group; PTPD1, protein tyrosine phosphatase D1; SAM50, sorting and assembly machinery component; StAR, steroidogenesis acute regulatory protein; TSPO, translocator protein; VDAC-1, voltage-dependent anion channel 1.

complexes (signalosomes) can be assembled at different subcellular locations where specific cAMP signaling is implemented locally (Greenwald and Saucerman, 2011).

RII subunits show higher affinity for AKAPs than RI subunits (Banky et al., 2003; Kritzer et al., 2012). This seems at odds with the idea of compartmentalized signaling. An untargeted, predominantly cytosolic and freely moving type I kinase that is activated at a lower cAMP concentration than required to activate the AKAP-bound type II PKA, (Taylor et al., 2012) would appear to nullify the advantage of having pools of cAMP and effector PKAII enzymes localised at at the same site. Multiple studies, however, have identified several type I selective AKAPs with high affinity for RI subunits—including sphingosine kinase interacting protein (SKIP) (Kovanich et al., 2010), small membrane AKAP (smAKAPs) (Means

et al., 2011; Burgers et al., 2012, 2016), and G protein-coupled receptor 161 (Gpr161) (Bachmann et al., 2016). In addition, a number of so-called dual-specific AKAPs (e.g., AKAP1/2, ezrin, merlin, and AKAP11) can also interact with RI dimers, although typically with lower affinity than with RII dimers (Herberg et al., 2000; Pawson and Scott, 2010; Skroblin et al., 2010; Torres-Quesada et al., 2017). Experiments using fluorescence recovery after photobleaching showed that in cardiomyocytes, RI, similarly to RII, is largely anchored to AKAPs (Di Benedetto et al., 2008). In the same study, cAMP FRET-based sensors selectively targeted to RI- or RII-binding AKAPs were used to demonstrate that PKA type I and type II define distinct intracellular signaling compartments in these cells. Activation of β-AR generates a cAMP response that is selectively confined to AKAP-anchored PKA type II. This cAMP
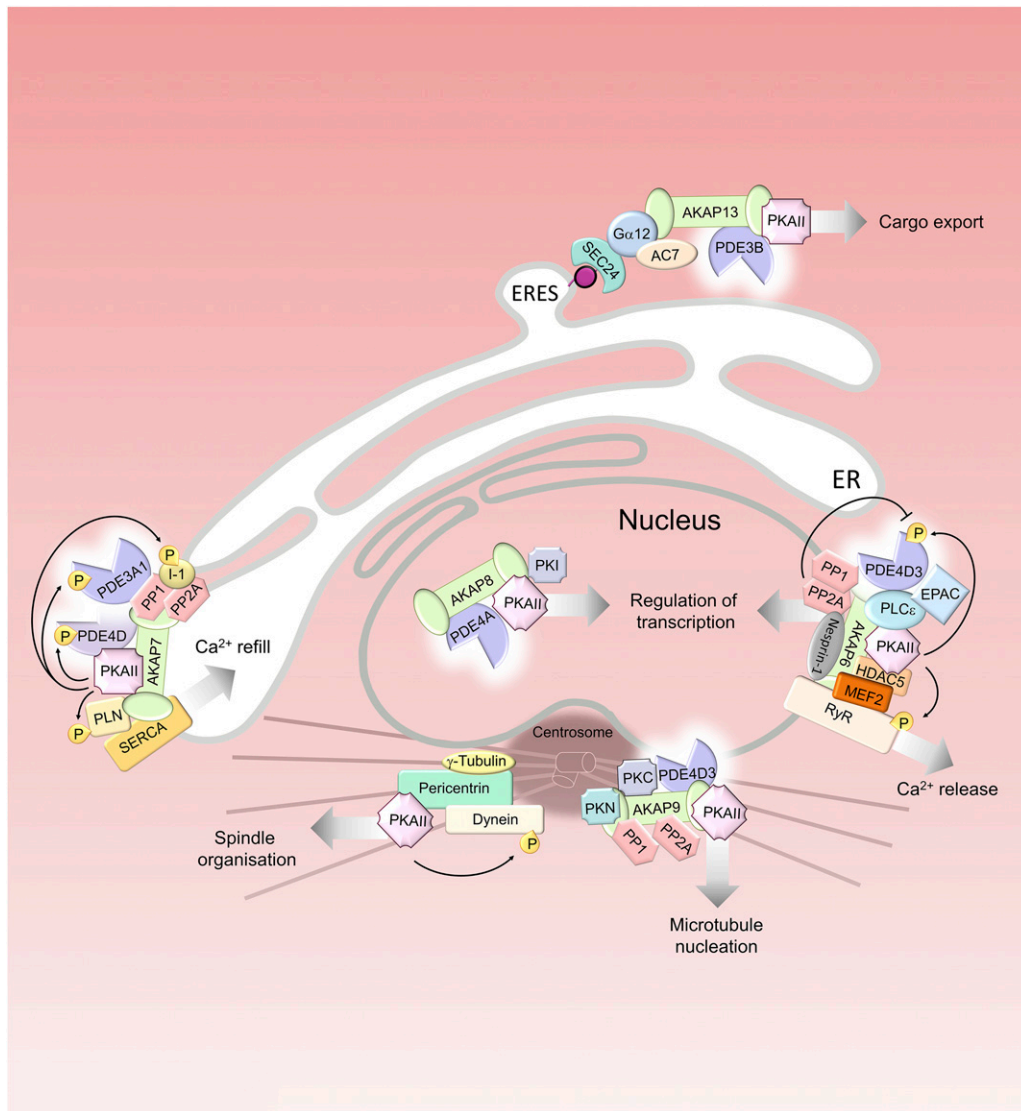
Fig. 3. cAMP signalosomes at the endoplasmic reticulum, nucleus, and centrosome. For clarity, multiprotein complexes illustrated here may not include all components that have been described in the literature. For identification of individual protein components, please refer to the main text. The red gradient illustrating cAMP highlights the fact that the nuclear membrane is permeable to cAMP, whereas the ER membrane is not. Lighter red halos surrounding PDEs indicate low local cAMP. P, phosphate group; SEC24, secretion 24.

pool leads to phospholamban (PLN) and troponin I phosphorylation and is sensitive to rolipram (a PDE4 selective inhibitor). Conversely, prostaglandin 1 and glucagon stimulation of cardiac myocytes generates a cAMP signal that is selectively confined to AKAP-anchored PKA type I. This pool of cAMP is selectively sensitive to the PDE2A inhibitor *erythro*-9-Amino-β-hexyl-α-methyl-9H-purine-9-ethanol (EHNA) and does not affect PLN or troponin I phosphorylation (Di Benedetto et al., 2008).

The study of the functional role of PKA-AKAP interactions has been facilitated by the development of several disruptor peptides and small molecules (Carr et al., 1992; Christian et al., 2011). For example, the peptide super A Kinase anchoring protein-in silico (SuperAKAP-IS) is 10,000-fold more selective for RII over RI (Gold et al., 2006), whereas the peptide RI anchoring disruptor

shows 1000-fold higher affinity for RI over RII (Gold et al., 2006). Disruptor peptides that displace PKA specifically from individual AKAPs have been more difficult to generate. Some success has been achieved using a screening approach based on high-resolution structural information. In this study, several engineered RII subunit fragments that show some preference in disrupting interaction of RII with specific AKAPs were isolated. These peptides, named R_{Select}, were shown to have some selectivity for targeting the cognate AKAP in living cells, although their affinity of binding remains lower than endogenous full-length RII subunits, limiting for the moment their applicability (Gold et al., 2013). One recent example that successfully used PKA-AKAP–disrupting peptides to study the multifaceted role that AKAPs play in the regulation of cAMP/PKA signaling has been reported by Gorshkov

et al. (2017), who observed cAMP gradients in developing hippocampal neurons, with higher cAMP levels in more distal regions of the axon compared with other regions of the cell; disruption of PKA-AKAP interactions using competing peptides enhanced the cAMP gradient and promoted axon elongation. The data suggest that, during development, AKAPs localized in the elongating axons organize a negative-feedback loop whereby AKAP-bound PKA activates a local PDE, resulting in reduced local cAMP levels (Gorshkov et al., 2017). Thus, depending on the different signaling elements that they assemble locally, AKAPs can play a complex role in the regulation of the cAMP/PKA pathway, in some cases facilitating and in others quenching signaling.

The localization of some AKAPs has been shown to be dynamic and to respond to changes in cellular conditions, resulting in translocation of the cAMP signalosome across the cell (Fields et al., 2016). One example is AKAP79/150, which targets both PKA and protein phosphatase 2B (PP2B)/calcineurin (CaN) to the plasma membrane to regulate the phosphorylation status of glutamate receptor 1. Activation of glutamate receptors results in the redistribution of AKAP79/150 and its anchored PKA-RII, but not of PP2B/CaN, from postsynaptic membranes to the cytoplasm in hippocampal slices (Smith et al., 2006).

Evidence is also emerging that alteration of AKAP-PKA interactions is linked to disease (Suryavanshi et al., 2018). Analysis of large datasets has uncovered genetic variation in AKAPs that correlates with susceptibility to a variety of conditions, including neurologic disorders, hypertension, cardiac arrhythmias, and certain cancers (Kammerer et al., 2003; Chen et al., 2007; Logue et al., 2014; Reggi and Diviani, 2017; Kjällquist et al., 2018). A systematic investigation of the properties of polymorphic variants in AKAPs that elicit changes of single amino acids in the helical region that binds to regulatory subunits type II of PKA has shown that a number of these amino acid substitutions disrupt PKA anchoring. In one example, a valine-to-methionine variant in the PKA-anchoring helix of AKAP18 was sufficient to reduce RII-binding affinity by over 8-fold, with functional consequences on the cAMP-dependent potentiation of L-type Ca^{2+} currents (Smith et al., 2018).

Although a large body of evidence supports a key role of AKAPs in directing context-specific cellular outputs of cAMP/PKA signals, one long-standing question is how, on PKA activation, dissociation of the C subunits from the R subunits dimer does not result in uncontrolled phosphorylation of PKA targets that are distal to the PKA-AKAP complex, thus nullifying the advantage of locally anchoring the kinase. A few recent studies have provided some possible answers. One hypothesis is that, for PKA to phosphorylate its substrates, complete dissociation of C from R subunits is not necessary. This idea was originally suggested after the observation that the rotational mobility of labeled C subunits complexed with dimeric R subunits was unchanged on addition of cAMP (Yang et al., 1995). More recent work by Smith et al. has provided fresh evidence in support of this hypothesis (Smith et al., 2013, 2017). Using pull-down experiments and native mass spectrometry of PKA holoenzyme in complex with AKAPs, the authors observed that, in the presence of cAMP, the AKAP-bound PKA holoenzyme remains largely intact (Smith et al., 2017). Using single-particle electron microscopy analysis of the AKAP-PKA complex, they observed that intrinsically disordered domains within the RII subunits allow a significant level of conformational plasticity within the AKAP-PKA complex (Smith et al., 2013). This molecular plasticity of the R subunit would provide a radius of motion to the associated C subunits of 15–25 nm, constraining phosphorylation to targets that are within the immediate vicinity of the AKAP-anchored PKA (Smith et al., 2013). An alternative hypothesis to explain how activation of PKA results in preferential phosphorylation of local targets is that C subunits that dissociate upon cAMP binding are rapidly recaptured by R subunits that are present at high density in the same neighborhood. A study revealed that the R subunits can be expressed in large molar excess of C subunits: up to 17-fold in the cells analyzed in that investigation (Walker-Gray et al., 2017). Clustering of AKAP complexes has also been reported—for example, at the plasma membrane (Mo et al., 2017)–which would effectively increase the local concentration of PKA R subunits. With increased local buffering capacity provided by free R subunits, the range over which the released C subunit can phosphorylate substrates would depend on its rate of diffusion and the rate of recapture.

In addition to AC species, AKAPs can also bind PDEs, thus providing the signaling nodes with machinery that allows them to both form and shape cAMP microenvironments (Sherpa et al., 2019).

E. Exchange Protein Directly Activated by cAMP

EPAC1 and EPAC2 proteins function as guanine nucleotide exchange factors for both Rap1 and Rap2 (de Rooij et al., 1998, 2000). Rap, a small Ras-like GTPase, belongs to the Ras superfamily of small G proteins, which cycle between an inactive GDP-bound state and an active GTP-bound state. Rap, whose activation by cAMP occurs independently of PKA, is activated by several extracellular stimuli and is involved in a large variety of cellular processes, including cell proliferation and adhesion, actin cytoskeleton dynamics, cell polarity, exocytosis, membrane protein recycling, and cell differentiation (Bos, 2006). Several studies support the involvement of EPAC in cell migration and proliferation in multiple types of cancer, making this protein a potential therapeutic target for cancer treatments (Wang et al., 2017; Kumar et al., 2018).

As for PKA, EPAC proteins are highly compartmentalized by different anchoring mechanisms. In response

to cAMP, EPAC1 translocates and anchors to the plasma membrane, where it tethers to phosphatidic acid or to phosphorylated cytoskeleton proteins to induce its downstream effectors (Ponsioen et al., 2009; Gloerich and Bos, 2010; Gloerich et al., 2010; Consonni et al., 2012). EPAC2 is targeted to the plasma membrane after binding to activated Ras proteins independently of its interaction with cAMP (Li et al., 2006). In thyroid cells, EPAC1 and PKA simultaneously bind to radixin, an actin filament binding protein that belongs to the ezrin/radixin/moesin family, and form a functional cAMP-sensing sub–plasma membrane domain in which the two effectors act synergistically to mediate efficient proliferation in response to the mitogenic action of thyroid stimulating hormone (Hochbaum et al., 2011). Epac1 and Epac2 show distinct activation patterns in response to cAMP-raising stimuli, indicating that, similarly to PKA, differently localized EPAC isoforms are under the control of distinct pools of cAMP. For example, in cardiac myocytes, EPAC1 has been shown to localize to the plasmalemma, sarcoplasm, and nuclear/perinuclear region (Cazorla et al., 2009; Pereira et al., 2015). Stimulation of β_1-AR, but not β_2-AR, induces the recruitment of a β-arrestin-EPAC1 signaling complex at the plasma membrane, whereby it activates a pro-hypertrophic signaling cascade involving the small GTPases Rap2, Ras, and CaMKII, triggering histone deacetylase (HDAC) type 4 nuclear export and initiation of a pro-hypertrophic gene program (Berthouze-Duquesnes et al., 2013). By contrast, EPAC2 has been found to localize at the T-tubules (Pereira et al., 2015), and its activation by cAMP in response to β_1-AR stimulation leads to CaMKII-dependent diastolic sarcoplasmic reticulum (SR) calcium release and arrhythmias via a pathway that involves EPAC-phosphoinositide 3-kinases (PI3K)-protein kinase B (Akt)-nitric oxide synthase (NOS1) (Pereira et al., 2013, 2017).

F. Phosphodiesterases

The different PDE isoforms have distinct tissue distribution, and each individual cell typically expresses several PDE isoforms. The diversity of binding affinities and regulatory and targeting features of the various PDE isoforms provides a sophisticated system for unique regulation of amplitude and duration of cAMP signals at different sites (Houslay and Milligan, 1997; Houslay, 2001, 2010; Houslay and Adams, 2003; Houslay et al., 2005; Lugnier, 2006; Zaccolo et al., 2006; Conti and Beavo, 2007; Francis et al., 2011a). The large number of PDE isoforms arises from a combination of multiple genes, alternative splicing sites, and multiple transcription starting points. Each member shares a similar overall structure but is functionally unique. The catalytic domain located at the C-terminal region of each PDE enzyme is highly conserved (Conti and Beavo, 2007). The N terminus of these proteins is quite diverse and confers unique regulatory properties and targeting

sequences that result in tethering of PDEs to specific subcellular locations (Bender and Beavo, 2006; Zaccolo, 2009; Kokkonen and Kass, 2017). The N-terminal region can carry post-translational modification sites. For example, phosphorylation of PDE3, PDE4, and PDE10 modulates their enzymatic activity and the ability of these enzymes to form complexes with other proteins. In addition, some PDEs can be regulated by cGMP (e.g., PDE2 and PDE3) (Zaccolo and Movsesian, 2007; Kokkonen and Kass, 2017). The UPS is also involved in the regulation of PDE degradation (e.g., PDE4D), which leads to upregulation of cAMP levels and to activation of PKA (Zhu et al., 2010). For long PDE4D isoforms, direct binding of the protein phosphatase calcineurin has been shown to inhibit proteasomal degradation of the PDE. Such degradation may occur in specific cellular compartments, including the nucleus, suggesting a complex regulatory mechanism in which both phosphatases and kinases are involved in the control of cAMP signaling (Zhu et al., 2010; Rinaldi et al., 2015). A detailed discussion of the regulatory mechanisms that control the activity of PDEs is beyond the scope of this article, but excellent reviews are available elsewhere (Conti and Beavo, 2007; Houslay, 2010; Cheepala et al., 2013; Baillie et al., 2019; Chao et al., 2019).

A large body of experimental evidence (Jurevicius and Fischmeister, 1996; Rich et al., 2001; Zaccolo and Pozzan, 2002; Mongillo et al., 2004; Rochais et al., 2004; Houslay et al., 2005, 2007; Lynch et al., 2006; Baillie, 2009; Houslay, 2010; Perera and Nikolaev, 2013; Lefkimmiatis and Zaccolo, 2014; Kokkonen and Kass, 2017) as well as computational studies (Oliveira et al., 2010; Saucerman et al., 2014; Chay et al., 2016; Yang et al., 2016; Lohse et al., 2017) indicate that PDEs play an important role in compartmentalization of cAMP signaling. However, how PDEs can accomplish this is still debated (Feinstein et al., 2012; Saucerman et al., 2014; Lohse et al., 2017). The heart of the problem is in the apparent contradiction between the high diffusivity of cAMP and the somewhat sluggish enzymatic activity of the PDEs (Koschinski and Zaccolo, 2017). The diffusion coefficient of cAMP has been experimentally calculated to be around 40 μm^2/s, only approximately one order of magnitude lower than in water (Nikolaev et al., 2004; Richards et al., 2016). Based on the fast intracellular diffusion of cAMP, one would predict fast equilibration of cAMP levels across the cell, in apparent conflict with the notion of cAMP compartmentalization (Terrin et al., 2006; Lefkimmiatis and Zaccolo, 2014; Kokkonen and Kass, 2017). Based on the reported K_M and V_{max} values for PDEs, it is difficult to envisage how PDEs may be able to maintain the concentration of cAMP below the activation threshold of PKA even at basal cAMP levels, measured to be around 1 μM in a number of cell types (Beavo et al., 1974; Börner et al., 2011; Koschinski and Zaccolo, 2017), let alone contribute

to compartmentalization of high concentrations of fast diffusing cAMP generated on hormonal stimulation. Inside a living cell, a number of factors may however come into play that slow down cAMP diffusion, including cAMP buffering (Chen et al., 1999; Lefkimmiatis et al., 2009), cytosol viscosity (Feinstein et al., 2012), and physical barriers (Richards et al., 2016). These factors may be particularly relevant within the restricted volume of a nanodomain, in which the stoichiometry of the molecules involved may be close to equimolar and enzymatic reactions do not follow Michaelis-Menten kinetics. In the confined volume of a nanodomain, local PDEs may be able to effectively reduce the concentration of free cAMP, keeping it below the level achieved a few tens of nanometers farther away (Musheshe et al., 2018; Chao et al., 2019).

Early evidence of a role for PDEs in compartmentalization of cAMP was provided by Hohl and Li, who showed that although approximately 45% of the cAMP generated upon isoproterenol stimulation of cardiac myocytes is found in the cellular particulate fraction, combining isoproterenol stimulation with inhibition of PDEs results in a decline to less than 20% of the proportion of total cAMP residing in the particulate fraction, in spite of a 3.4-fold total increase in cAMP. The authors concluded that this was a result of compartmentalized cAMP in the particulate fractions due to high PDE activity in the cytosol (Hohl and Li, 1991; Méry et al., 1991). cAMP-sensitive FRET-based sensors confirmed the central role of PDEs in shaping the intracellular cAMP nanodomains (Zaccolo and Pozzan, 2002; Nikolaev et al., 2004; Leroy et al., 2008; Surdo et al., 2017). PDE family–selective inhibitors are available and have contributed significantly to our understanding of the role of different PDEs in the compartmentalization of cAMP (Houslay et al., 2005; Lugnier, 2006; Francis et al., 2011b; Page and Spina, 2012; Kokkonen and Kass, 2017). For example, in rat cardiac myocytes, PDE3 and PDE4 enzymes show a very distinct localization, and inhibition of PDE4, but not inhibition of PDE3, results in significant increase in cAMP levels generated by β-agonists (Mongillo et al., 2004; Bedioune et al., 2017; Conti, 2017). Currently available pharmacological inhibitors do not discriminate between different isoforms within the same PDE family. To dissect the role of individual PDE isoforms in compartmentalization, an approach that has been used successfully relies on using isoform point mutants that are catalytically inactive. When overexpressed in the cell, the mutant PDE has a dominant negative effect by displacing the endogenous active enzyme from its anchor sites, effectively resulting in local ablation of cAMP hydrolytic activity (Lynch et al., 2007). Studies using this approach have shown that different isoforms of the same PDE family have a nonredundant and specific role in shaping local cAMP gradients, and their displacement can effectively disrupt local cAMP nanodomains (Baillie et al., 2003; Lynch et al., 2005; McCahill et al., 2005;

Zoccarato et al., 2015). For example, expression of the catalytically inactive mutant PDE4D5-D556A in cardiac myocytes was used to demonstrate that β-AR activation recruits a β-arrestin–sequestered subpopulation of the PDE4D5 isoform that specifically regulates the phosphorylation of the receptor by PKA and induces a switch of the receptor from G_s to G_i coupling (Baillie et al., 2003). In another study, overexpression in neonatal rat cardiac myocytes of PDE2A2, PDE3A2, and PDE4D3 catalytically inactive mutants showed that displacement of endogenous PDE4D3 and PDE3A2 results in cardiac myocyte hypertrophy, whereas displacement of PDE2A2 counteracts hypertrophic growth induced by chronic β-AR stimulation (Zoccarato et al., 2015).

G. Phosphatases

The phosphorylation state of the PKA targets in a subcellular domain depends on the dynamic balance between kinase activity and phosphatase activity available at that specific site (Virshup and Shenolikar, 2009), and phosphoprotein phosphatases (PPPs) are the last checkpoint control for termination of the cAMP/PKA signaling cascade (Heijman et al., 2013). A key defining feature of all PPPs is that they are multimeric enzymes in which the catalytic subunit is associated with numerous regulatory subunits. These regulators are key determinants of PPP subcellular localization, substrate specificity, and enzymatic activity, conferring high complexity to the phosphatase system and the ability to exert a highly diversified local action (Virshup and Shenolikar, 2009). Dephosphorylation of PKA targets is accomplished by the three most abundant serine/threonine phosphatases–namely, protein phosphatase 1 (PP1), PP2A, and CaN (or PP2B) (Virshup and Shenolikar, 2009). In cardiac myocytes, for example, PP1, PP2A, and CaN account for >90% of the total phosphatase activity (Heijman et al., 2013).

PP1 is ubiquitously expressed. It includes three types of catalytic subunits that are encoded in different genes (PP1α, PP1γ, and PP1δ, also known as PP1β) (Herzig and Neumann, 2000). Each PP1α and PP1γ isoform has two splice variants (PP1α1 and PP1α2; PP1γ1 and PP1γ2), and different isoforms localize to distinct cellular regions. For example, in the heart, the myofibrillar fraction contains mainly PP1α (Herzig and Neumann, 2000), whereas PP1β predominantly localizes to the longitudinal SR, and PP1γ is enriched in the junctional SR (Aoyama et al., 2011). PP1 catalytic subunits can dimerize with more than 200 different regulatory subunits (Heroes et al., 2013). Notably, PP1 has been found to be associated with AKAPs, in which it can locally modulate the phosphorylation status of PKA targets within the same AKAP complex. For example, in cardiac myocytes, PP1 interacts with the AKAP Yotiao and regulates the PKA-mediated phosphorylation of the KCNQ1 subunit of the slowly activating potassium channel I(Ks) (Marx et al., 2002). PP1 interaction with

AKAP11 (also called AKAP220) has been suggested to play a role in regulating the activity of glycogen synthase kinase 3b (Schillace et al., 2001), whereas anchoring of PP1 to AKAP1 has been proposed to be involved in efficient nuclear envelope assembly after mitosis (Steen et al., 2000). PP1 also interacts with AKAP7 (also called AKAP18), although indirectly. At this AKAP complex, PP1 binds Inhibitor-1 to promote its phosphorylation by PKA, and the net effect of this later phosphorylation event is to promote local inhibition of PP1c (Singh et al., 2011).

PP2A can form a dimer containing a catalytic (PP2A-C) and a structural scaffold (PP2A-A) subunit or a trimer consisting of scaffold, catalytic, and regulatory subunits. The scaffold and catalytic subunits consist of two isoforms each (PP2A-Aα and PP2A-Aβ; PP2A-Cα and PP2A-Cβ), whereas the regulatory PP2A subunits are organized into four families (PP2A-B, PP2A-B′, PP2A-B″, and PP2A-B‴), most of which contain multiple isoforms with different splice variants (Shi, 2009; DeGrande et al., 2013; Heijman et al., 2013). Cells can assemble more than 200 distinct protein complexes containing different combinations of PP2A-A, PP2A-B, PP2A-C, and other proteins (Virshup and Shenolikar, 2009). Although regulatory subunit α and β are mainly cytosolic, the γ subunit is enriched in the cytoskeletal fraction, conferring different subcellular localizations to the PP2A holoenzyme (Strack et al., 1998). In addition, PP2A localization can be mediated by interaction with AKAPs. For example, in striated muscle, PP2A is part of a complex organized by muscle-specific A-kinase anchoring protein (AKAP6 or mAKAP) and also includes multiple other signaling components (Dodge-Kafka et al., 2010).

CaN is a Ca^{2+}-dependent phosphatase consisting of one catalytic (CNA) and one regulatory (CNB) subunit. CNA is expressed by three genes (CNAα, CNAβ, and CNAγ), whereas CNB is expressed by two genes (CNBα and CNBβ) (Shi, 2009). Regulation of CaN activity toward specific substrates is accomplished primarily through protein-protein interactions, which notably include AKAP5 (AKAP79/150) (Dell'Acqua et al., 2002) and AKAP6 (mAKAP) (Li et al., 2010). For example, in the hippocampus, AKAP5-bound CaN modulates the phosphorylation state of Ser845 of the glutamate A1 (GluA1) subunit of the amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor and modulates synaptic plasticity (Woolfrey and Dell'Acqua, 2015). In the heart, the localization of CaN at the AKAP6 complex appears to be involved in efficient dephosphorylation of the nuclear factor of activated T-cells (NFAT), leading to increased gene transcription (Li et al., 2010).

A study comparing the spatiotemporal dynamics of PKA target phosphorylation in the bulk cytosol and at the mitochondrial outer membrane (MOM) using FRET reporters for cAMP and PKA activity showed that the bulk cytosol contains significantly higher phosphatase activity than the MOM (Burdyga et al., 2018). Based on this observation, the authors suggest that the excess of phosphatases in the cytosol may play a role in the compartmentalization of cAMP/PKA signaling by efficient suppression of accidental phosphorylation of PKA targets that may occur outside the designated signaling domain.

IV. cAMP Subcellular Nanodomains

Below, we describe cAMP signalosomes for which there is experimental evidence, grouping them based on their association with different cellular organelles or structures. The aim here is not to provide an exhaustive account of all multiprotein complexes reported in the literature, and relevant reviews have been published elsewhere (Malbon et al., 2004; Taskén and Aandahl, 2004; Skroblin et al., 2010; Langeberg and Scott, 2015; Torres-Quesada et al., 2017). With the following examples, we intend to highlight the fact that compartmentalization of cAMP occurs on a nanometer scale (macromolecular complex dimension) rather than within the micrometer range (organelle or subcellular structure dimension). Individual organelles within the same cell host multiple cAMP nanodomains, each potentially undergoing independent regulation and controlling different functions.

A. Plasma Membrane

The plasma membrane has been shown to host multiple cAMP signaling domains, which are often organized by large multiscaffolding protein complexes. The specific localization at the plasma membrane of individual G_s-coupled receptors affects the subcellular compartment that experiences the rise in cAMP and determines, at least in part, the functional response to receptor activation. This is supported, for example, by experiments in which membrane rafts are disrupted via cholesterol depletion (Calaghan et al., 2008; DiPilato and Zhang, 2009; Wright et al., 2018). How the arrangement of the receptors at the plasma membrane can affect compartmentalized cAMP signaling is not completely understood, but a contributing factor appears to be the coupling of a given receptor with a specific AC.

1. A-Kinase Anchoring Protein 9. The preferential coupling of receptor and AC at the plasma membrane can be mediated by isoform-specific interaction of the cyclase with an AKAP (Baldwin and Dessauer, 2018). For example, AKAP9 interacts with several ACs (AC1, AC2, AC3, and AC9), contributing to their regulation. Although binding of AKAP9 to AC2 does not alter the basal cyclase activity, this interaction significantly decreases AC2 activity in the presence of cAMP-raising agents (Piggott et al., 2008). The smallest splicing variant of AKAP9, called Yotiao, organizes a macromolecular complex containing AC9, PKA, PP1, PDE4D3, the NR1 subunit of the N-methyl-D-aspartate (NMDA) receptor, and the PKA-regulated α subunit (KCNQ1) of the slowly activating delayed rectifier potassium channel

I(Ks) (Malbon et al., 2004; Li et al., 2012) (Fig. 1). In the heart, recruitment of PKA to the Yotiao-I(Ks) complex facilitates phosphorylation of KCNQ1, whereas PP1 anchored to the same complex opposes the actions of PKA for tight temporal control (Lin et al., 1998; Marx et al., 2002; Kurokawa et al., 2004). Within the same signalosome, phosphodiesterase PDE4D3 locally degrades cAMP to both temporally and spatially regulate channel function (Terrenoire et al., 2009). AKAP9 is required for sympathetic regulation of I(Ks) currents, which shape the duration of action potentials (Chen and Kass, 2006), and inherited mutations in KCNQ1 and/or AKAP9 that disrupt their interaction are associated with long QT syndrome, a disease characterized by cardiac arrhythmias and sudden death (Chen et al., 2007). It should be noted that not all the proteins that can interact with AKAP9 will do so simultaneously. In particular, it is unlikely that NMDA and KCNQ1 are in the same complex; more likely, AKAP9 organizes a complex with NR1 in neurons and KCNQ1 in heart.

2. A-Kinase Anchoring Protein 5. In neurons, Yotiao binds directly to the NR1 subunit, but a second signalosome is organized by AKAP5 (or AKAP79/150) that interacts indirectly with NMDA receptors via postsynaptic density protein 95 (PSD-95) (Colledge et al., 2000). Both scaffolds, in addition to PKA, also anchor a phosphoprotein phosphatase—PP1 and CaN, respectively—that opposes the action of PKA on NMDA receptor (NMDAR) function. This allows a complex, tightly balanced, local regulation of NMDAR function by the interplay of PKA and these phosphatases. A study showed that, under basal conditions, constitutively active PP1 keeps NMDARs in a state of dephosphorylation and low activity. Upon activation by cAMP, PKA phosphorylates PP1, decreasing its activity. This, combined with direct phosphorylation of the channel, leads to a shift in the balance of NMDARs to a higher phosphorylation state and thus a higher activity state (Westphal et al., 1999).

AKAP5 can function as a scaffold for multiple signalosomes. In addition to the NMDA receptors, this AKAP anchors β-adrenergic receptors, AC5/6, PKA, PKC, the phosphatase CaN, and a variety of channels, including the calcium channel voltage-dependent (Cav1.2), the AMPA-type glutamate receptors, M-type K$^+$ channels, and the capsaicin receptor. Given its multivalent nature, the AKAP5 signalosome coordinates different enzyme combinations to modulate the activity of anchored channels depending on the specific context (Dessauer, 2009). This cluster of proteins enables a negative-feedback loop that temporally regulates cAMP production in the heart and in the brain (Fuller et al., 2014; Murphy et al., 2014) (Fig. 1). In cardiac myocytes, PKA stimulates Cav1.2 currents while also negatively regulating the action of adrenergic receptors and AC5/6. In neurons, AKAP5 forms a complex with synaptic glutamate receptor ion channels through the intermediary scaffolding proteins postsynaptic density protein 95 (PSD-95) and synapse-associated

protein 97 (SAP-97). At this molecular hub, AKAP5 anchors PKA and CaN, whose opposing actions regulate AMPA current through glutamate receptor 1 (Scott and Pawson, 2009; Welch et al., 2010).

3. Ezrin. In some circumstances, AKAPs can recruit both PKA and EPAC at the plasma membrane and control their coordinated activity. One example is the regulation of the cystic fibrosis transmembrane conductance regulator (CFTR) channel. A signalosome including the CFTR, the AKAP ezrin (or AKAP78), the Na$^+$/H$^+$ exchanger regulatory factor, PKA, and EPAC1 has been described at the plasma membrane (Sun et al., 2000). Both cAMP effectors regulate CFTR activity, although via distinct molecular mechanisms: PKA phosphorylates CFTR and promotes opening of the channel, whereas EPAC1 stabilizes CFTR at the plasma membrane (Moyer et al., 2000; Lobo et al., 2016). In human airway epithelial cells, cAMP-mediated regulation of CFTR activity requires the integrity of the actin cytoskeleton and compartmentalized cAMP and PKA activity in the sub–plasma membrane domain. CFTR expression is required to maintain an organized cortical cytoskeleton (Favia et al., 2010), and in turn the cortical cytoskeleton is essential for maintaining compartmentalized cAMP and PKA activity for optimal CFTR regulation (Monterisi et al., 2012). The subcortical cytoskeleton, with its densely packed structure, may contribute to limiting the diffusion of cAMP from the plasma membrane to the bulk cytosol, thus promoting activation of membrane-anchored subsets of PKA and EPAC (Rich et al., 2000; Monterisi et al., 2012). In patients with cystic fibrosis, mutations in the CFTR result in a misfolded protein and its defective trafficking to the cell surface, affecting chloride efflux. Pharmacological correctors that rescue the channel expression at the membrane and its activity were shown to also restore the subcortical actin organization as well as the subplasma membrane compartmentalization of cAMP and the local activity of PKA (Abbattiscianni et al., 2016).

4. A-Kinase Anchoring Protein 12. AKAP12 (or gravin) is targeted to lipid rafts, where it appears to interact with the β_2-AR (Fan et al., 2001). In prostate cancer cells, AKAP12 was shown to anchor PKA, PKC, calmodulin, cyclins, and Src-tyrosine kinase and to mediate suppression of the metastatic effects of the oncogenic kinase (Su et al., 2013). It has been suggested that in the presence of oxidative stress the lipids in the membrane are oxidized and change their biophysical properties, resulting in relocalization of AKAP12; as a consequence, Src-tyrosine kinase is released from lipid rafts, and the metastatic potential of the cell is increased (Su et al., 2013). In the forebrain, AKAP12 recruits a signaling complex containing PKA, PKC, calmodulin, and PDE4D to the β_2-adrenergic receptor that regulates long-lasting forms of hippocampal synaptic plasticity and long-term memory storage (Havekes et al., 2012).

5. A-Kinase Anchoring Protein 7. AKAP7 (or AKAP18) comprises a family of four isoforms: AKAP18α, -β, -γ, and -δ, (Trotter et al., 1999; Henn et al., 2005). The α- and β-isoforms of AKAP15/18 localize at the plasma membrane in complex with L-type Ca^{2+} channels (Gray et al., 1997; Fraser et al., 1998), whereas the a high-molecular-weight isoform AKAP15/18δ localizes at the sarcoplasmic reticulum (see below). A lipid modification of three residues in the N terminus of the protein mediates interaction with the inner leaflet of the plasma membrane, whereas a leucine zipper–like motif at its C terminus interacts with the cytoplasmic domain of the L-type Ca^{2+} channel (Hulme et al., 2002). Experimental evidence demonstrates that, in this arrangement, the PKA subset anchored to AKAP15/18 is optimally positioned to phosphorylate the Ca^{2+} channel in the heart (Gray et al., 1998) (Fig. 1) and the epithelial Na^+ channel in the brain (Johnson et al., 2012). However, the knockout of AKAP7 had no effect on L-type calcium channel currents in cardiac myocytes, and some question whether the α splice variant is expressed in heart. More recent work (Yu et al., 2018) suggests that the AKAP Cypher/Z-band alternatively spliced PDZ-motif (Zasp) is important for PKA regulation of Cav1.2 in the heart. Electrophysiological measurements demonstrated that a mutant of AKAP15/18α that lacks the sites of lipid modification reduced the rate of cAMP-dependent stimulation of L-type Ca^{2+} currents (Fraser et al., 1998), and disruption of the AKAP15/18α-channel interaction with a leucine zipper peptide mimetic had similar effects (Hulme et al., 2002). Compared with AKAP18α, AKAP18β carries an insert of 23 amino acid residues in its N terminus, which leads to apical plasma membrane localization of the AKAP-PKA complex; however, its function remains unknown.

B. Primary Cilium

The primary cilium, a nonmotile, microtubule-based protrusion of the plasma membrane present in most cells in the body, functions as a highly specialized sensory structure with a primary role in cell fate specification and cell proliferation (Ingham et al., 2011). In normal proliferating cells, it is transiently observed in G1 phase and is reabsorbed when the cell enters the cell cycle (Plotnikova et al., 2008). The primary cilium uses cAMP to transduce multiple signals, and given the spatially confined nature of this structure, it is not surprising to find that it hosts compartmentalized cAMP/PKA signaling modules (Moore et al., 2016; Mukherjee et al., 2016; Jiang et al., 2019; Sherpa et al., 2019).

1. G Protein Coupled Receptor 161. Multiple components of the cAMP signaling cascade localize to the cilium, including GPCRs, ACs, PKA, and PDEs. Recently, the orphan GPCR Gpr161 has been reported to act as an AKAP that selectively recruits PKA type to the cilium (Fig. 1). The complex has been shown to be involved in cAMP/PKA-dependent antagonism of Hedgehog-initiated signaling, a pathway that is essential for normal embryonic development (Chen et al., 2011; Bachmann et al., 2016). Gpr161 is the first example of a GPCR that directly recruits PKA holoenzymes to its C-terminal tail, and the receptor itself is a PKA substrate containing a PKA phosphorylation site at the C terminus. A phosphomimetic mutant of Gpr161 was found to significantly reduce the ciliary localization of the Gpr161/PKA complex, suggesting that PKA phosphorylation of Gpr161 affects PKA compartmentalization (Mukhopadhyay et al., 2013; Bachmann et al., 2016; Torres-Quesada et al., 2017).

2. A-Kinase Anchoring Protein 5. A protein complex comprising the protein polycystin-2 (PC-2), a transient receptor potential (TRP)-type, Ca^{2+}-permeable nonselective cation channel; AC5/6; PKA; PDE4C; and the A-kinase–anchoring protein AKAP5 has been described within the primary cilium of renal epithelial cells (Choi et al., 2011) (Fig. 1). PKA directly phosphorylates PC-2, which increases the channel mean open time and regulates PC-2–mediated cation transport. Mutations in PC-2 result in PKD, a genetic disorder characterized by the formation of cysts within the kidney tubules, altered molecular architecture of the cilium, and abnormally elevated cAMP production. Interestingly, treatment with PDE4 activator compounds was shown to lower intracellular cAMP levels and inhibit cyst formation in both animal and human cell models of the disease (Omar et al., 2019), suggesting that targeting cAMP signaling within this domain may be a valuable therapeutic strategy.

3. Pericentriolar Matrix Protein 1. A distinct multimeric complex nucleated by the pericentriolar matrix protein 1 is localized at the base of the cilium. This signalosome includes, in addition to PKA, the kinase NEK10 and the E3 ubiquitin ligase carboxy-terminus of Hsc70 interacting protein (CHIP). Within this multiprotein complex, PKA phosphorylation primes NEK10 for CHIP-mediated ubiquitination and proteolysis, resulting in cilium reabsorption (Fig. 1), a control mechanism for cilium dynamics that might be affected in specific human cancers and genetic disorders (Porpora et al., 2018).

C. Mitochondria

Mitochondria have been increasingly recognized as organelles with tightly regulated cAMP signaling. Indeed, every mitochondrial compartment, including the MOM, the mitochondrial intermembrane space (MIS), and the mitochondrial matrix, harbors one (or more) spatially and functionally distinct cAMP signalosome. cAMP signaling at the mitochondria contributes to the extreme adaptability of these organelles in response to changes in metabolic demands (Carlucci et al., 2008; Reggi and Diviani, 2017) via direct modulation of a variety of functions, including mitochondria biogenesis and morphology, gene expression, metabolism,

steroidogenesis, and survival (Zhang and Xu, 2016). Several components of the cAMP signaling pathway, including source of cAMP, effectors, terminators, and targets, have been identified in mitochondria (Monterisi and Zaccolo, 2017; Rinaldi et al., 2018).

PKA R and C subunits were isolated from purified mitochondria over 30 years ago (Schwoch *et al.*, 1990; Papa, 1999). Mass spectrometry analysis of the mitochondrial phosphoproteome identified more than 100 potential PKA targets in the mitochondrial phosphopeptide pool (Grimsrud et al., 2012). Most of these phosphorylation sites are yet to be validated, and it is therefore possible that the mitochondria may harbor additional cAMP signalosomes that remain to be characterized (Monterisi and Zaccolo, 2017). At least five different AKAPs have been found to associate with the MOM: AKAP1 (also known as AKAP121, AKAP149, and spermatid (S)-AKAP84, depending on the species) (Ma and Taylor, 2002; Affaitati et al., 2003), AKAP2 (Wang et al., 2001), Rab32 (Alto et al., 2002), peripheral-type benzodiazepine receptor–associated protein 7 (PAP7) (Li et al., 2001), and Wiskott-Aldrich syndrome protein verprolin homologous (WAVE) (Danial et al., 2003) (Fig. 2). The presence of several AKAPs at the MOM implies that this structure hosts several cAMP signaling domains with different roles in the regulation of mitochondrial function.

1. A-Kinase Anchoring Protein 1.   AKAP1 has two N-terminal splice variants, with the shorter form being targeted to the MOM through a functional tubulin-binding motif, which is essential for mitochondrial localization. A longer splice variant has been described that includes a hydrophobic segment upstream to the mitochondrial targeting domain and generates a protein that targets to the ER and may be potentially involved in coordinating Ca^{2+} homeostasis and cell respiration (Carlucci et al., 2008), although the existence of this longer variant is controversial (Merrill and Strack, 2014). A feature of AKAP1 is its ability to bind both type I and type II PKA holoenzymes (Huang et al., 1999; Ma and Taylor, 2002; Merrill et al., 2011; Torres-Quesada et al., 2017). Other components of the cAMP cascade that have been reported to interact with AKAP1 are CaN (Abrenica et al., 2009), PP1 (Bridges et al., 2006; Rogne et al., 2009), and PDE4A (Asirvatham et al., 2004) (Fig. 2). AKAP1 appears to be involved in multiple signalosomes at the MOM. Whether these are independent multiprotein complexes all simultaneously present on the mitochondria or whether AKAP1 can interact with different components in a dynamic manner remains to be established.

AKAP1 contains at its C terminus a heterogeneous nuclear ribonucleoprotein K-homology (KH) domain, which interacts with nuclear-encoded mRNAs (Chen et al., 1997). Tethering mRNA and ribosomes to the MOM promotes efficient translation and coimport of mitochondrial proteins into the organelle, with

important implications for global protein synthesis and mitochondrial physiology (Ranganathan et al., 2002; Dyson et al., 2008; Unal et al., 2008; Gabrovsek et al., 2020). This AKAP1 complex includes, in addition to PKA and mRNA, the protein tyrosine kinases Src and the phosphatases PP1 and protein tyrosine phosphatase D1 (PTPD1) (Fig. 2). This transduction unit supports mitochondrial homeostasis, metabolism, respiration, and survival (Cardone et al., 2004; Livigni et al., 2006).

AKAP1 overexpression stimulates PKA-dependent phosphorylation of the proapoptotic protein BCL2-associated agonist of cell death (BAD), inhibits release of cytochrome c from mitochondria, and protects cells from apoptosis (Harada et al., 1999; Affaitati et al., 2003) (Fig. 2). In addition to BAD, PKA can phosphorylate Bcl-2–associated X protein (BAX) and bcl-2–interacting mediator of cell death (BIM), two other proteins involved in regulation of apoptosis. PKA phosphorylation of these targets, however, has opposite effects. Although PKA phosphorylation of BAD inhibits its apoptotic activity, PKA-mediated phosphorylation of BAX promotes its translocation to mitochondria and triggers cytochrome c release and apoptosis (Srivastava et al., 1998; Kumar et al., 2009). Similarly, phosphorylation of BIM increases its stability and protects it from proteasome-dependent degradation, promoting apoptosis (Moujalled et al., 2011). This is an example of how cAMP can regulate opposing effects even when the targets involved are in relatively close proximity and supports the existence of functionally independent cAMP/PKA signalosomes operating at the mitochondria. Although AKAP1-tethered PKA has been linked to regulation of BAD, whether and how PKA activity is selectively targeted to BAX and BIM remains to be elucidated.

Overexpression of AKAP1 also promotes recruitment of PKA to the MOM and phosphorylation at serine 637 of dynamin-1–like protein (Drp1), a GTPase involved in regulating mitochondrial fusion-fission balance, resulting in inhibition of mitochondrial fission and cell survival (Fig. 2). Studies in cardiac myocytes have shown that downregulation or displacement of AKAP1 results in mitochondrial dysfunction and cell death, confirming the prosurvival actions of AKAP1-tethered PKA (Livigni et al., 2006; Perrino et al., 2010). A similar role has been described in neurons, in which the PKA/AKAP1 complex is involved in maintenance of mitochondrial integrity and protection from cell injury. In invasive breast cancer cells, disruption of this complex affects cell motility and mitochondrial movement toward the leading edge. Analysis of primary and metastatic samples revealed an inverse correlation between AKAP1 expression and mesenchymal markers, and depletion of this signalosome from the MOM was suggested to contribute to the metastatic growth of breast cancer (Aggarwal et al., 2019).

AKAP1 presence at the mitochondria is dynamic and is modulated by the UPS. Such regulation impacts mitochondrial dynamics and reduces mitochondrial oxidative stress in conditions of low oxygen levels, promoting mitochondrial adaptation to metabolic and environmental challenges. After a hypoxic insult, the Really Interesting New Gene (RING) domain E3-ubiquitin ligase seven in absentia homolog 2 (Siah2) ubiquitinates AKAP1, resulting in its proteolysis. Degradation of AKAP1 by the UPS rapidly attenuates the oxidative metabolism, promoting adaptation of cells to accidental hypoxic conditions (Carlucci et al., 2008). Similarly, proteolysis of AKAP1 by the hypoxia-induced Siah2 pathway removes the inhibitory constraint of PKA on the Drp1/fission machinery and leads to mitochondria fragmentation and cell death (Kim et al., 2011).

2. A-Kinase Anchoring Protein 2. AKAP2 also localizes to the MOM and anchors both type I and type II PKA (Wang et al., 2001) (Fig. 2). This signalosome may be cell type–dependent, as AKAP2 was not found in the mitochondria at the neuromuscular junction (Perkins et al., 2001). AKAP2 can also interact with components of the cytoskeleton via its C terminus (Sarma et al., 2015), and its overexpression promotes cell growth and is required for calcitonin-mediated migration of cancer cells (Thakkar et al., 2016; Li et al., 2017).

3.Ras-Related Protein 32. Ras-related protein 32 (Rab32), a member of the Ras superfamily of small-molecular-weight G proteins, was initially described as an AKAP for PKA type II involved in mitochondrial dynamics (Alto et al., 2002) (Fig. 2). More recent studies have shown that Rab32 localizes to mitochondria-associated membranes, a specialized subdomain at the interface between mitochondria and ER that acts as an intracellular signaling hub (Bui et al., 2010; van Vliet et al., 2014). At the mitochondria-associated membrane, Rab32 is involved in the modulation of ER Ca^{2+} handling; it regulates the levels of the chaperon protein calnexin and is involved in the regulation of the phosphorylation status of both BAD and Drp1 via PKA, affecting mitochondrial dynamics and cell death (Bui et al., 2010).

4. Peripheral-Type Benzodiazepine Receptor–Associated Protein. PAP7 is a mitochondrial AKAP that selectively binds and targets PKA type I to the MOM (Li et al., 2001; Liu et al., 2003). PAP7 is present in all mammalian tissues and cells, with predominant expression in steroidogenic tissues, such as testis and adrenal gland, and organizes a complex that includes translocator protein, the voltage-dependent anion channel 1 (VDAC-1), and type I PKA (Fig. 2). PAP7 myristylation is required for binding to MOM. At this site, PAP7 promotes PKA-directed phosphorylation and catalytic activation of steroidogenesis acute regulatory protein, leading to cholesterol transport into the organelle. Interference with PAP7-mediated cAMP signaling profoundly affects hormone-induced cholesterol transport into mitochondria and downregulates steroidogenesis in endocrine cells. In adrenal tumor cells, PAP7 participates in PKA-dependent mitogenic signaling, supporting tumorigenesis and hormone-independent hypercortisolism (Miller, 2013).

5. Wiskott-Aldrich Syndrome Protein Verprolin Homologous-1. WAVE1 controls actin polymerization and cytoskeletal remodeling (Kim et al., 2006). In neurons, WAVE1 is dephosphorylated and activated upon NMDA stimulation and translocates from the cytoskeleton to the mitochondria, where it induces organelle fission and trafficking along filopodia, promoting filopodia outgrowth and spine morphogenesis, a prerequisite for synaptic activity and plasticity. The mechanisms that regulate localization of WAVE1 to mitochondria seem to involve interaction with mitochondrial BAD family members. These nucleate multivalent complexes on mitochondria that include glucokinase, WAVE1, and other PKA-regulated substrates/effectors (Fig. 2). The complexes assembled by BAD on mitochondria act as metabolic sensors for nutrient availability and stress stimuli, optimally integrating glycolytic machinery and respiration with the organelle network and apoptotic pathway (Danial et al., 2003; Kim et al., 2006; Cheng et al., 2007).

6. Sphingosine Kinase Interacting Protein. The protein SKIP has been identified as an AKAP localized in the MIS. SKIP was shown to specifically interact with PKA RI subunits with high affinity (Kovanich et al., 2010; Means et al., 2011). As the MOM is permeable to cAMP (Acin-Perez et al., 2009), MOM and MIS experience the same level of cAMP. The presence of a SKIP-PKA type I complex in the MIS, however, provides a means for these two compartments to potentially act as distinct cAMP domains whereby the MIS-targeted PKA type I responds to levels of cAMP that are not sufficient to activate the MOM-tethered pools of PKA type II (Lefkimmiatis and Zaccolo, 2014). SKIP interacts with several proteins, including coiled-coil-helix-coiled-coil-helix domain containing (ChChd) 3, ChChd6, sorting and assembly machinery 50 (SAM50), metaxin 1 (MTX1), and MTX2. ChChd3 and ChChd6 are components of the mitochondrial inner membrane organizing system (MINOS), a multiprotein complex that is involved in the regulation of mitochondrial membrane architecture and formation of cristae junctions and contact sites between the outer and inner membranes (Pfanner et al., 2014). PKA phosphorylates ChChd3, and SKIP is required for this phosphorylation to take place (Schauble et al., 2007; Darshi et al., 2011; Means et al., 2011). PKA also phosphorylates another component of MINOS, the protein mitofilin [MICOS complex subunit 60 (MIC60)] (Akabane et al., 2016). A recent study showed that PKA-mediated phosphorylation of MIC60 and ChChd3 reduces phosphatase and tensin homolog (PTEN)-induced putative kinase 1 levels at the MOM and, consequently, negatively regulates Parkin

recruitment and mitochondrial clearance by mitophagy (Akabane et al., 2016). These findings suggest that cAMP in the MIS may regulate mitophagy via the MINOS complex, whereas the cAMP signalosomes at the MOM are mainly involved in the regulation of mitochondria fission-fusion dynamics, effected by Drp1, and apoptosis.

7. Mitochondrial Matrix. The mitochondrial matrix appears to define a cAMP compartment that is isolated from the rest of the cell. Cytosolic cAMP cannot reach the mitochondrial matrix, as unlike the MOM, the mitochondrial inner membrane (MIM) is not permeable to cytosolic cAMP (Acin-Perez et al., 2011; Di Benedetto et al., 2013), and studies suggest that the second messenger is generated within mitochondria by the carbon dioxide/bicarbonate-activated and Ca^{2+}-regulated AC10 in response to metabolically generated carbon dioxide (Fig. 2) (Acin-Perez et al., 2009). Therefore, MOM and MIS are physically separated from the mitochondrial matrix, which forms an independent cAMP domain distinct from the cytosol. Evidence supports a mechanism whereby a Ca^{2+} increase in the mitochondrial matrix stimulates a local cAMP increase that promotes mitochondrial ATP synthesis (Di Benedetto et al., 2013; Lefkimmiatis et al., 2013; Wang et al., 2016). These findings suggest that the mitochondrial matrix may host a cAMP signaling pathway that responds to metabolic changes and is not directly dependent on hormonal stimulation. The fact that PKA subunits lack any obvious mitochondrial targeting sequence challenges the idea of their presence in the matrix (Valsecchi et al., 2014). Indeed, presence of PKA as the effector of cAMP signaling in the matrix remains controversial, and experiments using FRET-based sensors targeted to the matrix failed to detect any PKA enzymatic activity in this compartment (Lefkimmiatis et al., 2013). Other studies also questioned the presence of a PKA pathway in the matrix, as no significant effect of Ca^{2+} stimulation on oxidative phosphorylation was observed when applying membrane-permeable cAMP analogs or PKA inhibitors (Covian et al., 2014).

8. Mitochondrial Exchange Protein Directly Activated by cAMP. EPAC1 was originally reported to localize to the mitochondria in a cell cycle–dependent manner, although its precise submitochondrial localization and function were unclear (Qiao et al., 2002). Treatment of isolated mitochondria with EPAC-specific activators showed no effect on oxygen consumption and oxidative phosphorylation activity. A recent study demonstrates that during hypoxia/reoxygenation, EPAC1 is activated by AC10-generated cAMP to increase Ca^{2+} uptake and reactive oxigen species (ROS) production, thereby promoting permeability transition pore (mitochondrial permeability transition pore) opening, cytochrome c release, and caspase-9 and -3 activation (Fazal et al., 2017). Another report, however, provides evidence that both isoform 1 and 2 of EPAC are present in the mitochondrial matrix in rat cardiac myocytes and that

cAMP generated by AC10 activates EPAC1 and prevents Ca^{2+}-induced opening of the mitochondrial permeability transition pore (Wang et al., 2016). Reports also suggest that during ischemia or acidosis, cytosolic AC10 translocates to the mitochondria and activates a local pool of PKA that phosphorylates and enhances the activity of the proapoptotic BAX protein (Kumar et al., 2009; Appukuttan et al., 2012).

9. Mitochondrial Phosphodiesterases. Mitochondria are also provided with their own supply of PDEs. The first report documenting localization of PDEs at this organelle dates back to 1982, when Cercek and Houslay, using cell fractionation experiments and PDE activity assays, found two distinct PDEs associate with the MOM and the MIM, respectively (Cercek and Houslay, 1982). More recent studies have confirmed that both PDE2A2 and PDE8 localize to mitochondria (Acin-Perez et al., 2011; Shimizu-Albergine et al., 2012). Although little is known on the specific role of PDE8 at the mitochondria, several recent studies have focused on mitochondrial PDE2A2. A study reports the presence of PDE2A2 in the matrix of mitochondria isolated from liver and brain lysates (Acin-Perez et al., 2011), in keeping with the idea of a cAMP pool confined to the mitochondrial matrix. These investigations showed that targeting of PDE2A2 to the matrix is mediated by its N terminus and that selective inhibition of PDE2A enhances oxidative phosphorylation and ATP synthesis (Acin-Perez et al., 2011). In contrast with these findings, another study based on protein fractionation, electron microscopy, and super-resolution imaging showed localization of PDE2A2 to the MOM and MIS rather than the mitochondrial matrix (Monterisi et al., 2017). No detectable PDE2A activity in the mitochondrial matrix was found using targeted FRET reporters, in agreement with other investigations in which nonspecific PDE inhibition had no effect on cAMP levels in the matrix (Di Benedetto et al., 2013; Lefkimmiatis et al., 2013). PDE2A2 at the MOM was shown to regulate local cAMP levels and PKA-dependent phosphorylation of Drp1, affecting mitochondria morphology and apoptotic cell death, effects that were recapitulated in $AC10^{-/-}$ cells, confirming a role of PDE2A2 at the mitochondria outside the matrix (Monterisi et al., 2017). A PDE2A2 interactome analysis also revealed interaction of this PDE isoform with the MINOS components MIC60 and ChChd3, consistent with its localization in the MIS. At this site, PDE2A2 was shown to regulate local levels of cAMP and control Parkin recruitment to the mitochondria and mitophagy (Lobo, 2020).

D. Golgi Apparatus

Several components of the cAMP signaling pathway have been found to localize at the Golgi, and several studies support compartmentalized cAMP/PKA signaling at this organelle (Godbole et al., 2017; Nash et al., 2018). For example, Yang et al. showed that in cardiac

myocytes, where the Golgi encircles or is adjacent to the nucleus, activation of β_1-AR stimulates Ca^{2+} release from this organelle. Release of Ca^{2+} from the Golgi seems to require activation of both EPAC and PKA and results in enhanced trafficking of proteins to the cell surface. The perinuclear release of Ca^{2+} appears to be distinct from the release of Ca^{2+} from the SR, an event that is also under the control of β_1-AR/cAMP and that regulates myocyte contraction. The amount of agonist required to induce release of Ca^{2+} from the Golgi is larger than the amount that promotes Ca^{2+} release from the SR. However, such difference is obliterated on inhibition of PDE3 or PDE4 (Yang et al., 2015), two enzymes that have also been shown to be associated with the Golgi fraction (Pooley et al., 1997; Geoffroy et al., 2001), but not by inhibition of PDE2 (Yang et al., 2015), which instead affects the amplitude of the Ca^{2+} transient in response to catecholamines (Mongillo et al., 2006). These findings are consistent with compartmentalization of cAMP signaling in the microenvironment surrounding the Golgi apparatus attained by local degradation of cAMP by PDE3 and PDE4 (Yang et al., 2015). Yang et al. did not investigate whether an AKAP-dependent signalosome is involved in the regulation of Ca^{2+} release from the Golgi. Interestingly, AKAP9 [AKAP350/AKAP450/centrosome and golgi localized protein kinase N-associated protein (CG-NAP)/Yotiao] has been found to localize to the Golgi apparatus in cerebellar granule cells (Shanks et al., 2002), where it brings PKA-RIIβ in close proximity to the IP$_3$ receptors (Collado-Hilly and Coquil, 2009). Several studies, predominantly in cell lines, have shown that Ca^{2+} efflux from the Golgi is mediated by IP$_3$ receptors (Pinton et al., 1998), and PKA is known to phosphorylate and activate IP$_3$ receptors and enhance Ca^{2+} release from the ER (Betzenhauser and Yule, 2010). However, in cardiac myocytes, Ca^{2+} release from the Golgi appears to be mediated by ryanodine receptors rather than IP$_3$ receptors (Yang et al., 2015).

E. Centrosome

The centrosome is the major microtubule-nucleating organelle in animal cells and is usually composed of a pair of centrioles surrounded by a protein matrix (Doxsey et al., 1994). In mitosis, the centrosome ensures fidelity of chromosome segregation through the formation of a bipolar spindle, whereas in differentiating cells, the centrosome acts as a platform for the formation of the primary cilium (Joukov and De Nicolo, 2019). Centrosomes contain all the components of the cAMP signaling pathway, including PKA (Nigg et al., 1985), at least two AKAPs (Schmidt et al., 1999; Takahashi et al., 1999; Witczak et al., 1999; Diviani et al., 2000), PDEs, and targets of PKA regulation, such as dynein, MAP2, and centrin, which are necessary for spindle formation, chromosome movement, and cytokinesis (Diviani and Scott, 2001).

1. A-Kinase Anchoring Protein 9. AKAP-450, one of the splice variants of AKAP9, is one of the anchoring proteins found at the centrosome and has been shown to be involved in microtubule nucleation (Schmidt et al., 1999; Takahashi et al., 1999; Witczak et al., 1999) and to affect cell migration but not cell polarization (Rivero et al., 2009). At the centrosome, AKAP9 organizes a multiprotein complex involving PKA type II, PDE4D3, PP1, and PP2A (Takahashi et al., 1999; McCahill et al., 2005; Taskén et al., 2001) (Fig. 3) and is required for centrosome integrity, centriole duplication, and cell cycle progression (Keryer et al., 2003). AKAP9 contains two distinct RII-binding domains, which could potentially recruit two PKA holoenzymes per scaffolding unit. Anchoring of PKA type II to AKAP9 was shown to lower the activation threshold of the enzyme as a consequence of increased RII autophosphorylation at S114. The AKAP9-anchored PDE4D3 provides local modulation of cAMP levels at the centrosome in a cell cycle–dependent manner (Terrin et al., 2012). Displacement of PDE4D3 from AKAP9 was shown to lead to accumulation of cells in prophase, indicating that cell cycle progression relies on unique regulation of centrosomal cAMP/PKA signals at the AKAP9 signalosome (Terrin et al., 2012).

2. Pericentrin. Another centrosomal protein that anchors PKA is pericentrin, although this interaction does not involve the canonical amphipathic helix found in other AKAPs (Diviani et al., 2000). Pericentrin is a highly conserved component of the centrosomal matrix and provides a structural scaffold for proper centrosomal architecture and microtubule nucleation during mitosis and meiosis (Dictenberg et al., 1998). In addition to PKA, pericentrin anchors γ-tubulin and dynein, the latter being a target for PKA-dependent phosphorylation (Inaba et al., 1998). Impairment of dynein function results in spindle fragmentation and disorganization (Purohit et al., 1999), defects that are also observed on disruption of AKAP/PKA interactions, and it has been speculated that PKA anchored at the centrosome through association with pericentrin might be involved in the regulation of dynein function (Diviani and Scott, 2001).

3. Centrosomal Exchange Protein Directly Activated by cAMP. Overexpression studies have shown dynamic localization of EPAC at the centrosome. Although in interphase EPAC is predominantly localized to mitochondria and the perinuclear region, in metaphase it dissociates from the nuclear membrane and localizes to the mitotic spindle and centrosomes. When cell division has completed, EPAC reassociates with the nuclear envelope and concentrates around the contractile ring. In breast cancer cells, inhibition of EPAC1 was shown to disrupt the association of AKAP9 with the microtubule cytoskeleton and to delocalize AKAP9 from the centrosome, with effects on cell migration and cell death (Kumar et al., 2017). Immunolocalization studies

have identified AC10 at the spindle poles and centrioles, where it can potentially act as a source of cAMP to direct centriole separation and mitotic spindle formation (Zippin et al., 2003).

F. Endo/Sarcoplasmic Reticulum

1. A-Kinase Anchoring Protein 7. A well studied example of compartmentalized cAMP signaling occurs at the SR of cardiac myocytes. A critical event that occurs in the flight-or-fight response upon adrenergic stimulation is cAMP/PKA-dependent enhancement of the amplitude of the Ca^{2+} transient during systole. One mechanism that contributes to this is more efficient Ca^{2+} reuptake in the SR during diastole via PKA-dependent modulation of the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2) activity. The pump is part of a signalosome organized by AKAP18δ that also includes PKA type II, PDE3A1, PDE4D, and the SERCA2-associated protein PLN (Fig. 3) (Kerfant et al., 2007; Lygren et al., 2007; Ahmad et al., 2015). In unstimulated myocytes, SERCA2 is inhibited by unphosphorylated PLN. Upon activation of β-ARs, PKA phosphorylates PLN and removes its inhibitory effect on SERCA2, promoting Ca^{2+} refilling of the SR and relaxation (Henn et al., 2004; Rigatti et al., 2015; Götz et al., 2016). Disruption of the PKA-AKAP18δ interaction using competing peptides or via AKAP18δ knockdown was shown to blunt phosphorylation of PLN and to abolish the effect of catecholamines on SR Ca^{2+} reuptake (Lygren et al., 2007), demonstrating that clustering of PKA next to PLN via anchoring to AKAP18δ is necessary for PLN phosphorylation and that other PKA subsets, either free in the cytosol or anchored to other AKAPs, cannot carry out this function. The cAMP pool that controls SERCA2 activity is modulated by PDE3A1 and PDE4D. Both enzymes have been shown to interact with SERCA2 and to modulate basal cAMP levels in SR microdomains containing SERCA2A-PLN, thus regulating basal cardiac contractility (Schwoerer et al., 2008; Beca et al., 2011, 2013). The hydrolytic activity of both PDE3A1 and PDE4D can be enhanced by PKA-dependent phosphorylation (Grant et al., 1988; Fertig and Baillie, 2018), providing a local negative-feedback loop. Furthermore, phosphorylation of PDE3A1 at a PKA site in its unique N-terminal region promotes incorporation of this PDE into the SERCA2/AKAP18δ complex by modulating phosphorylation-dependent protein-protein interactions between PDE3A1 and SERCA2 and between PDE3A1 and AKAP18δ (Ahmad et al., 2015).

PP1β is the most significant PP1 isoform involved in regulating SR Ca^{2+} levels (Aoyama et al., 2011). Phosphatase inhibitor-1 (I-1) regulates phosphatase activity and has also been described to be part of the signaling complex organized by AKAP18δ at the SR. PKA-mediated phosphorylation of I-1 induces potent inhibition of PP1, providing an additional level of local regulation of SERCA2 activity (Livigni et al., 2006; Nicolaou et al., 2009). As mentioned above, AKAP18δ is the longest isoform of the AKAP18 family (Henn et al., 2004), which consists of four splice variants with various involvement in intracellular Ca^{2+} regulation. A knockout mouse model for AKAP18 did not show altered regulation of Ca^{2+} handling in cardiomyocytes (Jones et al., 2012), questioning the unique involvement of AKAP18 in these processes. Potential confounding effects deriving from expression in the knock out model of a truncated AKAP18 protein lacking the PKA-binding domain have been suggested as a possible explanation for these contrasting findings (Smith et al., 2018).

2. A-Kinase Anchoring Protein 13. A signalosome organized by AKAP13 [also known as AKAP–lymphocyte blast crisis (Lbc)] has recently been described to operate at ER exit sites, the specialized region at the ER membrane where export of newly synthesized proteins is facilitated via coat protein complex II–coated vesicles. At these sites, AKAP13 is involved in a complex signaling cascade that includes Gα12, AC7, PDE3B, PKA, and other kinases. cAMP generated locally by AC7 activates the AKAP13 anchored PKA pool, and the ensuing substrate phosphorylation contributes to activate cargo export (Subramanian et al., 2019). The role of this signalosome is thought to be to ensure quick response to fluctuations in protein synthesis that might occur under different conditions and that, if not handled properly, may lead to aberrant cargo processing and sorting and potentially to disease.

G. Nucleus

Multiple components of the cAMP signaling cascade have been shown to localize to the nucleus and/or nuclear membrane, including GPCRs, G proteins, ACs, AKAPs, and PKA (Vaniotis et al., 2011).

1. A-Kinase Anchoring Protein 6. AKAP6 (or mAKAP) is expressed in excitable cells as two splice variants: mAKAPα is found in neurons, and mAKAPβ is found in striated muscle cells. Earlier studies described a macromolecular complex involving mAKAP and localized at the SR (Pare et al., 2005); however, subsequent studies have firmly established a localization of mAKAPβ at the outer nuclear membrane via interaction with the protein Nesprin-1α (Marx et al., 1998; Yang et al., 1998; Kapiloff et al., 1999). This signalosome is complex, and multiple elements have been found in different studies to associate with mAKAPβ. Whether all components are simultaneously present within this signalosome remains to be determined. In addition to PKA, there is evidence of direct interaction of mAKAPβ with RyR2 (Marx et al., 2001), PP2A (Dodge-Kafka et al., 2010) and CaN (PP2B) (Li et al., 2010), AC5 (Kapiloff et al., 2009), PLCε (Zhang et al., 2011), the nuclear HDAC5 (Dodge-Kafka et al., 2018), myocyte enhancer factor-2 (MEF2) (Vargas et al., 2012), 3-phosphoinositide-dependent kinase-1 (Michel et al., 2005), the p90 ribosomal S6 kinase 3, (Passariello et al., 2015), and PDE4D3 (Dodge et al., 2001) (Fig. 3). It should be noted that although some groups have claimed that PDE4D3 interacts

natively with mAKAP, in these studies, the identification of PDE4D3 relied solely on comigration of recombinant PDE4D3 with an immunoreactive species in cardiac cell extracts, as detected using "pan-PDE4" antisera able to detect all PDE4 isoforms. However, such studies were unable to take into account the subsequent identification, cloning, and characterization of several other PDE4D isoforms that were found to have a similar molecular weight to PDE4D3 and, indeed, were found to comigrate with PDE4D3 on SDS-PAGE—namely, PDE4D8, PDE4D9, and PDE4D11 (for a review, see Bolger, 2006). Thus, the PDE4D species in cardiac tissue that interacts with AKAPs could be any of PDE4D3, PDE4D8, PDE4D9, and PDE4D11. Furthermore, given the rarity of PDE4D3 transcripts, it is highly unlikely that the PDE4D interacting in heart tissues with mAKAP is PDE4D3. The concomitant assembly by mAKAP of cAMP-sensing and terminating enzymes is the basis for a negative-feedback loop regulation demonstrated in cardiac myocytes. PKA-mediated phosphorylation of Ser13 in PDE4D3 enhances its binding to mAKAP (Carlisle Michel et al., 2004), whereas phosphorylation of Ser54 enhances the cAMP hydrolytic activity of the catalytic domain of PDE4D3 (Sette and Conti, 1996) (but see comments above). Interestingly, mAKAP is expressed at very low levels in myocytes, such that loss of mAKAP does not even affect the localization of perinuclear PKA (Passariello et al., 2015). However, replacement of endogenous mAKAP with a mAKAP mutant that cannot bind PKA is sufficient to inhibit the induction of myocyte hypertrophy (Pare et al., 2005). AC5 activity is inhibited by PKA feedback phosphorylation when the cyclase is associated with the mAKAP complex. Disruption of mAKAP-AC5 interaction in neonatal cardiac myocytes results in increased cAMP and hypertrophy in the absence of agonist stimulation (Kapiloff et al., 2009). Lastly, through Rap1, EPAC1 can inhibit extracellular signal-regulated kinase 5 (ERK5) activity, thus preventing PDE4D3 inhibition by MAPK signaling, resulting in higher PDE4D3 activity due to concomitant PKA phosphorylation. As a result, EPAC1 provides a third negative-feedback loop that can attenuate cAMP levels in the proximity of mAKAP complexes (Dodge-Kafka et al., 2005; Passariello et al., 2015). Interestingly, mAKAP proteins containing nonsynonymous polymorphisms display different binding affinities for PKA and PDE4D3 (Rababa'h et al., 2013), suggesting potential individual differences on how cAMP signaling is handled at mAKAP by PDE4D3. Binding of PP2A to the C terminus of mAKAP provides an additional layer of regulation. PP2A dephosphorylates PDE4D3 at Ser54, inhibiting the PDE in the absence of upstream stimuli (Dodge-Kafka et al., 2010; Passariello et al., 2015) (Fig. 3).

As perhaps expected, given its perinuclear localization, the signalosome organized by mAKAPβ is involved in the modulation of transcriptional regulation (Dodge-Kafka et al., 2019). In cardiac myocytes, the mAKAPβ signalosome has been proposed to nucleate the effect of distinct pools of cAMP, which either activate or block transcription of pro-hypertrophic genes (Zhang et al., 2013; Zhang and Eggert, 2013; Kritzer et al., 2014). mAKAPβ-bound PKA can phosphorylate a small pool of perinuclear RyRs promoting Ca^{2+} released from perinuclear stores and local activation of CaN, which in turn induces nuclear factor of activated T-cells (NFAT) and MEF2-dependent gene expression associated with hypertrophy (Li et al., 2010). A distinct pro-hypertrophic pathway involves EPAC-PLCε–dependent PI4P hydrolysis at the Golgi, which results in diacylglycerol-dependent activation of mAKAPβ-bound PKCε and PKD, phosphorylation, and nuclear export of class IIa HDACs and induction of hypertrophy (Zhang et al., 2013). On the other hand, cAMP, via PKA activation, can mediate inhibition of PLCε-dependent PI4P hydrolysis, counteracting hypertrophy (Nash et al., 2018). mAKAPβ thus appears to act as a platform where the complex action of multiple pathways is locally coordinated to control the development of cardiac hypertrophy, although the full details of how cAMP/PKA/EPAC pathways operate in this context remains to be fully elucidated (Diviani et al., 2016).

2. A-Kinase Anchoring Protein 8. AKAP8 (or AKAP95) has been described to anchor PKA in the nuclear matrix in a cell cycle–dependent manner (Coghlan et al., 1994; Eide et al., 1998) (Fig. 3). Several different functions have been attributed to AKAP8, including DNA replication via interaction with minichromosome maintenance 2 protein, a function that appears to be independent of PKA (Eide et al., 2003), chromatin condensation through association with the RII subunit of PKA (Collas et al., 1999; Bomar et al., 2002), recruitment of HDACs to mitotic chromosomes (Li et al., 2006), and regulation of histone methylation and gene expression (Jiang et al., 2013; Hu et al., 2016). Additionally, AKAP8 has been shown to bind PDE4A (Asirvatham et al., 2004) and to be involved in the PKA-mediated phosphorylation of CREB (Lu et al., 2017). Tyrosine phosphorylation of AKAP8 promotes its dissociation from chromatin and the nuclear matrix, although the impact on local signaling remains unclear (Kubota et al., 2015).

Activation of the transcription factor CREB via PKA-mediated phosphorylation is one of the long-recognized functional effects of cAMP signals (Yamamoto et al., 1988). The conventional view is that upon cAMP binding to PKA holoenzyme, C subunits dissociate and translocate to the nucleus via diffusion (Harootunian et al., 1993). Once in the nucleus, PKA-mediated phosphorylation of CREB promotes its interaction with CREB binding protein, followed by enhanced transcription of target genes. Binding of protein kinase inhibitor to the C subunits induces exit of the complex from the nucleus (Dalton and Dewey, 2006), followed by dephosphorylation of CREB mainly by PP2A (Wadzinski et al., 1993). Recent

studies, however, suggest an alternative mechanism. Sample et al. reported the activation of a resident pool of PKA holoenzyme in the nuclei of HEK-293 cells by AC10. These studies suggest a model whereby the nuclear AKAP8 assembles PDE4D3 or PDE4D5 (Clister et al., 2019) and PKA to form a signaling complex in the nucleus that controls local cAMP levels and nuclear PKA activity. The nuclear envelope is permeable to cAMP produced at the plasma membrane (Terrin et al., 2006). When cAMP generated by ACs at the plasma membrane reaches the nucleus, PDE4D3/5 keeps cAMP concentration below the activation threshold of the local PKA holoenzyme; when cAMP is produced locally by AC10 and above that threshold, activation of the local PKA occurs, resulting in faster activation kinetics compared with slow kinetics associated with translocation of C subunit to the nucleus (Sample et al., 2012). Other studies support the presence of AC10 in the nucleus (Dodge-Kafka et al., 2005; Passariello et al., 2015). However, Yang et al. failed to reproduce these observations in primary rat neonatal cardiac myocytes. The authors propose that the different kinetics of nuclear PKA are explained by diffusion and differences in the nuclear expression of protein kinase inhibitor rather than being dependent on a nuclear pool of PKA holoenzyme (Yang et al., 2014).

V. Novel Insight into Cell Physiology from Nanodomain cAMP Signaling

The recent methodological advances allow analysis of cAMP dynamics and PKA activity with unprecedented spatial and temporal resolution. The data that emerge from studies using targeted versions of the cAMP probes suggest that cAMP signaling is complex. Compartmentalization of cAMP is more extreme than initially thought, as evidence suggests that distinct handling of cAMP is confined to domains that can have submicrometer dimensions. Whatever the source of cAMP (whether an AC at the plasma membrane, an AC localized at internal membranes, or the soluble AC), its activation generates a gradient of second messenger that propagates away from the site of synthesis and that dissipates as distance increases. The size of this gradient is variable and depends on the AC involved and the amplitude and duration of the stimulus. In some cases, the gradient may dissipate rapidly, and a significant amount of cAMP is achieved only at sites proximal to the site of synthesis. If the trigger persists and the activity of the AC is sufficiently high to counteract cAMP degradation by PDEs, the level of second messenger can progressively increase distally from the site of synthesis, and over time, cAMP equilibrates across the entire cell. However, superimposed to this overall cytosolic gradient, a more variegated pattern of multiple and diverse cAMP pools develops in which the level of the second messenger in each nanocompartment is dictated by the local activity of PDEs and other contributing factors, including local buffering, high viscosity, and physical obstacles. The amount of cAMP within these multiple subcellular nanodomains can be either lower or higher than the level of the second messenger in the bulk cytosol, depending on which modulators prevail. It is the concentration of cAMP within each of these nanodomains, then, that dictates whether the local cAMP effectors are activated and whether a certain function is triggered. Bulk cytosolic cAMP may be functionally irrelevant (Bers et al., 2019), or its unique spatiotemporal dynamics may also encode information that serves particular roles.

What is clear is that cAMP signal nanoheterogeneity is essential for regulation of function, and as research uncovers new details, novel facets of cell physiology emerge. A recent investigation in retinal ganglion cells analyzed the role of cAMP in the regulation of growth cone response to ephrine-A, a cue that during development triggers refinement of retinal axons that initially overshoot in the most caudal part of the superior colliculus. cAMP plays an important role in neuronal development, as it mediates the response to a variety of axon guidance molecules, including Netrin-1, Semaphorin-3A, and ephrin-A5. The appropriate development of neuronal connectivity relies on axon guidance molecules that promote axonal growth and orient the axon toward appropriate synaptic partners. Initially, axonal branches develop exuberant projections that must be pruned to establish a correct projection map. Using a combination of real-time imaging of cAMP and molecular tools to either generate or buffer cAMP within restricted domains, the study found that it is specifically a decrease in cAMP occurring at the plasma membrane proximally to lipid rafts that is responsible for the ephrine-A–induced axon retraction in vitro and for pruning of retinal ganglion cells arbors in the superior colliculus in vivo. Manipulation of cAMP at nonraft plasma membrane domains, in contrast, showed no effect on pruning. Interestingly, selective manipulation of cAMP at lipid rafts had no effect on axon outgrowth and early development of the retinal pathway, both process that are also dependent on cAMP, confirming in vivo that distinct pools of cAMP generated at the plasma membrane exhibit clear functional specificity (Averaimo et al., 2016).

Recent studies using targeted reporter also demonstrate that not only does activation of different GPCRs result in different cAMP signals in distinct subcellular compartments, but activation of a single receptor can generate multiple cAMP signals with different amplitude and kinetics at different subcellular locations. In ventricular myocytes, cAMP mediates the response to sympathetic activation of β-AR. Strength of contraction and efficiency of relaxation are regulated via PKA-dependent phosphorylation of the L-type Ca^{2+} channel at the plasmalemma and PLN at the sarcoplasmic reticulum, which rapidly enhance the amplitude of

the Ca^{2+} transients and contraction, and phosphorylation of troponin I at the myofilaments, which reduces the affinity of the troponin complex for Ca^{2+}, promoting relaxation. A recent study using targeted cAMP FRET-based reporters demonstrated that β-AR stimulation results in distinct signals at these sites and that the cAMP increase is delayed and attenuated at the myofilaments compared with the plasmalemma and sarcoplasmic reticulum (Surdo et al., 2017). The compartmentalized nature of the cAMP signal generated on β-AR activation appears to be required to achieve maximal effect of sympathetic stimulation, as demonstrated by the fact that upon application of PDE inhibitors, a maneuver that generates overall a larger increase in cAMP but abolishes local differences, a weaker contraction was detected. Interestingly, in experimental models of cardiac disease, such as cardiac hypertrophy and heart failure, which are characterized by downregulation of the adrenergic pathway, the cAMP signal triggered by β-AR stimulation was nearly abolished at the myofilament but was well preserved at plasmalemma and sarcoplasmic reticulum. These findings have important implications for cardiac pathophysiology and potentially for the treatment of cardiac disease (Bers et al., 2019). Firstly, the data suggest that maximal stimulated contractility is achieved at the cost of an intrinsic predisposition of the myofilament to Ca^{2+} sensitization, which derives from attenuated local cAMP signaling. The propensity of the myofilament to experience lower levels of cAMP may become dangerous in conditions of reduced adrenergic drive (as experienced in pathologic conditions) when no effective cAMP signal may be generated at the troponin complex, resulting in persistent Ca^{2+} binding to the myofilament and diastolic dysfunction. Secondly, the findings may explain the well established long-term adverse effects of PDE inhibitors in the treatment of heart failure (Packer et al., 1991). The data show that PDE inhibition equalizes cAMP levels in the different compartments and reduces efficiency of cardiac myocyte contraction: in a failing heart, this results in further reduction of cardiac reserve and may contribute to pathogenesis. These unexpected findings also provide a new perspective on how to approach treatment of heart failure, as they suggest that therapeutic interventions aimed at normalizing Ca^{2+} sensitivity may prove beneficial, particularly in early stages of the pathologic process.

VI. Open Questions and Future Directions

Although the recent findings represent important progress in understanding novel aspects of cAMP signaling, significant gaps remain to be bridged, and important questions remain to be answered. For example, additional factors that contribute to compartmentalizing cAMP (e.g., buffering, high viscosity, or physical obstacles) have been postulated, but what exactly is their nature and how they impact different subcellular sites remain largely to be defined. How activation of a given GPCR orchestrates the activity of downstream modulators to determine what part of the signaling network is activated to achieve the required pattern of cAMP nanosignals remains puzzling. Answers to these questions will emerge as we learn more about the organization, regulation, and function of individual signaling compartments. Defining how many cAMP domains there are in a cell, what their size is, what their architecture and molecular composition are, and how they are affected in pathologic conditions is now feasible. High-resolution imaging approaches combined with biochemical studies (Schleicher and Zaccolo, 2020) applied to different cell types will allow us to work out the unique topography and function of cell type–specific cAMP nanodomains. As probes that can monitor cAMP signaling with single-cell resolution in moving animals are being developed (Ma et al., 2018), the physiologic relevance of subcellular signaling will be established at the organ and organism level. Integration of this information will certainly benefit from development of mathematical models that incorporate local handling of cAMP at the nanometer scale.

Defining the details of local cAMP signaling is important. Multiple drugs currently in clinical use target this signaling pathway, and understanding the subcellular ramifications of these pharmacological interventions would facilitate dissection of the mechanisms leading to side effects and help devise mitigation strategies. Although assessment of global cellular cAMP responses is still the prevalent approach used in screening programs for new drugs that target GPCRs, consideration of subcellular local effects at early stages would help reduce the high attrition rates. Finally, the compartmentalized nature of the cAMP signaling pathway offers the unique opportunity to develop molecules that target individual cAMP domains rather than global intracellular cAMP (Baillie et al., 2019), and a detailed understanding of this complex signaling systems and its nanodomain organization will provide a framework for the development of precision medicine strategies.

References

Abbattiscianni AC, Favia M, Mancini MT, Cardone RA, Guerra L, Monterisi S, Castellani S, Laselva O, Di Sole F, Conese M, et al. (2016) Correctors of mutant CFTR enhance subcortical cAMP-PKA signaling through modulating ezrin phosphorylation and cytoskeleton organization. *J Cell Sci* **129**:1128–1140.
Abrenica B, AlShaaban M, and Czubryt MP (2009) The A-kinase anchor protein AKAP121 is a negative regulator of cardiomyocyte hypertrophy. *J Mol Cell Cardiol* **46**:674–681.
Acin-Perez R, Russwurm M, Günnewig K, Gertz M, Zoidl G, Ramos L, Buck J, Levin LR, Rassow J, Manfredi G, et al. (2011) A phosphodiesterase 2A isoform localized to mitochondria regulates respiration. *J Biol Chem* **286**:30423–30432.
Acin-Perez R, Salazar E, Kamenetsky M, Buck J, Levin LR, and Manfredi G (2009) Cyclic AMP produced inside mitochondria regulates oxidative phosphorylation. *Cell Metab* **9**:265–276.
Adams SR, Harootunian AT, Buechler YJ, Taylor SS, and Tsien RY (1991) Fluorescence ratio imaging of cyclic AMP in single cells. *Nature* **349**:694–697.

Affaitati A, Cardone L, de Cristofaro T, Carlucci A, Ginsberg MD, Varrone S, Gottesman ME, Avvedimento EV, and Feliciello A (2003) Essential role of A-kinase protein 121 for cAMP signaling to mitochondria. *J Biol Chem* **278**:4286–4294.

Agarwal SR, Gratwohl J, Cozad M, Yang PC, Clancy CE, and Harvey RD (2018) Compartmentalized cAMP signaling associated with lipid raft and non-raft membrane domains in adult ventricular myocytes. *Front Pharmacol* **9**:332.

Agarwal SR, Miyashiro K, Latt H, Ostrom RS, and Harvey RD (2017) Compartmentalized cAMP responses to prostaglandin EP₂ receptor activation in human airway smooth muscle cells. *Br J Pharmacol* **174**:2784–2796.

Aggarwal S, Gabrovsek L, Langeberg LK, Golkowski M, Ong SE, Smith FD, and Scott JD (2019) Depletion of dAKAP1-protein kinase A signaling islands from the outer mitochondrial membrane alters breast cancer cell metabolism and motility. *J Biol Chem* **294**:3152–3168.

Ahmad F, Shen W, Vandeput F, Szabo-Fresnais N, Krall J, Degerman E, Goetz F, Klussmann E, Movsesian M, and Manganiello V (2015) Regulation of sarcoplasmic reticulum Ca2+ ATPase 2 (SERCA2) activity by phosphodiesterase 3A (PDE3A) in human myocardium: phosphorylation-dependent interaction of PDE3A1 with SERCA2. *J Biol Chem* **290**:6763–6776.

Akabane S, Uno M, Tani N, Shimazaki S, Ebara N, Kato H, Kosako H, and Oka T (2016) PKA regulates PINK1 stability and parkin recruitment to damaged mitochondria through phosphorylation of MIC60. *Mol Cell* **62**:371–384.

Allen MD and Zhang J (2006) Subcellular dynamics of protein kinase A activity visualized by FRET-based reporters. *Biochem Biophys Res Commun* **348**: 716–721.

Alto NM, Soderling J, and Scott JD (2002) Rab32 is an A-kinase anchoring protein and participates in mitochondrial dynamics. *J Cell Biol* **158**:659–668.

Aoyama H, Ikeda Y, Miyazaki Y, Yoshimura K, Nishino S, Yamamoto T, Yano M, Inui M, Aoki H, and Matsuzaki M (2011) Isoform-specific roles of protein phosphatase 1 catalytic subunits in sarcoplasmic reticulum-mediated Ca(2+) cycling. *Cardiovasc Res* **89**:79–88.

Appukuttan A, Kasseckert SA, Micoogullari M, Flacke JP, Kumar S, Woste A, Abdallah Y, Pott L, Reusch HP, and Ladilov Y (2012) Type 10 adenylyl cyclase mediates mitochondrial Bax translocation and apoptosis of adult rat cardiomyocytes under simulated ischaemia/reperfusion. *Cardiovasc Res* **93**:340–349.

Asirvatham AL, Galligan SG, Schillace RV, Davey MP, Vasta V, Beavo JA, and Carr DW (2004) A-kinase anchoring proteins interact with phosphodiesterases in T lymphocyte cell lines. *J Immunol* **173**:4806–4814.

Averaimo S, Assali A, Ros O, Couvet S, Zagar Y, Genescu I, Rebsam A, and Nicol X (2016) A plasma membrane microdomain compartmentalizes ephrin-generated cAMP signals to prune developing retinal axon arbors. *Nat Commun* **7**:12896.

Bachmann VA, Mayrhofer JE, Ilouz R, Tschaikner P, Raffeiner P, Röck R, Courcelles M, Apelt F, Lu TW, Baillie GS, et al. (2016) Gpr161 anchoring of PKA consolidates GPCR and cAMP signaling. *Proc Natl Acad Sci USA* **113**:7786–7791.

Baillie GS (2009) Compartmentalized signalling: spatial regulation of cAMP by the action of compartmentalized phosphodiesterases. *FEBS J* **276**:1790–1799.

Baillie GS, Sood A, McPhee I, Gall I, Perry SJ, Lefkowitz RJ, and Houslay MD (2003) beta-Arrestin-mediated PDE4 cAMP phosphodiesterase recruitment regulates beta-adrenoceptor switching from Gs to Gi. *Proc Natl Acad Sci USA* **100**: 940–945.

Baillie GS, Tejeda GS, and Kelly MP (2019) Therapeutic targeting of 3′,5′-cyclic nucleotide phosphodiesterases: inhibition and beyond. *Nat Rev Drug Discov* **18**: 770–796.

Baldwin TA and Dessauer CW (2018) Function of adenylyl cyclase in heart: the AKAP connection. *J Cardiovasc Dev Dis* **5**:2.

Banky P, Huang LJ, and Taylor SS (1998) Dimerization/docking domain of the type Ialpha regulatory subunit of cAMP-dependent protein kinase. Requirements for dimerization and docking are distinct but overlapping. *J Biol Chem* **273**: 35048–35055.

Banky P, Roy M, Newlon MG, Morikis D, Haste NM, Taylor SS, and Jennings PA (2003) Related protein-protein interaction modules present drastically different surface topographies despite a conserved helical platform. *J Mol Biol* **330**: 1117–1129.

Bauman AL, Soughayer J, Nguyen BT, Willoughby D, Carnegie GK, Wong W, Hoshi N, Langeberg LK, Cooper DM, Dessauer CW, et al. (2006) Dynamic regulation of cAMP synthesis through anchored PKA-adenylyl cyclase V/VI complexes. *Mol Cell* **23**:925–931.

Beavo JA, Bechtel PJ, and Krebs EG (1974) Activation of protein kinase by physiological concentrations of cyclic AMP. *Proc Natl Acad Sci USA* **71**:3580–3583.

Beavo JA and Brunton LL (2002) Cyclic nucleotide research -- still expanding after half a century. *Nat Rev Mol Cell Biol* **3**:710–718.

Beavo JA, Hardman JG, and Sutherland EW (1971) Stimulation of adenosine 3′,5′-monophosphate hydrolysis by guanosine 3′,5′-monophosphate. *J Biol Chem* **246**: 3841–3846.

Beca S, Ahmad F, Shen W, Liu J, Makary S, Polidovitch N, Sun J, Hockman S, Chung YW, Movsesian M, et al. (2013) Phosphodiesterase type 3A regulates basal myocardial contractility through interacting with sarcoplasmic reticulum calcium ATPase type 2a signaling complexes in mouse heart. *Circ Res* **112**:289–297.

Beca S, Helli PB, Simpson JA, Zhao D, Farman GP, Jones P, Tian X, Wilson LS, Ahmad F, Chen SRW, et al. (2011) Phosphodiesterase 4D regulates baseline sarcoplasmic reticulum Ca2+ release and cardiac contractility, independently of L-type Ca2+ current. *Circ Res* **109**:1024–1030.

Bedioune I, Bobin P, Leroy J, Fischmeister R, and Vandecasteele G (2017) Cyclic nucleotide phosphodiesterases and compartmentation in normal and diseased heart, in *Microdomains in the Cardiovascular System* (Nikolaev V and Zaccolo M eds), Springer International Publishing, Cham, Switzerland.

Bender AT and Beavo JA (2006) Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol Rev* **58**:488–520.

Bers DM, Xiang YK, and Zaccolo M (2019) Whole-cell cAMP and PKA activity are epiphenomena, nanodomain signaling matters. *Physiology (Bethesda)* **34**:240–249.

Berthouze-Duquesnes M, Lucas A, Saulière A, Sin YY, Laurent AC, Galés C, Baillie G, and Lezoualc'h F (2013) Specific interactions between Epac1, β-arrestin2 and

PDE4D5 regulate β-adrenergic receptor subtype differential effects on cardiac hypertrophic signaling. *Cell Signal* **25**:970–980.

Betzenhauser MJ and Yule DI (2010) Regulation of inositol 1,4,5-trisphosphate receptors by phosphorylation and adenine nucleotides. *Curr Top Membr* **66**: 273–298.

Bitterman JL, Ramos-Espiritu L, Diaz A, Levin LR, and Buck J (2013) Pharmacological distinction between soluble and transmembrane adenylyl cyclases. *J Pharmacol Exp Ther* **347**:589–598.

Bloom JR, Ross RD, and Burnell G (1978) The effect of social support on patient adjustment after breast surgery. *Patient Couns Health Educ* **1**:50–59.

Bolger GB (2006) Phosphodiesterase isoforms – an annotated list, in *Cyclic Nucleotide Phosphodiesterases in Health and Disease* (Beavo JA, Francis SH, and Houslay MD eds) pp 19–31, CRC Press, Boca Raton, FL.

Bomar J, Moreira P, Balise JJ, and Collas P (2002) Differential regulation of maternal and paternal chromosome condensation in mitotic zygotes. *J Cell Sci* **115**: 2931–2940.

Börner S, Schwede F, Schlipp A, Berisha F, Calebiro D, Lohse MJ, and Nikolaev VO (2011) FRET measurements of intracellular cAMP concentrations and cAMP analog permeability in intact cells. *Nat Protoc* **6**:427–438.

Bos JL (2006) Epac proteins: multi-purpose cAMP targets. *Trends Biochem Sci* **31**: 680–686.

Brand T (2005) The Popeye domain-containing gene family. *Cell Biochem Biophys* **43**: 95–103.

Brandon EP, Zhuo M, Huang YY, Qi M, Gerhold KA, Burton KA, Kandel ER, McKnight GS, and Idzerda RL (1995) Hippocampal long-term depression and depotentiation are defective in mice carrying a targeted disruption of the gene encoding the RI beta subunit of cAMP-dependent protein kinase. *Proc Natl Acad Sci USA* **92**:8851–8855.

Braun T (1975) The effect of divalent cations on bovine spermatozoal adenylate cyclase activity. *J Cyclic Nucleotide Res* **1**:271–281.

Braun T and Dods RF (1975) Development of a Mn-2+-sensitive, "soluble" adenylate cyclase in rat testis. *Proc Natl Acad Sci USA* **72**:1097–1101.

Bridges D, MacDonald JA, Wadzinski B, and Moorhead GB (2006) Identification and characterization of D-AKAP1 as a major adipocyte PKA and PP1 binding protein. *Biochem Biophys Res Commun* **346**:351–357.

Brisson GR, Malaisse-Lagae F, and Malaisse WJ (1972) The stimulus-secretion coupling of glucose-induced insulin release. VII. A proposed site of action for adenosine-3′,5′-cyclic monophosphate. *J Clin Invest* **51**:232–241.

Bui M, Gilady SY, Fitzsimmons RE, Benson MD, Lynes EM, Gesson K, Alto NM, Strack S, Scott JD, and Simmen T (2010) Rab32 modulates apoptosis onset and mitochondria-associated membrane (MAM) properties. *J Biol Chem* **285**: 31590–31602.

Bundey RA and Insel PA (2006) Adenylyl cyclase 6 overexpression decreases the permeability of endothelial monolayers via preferential enhancement of prostacyclin receptor function. *Mol Pharmacol* **70**:1700–1707.

Burdyga A, Surdo NC, Monterisi S, Di Benedetto G, Grisan F, Penna E, Pellegrini L, Zaccolo M, Bortolozzi M, Swietach P, et al. (2018) Phosphatases control PKA-dependent functional microdomains at the outer mitochondrial membrane. *Proc Natl Acad Sci USA* **115**:E6497–E6506.

Burgers PP, Bruystens J, Burnley RJ, Nikolaev VO, Keshwani M, Wu J, Janssen BJC, Taylor SS, Heck AJR, and Scholten A (2016) Structure of smAKAP and its regulation by PKA-mediated phosphorylation. *FEBS J* **283**:2132–2148.

Burgers PP, Ma Y, Margarucci L, Mackey M, van der Heyden MA, Ellisman M, Scholten A, Taylor SS, and Heck AJ (2012) A small novel A-kinase anchoring protein (AKAP) that localizes specifically protein kinase A-regulatory subunit I (PKA-RI) to the plasma membrane. *J Biol Chem* **287**:43789–43797.

Calaghan S, Kozera L, and White E (2008) Compartmentalisation of cAMP-dependent signalling by caveolae in the adult cardiac myocyte. *J Mol Cell Cardiol* **45**:88–92.

Calebiro D, de Filippis T, Lucchi S, Martinez F, Porazzi P, Trivellato R, Locati M, Beck-Peccoz P, and Persani L (2006) Selective modulation of protein kinase A I and II reveals distinct roles in thyroid cell gene expression and growth. *Mol Endocrinol* **20**:3196–3211.

Calebiro D, Nikolaev VO, Gagliani MC, de Filippis T, Dees C, Tacchetti C, Persani L, and Lohse MJ (2009) Persistent cAMP-signals triggered by internalized G-protein-coupled receptors. *PLoS Biol* **7**:e1000172.

Calebiro D, Nikolaev VO, and Lohse MJ (2010) Imaging of persistent cAMP signaling by internalized G protein-coupled receptors. *J Mol Endocrinol* **45**: 1–8.

Cardone L, Carlucci A, Affaitati A, Livigni A, DeCristofaro T, Garbi C, Varrone S, Ullrich A, Gottesman ME, Avvedimento EV, et al. (2004) Mitochondrial AKAP121 binds and targets protein tyrosine phosphatase D1, a novel positive regulator of src signaling. *Mol Cell Biol* **24**:4613–4626.

Carlisle Michel JJ, Dodge KL, Wong W, Mayer NC, Langeberg LK, and Scott JD (2004) PKA-phosphorylation of PDE4D3 facilitates recruitment of the mAKAP signalling complex. *Biochem J* **381**:587–592.

Carlucci A, Lignitto L, and Feliciello A (2008) Control of mitochondria dynamics and oxidative metabolism by cAMP, AKAPs and the proteasome. *Trends Cell Biol* **18**: 604–613.

Carr DW, Hausken ZE, Fraser ID, Stofko-Hahn RE, and Scott JD (1992) Association of the type II cAMP-dependent protein kinase with a human thyroid RII-anchoring protein. Cloning and characterization of the RII-binding domain. *J Biol Chem* **267**: 13376–13382.

Cazorla O, Lucas A, Poirier F, Lacampagne A, and Lezoualc'h F (2009) The cAMP binding protein Epac regulates cardiac myofilament function. *Proc Natl Acad Sci USA* **106**:14144–14149.

Cercek B and Houslay MD (1982) Submitochondrial localization and asymmetric disposition of two peripheral cyclic nucleotide phosphodiesterases. *Biochem J* **207**: 123–132.

Chao YC, Surdo NC, Pantano S, and Zaccolo M (2019) Imaging cAMP nanodomains in the heart. *Biochem Soc Trans* **47**:1383–1392.

Chay A, Zamparo I, Koschinski A, Zaccolo M, and Blackwell KT (2016) Control of βAR- and N-methyl-D-aspartate (NMDA) receptor-dependent cAMP dynamics in hippocampal neurons. *PLOS Comput Biol* 12:e1004735.

Cheepala S, Hulot JS, Morgan JA, Sassi Y, Zhang W, Naren AP, and Schuetz JD (2013) Cyclic nucleotide compartmentalization: contributions of phosphodiesterases and ATP-binding cassette transporters. *Annu Rev Pharmacol Toxicol* 53: 231–253.

Chen C, Nakamura T, and Koutalos Y (1999) Cyclic AMP diffusion coefficient in frog olfactory cilia. *Biophys J* 76:2861–2867.

Chen L and Kass RS (2006) Dual roles of the A kinase-anchoring protein Yotiao in the modulation of a cardiac potassium channel: a passive adaptor versus an active regulator. *Eur J Cell Biol* 85:623–626.

Chen L, Marquardt ML, Tester DJ, Sampson KJ, Ackerman MJ, and Kass RS (2007) Mutation of an A-kinase-anchoring protein causes long-QT syndrome. *Proc Natl Acad Sci USA* 104:20990–20995.

Chen Q, Lin RY, and Rubin CS (1997) Organelle-specific targeting of protein kinase AII (PKAII). Molecular and in situ characterization of murine A kinase anchor proteins that recruit regulatory subunits of PKAII to the cytoplasmic surface of mitochondria. *J Biol Chem* 272:15247–15257.

Chen W, Levine H, and Rappel WJ (2008) A mathematical analysis of second messenger compartmentalization. *Phys Biol* 5:046006.

Chen W, Levine H, and Rappel WJ (2009) Compartmentalization of second messengers in neurons: a mathematical analysis. *Phys Rev E Stat Nonlin Soft Matter Phys* 80:041901.

Chen Y, Cann MJ, Litvin TN, Iourgenko V, Sinclair ML, Levin LR, and Buck J (2000) Soluble adenylyl cyclase as an evolutionarily conserved bicarbonate sensor. *Science* 289:625–628.

Chen Y, Yue S, Xie L, Pu XH, Jin T, and Cheng SY (2011) Dual phosphorylation of suppressor of fused (Sufu) by PKA and GSK3beta regulates its stability and localization in the primary cilium. *J Biol Chem* 286:13502–13511.

Cheng A, Arumugam TV, Liu D, Khatri RG, Mustafa K, Kwak S, Ling HP, Gonzales C, Xin O, Jo DG, et al. (2007) Pancortin-2 interacts with WAVE1 and Bcl-xL in a mitochondria-associated protein complex that mediates ischemic neuronal death. *J Neurosci* 27:1519–1528.

Cheung WY (1971) Cyclic 3′,5′-nucleotide phosphodiesterase. Evidence for and properties of a protein activator. *J Biol Chem* 246:2859–2869.

Choi YH, Suzuki A, Hajarnis S, Ma Z, Chapin HC, Caplan MJ, Pontoglio M, Somlo S, and Igarashi P (2011) Polycystin-2 and phosphodiesterase 4C are components of a ciliary A-kinase anchoring protein complex that is disrupted in cystic kidney diseases. *Proc Natl Acad Sci USA* 108:10679–10684.

Christian F, Szaszák M, Friedl S, Drewianka S, Lorenz D, Goncalves A, Furkert J, Vargas C, Schmieder P, Götz F, et al. (2011) Small molecule AKAP-protein kinase A (PKA) interaction disruptors that activate PKA interfere with compartmentalized cAMP signaling in cardiac myocytes. *J Biol Chem* 286: 9079–9096.

Clister T, Greenwald EC, Baillie GS, and Zhang J (2019) AKAP95 organizes a nuclear microdomain to control local cAMP for regulating nuclear PKA. *Cell Chem Biol* 26:885–891.e4.

Coghlan VM, Langeberg LK, Fernandez A, Lamb NJ, and Scott JD (1994) Cloning and characterization of AKAP 95, a nuclear protein that associates with the regulatory subunit of type II cAMP-dependent protein kinase. *J Biol Chem* 269: 7658–7665.

Coghlan VM, Perrino BA, Howard M, Langeberg LK, Hicks JB, Gallatin WM, and Scott JD (1995) Association of protein kinase A and protein phosphatase 2B with a common anchoring protein. *Science* 267:108–111.

Collado-Hilly M and Coquil J-F (2009) Ins(1,4,5)P3 receptor type 1 associates with AKAP9 (AKAP450 variant) and protein kinase A type IIbeta in the Golgi apparatus in cerebellar granule cells. *Biol Cell* 101:469–480.

Collas P, Le Guellec K, and Taskén K (1999) The A-kinase-anchoring protein AKAP95 is a multivalent protein with a key role in chromatin condensation at mitosis. *J Cell Biol* 147:1167–1180.

Colledge M, Dean RA, Scott GK, Langeberg LK, Huganir RL, and Scott JD (2000) Targeting of PKA to glutamate receptors through a MAGUK-AKAP complex. *Neuron* 27:107–119.

Consonni SV, Gloerich M, Spanjaard E, and Bos JL (2012) cAMP regulates DEP domain-mediated binding of the guanine nucleotide exchange factor Epac1 to phosphatidic acid at the plasma membrane. *Proc Natl Acad Sci USA* 109: 3814–3819.

Conti M (2017) Subcellular targeting of PDE4 in cardiac myocytes and generation of signaling compartments, in *Microdomains in the Cardiovascular System* (Nikolaev V and Zaccolo M eds), Springer International Publishing, Cham, Switzerland.

Conti M and Beavo J (2007) Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling. *Annu Rev Biochem* 76:481–511.

Conti M, Kasson BG, and Hsueh AJ (1984) Hormonal regulation of 3′,5′-adenosine monophosphate phosphodiesterases in cultured rat granulosa cells. *Endocrinology* 114:2361–2368.

Cooper DM and Tabbasum VG (2014) Adenylate cyclase-centred microdomains. *Biochem J* 462:199–213.

Corbin JD and Keely SL (1977) Characterization and regulation of heart adenosine 3′:5′-monophosphate-dependent protein kinase isozymes. *J Biol Chem* 252: 910–918.

Covian R, French S, Kusnetz H, and Balaban RS (2014) Stimulation of oxidative phosphorylation by calcium in cardiac mitochondria is not influenced by cAMP and PKA activity. *Biochim Biophys Acta* 1837:1913–1921.

Crosthwaite AJ, Ciruela A, Rayner TF, and Cooper DMF (2006) A direct interaction between the N terminus of adenylyl cyclase AC8 and the catalytic subunit of protein phosphatase 2A. *Mol Pharmacol* 69:608–617.

Cummings DE, Brandon EP, Planas JV, Motamed K, Idzerda RL, and McKnight GS (1996) Genetically lean mice result from targeted disruption of the RII beta subunit of protein kinase A. *Nature* 382:622–626.

Dahl EF, Wu SC, Healy CL, Harsch BA, Shearer GC, and O'Connell TD (2018) Subcellular compartmentalization of proximal Gα_q-receptor signaling produces unique hypertrophic phenotypes in adult cardiac myocytes. *J Biol Chem* 293: 8734–8749.

Dalton GD and Dewey WL (2006) Protein kinase inhibitor peptide (PKI): a family of endogenous neuropeptides that modulate neuronal cAMP-dependent protein kinase function. *Neuropeptides* 40:23–34.

Danial NN, Gramm CF, Scorrano L, Zhang CY, Krauss S, Ranger AM, Datta SR, Greenberg ME, Licklider LJ, Lowell BB, et al. (2003) BAD and glucokinase reside in a mitochondrial complex that integrates glycolysis and apoptosis. *Nature* 424: 952–956.

Darshi M, Mendiola VL, Mackey MR, Murphy AN, Koller A, Perkins GA, Ellisman MH, and Taylor SS (2011) ChChd3, an inner mitochondrial membrane protein, is essential for maintaining crista integrity and mitochondrial function. *J Biol Chem* 286:2918–2932.

DeGrande ST, Little SC, Nixon DJ, Wright P, Snyder J, Dun W, Murphy N, Kilic A, Higgins R, Binkley PF, et al. (2013) Molecular mechanisms underlying cardiac protein phosphatase 2A regulation in heart. *J Biol Chem* 288:1032–1046.

Dell'Acqua ML, Dodge KL, Tavalin SJ, and Scott JD (2002) Mapping the protein phosphatase-2B anchoring site on AKAP79. Binding and inhibition of phosphatase activity are mediated by residues 315-360. *J Biol Chem* 277:48796–48802.

de Rooij J, Rehmann H, van Triest M, Cool RH, Wittinghofer A, and Bos JL (2000) Mechanism of regulation of the Epac family of cAMP-dependent RapGEFs. *J Biol Chem* 275:20829–20836.

de Rooij J, Zwartkruis FJ, Verheijen MH, Cool RH, Nijman SM, Wittinghofer A, and Bos JL (1998) Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature* 396:474–477.

Dessauer CW (2009) Adenylyl cyclase--A-kinase anchoring protein complexes: the next dimension in cAMP signaling. *Mol Pharmacol* 76:935–941.

Dessauer CW, Watts VJ, Ostrom RS, Conti M, Dove S, and Seifert R (2017) International Union of Basic and Clinical Pharmacology. CI. Structures and small molecule modulators of mammalian adenylyl cyclases. *Pharmacol Rev* 69:93–139.

Di Benedetto G, Scalzotto E, Mongillo M, and Pozzan T (2013) Mitochondrial Ca^{2+} uptake induces cyclic AMP generation in the matrix and modulates organelle ATP levels. *Cell Metab* 17:965–975.

Di Benedetto G, Zoccarato A, Lissandron V, Terrin A, Li X, Houslay MD, Baillie GS, and Zaccolo M (2008) Protein kinase A type I and type II define distinct intracellular signaling compartments. *Circ Res* 103:836–844.

Dictenberg JB, Zimmerman W, Sparks CA, Young A, Vidair C, Zheng Y, Carrington W, Fay FS, and Doxsey SJ (1998) Pericentrin and gamma-tubulin form a protein complex and are organized into a novel lattice at the centrosome. *J Cell Biol* 141: 163–174.

DiPilato LM, Cheng X, and Zhang J (2004) Fluorescent indicators of cAMP and Epac activation reveal differential dynamics of cAMP signaling within discrete subcellular compartments. *Proc Natl Acad Sci USA* 101:16513–16518.

DiPilato LM and Zhang J (2009) The role of membrane microdomains in shaping beta2-adrenergic receptor-mediated cAMP dynamics. *Mol Biosyst* 5:832–837.

Diskar M, Zenn HM, Kaupisch A, Kaufholz M, Brockmeyer S, Sohmen D, Berrera M, Zaccolo M, Boshart M, Herberg FW, et al. (2010) Regulation of cAMP-dependent protein kinases: the human protein kinase X (PrKX) reveals the role of the catalytic subunit alphaH-alphaI loop. *J Biol Chem* 285:35910–35918.

Diviani D, Langeberg LK, Doxsey SJ, and Scott JD (2000) Pericentrin anchors protein kinase A at the centrosome through a newly identified RII-binding domain. *Curr Biol* 10:417–420.

Diviani D, Reggi E, Arambasic M, Caso S, and Maric D (2016) Emerging roles of A-kinase anchoring proteins in cardiovascular pathophysiology. *Biochim Biophys Acta* 1863:1926–1936.

Diviani D and Scott JD (2001) AKAP signaling complexes at the cytoskeleton. *J Cell Sci* 114:1431–1437.

Dodge KL, Khouangsathiene S, Kapiloff MS, Mouton R, Hill EV, Houslay MD, Langeberg LK, and Scott JD (2001) mAKAP assembles a protein kinase A/PDE4 phosphodiesterase cAMP signaling module. *EMBO J* 20:1921–1930.

Dodge-Kafka K, Gildart M, Tokarski K, and Kapiloff MS (2019) mAKAPβ signalosomes - a nodal regulator of gene transcription associated with pathological cardiac remodeling. *Cell Signal* 63:109357.

Dodge-Kafka KL, Bauman A, Mayer N, Henson E, Heredia L, Ahn J, McAvoy T, Nairn AC, and Kapiloff MS (2010) cAMP-stimulated protein phosphatase 2A activity associated with muscle A kinase-anchoring protein (mAKAP) signaling complexes inhibits the phosphorylation and activity of the cAMP-specific phosphodiesterase PDE4D3. *J Biol Chem* 285:11078–11086.

Dodge-Kafka KL, Gildart M, Li J, Thakur H, and Kapiloff MS (2018) Bidirectional regulation of HDAC5 by mAKAPβ signalosomes in cardiac myocytes. *J Mol Cell Cardiol* 118:13–25.

Dodge-Kafka KL, Soughayer J, Pare GC, Carlisle Michel JJ, Langeberg LK, Kapiloff MS, and Scott JD (2005) The protein kinase A anchoring protein mAKAP coordinates two integrated cAMP effector pathways. *Nature* 437:574–578.

Dostmann WR, Taylor SS, Genieser HG, Jastorff B, Døskeland SO, and Ogreid D (1990) Probing the cyclic nucleotide binding sites of cAMP-dependent protein kinases I and II with analogs of adenosine 3′,5′-cyclic phosphorothioates. *J Biol Chem* 265:10484–10491.

Doxsey SJ, Stein P, Evans L, Calarco PD, and Kirschner M (1994) Pericentrin, a highly conserved centrosome protein involved in microtubule organization. *Cell* 76:639–650.

Dschietzig T, Alexiou K, Kinkel H-T, Baumann G, Matschke K, and Stangl K (2011) The positive inotropic effect of relaxin-2 in human atrial myocardium is preserved in end-stage heart failure: role of G(i)-phosphoinositide-3 kinase signaling. *J Card Fail* 17:158–166.

Dupré DJ, Baragli A, Rebois RV, Ethier N, and Hébert TE (2007) Signalling complexes associated with adenylyl cyclase II are assembled during their biosynthesis. *Cell Signal* 19:481–489.

Dyson MT, Jones JK, Kowalewski MP, Manna PR, Alonso M, Gottesman ME, and Stocco DM (2008) Mitochondrial A-kinase anchoring protein 121 binds type II protein kinase A and enhances steroidogenic acute regulatory protein-mediated steroidogenesis in MA-10 mouse leydig tumor cells. *Biol Reprod* **78**:267–277.

Eccles RL, Czajkowski MT, Barth C, Müller PM, McShane E, Grunwald S, Beaudette P, Mecklenburg N, Volkmer R, Zühlke K, et al. (2016) Bimodal antagonism of PKA signalling by ARHGAP36. *Nat Commun* **7**:12963.

Efendiev R, Bavencoffe A, Hu H, Zhu MX, and Dessauer CW (2013) Scaffolding by A-kinase anchoring protein enhances functional coupling between adenylyl cyclase and TRPV1 channel. *J Biol Chem* **288**:3929–3937.

Eide T, Coghlan V, Ørstavik S, Holsve C, Solberg R, Skålhegg BS, Lamb NJC, Langeberg L, Fernandez A, Scott JD, et al. (1998) Molecular cloning, chromosomal localization, and cell cycle-dependent subcellular distribution of the A-kinase anchoring protein, AKAP95. *Exp Cell Res* **238**:305–316.

Eide T, Taskén KA, Carlson C, Williams G, Jahnsen T, Taskén K, and Collas P (2003) Protein kinase A-anchoring protein AKAP95 interacts with MCM2, a regulator of DNA replication. *J Biol Chem* **278**:26750–26756.

Emery AC, Liu X-H, Xu W, Eiden MV, and Eiden LE (2015) Cyclic adenosine 3′,5′-monophosphate elevation and biological signaling through a secretin family Gs-coupled G protein-coupled receptor are restricted to a single adenylate cyclase isoform. *Mol Pharmacol* **87**:928–935.

Engelhardt S (2007) Alternative signaling: cardiomyocyte beta1-adrenergic receptors signal through EGFRs. *J Clin Invest* **117**:2396–2398.

Esposito G, Jaiswal BS, Xie F, Krajnc-Franken MA, Robben TJ, Strik AM, Kuil C, Philipsen RL, van Duin M, Conti M, et al. (2004) Mice deficient for soluble adenylyl cyclase are infertile because of a severe sperm-motility defect [published correction appears in *Proc Natl Acad Sci USA* (2004) 101:5180]. *Proc Natl Acad Sci USA* **101**: 2993–2998.

Fan G, Shumay E, Wang H, and Malbon CC (2001) The scaffold protein gravin (cAMP-dependent protein kinase-anchoring protein 250) binds the beta 2-adrenergic receptor via the receptor cytoplasmic Arg-329 to Leu-413 domain and provides a mobile scaffold during desensitization. *J Biol Chem* **276**:24005–24014.

Favia M, Guerra L, Fanelli T, Cardone RA, Monterisi S, Di Sole F, Castellani S, Chen M, Seidler U, Reshkin SJ, et al. (2010) Na+/H+ exchanger regulatory factor 1 overexpression-dependent increase of cytoskeleton organization is fundamental in the rescue of F508del cystic fibrosis transmembrane conductance regulator in human airway CFBE41o- cells. *Mol Biol Cell* **21**:73–86.

Fazal L, Laudette M, Paula-Gomes S, Pons S, Conte C, Tortosa F, Sicard P, Sainte-Marie Y, Bisserier M, Lairez O, et al. (2017) Multifunctional mitochondrial Epac1 controls myocardial cell death. *Circ Res* **120**:645–657.

Feinstein TN, Wehbi VL, Ardura JA, Wheeler DS, Ferrandon S, Gardella TJ, and Vilardaga JP (2011) Retromer terminates the generation of cAMP by internalized PTH receptors. *Nat Chem Biol* **7**:278–284.

Feinstein WP, Zhu B, Leavesley SJ, Sayner SL, and Rich TC (2012) Assessment of cellular mechanisms contributing to cAMP compartmentalization in pulmonary microvascular endothelial cells. *Am J Physiol Cell Physiol* **302**: C839–C852.

Ferrandon S, Feinstein TN, Castro M, Wang B, Bouley R, Potts JT, Gardella TJ, and Vilardaga JP (2009) Sustained cyclic AMP production by parathyroid hormone receptor endocytosis. *Nat Chem Biol* **5**:734–742.

Fertig BA and Baillie GS (2018) PDE4-mediated cAMP signalling. *J Cardiovasc Dev Dis* **5**:8.

Fesenko EE, Kolesnikov SS, and Lyubarsky AL (1985) Induction by cyclic GMP of cationic conductance in plasma membrane of retinal rod outer segment. *Nature* **313**:310–313.

Fields LA, Koschinski A, and Zaccolo M (2016) Sustained exposure to catecholamines affects cAMP/PKA compartmentalised signalling in adult rat ventricular myocytes. *Cell Signal* **28**:725–732.

Francis SH, Blount MA, and Corbin JD (2011a) Mammalian cyclic nucleotide phosphodiesterases: molecular mechanisms and physiological functions. *Physiol Rev* **91**: 651–690.

Francis SH, Houslay MD, and Conti M (2011b) Phosphodiesterase inhibitors: factors that influence potency, selectivity, and action. *Handb Exp Pharmacol*:47–84.

Fraser ID, Tavalin SJ, Lester LB, Langeberg LK, Westphal AM, Dean RA, Marrion NV, and Scott JD (1998) A novel lipid-anchored A-kinase anchoring protein facilitates cAMP-responsive membrane events. *EMBO J* **17**:2261–2272.

Freedman NJ and Lefkowitz RJ (1996) Desensitization of G protein-coupled receptors. *Recent Prog Horm Res* **51**:319–351, discussion 352–353.

Froese A and Nikolaev VO (2015) Imaging alterations of cardiomyocyte cAMP microdomains in disease. *Front Pharmacol* **6**:172.

Fuller MD, Fu Y, Scheuer T, and Catterall WA (2014) Differential regulation of CaV1.2 channels by cAMP-dependent protein kinase bound to A-kinase anchoring proteins 15 and 79/150. *J Gen Physiol* **143**:315–324.

Gabrovsek L, Collins KB, Aggarwal S, Saunders LM, Lau H-T, Suh D, Sancak Y, Trapnell C, Ong SE, Smith FD, et al. (2020) A-kinase-anchoring protein 1 (dAKAP1)-based signaling complexes coordinate local protein synthesis at the mitochondrial surface. *J Biol Chem* **295**:10749–10765.

Geelen MJ and Vaartjes WJ (1977) Levels of cyclic 3′-5′ -adenosine monophosphate (cAMP) in maintenance cultures of rat hepatocytes in response to insulin and glucagon. *Lipids* **12**:577–580.

Geoffroy V, Fouque F, Lugnier C, Desbuquois B, and Benelli C (2001) Characterization of an in vivo hormonally regulated phosphodiesterase 3 (PDE3) associated with a liver Golgi-endosomal fraction. *Arch Biochem Biophys* **387**:154–162.

Gidon A, Al-Bataineh MM, Jean-Alphonse FG, Stevenson HP, Watanabe T, Louet C, Khatri A, Calero G, Pastor-Soler NM, Gardella TJ, et al. (2014) Endosomal GPCR signaling turned off by negative feedback actions of PKA and v-ATPase. *Nat Chem Biol* **10**:707–709.

Gloerich M and Bos JL (2010) Epac: defining a new mechanism for cAMP action. *Annu Rev Pharmacol Toxicol* **50**:355–375.

Gloerich M, Ponsioen B, Vliem MJ, Zhang Z, Zhao J, Kooistra MR, Price LS, Ritsma L, Zwartkruis FJ, Rehmann H, et al. (2010) Spatial regulation of cyclic

AMP-Epac1 signaling in cell adhesion by ERM proteins. *Mol Cell Biol* **30**: 5421–5431.

Godbole A, Lyga S, Lohse MJ, and Calebiro D (2017) Internalized TSH receptors en route to the TGN induce local Gs-protein signaling and gene transcription. *Nat Commun* **8**:443.

Gold MG, Fowler DM, Means CK, Pawson CT, Stephany JJ, Langeberg LK, Fields S, and Scott JD (2013) Engineering A-kinase anchoring protein (AKAP)-selective regulatory subunits of protein kinase A (PKA) through structure-based phage selection. *J Biol Chem* **288**:17111–17121.

Gold MG, Lygren B, Dokurno P, Hoshi N, McConnachie G, Taskén K, Carlson CR, Scott JD, and Barford D (2006) Molecular basis of AKAP specificity for PKA regulatory subunits. *Mol Cell* **24**:383–395.

Gorshkov K, Mehta S, Ramamurthy S, Ronnett GV, Zhou FQ, and Zhang J (2017) AKAP-mediated feedback control of cAMP gradients in developing hippocampal neurons. *Nat Chem Biol* **13**:425–431.

Götz F, Roske Y, Schulz MS, Autenrieth K, Bertinetti D, Faelber K, Zühlke K, Kreuchwig A, Kennedy EJ, Krause G, et al. (2016) AKAP18:PKA-RIIα structure reveals crucial anchor points for recognition of regulatory subunits of PKA. *Biochem J* **473**:1881–1894.

Grant PG, Mannarino AF, and Colman RW (1988) cAMP-mediated phosphorylation of the low-Km cAMP phosphodiesterase markedly stimulates its catalytic activity. *Proc Natl Acad Sci USA* **85**:9071–9075.

Gray PC, Johnson BD, Westenbroek RE, Hays LG, Yates JR III, Scheuer T, Catterall WA, and Murphy BJ (1998) Primary structure and function of an A kinase anchoring protein associated with calcium channels. *Neuron* **20**:1017–1026.

Gray PC, Tibbs VC, Catterall WA, and Murphy BJ (1997) Identification of a 15-kDa cAMP-dependent protein kinase-anchoring protein associated with skeletal muscle L-type calcium channels. *J Biol Chem* **272**:6297–6302.

Greenwald EC and Saucerman JJ (2011) Bigger, better, faster: principles and models of AKAP anchoring protein signaling. *J Cardiovasc Pharmacol* **58**:462–469.

Grimsrud PA, Carson JJ, Hebert AS, Hubler SL, Niemi NM, Bailey DJ, Jochem A, Stapleton DS, Keller MP, Westphall MS, et al. (2012) A quantitative map of the liver mitochondrial phosphoproteome reveals posttranslational control of ketogenesis. *Cell Metab* **16**:672–683.

Grundmann M and Kostenis E (2017) Temporal bias: time-encoded dynamic GPCR signaling. *Trends Pharmacol Sci* **38**:1110–1124.

Guellich A, Mehel H, and Fischmeister R (2014) Cyclic AMP synthesis and hydrolysis in the normal and failing heart. *Pflugers Arch* **466**:1163–1175.

Halls ML (2019) Localised GPCR signalling as revealed by FRET biosensors. *Curr Opin Cell Biol* **57**:48–56.

Halls ML, Bathgate RA, and Summers RJ (2006) Relaxin family peptide receptors RXFP1 and RXFP2 modulate cAMP signaling by distinct mechanisms. *Mol Pharmacol* **70**:214–226.

Halls ML, Bathgate RA, and Summers RJ (2007) Comparison of signaling pathways activated by the relaxin family peptide receptors, RXFP1 and RXFP2, using reporter genes. *J Pharmacol Exp Ther* **320**:281–290.

Halls ML and Cooper DMF (2017) Adenylyl cyclase signalling complexes - Pharmacological challenges and opportunities. *Pharmacol Ther* **172**:171–180.

Harada H, Becknell B, Wilm M, Mann M, Huang LJ, Taylor SS, Scott JD, and Korsmeyer SJ (1999) Phosphorylation and inactivation of BAD by mitochondria-anchored protein kinase A. *Mol Cell* **3**:413–422.

Harootunian AT, Adams SR, Wen W, Meinkoth JL, Taylor SS, and Tsien RY (1993) Movement of the free catalytic subunit of cAMP-dependent protein kinase into and out of the nucleus can be explained by diffusion. *Mol Biol Cell* **4**:993–1002.

Havekes R, Canton DA, Park AJ, Huang T, Nie T, Day JP, Guercio LA, Grimes Q, Luczak V, Gelman IH, et al. (2012) Gravin orchestrates protein kinase A and β2-adrenergic receptor signaling critical for synaptic plasticity and memory. *J Neurosci* **32**:18137–18149.

Hayes JS, Brunton LL, and Mayer SE (1980) Selective activation of particulate cAMP-dependent protein kinase by isoproterenol and prostaglandin E1. *J Biol Chem* **255**:5113–5119.

Heijman J, Dewenter M, El-Armouche A, and Dobrev D (2013) Function and regulation of serine/threonine phosphatases in the healthy and diseased heart. *J Mol Cell Cardiol* **64**:90–98.

Henn V, Edemir B, Stefan E, Wiesner B, Lorenz D, Theilig F, Schmitt R, Vossebein L, Tamma G, Beyermann M, et al. (2004) Identification of a novel A-kinase anchoring protein 18 isoform and evidence for its role in the vasopressin-induced aquaporin-2 shuttle in renal principal cells. *J Biol Chem* **279**:26654–26665.

Henn V, Stefan E, Baillie GS, Houslay MD, Rosenthal W, and Klussmann E (2005) Compartmentalized cAMP signalling regulates vasopressin-mediated water reabsorption by controlling aquaporin-2. *Biochem Soc Trans* **33**:1316–1318.

Hensch TK, Gordon JA, Brandon EP, McKnight GS, Idzerda RL, and Stryker MP (1998) Comparison of plasticity in vivo and in vitro in the developing visual cortex of normal and protein kinase A RIbeta-deficient mice. *J Neurosci* **18**: 2108–2117.

Hepler JR and Gilman AG (1992) G proteins. *Trends Biochem Sci* **17**:383–387.

Herberg FW, Maleszka A, Eide T, Vossebein L, and Tasken K (2000) Analysis of A-kinase anchoring protein (AKAP) interaction with protein kinase A (PKA) regulatory subunits: PKA isoform specificity in AKAP binding. *J Mol Biol* **298**: 329–339.

Heroes E, Lesage B, Görnemann J, Beullens M, Van Meervelt L, and Bollen M (2013) The PP1 binding code: a molecular-lego strategy that governs specificity. *FEBS J* **280**:584–595.

Herzig S and Neumann J (2000) Effects of serine/threonine protein phosphatases on ion channels in excitable membranes. *Physiol Rev* **80**:173–210.

Hindson VJ (2003) Serine acetyltransferase of Escherichia coli: substrate specificity and feedback control by cysteine. *Biochem J* **375**:745–752.

Hochbaum D, Barila G, Ribeiro-Neto F, and Altschuler DL (2011) Radixin assembles cAMP effectors Epac and PKA into a functional cAMP compartment: role in cAMP-dependent cell proliferation. *J Biol Chem* **286**:859–866.

Hohl CM and Li QA (1991) Compartmentation of cAMP in adult canine ventricular myocytes. Relation to single-cell free Ca2+ transients. *Circ Res* **69**: 1369–1379.

Houslay MD (2001) PDE4 cAMP-specific phosphodiesterases. *Prog Nucleic Acid Res Mol Biol* **69**:249–315.

Houslay MD (2010) Underpinning compartmentalised cAMP signalling through targeted cAMP breakdown. *Trends Biochem Sci* **35**:91–100.

Houslay MD and Adams DR (2003) PDE4 cAMP phosphodiesterases: modular enzymes that orchestrate signalling cross-talk, desensitization and compartmentalization. *Biochem J* **370**:1–18.

Houslay MD, Baillie GS, and Maurice DH (2007) cAMP-specific phosphodiesterase-4 enzymes in the cardiovascular system: a molecular toolbox for generating compartmentalized cAMP signaling. *Circ Res* **100**:950–966.

Houslay MD and Milligan G (1997) Tailoring cAMP-signalling responses through isoform multiplicity. *Trends Biochem Sci* **22**:217–224.

Houslay MD, Schafer P, and Zhang KY (2005) Keynote review: phosphodiesterase-4 as a therapeutic target. *Drug Discov Today* **10**:1503–1519.

Hu J, Khodadadi-Jamayran A, Mao M, Shah K, Yang Z, Nasim MT, Wang Z, and Jiang H (2016) AKAP95 regulates splicing through scaffolding RNAs and RNA processing factors. *Nat Commun* **7**:13347.

Huang LJ, Wang L, Ma Y, Durick K, Perkins G, Deerinck TJ, Ellisman MH, and Taylor SS (1999) NH2-Terminal targeting motifs direct dual specificity A-kinase-anchoring protein 1 (D-AKAP1) to either mitochondria or endoplasmic reticulum. *J Cell Biol* **145**:951–959.

Hulme JT, Ahn M, Hauschka SD, Scheuer T, and Catterall WA (2002) A novel leucine zipper targets AKAP15 and cyclic AMP-dependent protein kinase to the C terminus of the skeletal muscle Ca2+ channel and modulates its function. *J Biol Chem* **277**:4079–4087.

Hulme JT, Lin TWC, Westenbroek RE, Scheuer T, and Catterall WA (2003) Beta-adrenergic regulation requires direct anchoring of PKA to cardiac CaV1.2 channels via a leucine zipper interaction with A kinase-anchoring protein 15. *Proc Natl Acad Sci USA* **100**:13093–13098.

Iancu RV, Jones SW, and Harvey RD (2007) Compartment of cAMP signaling in cardiac myocytes: a computational study. *Biophys J* **92**:3317–3331.

Inaba K, Morisawa S, and Morisawa M (1998) Proteasomes regulate the motility of salmonid fish sperm through modulation of cAMP-dependent phosphorylation of an outer arm dynein light chain. *J Cell Sci* **111**:1105–1115.

Inda C, Dos Santos Claro PA, Bonfiglio JJ, Senin SA, Maccarrone G, Turck CW, and Silberstein S (2016) Different cAMP sources are critically involved in G protein-coupled receptor CRHR1 signaling. *J Cell Biol* **214**:181–195.

Ingebritsen TS and Cohen P (1983) Protein phosphatases: properties and role in cellular regulation. *Science* **221**:331–338.

Ingham PW, Nakano Y, and Seger C (2011) Mechanisms and functions of Hedgehog signalling across the metazoa. *Nat Rev Genet* **12**:393–406.

Insel PA, Head BP, Patel HH, Roth DM, Bundey RA, and Swaney JS (2005) Compartmentation of G-protein-coupled receptors and their signalling components in lipid rafts and caveolae. *Biochem Soc Trans* **33**:1131–1134.

Irannejad R, Pessino V, Mika D, Huang B, Wedegaertner PB, Conti M, and von Zastrow M (2017) Functional selectivity of GPCR-directed drug action through location bias. *Nat Chem Biol* **13**:799–806.

Irannejad R, Tomshine JC, Tomshine JR, Chevalier M, Mahoney JP, Steyaert J, Rasmussen SG, Sunahara RK, El-Samad H, Huang B, et al. (2013) Conformational biosensors reveal GPCR signalling from endosomes. *Nature* **495**: 534–538.

Jiang H, Lu X, Shimada M, Dou Y, Tang Z, and Roeder RG (2013) Regulation of transcription by the MLL2 complex and MLL complex-associated AKAP95. *Nat Struct Mol Biol* **20**:1156–1163.

Jiang JY, Falcone JL, Curci S, and Hofer AM (2019) Direct visualization of cAMP signaling in primary cilia reveals up-regulation of ciliary GPCR activity following Hedgehog activation. *Proc Natl Acad Sci USA* **116**:12066–12071.

Johnson KR, Nicodemus-Johnson J, Carnegie GK, and Danziger RS (2012) Molecular evolution of A-kinase anchoring protein (AKAP)-7: implications in comparative PKA compartmentalization. *BMC Evol Biol* **12**:125.

Johnstone TB, Agarwal SR, Harvey RD, and Ostrom RS (2018) cAMP signaling compartment: adenylyl cyclases as anchors of dynamic signaling complexes. *Mol Pharmacol* **93**:270–276.

Jones BW, Brunet S, Gilbert ML, Nichols CB, Su T, Westenbroek RE, Scott JD, Catterall WA, and McKnight GS (2012) Cardiomyocytes from AKAP7 knockout mice respond normally to adrenergic stimulation. *Proc Natl Acad Sci USA* **109**: 17099–17104.

Joukov V and De Nicolo A (2019) The centrosome and the primary cilium: the Yin and Yang of a hybrid organelle. *Cells* **8**:701.

Jurevicius J and Fischmeister R (1996) cAMP compartmentation is responsible for a local activation of cardiac Ca2+ channels by beta-adrenergic agonists. *Proc Natl Acad Sci USA* **93**:295–299.

Kammerer S, Burns-Hamuro LL, Ma Y, Hamon SC, Canaves JM, Shi MM, Nelson MR, Sing CF, Cantor CR, Taylor SS, et al. (2003) Amino acid variant in the kinase binding domain of dual-specific A kinase-anchoring protein 2: a disease susceptibility polymorphism. *Proc Natl Acad Sci USA* **100**:4066–4071.

Kapiloff MS, Piggott LA, Sadana R, Li J, Heredia LA, Henson E, Efendiev R, and Dessauer CW (2009) An adenylyl cyclase-mAKAPbeta signaling complex regulates cAMP levels in cardiac myocytes. *J Biol Chem* **284**:23540–23546.

Kapiloff MS, Schillace RV, Westphal AM, and Scott JD (1999) mAKAP: an A-kinase anchoring protein targeted to the nuclear membrane of differentiated myocytes. *J Cell Sci* **112**:2725–2736.

Kerfant BG, Zhao D, Lorenzen-Schmidt I, Wilson LS, Cai S, Chen SR, Maurice DH, and Backx PH (2007) PI3Kgamma is required for PDE4, not PDE3, activity in subcellular microdomains containing the sarcoplasmic reticular calcium ATPase in cardiomyocytes. *Circ Res* **101**:400–408.

Keryer G, Witczak O, Delouvée A, Kemmner WA, Rouillard D, Tasken K, and Bornens M (2003) Dissociating the centrosomal matrix protein AKAP450 from centrioles impairs centriole duplication and cell cycle progression. *Mol Biol Cell* **14**: 2436–2446.

Khandelwal RL, Vandenheede JR, and Krebs EG (1976) Purification, properties, and substrate specificities of phosphoprotein phosphatase(s) from rabbit liver. *J Biol Chem* **251**:4850–4858.

Kim H, Scimia MC, Wilkinson D, Trelles RD, Wood MR, Bowtell D, Dillin A, Mercola M, and Ronai ZA (2011) Fine-tuning of Drp1/Fis1 availability by AKAP121/Siah2 regulates mitochondrial adaptation to hypoxia. *Mol Cell* **44**:532–544.

Kim Y, Sung JY, Ceglia I, Lee KW, Ahn JH, Halford JM, Kim AM, Kwak SP, Park JB, Ho Ryu S, et al. (2006) Phosphorylation of WAVE1 regulates actin polymerization and dendritic spine morphology. *Nature* **442**:814–817.

Kjällquist U, Erlandsson R, Tobin NP, Alkodsi A, Ullah I, Stålhammar G, Karlsson E, Hatschek T, Hartman J, Linnarsson S, et al. (2018) Exome sequencing of primary breast cancers with paired metastatic lesions reveals metastasis-enriched mutations in the A-kinase anchoring protein family (AKAPs). *BMC Cancer* **18**:174.

Klauck TM, Faux MC, Labudda K, Langeberg LK, Jaken S, and Scott JD (1996) Coordination of three signaling enzymes by AKAP79, a mammalian scaffold protein. *Science* **271**:1589–1592.

Kokkonen K and Kass DA (2017) Nanodomain regulation of cardiac cyclic nucleotide signaling by phosphodiesterases. *Annu Rev Pharmacol Toxicol* **57**:455–479.

Koschinski A and Zaccolo M (2015) A novel approach combining real-time imaging and the patch-clamp technique to calibrate FRET-based reporters for cAMP in their cellular microenvironment, in *cAMP Signaling: Methods and Protocols* (Zaccolo M ed), Springer, New York.

Koschinski A and Zaccolo M (2017) Activation of PKA in cell requires higher concentration of cAMP than in vitro: implications for compartmentalization of cAMP signalling. *Sci Rep* **7**:14090.

Kovanich D, van der Heyden MA, Aye TT, van Veen TA, Heck AJ, and Scholten A (2010) Sphingosine kinase interacting protein is an A-kinase anchoring protein specific for type I cAMP-dependent protein kinase. *ChemBioChem* **11**:963–971.

Krebs EG and Beavo JA (1979) Phosphorylation-dephosphorylation of enzymes. *Annu Rev Biochem* **48**:923–959.

Kritzer MD, Li J, Dodge-Kafka K, and Kapiloff MS (2012) AKAPs: the architectural underpinnings of local cAMP signaling. *J Mol Cell Cardiol* **52**:351–358.

Kritzer MD, Li J, Passariello CL, Gayanilo M, Thakur H, Dayan J, Dodge-Kafka K, and Kapiloff MS (2014) The scaffold protein muscle A-kinase anchoring protein β orchestrates cardiac myocyte hypertrophic signaling required for the development of heart failure. *Circ Heart Fail* **7**:663–672.

Kubota S, Morii M, Yuki R, Yamaguchi N, Yamaguchi H, Aoyama K, Kuga T, Tomonaga T, and Yamaguchi N (2015) Role for tyrosine phosphorylation of A-kinase anchoring protein 8 (AKAP8) in its sissociation from chromatin and the nuclear matrix. *J Biol Chem* **290**:10891–10904.

Kumar N, Gupta S, Dabral S, Singh S, and Sehrawat S (2017) Role of exchange protein directly activated by cAMP (EPAC1) in breast cancer cell migration and apoptosis. *Mol Cell Biochem* **430**:115–125.

Kumar N, Prasad P, Jash E, Saini M, Husain A, Goldman A, and Sehrawat S (2018) Insights into exchange factor directly activated by cAMP (EPAC) as potential target for cancer treatment. *Mol Cell Biochem* **447**:77–92.

Kumar S, Kostin S, Flacke J-P, Reusch HP, and Ladilov Y (2009) Soluble adenylyl cyclase controls mitochondria-dependent apoptosis in coronary endothelial cells. *J Biol Chem* **284**:14760–14768.

Kumar V, Jong YJ, and O'Malley KL (2008) Activated nuclear metabotropic glutamate receptor mGlu5 couples to nuclear Gq/11 proteins to generate inositol 1,4,5-trisphosphate-mediated nuclear Ca2+ release. *J Biol Chem* **283**:14072–14083.

Kuo JF and Greengard P (1969) Cyclic nucleotide-dependent protein kinases. IV. Widespread occurrence of adenosine 3′,5′-monophosphate-dependent protein kinase in various tissues and phyla of the animal kingdom. *Proc Natl Acad Sci USA* **64**:1349–1355.

Kurokawa J, Motoike HK, Rao J, and Kass RS (2004) Regulatory actions of the A-kinase anchoring protein Yotiao on a heart potassium channel downstream of PKA phosphorylation. *Proc Natl Acad Sci USA* **101**:16374–16378.

Ladilov Y and Appukuttan A (2014) Role of soluble adenylyl cyclase in cell death and growth. *Biochim Biophys Acta* **1842**:2646–2655.

Lan TH, Liu Q, Li C, Wu G, and Lambert NA (2012) Sensitive and high resolution localization and tracking of membrane proteins in live cells with BRET. *Traffic* **13**: 1450–1456.

Langeberg LK and Scott JD (2015) Signalling scaffolds and local organization of cellular behaviour. *Nat Rev Mol Cell Biol* **16**:232–244.

Lazar AM, Irannejad R, Baldwin TA, Sundaram AB, Gutkind JS, Inoue A, Dessauer CW, and Von Zastrow M (2020) G protein-regulated endocytic trafficking of adenylyl cyclase type 9. *eLife* **9**:e58039.

Lees-Miller SP and Anderson CW (1989) The human double-stranded DNA-activated protein kinase phosphorylates the 90-kDa heat-shock protein, hsp90 alpha at two NH2-terminal threonine residues. *J Biol Chem* **264**:17275–17280.

Lefkimmiatis K, Leronni D, and Hofer AM (2013) The inner and outer compartments of mitochondria are sites of distinct cAMP/PKA signaling dynamics. *J Cell Biol* **202**:453–462.

Lefkimmiatis K, Moyer MP, Curci S, and Hofer AM (2009) "cAMP sponge": a buffer for cyclic adenosine 3′, 5′-monophosphate. *PLoS One* **4**:e7649.

Lefkimmiatis K and Zaccolo M (2014) cAMP signaling in subcellular compartments. *Pharmacol Ther* **143**:295–304.

Leroy J, Abi-Gerges A, Nikolaev VO, Richter W, Lechêne P, Mazet JL, Conti M, Fischmeister R, and Vandecasteele G (2008) Spatiotemporal dynamics of beta-adrenergic cAMP signals and L-type Ca2+ channel regulation in adult rat ventricular myocytes: role of phosphodiesterases. *Circ Res* **102**:1091–1100.

Levitzki A (1988) From epinephrine to cyclic AMP. *Science* **241**:800–806.

Li H, Degenhardt B, Tobin D, Yao ZX, Tasken K, and Papadopoulos V (2001) Identification, localization, and function in steroidogenesis of PAP7: a peripheral-type benzodiazepine receptor- and PKA (RIalpha)-associated protein. *Mol Endocrinol* **15**:2211–2228.

Li J, Negro A, Lopez J, Bauman AL, Henson E, Dodge-Kafka K, and Kapiloff MS (2010) The mAKAPbeta scaffold regulates cardiac myocyte hypertrophy via recruitment of activated calcineurin. *J Mol Cell Cardiol* **48**:387–394.

Li X, Wang C, Zhang G, Liang M, and Zhang B (2017) AKAP2 is upregulated in ovarian cancer, and promotes growth and migration of cancer cells. *Mol Med Rep* **16**:5151–5156.

Li Y, Chen L, Kass RS, and Dessauer CW (2012) The A-kinase anchoring protein Yotiao facilitates complex formation between adenylyl cyclase type 9 and the IKs potassium channel in heart. *J Biol Chem* **287**:29815–29824.

Li Y, Kao GD, Garcia BA, Shabanowitz J, Hunt DF, Qin J, Phelan C, and Lazar MA (2006) A novel histone deacetylase pathway regulates mitosis by modulating Aurora B kinase activity. *Genes Dev* **20**:2566–2579.

Lignitto L, Carlucci A, Sepe M, Stefan E, Cuomo O, Nisticò R, Scorziello A, Savoia C, Garbi C, Annunziato L, et al. (2011) Control of PKA stability and signalling by the RING ligase praja2. *Nat Cell Biol* **13**:412–422.

Lin JW, Wyszynski M, Madhavan R, Sealock R, Kim JU, and Sheng M (1998) Yotiao, a novel protein of neuromuscular junction and brain that interacts with specific splice variants of NMDA receptor subunit NR1. *J Neurosci* **18**:2017–2027.

Liu J, Li H, and Papadopoulos V (2003) PAP7, a PBR/PKA-RIalpha-associated protein: a new element in the relay of the hormonal induction of steroidogenesis. *J Steroid Biochem Mol Biol* **85**:275–283.

Liu S, Zhang J, and Xiang YK (2011) FRET-based direct detection of dynamic protein kinase A activity on the sarcoplasmic reticulum in cardiomyocytes. *Biochem Biophys Res Commun* **404**:581–586.

Livigni A, Scorziello A, Agnese S, Adornetto A, Carlucci A, Garbi C, Castaldo I, Annunziato L, Avvedimento EV, and Feliciello A (2006) Mitochondrial AKAP121 links cAMP and src signaling to oxidative metabolism. *Mol Biol Cell* **17**:263–271.

Lobo Miguel J (2020) Phosphodiesterase 2A2 regulates mitochondria clearance through Parkin-dependent mitophagy. *Commun Biol* **3**:596.

Lobo MJ, Amaral MD, Zaccolo M, and Farinha CM (2016) EPAC1 activation by cAMP stabilizes CFTR at the membrane by promoting its interaction with NHERF1. *J Cell Sci* **129**:2599–2612.

Logue MW, Schu M, Vardarajan BN, Farrell J, Bennett DA, Buxbaum JD, Byrd GS, Ertekin-Taner N, Evans D, Foroud T, et al.; Alzheimer Disease Genetics Consortium; Alzheimer Disease Genetics Consortium (2014) Two rare AKAP9 variants are associated with Alzheimer's disease in African Americans. *Alzheimers Dement* **10**:609–618.e11.

Lohse C, Bock A, Maiellaro I, Hannawacker A, Schad LR, Lohse MJ, and Bauer WR (2017) Experimental and mathematical analysis of cAMP nanodomains. *PLoS One* **12**:e0174856.

Lu J, Wang W, Mi Y, Zhang C, Ying H, Wang L, Wang Y, Myatt L, and Sun K (2017) AKAP95-mediated nuclear anchoring of PKA regulates cortisol-induced *PTGS2* expression in human amnion fibroblasts. *Sci Signal* **10**:eaac6160.

Lugnier C (2006) Cyclic nucleotide phosphodiesterase (PDE) superfamily: a new target for the development of specific therapeutic agents. *Pharmacol Ther* **109**:366–398.

Lyga S, Volpe S, Werthmann RC, Götz K, Sungkaworn T, Lohse MJ, and Calebiro D (2016) Persistent cAMP signaling by internalized LH receptors in ovarian follicles. *Endocrinology* **157**:1613–1621.

Lygren B, Carlson CR, Santamaria K, Lissandron V, McSorley T, Litzenberg J, Lorenz D, Wiesner B, Rosenthal W, Zaccolo M, et al. (2007) AKAP complex regulates Ca2+ re-uptake into heart sarcoplasmic reticulum. *EMBO Rep* **8**:1061–1067.

Lynch MJ, Baillie GS, and Houslay MD (2007) cAMP-specific phosphodiesterase-4D5 (PDE4D5) provides a paradigm for understanding the unique non-redundant roles that PDE4 isoforms play in shaping compartmentalized cAMP cell signalling. *Biochem Soc Trans* **35**:938–941.

Lynch MJ, Baillie GS, Mohamed A, Li X, Maisonneuve C, Klussmann E, van Heeke G, and Houslay MD (2005) RNA silencing identifies PDE4D5 as the functionally relevant cAMP phosphodiesterase interacting with beta arrestin to control the protein kinase A/AKAP79-mediated switching of the beta2-adrenergic receptor to activation of ERK in HEK293B2 cells. *J Biol Chem* **280**:33178–33189.

Lynch MJ, Hill EV, and Houslay MD (2006) Intracellular targeting of phosphodiesterase-4 underpins compartmentalized cAMP signaling. *Curr Top Dev Biol* **75**:225–259.

Ma L, Jongbloets BC, Xiong WH, Melander JB, Qin M, Lameyer TJ, Harrison MF, Zemelman BV, Mao T, and Zhong H (2018) A highly sensitive A-kinase activity reporter for imaging neuromodulatory events in awake mice. *Neuron* **99**:665–679.e5.

Ma Y and Taylor S (2002) A 15-residue bifunctional element in D-AKAP1 is required for both endoplasmic reticulum and mitochondrial targeting. *J Biol Chem* **277**:27328–27336.

Magalhaes AC, Dunn H, and Ferguson SS (2012) Regulation of GPCR activity, trafficking and localization by GPCR-interacting proteins. *Br J Pharmacol* **165**:1717–1736.

Malbon CC (2007) A-kinase anchoring proteins: trafficking in G-protein-coupled receptors and the proteins that regulate receptor biology. *Curr Opin Drug Discov Devel* **10**:573–579.

Malbon CC, Tao J, and Wang HY (2004) AKAPs (A-kinase anchoring proteins) and molecules that compose their G-protein-coupled receptor signalling complexes. *Biochem J* **379**:1–9.

Marchmont RJ and Houslay MD (1980) Insulin trigger, cyclic AMP-dependent activation and phosphorylation of a plasma membrane cyclic AMP phosphodiesterase. *Nature* **286**:904–906.

Marx SO, Kurokawa J, Reiken S, Motoike H, D'Armiento J, Marks AR, and Kass RS (2002) Requirement of a macromolecular signaling complex for beta adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. *Science* **295**:496–499.

Marx SO, Ondrias K, and Marks AR (1998) Coupled gating between individual skeletal muscle Ca2+ release channels (ryanodine receptors). *Science* **281**:818–821.

Marx SO, Reiken S, Hisamatsu Y, Gaburjakova M, Gaburjakova J, Yang YM, Rosemblit N, and Marks AR (2001) Phosphorylation-dependent regulation of ryanodine receptors: a novel role for leucine/isoleucine zippers. *J Cell Biol* **153**:699–708.

McCahill A, McSorley T, Huston E, Hill EV, Lynch MJ, Gall I, Keryer G, Lygren B, Tasken K, van Heeke G, et al. (2005) In resting COS1 cells a dominant negative approach shows that specific, anchored PDE4 cAMP phosphodiesterase isoforms gate the activation, by basal cyclic AMP production, of AKAP-tethered protein kinase A type II located in the centrosomal region. *Cell Signal* **17**:1158–1173.

Means CK, Lygren B, Langeberg LK, Jain A, Dixon RE, Vega AL, Gold MG, Petrosyan S, Taylor SS, Murphy AN, et al. (2011) An entirely specific type I A-kinase anchoring protein that can sequester two molecules of protein kinase A at mitochondria. *Proc Natl Acad Sci USA* **108**:E1227–E1235.

Merrill RA, Dagda RK, Dickey AS, Cribbs JT, Green SH, Usachev YM, and Strack S (2011) Mechanism of neuroprotective mitochondrial remodeling by PKA/AKAP1. *PLoS Biol* **9**:e1000612.

Merrill RA and Strack S (2014) Mitochondria: a kinase anchoring protein 1, a signaling platform for mitochondrial form and function. *Int J Biochem Cell Biol* **48**:92–96.

Méry PF, Lohmann SM, Walter U, and Fischmeister R (1991) Ca2+ current is regulated by cyclic GMP-dependent protein kinase in mammalian cardiac myocytes. *Proc Natl Acad Sci USA* **88**:1197–1201.

Michel JJ, Townley IK, Dodge-Kafka KL, Zhang F, Kapiloff MS, and Scott JD (2005) Spatial restriction of PDK1 activation cascades by anchoring to mAKAPalpha. *Mol Cell* **20**:661–672.

Mika D, Leroy J, Vandecasteele G, and Fischmeister R (2012) PDEs create local domains of cAMP signaling. *J Mol Cell Cardiol* **52**:323–329.

Miller WL (2013) Steroid hormone synthesis in mitochondria. *Mol Cell Endocrinol* **379**:62–73.

Mo GC, Ross B, Hertel F, Manna P, Yang X, Greenwald E, Booth C, Plummer AM, Tenner B, Chen Z, et al. (2017) Genetically encoded biosensors for visualizing live-cell biochemical activity at super-resolution. *Nat Methods* **14**:427–434.

Moll D, Prinz A, Brendel CM, Berrera M, Guske K, Zaccolo M, Genieser H-G, and Herberg FW (2008) Biochemical characterization and cellular imaging of a novel, membrane permeable fluorescent cAMP analog. *BMC Biochem* **9**:18.

Mongillo M, McSorley T, Evellin S, Sood A, Lissandron V, Terrin A, Huston E, Hannawacker A, Lohse MJ, Pozzan T, et al. (2004) Fluorescence resonance energy transfer-based analysis of cAMP dynamics in live neonatal rat cardiac myocytes reveals distinct functions of compartmentalized phosphodiesterases. *Circ Res* **95**:67–75.

Mongillo M, Tocchetti CG, Terrin A, Lissandron V, Cheung YF, Dostmann WR, Pozzan T, Kass DA, Paolocci N, Houslay MD, et al. (2006) Compartmentalized phosphodiesterase-2 activity blunts beta-adrenergic cardiac inotropy via an NO/cGMP-dependent pathway. *Circ Res* **98**:226–234.

Mons N, Decorte L, Jaffard R, and Cooper DM (1998) Ca2+-sensitive adenylyl cyclases, key integrators of cellular signalling. *Life Sci* **62**:1647–1652.

Monterisi S, Favia M, Guerra L, Cardone RA, Marzulli D, Reshkin SJ, Casavola V, and Zaccolo M (2012) CFTR regulation in human airway epithelial cells requires integrity of the actin cytoskeleton and compartmentalized cAMP and PKA activity. *J Cell Sci* **125**:1106–1117.

Monterisi S, Lobo MJ, Livie C, Castle JC, Weinberger M, Baillie G, Surdo NC, Musheshe N, Stangherlin A, Gottlieb E, et al. (2017) PDE2A2 regulates mitochondria morphology and apoptotic cell death via local modulation of cAMP/PKA signalling. *eLife* **6**:e21374.

Monterisi S and Zaccolo M (2017) Components of the mitochondrial cAMP signalosome. *Biochem Soc Trans* **45**:269–274.

Moore BS, Stepanchick AN, Tewson PH, Hartle CM, Zhang J, Quinn AM, Hughes TE, and Mirshahi T (2016) Cilia have high cAMP levels that are inhibited by Sonic Hedgehog-regulated calcium dynamics. *Proc Natl Acad Sci USA* **113**:13069–13074.

Moujalled D, Weston R, Anderton H, Ninnis R, Goel P, Coley A, Huang DC, Wu L, Strasser A, and Puthalakath H (2011) Cyclic-AMP-dependent protein kinase A regulates apoptosis by stabilizing the BH3-only protein Bim. *EMBO Rep* **12**:77–83.

Moyer BD, Duhaime M, Shaw C, Denton J, Reynolds D, Karlson KH, Pfeiffer J, Wang S, Mickle JE, Milewski M, et al. (2000) The PDZ-interacting domain of cystic fibrosis transmembrane conductance regulator is required for functional expression in the apical plasma membrane. *J Biol Chem* **275**:27069–27074.

Mukherjee S, Jansen V, Jikeli JF, Hamzeh H, Alvarez L, Dombrowski M, Balbach M, Strünker T, Seifert R, Kaupp UB, et al. (2016) A novel biosensor to study cAMP dynamics in cilia and flagella. *eLife* **5**:e14052.

Mukhopadhyay S, Wen X, Ratti N, Loktev A, Rangell L, Scales SJ, and Jackson PK (2013) The ciliary G-protein-coupled receptor Gpr161 negatively regulates the Sonic hedgehog pathway via cAMP signaling. *Cell* **152**:210–223.

Murphy JG, Sanderson JL, Gorski JA, Scott JD, Catterall WA, Sather WA, and Dell'Acqua ML (2014) AKAP-anchored PKA maintains neuronal L-type calcium channel activity and NFAT transcriptional signaling. *Cell Rep* **7**:1577–1588.

Musheshe N, Schmidt M, and Zaccolo M (2018) cAMP: from long-range second messenger to nanodomain signalling. *Trends Pharmacol Sci* **39**:209–222.

Namkung Y, Le Gouill C, Lukashova V, Kobayashi H, Hogue M, Khoury E, Song M, Bouvier M, and Laporte SA (2016) Monitoring G protein-coupled receptor and β-arrestin trafficking in live cells using enhanced bystander BRET. *Nat Commun* **7**:12178.

Nash CA, Brown LM, Malik S, Cheng X, and Smrcka AV (2018) Compartmentalized cyclic nucleotides have opposing effects on regulation of hypertrophic phospholipase Cε signaling in cardiac myocytes. *J Mol Cell Cardiol* **121**:51–59.

Nash CA, Wei W, Irannejad R, and Smrcka AV (2019) Golgi localized β1-adrenergic receptors stimulate Golgi PI4P hydrolysis by PLCε to regulate cardiac hypertrophy. *eLife* **8**:e48167.

Nelson CP, Rainbow RD, Brignell JL, Perry MD, Willets JM, Davies NW, Standen NB, and Challiss RA (2011) Principal role of adenylyl cyclase 6 in K+ channel

regulation and vasodilator signalling in vascular smooth muscle cells. *Cardiovasc Res* 91:694–702.

Nguyen BT and Dessauer CW (2005) Relaxin stimulates cAMP production in MCF-7 cells upon overexpression of type V adenylyl cyclase. *Ann N Y Acad Sci* 1041: 296–299.

Nicolaou P, Hajjar RJ, and Kranias EG (2009) Role of protein phosphatase-1 inhibitor-1 in cardiac physiology and pathophysiology. *J Mol Cell Cardiol* 47: 365–371.

Nigg EA, Schäfer G, Hilz H, and Eppenberger HM (1985) Cyclic-AMP-dependent protein kinase type II is associated with the Golgi complex and with centrosomes. *Cell* 41:1039–1051.

Nikolaev VO, Bünemann M, Hein L, Hannawacker A, and Lohse MJ (2004) Novel single chain cAMP sensors for receptor-induced signal propagation. *J Biol Chem* 279:37215–37218.

Nikolaev VO, Bünemann M, Schmittteckert E, Lohse MJ, and Engelhardt S (2006) Cyclic AMP imaging in adult cardiac myocytes reveals far-reaching beta1-adrenergic but locally confined beta2-adrenergic receptor-mediated signaling. *Circ Res* 99:1084–1091.

Nikolaev VO, Moshkov A, Lyon AR, Miragoli M, Novak P, Paur H, Lohse MJ, Korchev YE, Harding SE, and Gorelik J (2010) Beta2-adrenergic receptor redistribution in heart failure changes cAMP compartmentation. *Science* 327: 1653–1657.

Oliveira RF, Terrin A, Di Benedetto G, Cannon RC, Koh W, Kim M, Zaccolo M, and Blackwell KT (2010) The role of type 4 phosphodiesterases in generating microdomains of cAMP: large scale stochastic simulations. *PLoS One* 5: e11725.

Omar F, Findlay JE, Carfray G, Allcock RW, Jiang Z, Moore C, Muir AL, Lannoy M, Fertig BA, Mai D, et al. (2019) Small-molecule allosteric activators of PDE4 long form cyclic AMP phosphodiesterases. *Proc Natl Acad Sci USA* 116:13320–13329.

Onfroy L, Galandrin S, Pontier SM, N'Guyen D, and Gales C (2017) G protein stoichiometry dictates biased agonism through distinct receptor-G protein partitioning. *Scientific Reports* 7:7885.

Ostrom RS, Liu X, Head BP, Gregorian C, Seasholtz TM, and Insel PA (2002) Localization of adenylyl cyclase isoforms and G protein-coupled receptors in vascular smooth muscle cells: expression in caveolin-rich and noncaveolin domains. *Mol Pharmacol* 62:983–992.

Packer M, Carver JR, Rodeheffer RJ, Ivanhoe RJ, DiBianco R, Zeldis SM, Hendrix GH, Bommer WJ, Elkayam U, Kukin ML, et al.; The PROMISE Study Research Group (1991) Effect of oral milrinone on mortality in severe chronic heart failure. *N Engl J Med* 325:1468–1475.

Page CP and Spina D (2012) Selective PDE inhibitors as novel treatments for respiratory diseases. *Curr Opin Pharmacol* 12:275–286.

Papa S (1999) cAMP-dependent protein kinase and phosphoproteins in mammalian mitochondria. An extension of the cAMP-mediated intracellular signal transduction. *FEBS Lett*, doi: 10.1016/s0014-5793(99)00070-8.

Pare GC, Easlick JL, Mislow JM, McNally EM, and Kapiloff MS (2005) Nesprin-1alpha contributes to the targeting of mAKAP to the cardiac myocyte nuclear envelope. *Exp Cell Res* 303:388–399.

Passariello CL, Li J, Dodge-Kafka K, and Kapiloff MS (2015) mAKAP-a master scaffold for cardiac remodeling. *J Cardiovasc Pharmacol* 65:218–225.

Pavlos NJ and Friedman PA (2017) GPCR signaling and trafficking: the long and short of it. *Trends Endocrinol Metab* 28:213–226.

Pawson CT and Scott JD (2010) Signal integration through blending, bolstering and bifurcating of intracellular information. *Nat Struct Mol Biol* 17:653–658.

Pendin D, Greotti E, Lefkimmiatis K, and Pozzan T (2017) Exploring cells with targeted biosensors. *J Gen Physiol* 149:1–36.

Pereira L, Bare DJ, Galice S, Shannon TR, and Bers DM (2017) β-Adrenergic induced SR Ca^{2+} leak is mediated by an Epac-NOS pathway. *J Mol Cell Cardiol* 108:8–16.

Pereira L, Cheng H, Lao DH, Na L, van Oort RJ, Brown JH, Wehrens XH, Chen J, and Bers DM (2013) Epac2 mediates cardiac β1-adrenergic-dependent sarcoplasmic reticulum Ca2+ leak and arrhythmia. *Circulation* 127:913–922.

Pereira L, Rehmann H, Lao DH, Erickson JR, Bossuyt J, Chen J, and Bers DM (2015) Novel Epac fluorescent ligand reveals distinct Epac1 vs. Epac2 distribution and function in cardiomyocytes. *Proc Natl Acad Sci USA* 112:3991–3996.

Perera RK and Nikolaev VO (2013) Compartmentation of cAMP signalling in cardiomyocytes in health and disease. *Acta Physiol (Oxf)* 207:650–662.

Perkins GA, Wang L, Huang LJ, Humphries K, Yao VJ, Martone M, Deerinck TJ, Barraclough DM, Violin JD, Smith D, et al. (2001) PKA, PKC, and AKAP localization in and around the neuromuscular junction. *BMC Neurosci* 2:17.

Perrino C, Feliciello A, Schiattarella GG, Esposito G, Guerriero R, Zaccaro L, Del Gatto A, Saviano M, Garbi C, Carangi R, et al. (2010) AKAP121 downregulation impairs protective cAMP signals, promotes mitochondrial dysfunction, and increases oxidative stress. *Cardiovasc Res* 88:101–110.

Pfanner N, van der Laan M, Amati P, Capaldi RA, Caudy AA, Chacinska A, Darshi M, Deckers M, Hoppins S, Icho T, et al. (2014) Uniform nomenclature for the mitochondrial contact site and cristae organizing system. *J Cell Biol* 204: 1083–1086.

Piggott LA, Bauman AL, Scott JD, and Dessauer CW (2008) The A-kinase anchoring protein Yotiao binds and regulates adenylyl cyclase in brain. *Proc Natl Acad Sci USA* 105:13835–13840.

Pinton P, Pozzan T, and Rizzuto R (1998) The Golgi apparatus is an inositol 1,4,5-trisphosphate-sensitive Ca2+ store, with functional properties distinct from those of the endoplasmic reticulum. *EMBO J* 17:5298–5308.

Plotnikova OV, Golemis EA, and Pugacheva EN (2008) Cell cycle-dependent ciliogenesis and cancer. *Cancer Res* 68:2058–2061.

Ponsioen B, Gloerich M, Ritsma L, Rehmann H, Bos JL, and Jalink K (2009) Direct spatial control of Epac1 by cyclic AMP. *Mol Cell Biol* 29:2521–2531.

Ponsioen B, Zhao J, Riedl J, Zwartkruis F, van der Krogt G, Zaccolo M, Moolenaar WH, Bos JL, and Jalink K (2004) Detecting cAMP-induced Epac activation by fluorescence resonance energy transfer: Epac as a novel cAMP indicator. *EMBO Rep* 5:1176–1180.

Pontier SM, Percherancier Y, Galandrin S, Breit A, Galés C, and Bouvier M (2008) Cholesterol-dependent separation of the beta2-adrenergic receptor from its partners determines signaling efficacy: insight into nanoscale organization of signal transduction. *J Biol Chem* 283:24659–24672.

Pooley L, Shakur Y, Rena G, and Houslay MD (1997) Intracellular localization of the PDE4A cAMP-specific phosphodiesterase splice variant RD1 (RNPDE4A1A) in stably transfected human thyroid carcinoma FTC cell lines. *Biochem J* 321: 177–185.

Porpora M, Sauchella S, Rinaldi L, Delle Donne R, Sepe M, Torres-Quesada O, Intartaglia D, Garbi C, Insabato L, Santoriello M, et al. (2018) Counterregulation of cAMP-directed kinase activities controls ciliogenesis. *Nat Commun* 9:1224.

Purohit A, Tynan SH, Vallee R, and Doxsey SJ (1999) Direct interaction of pericentrin with cytoplasmic dynein light intermediate chain contributes to mitotic spindle organization. *J Cell Biol* 147:481–492.

Qiao J, Mei FC, Popov VL, Vergara LA, and Cheng X (2002) Cell cycle-dependent subcellular localization of exchange factor directly activated by cAMP. *J Biol Chem* 277:26581–26586.

Rababa'h A, Craft JW Jr, Wijaya CS, Atrooz F, Fan Q, Singh S, Guillory AN, Katsonis P, Lichtarge O, and McConnell BK (2013) Protein kinase A and phosphodiesterase-4D3 binding to coding polymorphisms of cardiac muscle anchoring protein (mAKAP). *J Mol Biol* 425:3277–3288.

Rahman N, Ramos-Espiritu L, Milner TA, Buck J, and Levin LR (2016) Soluble adenylyl cyclase is essential for proper lysosomal acidification. *J Gen Physiol* 148: 325–339.

Ranganathan G, Phan D, Pokrovskaya ID, McEwen JE, Li C, and Kern PA (2002) The translational regulation of lipoprotein lipase by epinephrine involves an RNA binding complex including the catalytic subunit of protein kinase A. *J Biol Chem* 277:43281–43287.

Reggi E and Diviani D (2017) The role of A-kinase anchoring proteins in cancer development. *Cell Signal* 40:143–155.

Rich TC, Fagan KA, Nakata H, Schaack J, Cooper DM, and Karpen JW (2000) Cyclic nucleotide-gated channels colocalize with adenylyl cyclase in regions of restricted cAMP diffusion. *J Gen Physiol* 116:147–161.

Rich TC, Fagan KA, Tse TE, Schaack J, Cooper DM, and Karpen JW (2001) A uniform extracellular stimulus triggers distinct cAMP signals in different compartments of a simple cell. *Proc Natl Acad Sci USA* 98:13049–13054.

Richards M, Lomas O, Jalink K, Ford KL, Vaughan-Jones RD, Lefkimmiatis K, and Swietach P (2016) Intracellular tortuosity underlies slow cAMP diffusion in adult ventricular myocytes. *Cardiovasc Res* 110:395–407.

Rigatti M, Le AV, Gerber C, Moraru II, and Dodge-Kafka KL (2015) Phosphorylation state-dependent interaction between AKAP7δ/γ and phospholamban increases phospholamban phosphorylation. *Cell Signal* 27:1807–1815.

Rinaldi L, Delle Donne R, Borzacchiello D, Insabato L, and Feliciello A (2018) The role of compartmentalized signaling pathways in the control of mitochondrial activities in cancer cells. *Biochim Biophys Acta Rev Cancer* 1869:293–302.

Rinaldi L, Sepe M, Donne RD, and Feliciello A (2015) A dynamic interface between ubiquitylation and cAMP signaling. *Front Pharmacol* 6:177.

Rivero S, Cardenas J, Bornens M, and Rios RM (2009) Microtubule nucleation at the cis-side of the Golgi apparatus requires AKAP450 and GM130. *EMBO J* 28: 1016–1028.

Rochais F, Vandecasteele G, Lefebvre F, Lugnier C, Lum H, Mazet JL, Cooper DM, and Fischmeister R (2004) Negative feedback exerted by cAMP-dependent protein kinase and cAMP phosphodiesterase on subsarcolemmal cAMP signals in intact cardiac myocytes: an in vivo study using adenovirus-mediated expression of CNG channels. *J Biol Chem* 279:52095–52105.

Rodbell M, Birnbaumer L, Pohl SL, and Krans HM (1971) The glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. V. An obligatory role of guanylnucleotides in glucagon action. *J Biol Chem* 246:1877–1882.

Rogne M, Stokka AJ, Taskén K, Collas P, and Küntziger T (2009) Mutually exclusive binding of PP1 and RNA to AKAP149 affects the mitochondrial network. *Hum Mol Genet* 18:978–987.

Ross EM, Howlett AC, Ferguson KM, and Gilman AG (1978) Reconstitution of hormone-sensitive adenylate cyclase activity with resolved components of the enzyme. *J Biol Chem* 253:6401–6412.

Ross EM and Wilkie TM (2000) GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. *Annu Rev Biochem* 69:795–827.

Rous S (1970) Effect of dibutyryl cAMP on the enzymes of fatty acid synthesis and of glycogen metabolism. *FEBS Lett* 12:45–48.

Rybin VO, Xu X, Lisanti MP, and Steinberg SF (2000) Differential targeting of beta-adrenergic receptor subtypes and adenylyl cyclase to cardiomyocyte caveolae. A mechanism to functionally regulate the cAMP signaling pathway. *J Biol Chem* 275: 41447–41457.

Sample V, DiPilato LM, Yang JH, Ni Q, Saucerman JJ, and Zhang J (2012) Regulation of nuclear PKA revealed by spatiotemporal manipulation of cyclic AMP. *Nat Chem Biol* 8:375–382.

Sarma GN, Moody IS, Ilouz R, Phan RH, Sankaran B, Hall RA, and Taylor SS (2015) D-AKAP2:PKA RII:PDZK1 ternary complex structure: insights from the nucleation of a polyvalent scaffold. *Protein Sci* 24:105–116.

Saucerman JJ, Greenwald EC, and Polanowska-Grabowska R (2014) Mechanisms of cyclic AMP compartmentation revealed by computational models. *J Gen Physiol* 143:39–48.

Schauble S, King CC, Darshi M, Koller A, Shah K, and Taylor SS (2007) Identification of ChChd3 as a novel substrate of the cAMP-dependent protein kinase (PKA) using an analog-sensitive catalytic subunit. *J Biol Chem* 282: 14952–14959.

Schillace RV, Voltz JW, Sim AT, Shenolikar S, and Scott JD (2001) Multiple interactions within the AKAP220 signaling complex contribute to protein phosphatase 1 regulation. *J Biol Chem* 276:12128–12134.

Schleicher K and Zaccolo M (2020) Defining a cellular map of cAMP nanodomains. *Mol Pharmacol* DOI: 10.1124/mol.119.118869 [published ahead of print].

Schmidt PH, Dransfield DT, Claudio JO, Hawley RG, Trotter KW, Milgram SL, and Goldenring JR (1999) AKAP350, a multiply spliced protein kinase A-anchoring protein associated with centrosomes. *J Biol Chem* **274**:3055–3066.

Schwoch G, Trinczek B, and Bode C (1990) Localization of catalytic and regulatory subunits of cyclic AMPdependent protein kinases in mitochondria from various rat tissues. *Biochem J* **270**:181–188.

Schwoerer AP, Neuber C, Schmechel A, Melnychenko I, Mearini G, Boknik P, Kirchhefer U, Schmitz W, Ehmke H, Eschenhagen T, et al. (2008) Mechanical unloading of the rat heart involves marked changes in the protein kinase-phosphatase balance. *J Mol Cell Cardiol* **45**:846–852.

Scott JD, Dessauer CW, and Taskén K (2013) Creating order from chaos: cellular regulation by kinase anchoring. *Annu Rev Pharmacol Toxicol* **53**:187–210.

Scott JD and Pawson T (2009) Cell signaling in space and time: where proteins come together and when they're apart. *Science* **326**:1220–1224.

Scott JD, Stofko RE, McDonald JR, Comer JD, Vitalis EA, and Mangili JA (1990) Type II regulatory subunit dimerization determines the subcellular localization of the cAMP-dependent protein kinase. *J Biol Chem* **265**:21561–21566.

Selbie LA and Hill SJ (1998) G protein-coupled-receptor cross-talk: the fine-tuning of multiple receptor-signalling pathways. *Trends Pharmacol Sci* **19**:87–93.

Sette C and Conti M (1996) Phosphorylation and activation of a cAMP-specific phosphodiesterase by the cAMP-dependent protein kinase. Involvement of serine 54 in the enzyme activation. *J Biol Chem* **271**:16526–16534.

Shanks RA, Steadman BT, Schmidt PH, and Goldenring JR (2002) AKAP350 at the Golgi apparatus. I. Identification of a distinct Golgi apparatus targeting motif in AKAP350. *J Biol Chem* **277**:40967–40972.

Shcherbakova OG, Hurt CM, Xiang Y, Dell'Acqua ML, Zhang Q, Tsien RW, and Kobilka BK (2007) Organization of beta-adrenoceptor signaling compartments by sympathetic innervation of cardiac myocytes. *J Cell Biol* **176**:521–533.

Shen A, Chen D, Kaur M, Bartels P, Xu B, Shi Q, Martinez JM, Man KM, Nieves-Cintron M, Hell JW, et al. (2019) β-blockers augment L-type Ca^{2+} channel activity by targeting spatially restricted β$_2$AR signaling in neurons. *eLife* **8**:e49464.

Shen JX and Cooper DM (2013) AKAP79, PKC, PKA and PDE4 participate in a Gq-linked muscarinic receptor and adenylate cyclase 2 cAMP signalling complex. *Biochem J* **455**:47–56.

Sherpa RT, Mohieldin AM, Pala R, Wachten D, Ostrom RS, and Nauli SM (2019) Sensory primary cilium is a responsive cAMP microdomain in renal epithelia. *Sci Rep* **9**:6523.

Shi Y (2009) Serine/threonine phosphatases: mechanism through structure. *Cell* **139**:468–484.

Shimizu-Albergine M, Tsai LC, Patrucco E, and Beavo JA (2012) cAMP-specific phosphodiesterases 8A and 8B, essential regulators of Leydig cell steroidogenesis. *Mol Pharmacol* **81**:556–566.

Singh A, Redden JM, Kapiloff MS, and Dodge-Kafka KL (2011) The large isoforms of A-kinase anchoring protein 18 mediate the phosphorylation of Inhibitor-1 by protein kinase A and the inhibition of protein phosphatase 1 activity. *Mol Pharmacol* **79**:533–540 DOI: 10.1124/mol.110.065425.

Skroblin P, Grossmann S, Schäfer G, Rosenthal W, and Klussmann E (2010) Mechanisms of protein kinase A anchoring. *Int Rev Cell Mol Biol* **283**:235–330.

Smith FD, Esseltine JL, Nygren PJ, Veesler D, Byrne DP, Vonderach M, Strashnov I, Eyers CE, Eyers PA, Langeberg LK, et al. (2017) Local protein kinase A action proceeds through intact holoenzymes. *Science* **356**:1288–1293.

Smith FD, Omar MH, Nygren PJ, Soughayer J, Hoshi N, Lau H-T, Snyder CG, Branon TC, Ghosh D, Langeberg LK, et al. (2018) Single nucleotide polymorphisms alter kinase anchoring and the subcellular targeting of A-kinase anchoring proteins. *Proc Natl Acad Sci USA* **115**:E11465–E11474.

Smith FD, Reichow SL, Esseltine JL, Shi D, Langeberg LK, Scott JD, and Gonen T (2013) Intrinsic disorder within an AKAP-protein kinase A complex guides local substrate phosphorylation. *eLife* **2**:e01319.

Smith KE, Gibson ES, and Dell'Acqua ML (2006) cAMP-dependent protein kinase postsynaptic localization regulated by NMDA receptor activation through translocation of an A-kinase anchoring protein scaffold protein. *J Neurosci* **26**:2391–2402.

Sowadski JM, Nguyen HX, Anderson D, and Taylor SS (1985) Crystallization studies of cAMP-dependent protein kinase. Crystals of catalytic subunit diffract to 3.5 A resolution. *J Mol Biol* **182**:617–620.

Sprenger JU, Perera RK, Steinbrecher JH, Lehnart SE, Maier LS, Hasenfuss G, and Nikolaev VO (2015) In vivo model with targeted cAMP biosensor reveals changes in receptor-microdomain communication in cardiac disease. *Nat Commun* **6**:6965.

Srivastava RK, Srivastava AR, Korsmeyer SJ, Nesterova M, Cho-Chung YS, and Longo DL (1998) Involvement of microtubules in the regulation of Bcl2 phosphorylation and apoptosis through cyclic AMP-dependent protein kinase. *Mol Cell Biol* **18**:3509–3517.

Stangherlin A, Koschinski A, Terrin A, Zoccarato A, Jiang H, Fields LA, and Zaccolo M (2014) Analysis of compartmentalized cAMP: a method to compare signals from differently targeted FRET reporters, in *Fluorescent Protein-Based Biosensors: Methods and Protocols* (Zhang J, Ni Q, and Newman RH eds), Humana Press, Totowa, NJ.

Stangherlin A and Zaccolo M (2012) cGMP-cAMP interplay in cardiac myocytes: a local affair with far-reaching consequences for heart function. *Biochem Soc Trans* **40**:11–14.

Steen RL, Martins SB, Taskén K, and Collas P (2000) Recruitment of protein phosphatase 1 to the nuclear envelope by A-kinase anchoring protein AKAP149 is a prerequisite for nuclear lamina assembly. *J Cell Biol* **150**:1251–1262.

Steinberg SF and Brunton LL (2001) Compartmentation of G protein-coupled signaling pathways in cardiac myocytes. *Annu Rev Pharmacol Toxicol* **41**:751–773.

Stoddart LA, Johnstone EKM, Wheal AJ, Goulding J, Robers MB, Machleidt T, Wood KV, Hill SJ, and Pfleger KDG (2015) Application of BRET to monitor ligand binding to GPCRs. *Nat Methods* **12**:661–663.

Stork PJ and Schmitt JM (2002) Crosstalk between cAMP and MAP kinase signaling in the regulation of cell proliferation. *Trends Cell Biol* **12**:258–266.

Strack S, Zaucha JA, Ebner FF, Colbran RJ, and Wadzinski BE (1998) Brain protein phosphatase 2A: developmental regulation and distinct cellular and subcellular localization by B subunits. *J Comp Neurol* **392**:515–527.

Su B, Gao L, Meng F, Guo LW, Rothschild J, and Gelman IH (2013) Adhesion-mediated cytoskeletal remodeling is controlled by the direct scaffolding of Src from FAK complexes to lipid rafts by SSeCKS/AKAP12. *Oncogene* **32**:2016–2026.

Subramanian A, Capalbo A, Iyengar NR, Rizzo R, di Campli A, Di Martino R, Lo Monte M, Beccari AR, Yerudkar A, Del Vecchio C, et al. (2019) Auto-regulation of secretory flux by sensing and responding to the folded cargo protein load in the endoplasmic reticulum. *Cell* **176**:1461–1476.e23.

Suen DF, Norris KL, and Youle RJ (2008) Mitochondrial dynamics and apoptosis. *Genes Dev* **22**:1577–1590.

Sun F, Hug MJ, Bradbury NA, and Frizzell RA (2000) Protein kinase A associates with cystic fibrosis transmembrane conductance regulator via an interaction with ezrin. *J Biol Chem* **275**:14360–14366.

Sunahara RK, Dessauer CW, and Gilman AG (1996) Complexity and diversity of mammalian adenylyl cyclases. *Annu Rev Pharmacol Toxicol* **36**:461–480.

Suofu Y, Li W, Jean-Alphonse FG, Jia J, Khattar NK, Li J, Baranov SV, Leronni D, Mihalik AC, He Y, et al. (2017) Dual role of mitochondria in producing melatonin and driving GPCR signaling to block cytochrome c release. *Proc Natl Acad Sci USA* **114**:E7997–E8006.

Surdo NC, Berrera M, Koschinski A, Brescia M, Machado MR, Carr C, Wright P, Gorelik J, Morotti S, Grandi E, et al. (2017) FRET biosensor uncovers cAMP nano-domains at β-adrenergic targets that dictate precise tuning of cardiac contractility. *Nat Commun* **8**:15031.

Suryavanshi SV, Jadhav SM, and McConnell BK (2018) Polymorphisms/mutations in A-kinase anchoring proteins (AKAPs): role in the cardiovascular system. *J Cardiovasc Dev Dis* **5**:7.

Sutherland EW and Rall TW (1958) Fractionation and characterization of a cyclic adenine ribonucleotide formed by tissue particles. *J Biol Chem* **232**:1077–1091.

Syed AU, Reddy GR, Ghosh D, Prada MP, Nystoriak MA, Morotti S, Grandi E, Sirish P, Chiamvimonvat N, Hell JW, et al. (2019) Adenylyl cyclase 5-generated cAMP controls cerebral vascular reactivity during diabetic hyperglycemia. *J Clin Invest* **129**:3140–3152.

Takahashi M, Shibata H, Shimakawa M, Miyamoto M, Mukai H, and Ono Y (1999) Characterization of a novel giant scaffolding protein, CG-NAP, that anchors multiple signaling enzymes to centrosome and the golgi apparatus. *J Biol Chem* **274**:17267–17274.

Tang T, Lai NC, Wright AT, Gao MH, Lee P, Guo T, Tang R, McCulloch AD, and Hammond HK (2013) Adenylyl cyclase 6 deletion increases mortality during sustained β-adrenergic receptor stimulation. *J Mol Cell Cardiol* **60**:60–67.

Taskén K and Aandahl EM (2004) Localized effects of cAMP mediated by distinct routes of protein kinase A. *Physiol Rev* **84**:137–167.

Taskén KA, Collas P, Kemmner WA, Witczak O, Conti M, and Taskén K (2001) Phosphodiesterase 4D and protein kinase a type II constitute a signaling unit in the centrosomal area. *J Biol Chem* **276**:21999–22002.

Taylor SS, Buechler JA, and Yonemoto W (1990) cAMP-dependent protein kinase: framework for a diverse family of regulatory enzymes. *Annu Rev Biochem* **59**:971–1005.

Taylor SS, Ilouz R, Zhang P, and Kornev AP (2012) Assembly of allosteric macro-molecular switches: lessons from PKA. *Nat Rev Mol Cell Biol* **13**:646–658.

Taylor SS, Kim C, Vigil D, Haste NM, Yang J, Wu J, and Anand GS (2005) Dynamics of signaling by PKA. *Biochim Biophys Acta* **1754**:25–37.

Taylor SS, Zhang P, Steichen JM, Keshwani MM, and Kornev AP (2013) PKA: lessons learned after twenty years. *Biochim Biophys Acta* **1834**:1271–1278.

Terrenoire C, Houslay MD, Baillie GS, and Kass RS (2009) The cardiac IKs potassium channel macromolecular complex includes the phosphodiesterase PDE4D3. *J Biol Chem* **284**:9140–9146.

Terrin A, Di Benedetto G, Pertegato V, Cheung YF, Baillie G, Lynch MJ, Elvassore N, Prinz A, Herberg FW, Houslay MD, et al. (2006) PGE(1) stimulation of HEK293 cells generates multiple contiguous domains with different [cAMP]: role of compartmentalized phosphodiesterases. *J Cell Biol* **175**:441–451.

Terrin A, Monterisi S, Stangherlin A, et al. (2012) PKA and PDE4D3 anchoring to AKAP9 provides distinct regulation of cAMP signals at the centrosome. *J Cell Biol* **198**:607–621.

Thakkar A, Aljameeli A, Thomas S, and Shah GV (2016) A-kinase anchoring protein 2 is required for calcitonin-mediated invasion of cancer cells. *Endocr Relat Cancer* **23**:1–14.

Timofeyev V, Myers RE, Kim HJ, Woltz RL, Sirish P, Heiserman JP, Li N, Singapuri A, Tang T, Yarov-Yarovoy V, et al. (2013) Adenylyl cyclase subtype-specific compartmentalization: differential regulation of L-type Ca2+ current in ventricular myocytes. *Circ Res* **112**:1567–1576.

Torres-Quesada O, Mayrhofer JE, and Stefan E (2017) The many faces of compartmentalized PKA signalosomes. *Cell Signal* **37**:1–11.

Trotter KW, Fraser IDC, Scott GK, Stutts MJ, Scott JD, and Milgram SL (1999) Alternative splicing regulates the subcellular localization of A-kinase anchoring protein 18 isoforms. *J Cell Biol* **147**:1481–1492.

Tsvetanova NG and von Zastrow M (2014) Spatial encoding of cyclic AMP signaling specificity by GPCR endocytosis. *Nat Chem Biol* **10**:1061–1065.

Unal R, Pokrovskaya I, Tripathi P, Monia BP, Kern PA, and Ranganathan G (2008) Translational regulation of lipoprotein lipase in adipocytes: depletion of cellular protein kinase Calpha activates the C subunit of protein kinase A to the 3'-untranslated region of the lipoprotein lipase mRNA. *Biochem J* **413**:315–322.

Valsecchi F, Konrad C, and Manfredi G (2014) Role of soluble adenylyl cyclase in mitochondria. *Biochim Biophys Acta* **1842**:2555–2560.

Vaniotis G, Allen BG, and Hébert TE (2011) Nuclear GPCRs in cardiomyocytes: an insider's view of β-adrenergic receptor signaling. *Am J Physiol Heart Circ Physiol* **301**:H1754–H1764.

van Vliet AR, Verfaillie T, and Agostinis P (2014) New functions of mitochondria associated membranes in cellular signaling. *Biochim Biophys Acta* **1843**:2253–2262.

Vargas MA, Tirnauer JS, Glidden N, Kapiloff MS, and Dodge-Kafka KL (2012) Myocyte enhancer factor 2 (MEF2) tethering to muscle selective A-kinase anchoring protein (mAKAP) is necessary for myogenic differentiation. *Cell Signal* **24**: 1496–1503.

Virshup DM and Shenolikar S (2009) From promiscuity to precision: protein phosphatases get a makeover. *Mol Cell* **33**:537–545.

Wachten S, Masada N, Ayling LJ, Ciruela A, Nikolaev VO, Lohse MJ, and Cooper DM (2010) Distinct pools of cAMP centre on different isoforms of adenylyl cyclase in pituitary-derived GH3B6 cells. *J Cell Sci* **123**:95–106.

Wadzinski BE, Wheat WH, Jaspers S, Peruski LF Jr, Lickteig RL, Johnson GL, and Klemm DJ (1993) Nuclear protein phosphatase 2A dephosphorylates protein kinase A-phosphorylated CREB and regulates CREB transcriptional stimulation. *Mol Cell Biol* **13**:2822–2834.

Walker-Gray R, Stengel F, and Gold MG (2017) Mechanisms for restraining cAMP-dependent protein kinase revealed by subunit quantitation and cross-linking approaches. *Proc Natl Acad Sci USA* **114**:10414–10419.

Walsh DA, Perkins JP, and Krebs EG (1968) An adenosine 3′,5′-monophosphate-dependant protein kinase from rabbit skeletal muscle. *J Biol Chem* **243**:3763–3765.

Wang L, Sunahara RK, Krumins A, Perkins G, Crochiere ML, Mackey M, Bell S, Ellisman MH, and Taylor SS (2001) Cloning and mitochondrial localization of full-length D-AKAP2, a protein kinase A anchoring protein. *Proc Natl Acad Sci USA* **98**:3220–3225.

Wang P, Liu Z, Chen H, Ye N, Cheng X, and Zhou J (2017) Exchange proteins directly activated by cAMP (EPACs): emerging therapeutic targets. *Bioorg Med Chem Lett* **27**:1633–1639.

Wang Z, Liu D, Varin A, Nicolas V, Courilleau D, Mateo P, Caubere C, Rouet P, Gomez AM, Vandecasteele G, et al. (2016) A cardiac mitochondrial cAMP signaling pathway regulates calcium accumulation, permeability transition and cell death. *Cell Death Dis* **7**:e2198.

Wei J, Zhao AZ, Chan GC, Baker LP, Impey S, Beavo JA, and Storm DR (1998) Phosphorylation and inhibition of olfactory adenylyl cyclase by CaM kinase II in neurons: a mechanism for attenuation of olfactory signals. *Neuron* **21**:495–504.

Weissinger EM, Eissner G, Grammer C, Fackler S, Haefner B, Yoon LS, Lu KS, Bazarov A, Sedivy JM, Mischak H, et al. (1997) Inhibition of the Raf-1 kinase by cyclic AMP agonists causes apoptosis of v-abl-transformed cells. *Mol Cell Biol* **17**: 3229–3241.

Welch EJ, Jones BW, and Scott JD (2010) Networking with AKAPs: context-dependent regulation of anchored enzymes. *Mol Interv* **10**:86–97.

Westphal RS, Tavalin SJ, Lin JW, Alto NM, Fraser ID, Langeberg LK, Sheng M, and Scott JD (1999) Regulation of NMDA receptors by an associated phosphatase-kinase signaling complex. *Science* **285**:93–96.

Wettschureck N and Offermanns S (2005) Mammalian G proteins and their cell type specific functions. *Physiol Rev* **85**:1159–1204.

Wiggins SV, Steegborn C, Levin LR, and Buck J (2018) Pharmacological modulation of the $CO_2/HCO_3^-/pH-$, calcium-, and ATP-sensing soluble adenylyl cyclase. *Pharmacol Ther* **190**:173–186.

Willoughby D, Halls ML, Everett KL, Ciruela A, Skroblin P, Klussmann E, and Cooper DMF (2012) A key phosphorylation site in AC8 mediates regulation of Ca(2+)-dependent cAMP dynamics by an AC8-AKAP79-PKA signalling complex. *J Cell Sci* **125**:5850–5859.

Witczak O, Skålhegg BS, Keryer G, Bornens M, Taskén K, Jahnsen T, and Orstavik S (1999) Cloning and characterization of a cDNA encoding an A-kinase anchoring protein located in the centrosome, AKAP450. *EMBO J* **18**:1858–1868.

Woolfrey KM and Dell'Acqua ML (2015) Coordination of protein phosphorylation and dephosphorylation in synaptic plasticity. *J Biol Chem* **290**:28604–28612.

Wright PT, Bhogal NK, Diakonov I, Pannell LMK, Perera RK, Bork NI, Schobesberger S, Lucarelli C, Faggian G, Alvarez-Laviada A, et al. (2018) Cardiomyocyte membrane structure and cAMP compartmentation produce anatomical variation in β2AR-cAMP responsiveness in murine hearts. *Cell Rep* **23**:459–469.

Wright PT, Nikolaev VO, O'Hara T, Diakonov I, Bhargava A, Tokar S, Schobesberger S, Shevchuk AI, Sikkel MB, Wilkinson R, et al. (2014) Caveolin-3 regulates compartmentation of cardiomyocyte beta2-adrenergic receptor-mediated cAMP signaling. *J Mol Cell Cardiol* **67**:38–48.

Yamamoto KK, Gonzalez GA, Biggs WH III, and Montminy MR (1988) Phosphorylation-induced binding and transcriptional efficacy of nuclear factor CREB. *Nature* **334**: 494–498.

Yan L, Vatner DE, O'Connor JP, Ivessa A, Ge H, Chen W, Hirotani S, Ishikawa Y, Sadoshima J, and Vatner SF (2007) Type 5 adenylyl cyclase disruption increases longevity and protects against stress. *Cell* **130**:247–258.

Yang J, Drazba JA, Ferguson DG, and Bond M (1998) A-kinase anchoring protein 100 (AKAP100) is localized in multiple subcellular compartments in the adult rat heart. *J Cell Biol* **142**:511–522.

Yang JH, Polanowska-Grabowska RK, Smith JS, Shields CWT IV, and Saucerman JJ (2014) PKA catalytic subunit compartmentation regulates contractile and hypertrophic responses to β-adrenergic signaling. *J Mol Cell Cardiol* **66**:83–93.

Yang PC, Boras BW, Jeng MT, Docken SS, Lewis TJ, McCulloch AD, Harvey RD, and Clancy CE (2016) A computational modeling and simulation approach to investigate mechanisms of subcellular cAMP compartmentation. *PLOS Comput Biol* **12**:e1005005.

Yang S, Fletcher WH, and Johnson DA (1995) Regulation of cAMP-dependent protein kinase: enzyme activation without dissociation. *Biochemistry* **34**:6267–6271.

Yang Z, Kirton HM, MacDougall DA, Boyle JP, Deuchars J, Frater B, Ponnambalam S, Hardy ME, White E, Calaghan SC, et al. (2015) The Golgi apparatus is a functionally distinct Ca2+ store regulated by the PKA and Epac branches of the β1-adrenergic signaling pathway. *Sci Signal* **8**:ra101.

Yano N, Suzuki D, Endoh M, Tseng A, Stabila JP, McGonnigal BG, Zhao TC, Padbury JF, and Tseng YT (2008) Beta-adrenergic receptor mediated protection against doxorubicin-induced apoptosis in cardiomyocytes: the impact of high ambient glucose. *Endocrinology* **149**:6449–6461.

Yu H, Yuan C, Westenbroek RE, and Catterall WA (2018) The AKAP Cypher/Zasp contributes to β-adrenergic/PKA stimulation of cardiac Ca$_V$1.2 calcium channels. *J Gen Physiol* **150**:883–889.

Yu P, Chen Y, Tagle DA, and Cai T (2002) PJA1, encoding a RING-H2 finger ubiquitin ligase, is a novel human X chromosome gene abundantly expressed in brain. *Genomics* **79**:869–874.

Zaccolo M (2009) cAMP signal transduction in the heart: understanding spatial control for the development of novel therapeutic strategies. *Br J Pharmacol* **158**: 50–60.

Zaccolo M, De Giorgi F, Cho CY, Feng L, Knapp T, Negulescu PA, Taylor SS, Tsien RY, and Pozzan T (2000) A genetically encoded, fluorescent indicator for cyclic AMP in living cells. *Nat Cell Biol* **2**:25–29.

Zaccolo M, Di Benedetto G, Lissandron V, Mancuso L, Terrin A, and Zamparo I (2006) Restricted diffusion of a freely diffusible second messenger: mechanisms underlying compartmentalized cAMP signalling. *Biochem Soc Trans* **34**:495–497.

Zaccolo M and Movsesian MA (2007) cAMP and cGMP signaling cross-talk: role of phosphodiesterases and implications for cardiac pathophysiology. *Circ Res* **100**: 1569–1578.

Zaccolo M and Pozzan T (2002) Discrete microdomains with high concentration of cAMP in stimulated rat neonatal cardiac myocytes. *Science* **295**:1711–1715.

Zhang L, Malik S, Kelley GG, Kapiloff MS, and Smrcka AV (2011) Phospholipase C epsilon scaffolds to muscle-specific A kinase anchoring protein (mAKAPbeta) and integrates multiple hypertrophic stimuli in cardiac myocytes. *J Biol Chem* **286**: 23012–23021.

Zhang L, Malik S, Pang J, Wang H, Park KM, Yule DI, Blaxall BC, and Smrcka AV (2013) Phospholipase Cε hydrolyzes perinuclear phosphatidylinositol 4-phosphate to regulate cardiac hypertrophy. *Cell* **153**:216–227.

Zhang X and Eggert US (2013) Non-traditional roles of G protein-coupled receptors in basic cell biology. *Mol Biosyst* **9**:586–595.

Zhang Y and Xu H (2016) Translational regulation of mitochondrial biogenesis. *Biochem Soc Trans* **44**:1717–1724.

Zhou XB, Lutz S, Steffens F, Korth M, and Wieland T (2007) Oxytocin receptors differentially signal via Gq and Gi proteins in pregnant and nonpregnant rat uterine myocytes: implications for myometrial contractility. *Mol Endocrinol* **21**: 740–752.

Zhou XB, Wang GX, Huneke B, Wieland T, and Korth M (2000) Pregnancy switches adrenergic signal transduction in rat and human uterine myocytes as probed by BKCa channel activity. *J Physiol* **524**:339–352.

Zhu H, Suk HY, Yu RYL, Brancho D, Olabisi O, Yang TTC, Yang X, Zhang J, Moussaif M, Durand JL, et al. (2010) Evolutionarily conserved role of calcineurin in phosphodegron-dependent degradation of phosphodiesterase 4D. *Mol Cell Biol* **30**: 4379–4390.

Zippin JH, Chen Y, Nahirney P, Kamenetsky M, Wuttke MS, Fischman DA, Levin LR, and Buck J (2003) Compartmentalization of bicarbonate-sensitive adenylyl cyclase in distinct signaling microdomains. *FASEB J* **17**:82–84.

Zippin JH, Chen Y, Straub SG, Hess KC, Diaz A, Lee D, Tso P, Holz GG, Sharp GW, Levin LR, et al. (2013) CO2/HCO3(-)- and calcium-regulated soluble adenylyl cyclase as a physiological ATP sensor. *J Biol Chem* **288**:33283–33291.

Zoccarato A, Surdo NC, Aronsen JM, Fields LA, Mancuso L, Dodoni G, Stangherlin A, Livie C, Jiang H, Sin YY, et al. (2015) Cardiac hypertrophy is inhibited by a local pool of cAMP regulated by phosphodiesterase 2. *Circ Res* **117**:707–719.