Published in final edited form as:

Pflugers Arch. 2013 October ; 465(10): 1397–1407. doi:10.1007/s00424-013-1280-6.

Compartmentalization of cyclic nucleotide signaling: A question of when, where, and why?

Kavisha Arora¹, Chandrima Sinha¹, Weiqiang Zhang^{1,2}, Aixia Ren¹, Chang Suk Moon¹, Sunitha Yarlagadda¹, and Anjaparavanda P. Naren^{1,*}

¹Department of Physiology, University of Tennessee Health Science Center, Memphis, TN 38163, USA

²University of Tennessee Cystic Fibrosis Care and Research Center at Le Bonheur Children's Hospital, Memphis, TN 38103, USA

Abstract

Preciseness of cellular behavior depends upon how an extracellular cue mobilizes a correct orchestra of cellular messengers and effector proteins spatially and temporally. This concept, termed compartmentalization of cellular signaling, is now known to form the molecular basis of many aspects of cellular behavior in health and disease. The cyclic nucleotides cAMP and cGMP are ubiquitous cellular messengers that can be compartmentalized in three ways: first, by their physical containment; second, by formation of multiple protein signaling complexes; and third, by their selective depletion. Compartmentalized cyclic nucleotide signaling is a very prevalent response among all cell types. In order to understand how it becomes relevant to cellular behavior, it is important to know how it is executed in cells to regulate physiological responses and, also, how its execution or dysregulation can lead to a pathophysiological condition, which forms the current scope of the presented review.

Keywords

Compartmentalization of cell signaling; cyclic-nucleotide signaling

Introduction

It is important that a cell has appropriate machinery to respond precisely to extracellular signals. The fundamental operators of this machinery are the cellular messengers, which are small molecules that primarily act to rapidly transduce information obtained from outside the cell into an internal signaling cascade. The cyclic nucleotides cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are important cellular messengers. There is a huge network of signaling pathways that primarily runs through cyclic nucleotides controlling a wide array of cellular events: memory, metabolism, cell proliferation, differentiation, death, migration, and fluid secretion [6,12,95,99]. The question arises of how a cell can generate such diverse responses that work through the same messengers. The answer lies in the precise segregation of the pathways by spatial and temporal regulation of the generation and catabolism of these messengers (termed compartmentalization). This concept first came to notice with the elegant observation by Buxton and Brunton in the early 1980s in cardiac myocytes, in which they observed a

*To whom correspondence should be addressed (anaren@uthsc.edu).

Conflict of interest

Authors declare no conflicts of interest.

differentiation of signaling processes inside the cell mediated by different sets of ligands with the fundamental process of cAMP elevation being common [9,12]. Now it is understood that the basis of most aspects of cellular behavior is not homogenous signaling processes but rather discrete ones involving a localized signaling triad of receptor/transducer/effector [102]. Prokaryotes and eukaryotes can be distinguished based on plentiful complexities associated with the signaling process; but if we contemplate more deeply, it fundamentally derives from the advantage that eukaryotes possess over prokaryotes in engaging in highly sophisticated compartmentalized signaling. Apparently, compartmentalized signaling came into existence as an evolutionary response to selection pressure as eukaryotic cells felt the need to respond rapidly to fast-changing conditions by segregating parallel-running signaling processes and expanding the signal repertoire. This is the typical advantage associated with compartmentalized signaling, i.e., to allow high velocity, specificity, and efficiency of a signaling event. It is known that cAMP effectors exhibit micromolar affinities for cAMP [20,82]. In such a scenario, compartmental cAMP would enable faster activation of the targets with a limited pool of cAMP without the need of generating large amounts of the molecule. It must be noted that excessive generation of cAMP leads to dysregulated cellular functions and can eventually cause cell toxicity and death [52,58]. A question of interest would be are all the signaling mechanisms compartmentalized? Whether a signaling process becomes compartmentalized or not is largely determined by how a charged receptor synchronizes and integrates with the downstream effector machinery in space and time so that spatiotemporally restricted signal nodes are created. Opting for receptor mediated compartmentalization is more of a preference than obligation. It is based on a simple fact of slower mobilities and the planar movement of the receptors in the membrane and the immense heterogeneity of the plasma membrane that promote their spatial patterning; thus allowing them to selectively communicate with certain set of enzymes and scaffolds in an whole ensemble of cytosolic proteins. Counter intuitively, it is now being known that the process of compartmentalization need not start at the level of surface receptors which might merely serve to mobilize an intracellular signal and the process can get compartmentalized at subsequent events. Recent studies also suggest that receptor embedded plasma membrane is not the sole platform of compartmentalized signaling. Spatial analysis of signaling processes have unraveled the existence of highly compartment restricted pathways like Ras/MAPK signaling existing on intracellular membranes [68]. Internalized receptor tyrosine kinase receptors in the ligated state such as epidermal growth factor receptor existing in the endosomes can phosphorylate phospholipase C and phosphoinositide 3-kinase but is contained to cause phosphatidylinositol-(4,5)-biphosphate [PI(4,5)P₂] hydrolysis usually mediated by the surface receptor [43]. Therefore, containment of a receptor is also a dimension to signal compartmentalization.

In this review, we discuss integral components of compartmentalized cyclic nucleotide signaling, how they work to transduce signals to control cell physiology, and how its dysregulation can lead to a pathophysiological condition. Due to space limitation, this review does not focus on specific physiological systems or proteins, and only a few relevant examples are provided for the better understanding of the concepts. Although the review focuses on cyclic-nucleotide signaling, considering the importance of calcium as a cellular messenger, we also briefly explain the process of calcium compartmentalization.

G protein-coupled receptors and adenylyl cyclases: the starting point of cAMP compartments

The canonical pathway for the generation of cAMP is the stimulation of the membrane-bound adenylyl cyclases (ACs) by active GTP-bound G protein, G_s, integrated to G protein-coupled receptor (GPCR) signaling. One soluble AC isoform has been identified that

acts independently of GPCR signaling and is responsive to localized bicarbonate gradients [14,101,114]. It has been observed that distribution of ACs and GPCRs is not homogenous throughout the cell and can be found enriched in certain pockets of the plasma membrane [21,49,76]. Also, homo- and heteromeric oligomerizations of certain ACs and many GPCRs act obligatorily to their plasma membrane localization, which would broadly determine signal amplification and segregation of their functional outputs [7,18]. Certain membrane-bound AC isoforms (AC3, AC5, AC6, and AC8) are selectively delivered to membrane rafts where they respond to local sub-micromolar calcium gradients [18,23,34,76,94]. AC6 is present in caveolin-rich domains where it can selectively communicate with co-localizing α_1/α_2 -adrenergic receptors (ARs), (commonly expressed GPCRs in cardiac myocytes) at the plasma membrane [73,75]. It is also known that olfactory cyclic nucleotide-gated (oCNG) channels co-localize with ACs and the cAMP generated in their vicinity exhibit highly restricted diffusion [84]. The ACs co-localizing with oCNG channels have been determined to be Ca^{2+} -sensitive, and, therefore, the whole protein assembly is reciprocally regulated [33]. Also, it is speculated that because endoplasmic reticulum (ER) can appose with the plasma membrane at certain regions, caveolae and ER can function as a diffusion barrier for cAMP [77,84]. Thus the selective patching of GPCR-AC signaling components with an inherent role of heterogeneous physical barriers provides the first level of compartmentalization of cAMP messages.

With more than 1,000 GPCRs being present in a mammalian cell [36], it becomes imperative that cells possess mechanisms to preserve signal specificity. The formation of high order complexes of GPCRs and downstream effectors is an integral component of such mechanisms [76]. This requires that the triad of receptors, transducers, and effectors be assembled together as part of a large complex, allowing the process of signal transduction to be instantaneous, specific, and switchable. It is now generally accepted that the formation of many of these complexes is not randomized (collision coupling). Rather, the interacting proteins may exist as prearranged stable complexes prior to ligand binding [76]. Despite the fact that β -ARs are ubiquitous and well-expressed GPCRs, α_2 -AR can complex with the Ca^{2+} channel Cav1.2 in certain plasma membrane microdomains [21]. The epithelial membrane protein cystic fibrosis transmembrane conductance regulator (CFTR), which is a cAMP-activated chloride channel, is regulated by specific protein complexes governed by GPCRs that modulate its chloride secretory function. That CFTR is in communication with certain GPCRs has been validated by the identification of macromolecular signaling complexes of CFTR with the α_2 -AR and lysophosphatidic acid receptor 2 (LPA₂) at the plasma membrane [57,70]. In these cases, CFTR and GPCRs possess consensus motifs to bind to common scaffolds. Indulgence of GPCR activity in the CFTR can also form the basis of cholera toxin (CTX)-induced diarrhea, as CTX causes constitutive activation of the ACs via G protein and excessive cAMP generation overstimulates CFTR activity [38,89].

An elegant functional readout of compartmentalized regulation of cyclic nucleotide-regulated channels comes from single-channel recordings. It is based on the discrete gating of the channel depending upon the agonist application in the bath vs. pipette solution. The α_2 -AR agonist albuterol and adenosine receptor agonist failed to stimulate Cav1.2 and CFTR channel activities, respectively, when applied outside of the recording pipette [21,50,57]. These studies help us appreciate the idea that a given protein can be regulated specifically in a macromolecular complex existing within discrete microdomains of the plasma membrane.

Cyclic nucleotide scavengers

Phosphodiesterases

It is known that cAMP diffuses quite rapidly ($200\text{--}700\ \mu\text{m s}^{-1}$) and can elicit a global intracellular response in a fraction of second. Cyclic nucleotide-degrading enzymes,

phosphodiesterases (PDEs), are set in place to ensure that the signaling process is spatially restricted. PDEs are very critical enzymes because they maintain the precise concentrations of cyclic nucleotides required for cell homeostasis. PDEs break the phosphodiester bond in cAMP and cGMP, resulting in the formation of linear adenosine-5-monophosphate (AMP) and guanine-5-monophosphate (GMP), respectively. Does this imply that PDEs completely annul the cyclic nucleotide response to terminate the signaling process? If not what would be the fate of leaked cyclic-nucleotides? Hypothetically the buffering process is progressive and the signals will be attenuated at a level that falls below the activation threshold of the non-specific targets. It is also speculated that cAMP signals are rapidly degraded in the microdomains [88]. However, the activated cAMP-dependent protein kinase A (PKA) catalytic subunits may persist and get translocated to the nucleus to mediate long-term durable changes. This feature of cAMP-signaling also forms the basis of processes of learning and memory.

There are eleven mammalian PDE gene families, three cAMP-specific ones (PDE4, PDE7, and PDE8), three cGMP-specific ones (PDE5, PDE6, and PDE9), and five that exhibit dual specificities (PDE1, PDE2, PDE3, PDE10, and PDE11) [12,17,103]. The diversity and specificities of PDE-regulated signaling are derived from the variability of different isoforms in tissue-type expression, subcellular localization, and specific associations with adaptor and effector proteins [12,17,103]. With these characteristics, PDEs can control multiple dimensions of a signaling process and fine-tune many signaling events by acting as functional switches. One such example is the role of PDE4D5 in γ -AR signaling. Occupancy of the receptor by PDE4D5 regulates the phosphorylation status of the γ -AR that causes γ -AR to switch to the extracellular signal-regulated kinases (ERK) pathway and PDE4D5/ γ -arrestin complex-mediated receptor desensitization [3,17,62]. The significance of the PDE4/arrestin complex has been recently demonstrated in regulation of cytokine production of activated T-cells where it serves to counteract negative feedback of cAMP on T-cell receptors [1]. Many adaptor proteins and the signaling complexes, mostly for PDE4, have been elucidated and detailed elsewhere [17,62]. The PDE3 physical complex with CFTR allows regulation of its chloride channel activity by microdomain cAMP and has been shown to improve tracheal secretion upon PDE3 inhibition [78].

An added dimension to the PDE regulation of cyclic nucleotide signaling is the generation of low-frequency cAMP pulses in response to Ca^{2+} oscillations at the plasma membrane [39,55,105]. Importantly, cAMP oscillations are dependent upon the calcium sensitivities of AC and PDE, and such response profile has been found to correspond to glucagon-mediated stimulation of insulin-secreting pancreatic cells [31].

With the revolutionary development of genetically engineered fluorescent probes based on cAMP/cGMP-dependent protein kinases and effector backbones, it is possible to determine the cyclic nucleotide dynamics of a compartmentalized response [45,81,111]. Studies with fluorescence resonance energy transfer (FRET)-based sensors have elucidated highly compartmentalized generation of cyclic nucleotides upon pharmacological inhibition of PDEs [66]. Also, this approach has helped identify subcellular hotspots of specific PDEs. PDE-inhibition has been vigorously pursued for therapeutic interventions. Many PDE-inhibitors have been developed and used in the treatment of pulmonary hypertension, chronic obstructive pulmonary disease (COPD), heart failure, intermittent claudication, and erectile dysfunction [22,78,85,103].

Cyclic nucleotide efflux transporters: extrusion as an alternative to degradation

An interesting observation by Davoren and Sutherland showed that hormonal stimulation of the cells not only increased the intracellular cAMP levels but also their levels outside the cell [12]. The obvious rationale to account for this observation was that cells possess a

cyclic nucleotide extrusion system to control the intracellular cAMP levels. It was later confirmed in 1999 and subsequent years, first based on export studies of antiviral nucleoside monophosphate analogs and later on cyclic nucleotides themselves, that a transporter, the multidrug resistance protein 4 (MRP4; also known as ABCC4), a member of the ABC transporter family, can efflux cAMP as well as cGMP from the cells by extracting energy from ATP. Interestingly, the affinity constant of MRP4 is lower for cGMP (10 μ M) than for cAMP (45 μ M), implying that MRP4 can dictate differential absolute concentrations and ratios of cyclic-nucleotides [91]. It has also been shown that the localized extruded cAMP is converted to adenosine in the membrane biophase and can regulate, via adenosine receptors, numerous cellular responses, including regulation of vascular tone, renal transport, renin release from juxtaglomerular cells, cardiac fibroblast proliferation, and collagen synthesis [51]. Recently, we demonstrated that MRP4 maintains distinct PKA activity status at the leading edge vs. lagging edge to enable directional migration of a fibroblast and to facilitate the wound healing process [91]. MRP4 is ubiquitously expressed in many polarized and non-polarized cell types [12,58]. In polarized epithelial cells, MRP4 is expressed on both apical and basolateral membranes [58]. Li et al. reported an important role of apical MRP4 in regulating CFTR function in a highly compartmentalized manner in gut epithelial cells [58]. CFTR chloride channel function was potentiated upon inhibition or silencing of MRP4 in the presence of a submaximal dose of adenosine (< 20 μ M). Recent studies have reported that caveolins can physically anchor MRP4 in plasma membrane microdomains, rendering MRP4 to participate in segregated signaling modules [87]. Other ABC transporters, including MRP5, ABCG2, and MRP8, have also been identified as exporters of cyclic nucleotides [12]. Therefore, in addition to hydrolysis, the extrusion from cells by certain ABC transporters represents another mechanism for regulating intracellular levels of cyclic nucleotides. Also, there have been observations suggesting that these two mechanisms may act compensatory or in concert [12]. However, which mode of cyclic-nucleotide neutralization is more active and predominates or under which circumstances they act together largely depends upon the cell type and relative expression and type of efflux transporters and PDEs in these cells. Certain PDE inhibitors such as trequinsin and sildenafil have been found to significantly inhibit MRP4 function [12]. However, it is not clear whether this involves the binding of drugs to MRP4 directly or there is a communication between PDEs and MRP4, or MRP4 in the vicinity of a PDE merely needs cyclic nucleotide gradient at a particular threshold that turns on MRP4 function. Nevertheless, the concomitant inhibition of MRP4 and PDE seems to be a therapeutic advantage for PDE inhibitors, which makes it possible to achieve desirable clinical efficacy at lower doses.

A-kinase anchoring proteins: signal organizing structures

A-kinase anchoring proteins (AKAPs) can be described as central unit of dissemination and integration of PKA activity. Most AKAPs recognize the N-terminal of the regulatory subunit II of PKA, which causes the release of the active catalytic core of the enzyme [64,92]. It has been estimated that tethering of PKA by AKAPs hastens the phosphorylation process by 3–4 fold [64,113]. Interestingly, new findings indicate that many of the central components of compartmentalized signaling can assemble at one place [64,79,93]. Elucidation of AKAP-PDE complexes within a microdomain is the most sophisticated addition to spatiotemporal regulation of cAMP gradients, and the field is certainly set to expand. It has also been concluded that PDEs, AKAPs, kinases, and phosphatases have analogous targeting motifs (specific for protein-protein or protein-lipid interactions) [93]. PDE4D3 and PKA run a concerted negative feedback loop for cAMP signaling within a muscle-specific AKAP (mA-KAP)-maintained signaling module that controls the output of ventricular hypertrophy [28]. Also, PDE4 phosphorylation within the complex by PKA increases its activity by 2–3 fold [47]. Later, additional components of this complex were identified that include another cAMP-dependent enzyme, the Rap1GTPase exchange factor (GEF) Epac [27]. This signifies

how AKAP integrates and allows cross-communication of discrete cAMP-effectors pathways. Other elements of AKAP –centered complexes have been identified. AKAP79 binds to the phosphatase calcineurin and diminishes the phosphorylation of PKA substrates to affect downstream transcriptional effectors, including nuclear factor of activated T cells (NFAT) [15,65]. AKAP-calcineurin-NFAT functional status has been demonstrated to determine cardiac hypertrophic response. Later, AKAP79 was identified as binding to a second kinase, PKC, which can act on an overlapping but distinct set of substrates [54]. High amounts of AKAP79 are present at the site of mammalian excitatory synapses [4,16]. AKAP79 exists in a macromolecular complex with the synaptic AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) glutamate receptor to regulate postsynaptic excitatory transmission and AKAP-directed kinase/phosphatase complexes control synaptic plasticity [4,16,100]. These AKAP-centered mechanisms form the very basis of the process of learning and memory. It has been recently found that many AKAP-centered complexes regulate mitochondrial respiration, dynamics, and cellular apoptosis and determine cellular response to hypoxic conditions [79,106].

It is interesting that certain scaffolding proteins can tether AKAPs, allowing a macromolecular assembly to be functionally regulated in a highly compartmentalized manner. Scaffolding proteins Na^+/H^+ exchange regulatory factors (NHERFs) can bind ezrin, an AKAP, via its C-terminal ezrin/radixin/moesin (ERM) domain that positions PKA close to the CFTR and enables instantaneous phosphorylation-dependent channel regulation [30,70]. AKAP79 is tethered close to AMPA receptors by synaptic scaffolding proteins, SAP97 and PSD-95 and fine-tunes receptor activity[4].

AKAP-Lbc is a large, multifunctional anchoring protein that binds and is regulated by multiple kinases and can also act as a GEF for the small GTPase RhoA [10,25,26,29,93,108]. It is speculated that phosphatase or PDE activity may be associated with the AKAP-Lbc complex [93]. AKAP-Lbc is a signalosome with many associated functionalities and definitely represents the next step in more efficient and rapid signaling processes.

Compartmentalization of calcium signaling: a definite mention

Calcium is a vital molecule inside the cell which as we already stated participates in many cAMP-effector pathways. In order to accentuate how critical this molecule is for life processes, let us briefly concentrate on pathways governed by calcium and how the process of compartmentalization can be incorporated into those pathways. Similar to cyclic-nucleotides, compartmentalization of calcium forms the basis of large diversity of calcium regulated functions. Calcium signaling fundamentally starts with calcium mobilizing events essentially a function of coordination between release of internal calcium from ER localized channels (primarily inositol 1,4,5-trisphosphate receptor; IP3R) and external entry from voltage-operated, receptor-operated and store-operated channels[5,19]. Amounts of calcium inside the cell can be quite dramatic and can reach upto 1 μM upon stimulation. Therefore, it is imperative that the calcium signaling is well-regulated, controlled and compartmentalized. The components that enable calcium-dependent signaling being so discrete results from the variance in parameters of calcium waves (in terms of amplitude, duration, frequency and spatial patterning) and the spatial arrangements of the targets and the calcium sources [19]. Calcium can acquire different signal forms including Ca^{2+} blips, Ca^{2+} quarks, Ca^{2+} sparks, Ca^{2+} flickers and Ca^{2+} puffs largely dependent upon the mechanism and intensity of the calcium trigger[46]. It is also known that certain caveolae are enriched with calcium-responsive proteins and serve as site of highly localized calcium signaling events[46]. Calcium-sensitive enzyme endothelial NOS (eNOS) is more responsive to calcium flux via store-operated channels at the plasma membrane (referred to as capacitative calcium entry)

than from internal ER stores. It has also been proposed that store-operated channels colocalize with eNOS within these caveolae [46]. The seminal examples of how discrete calcium signals are, include regulation of smooth muscle contraction and relaxation by global and local calcium signals respectively, and distinct routes of compartmentalized calcium signaling that would control neuronal cell death and survival [46]. Functional relevance of compartmental calcium has been elaborated in dendritic spines where sub-threshold synaptic stimulation would generate highly localized calcium transients dependent upon coordinated actions of glutamate receptors, intracellular calcium buffers and intracellular sequestration and efflux mechanisms [109]. A recently published study highlighted the compartmentalization of intracellular calcium signals as the basis to compartmentalized axonal activity of RIA interneurons that regulate head movement in *C. elegans* [44]. Wei et al. proposed that spatiotemporal organization of calcium microdomains steers fibroblast migration [104]. In the polarized exocrine cells calcium spikes are concentrated in the apical membrane (due to clustering of IP3Rs near the apical pole) and these spikes act fundamental to initiation of enzymatic secretions [2]. There are limitations to quantitative characterization of the microdomain calcium signaling using currently available calcium sensors and therefore, mathematical models are needed for precise spatial and temporal scale calculations of calcium signaling.

Compartmentalized cGMP signaling: an overview

cGMP is another important cyclic nucleotide messenger that shares many overlapping signaling pathways with cAMP and also owns many distinct pathways. Studies related to cGMP signaling have often been set aside due to the highly invested focus on studying cAMP pathways. However, this could not deter underscoring the significance of cGMP pathways in living systems. Robert F. Furchgott, Louis J. Ignarro and Ferid Murad won 1998 Nobel Prize in Medicine and Physiology for studying the significance of the nitric oxide (NO)-cGMP pathway in cardiovascular health. cGMP is an important mediator of biological functions such as vascular toning, cell proliferation and differentiation, epithelial electrolyte transport, migration, neurotransmission, and immunomodulation [61,103]. Therefore, it is critical to precisely regulate cGMP levels and cGMP-mediated effects inside the cells.

cGMP is synthesized by soluble (sGC) and particulate (pGC) forms of guanylyl cyclases (GCs). sGC is ubiquitously expressed and stimulated by NO [61]. Seven isoforms of pGCs have been identified, including the natriuretic peptide receptors GC-A, GC-B, and GC-C (which are responsive to bacterial heat-stable enterotoxins, guanylin, and uroguanylin) and the orphan receptors GC-D to GC-G which have unidentified ligands [61].

The process of cGMP compartmentalization is less understood compared to that of cAMP. It is likely that the compartmentalization of cGMP signaling in cells is enabled by mechanisms similar to those for the compartmentalization of cAMP. Similar to ACs, GCs can maintain distinct pools of cGMP as validated by more rapid natriuretic peptide-mediated stimulation of cyclic nucleotide-gated channels in smooth muscle cells compared to stimulation by NO donors [80]. Furthermore, responses of cGMP from the sGC pool and the pGC pool are well discriminated and can be quite distinct [80,97,103]. Recent studies have reported that GC-C can also communicate with intracellular p21-activated kinase via Rac GTPase to regulate cell migration and actin cytoskeletal restructuring [41]. This process would involve directed and localized cGMP signaling, as it is now being understood that asymmetric and segregated signaling processes are critical in the directed cell migration process [41,90]. Also, such cGMP modules have been shown to be vital to angiogenesis and vascular permeability in endothelial cells [103].

Despite the fact that sGC is cytosolic, recent studies have reported the existence of functional NOS-NO-sGC-cGMP pathways in plasmalemmal microdomains based on the observation that cellular membranes provide the predominant site of calcium-dependent NO synthesis [110]. A certain fraction of sGC translocalizes to the cellular membrane upon the calcium flux in cells, which renders it more sensitive to NO. Such translocalized sGC has been found to be active in caveolae, causing compartmentalized activation of PKG and PKA within endothelial caveolae upon eNOS activation [59,110]. Recent studies have indicated that a functional NO-cGMP signaling system exists during the early differentiation process of embryonic stem cells and that their spatiotemporal regulation would generate specific signals for important cell fate decisions and lineage determination [63].

cGMP-dependent protein kinases (PKGs) are the most important effectors of cGMP response. PKGs regulate many critical cellular functions and are fundamental to intracellular Ca^{2+} regulation and thus smooth muscle contraction and relaxation [103]. However, the field of G-kinase anchoring protein (GKAP) for the dissemination of PKG signaling is very new, and limited information is available. The effect of compartmentalization of cGMP on Na^+ absorption in the small intestine has been elucidated. The scaffolding protein NHERF2 has been shown to function as a GKAP to mediate cGMP-dependent regulation of NHE3 [11]. Membrane-anchored kinase cGK II can regulate cGMP-dependent activation of the CFTR, which likely occurs in restricted compartments. Given the fact that CFTR is a NHERF interacting protein, NHERF2 can act as GKAP for cGMP-mediated potentiation of CFTR chloride channel function [57]. Few other GKAPs that bind to cGKI or -II have been described, especially in the cardiovascular system. Vimentin, guanylate cyclase A, troponin T, myosin light chain kinase, and testicular golgi protein GKAP42 seem to act as GKAPs for cGK I [11].

Other indispensable components of compartmentalized cGMP signaling, the cGMP-catabolizing PDEs, have also been identified, including three cGMP-specific PDEs (PDE5, PDE6, and PDE9) and five dual-specific PDEs (PDE1, PDE2, PDE3, PDE10, and PDE11). In many instances, the cGMP pathway cross-talks with the cAMP pathway, usually facilitated by dual-specific PDEs such as PDE2 and PDE3 [83,112]. Through PDE2, cGMP negatively regulates cAMP signals and thus affects cardiac function. PDE2 localizes at the membrane rafts and can influence the activity of other raft-localized proteins, including - AR receptors, ACs, and NO synthases, which also allows cAMP and cGMP cross-communication [67]. PDE5 is specific for cGMP and localizes proximal to PKG and other Z-band localized proteins in cardiac myocytes [53,69]. This subcellular localization of PDE5 is dependent upon NOS-NO-cGMP signaling in the myocyte Z-band.

Compartmentalization of cGMP is as critical for cellular function as compartmentalization of cAMP. Apparently, compartmentalization of any rapidly diffusible, ubiquitous and prevalent moieties, which are fundamentally carriers of information inside the cell, is a necessity for a cell to function properly.

Consequences of dysregulated compartmentalized cyclic nucleotide signaling

The pathophysiological consequences of dysregulated protein signaling complexes involving compartmentalized cAMP or cGMP signaling have been mostly inferred from knockout animal studies. Most of the studies pertain to cardiovascular dysfunctions. PDE4 is a major cAMP-catabolizing enzyme of the cardiovascular system [12,17]. PDE4D has been found to closely correlate to stroke-susceptibility [40]. PDE4D null mice suffer from late-onset dilated cardiomyopathy [56]. It is understood that signal termination is equally critical as signal onset. Absence of PDE4D in a macromolecular complex of PKA, mAkap, and the

ryanodine receptor causes constitutive phosphorylation of this channel which renders this calcium channel leaky [107]. Simultaneous malfunction of excitation-contraction coupling regulated by PDE4D and β -AR leads to progressive cardiac failure and increased incidence of arrhythmias upon exercise/stress in PDE4D null-mice [17,107]. It is also noted that uncoupling of β -AR from the localized pool of PKA activity in cardiac myocytes can result in heart failure [72]. Interestingly, PDE4D activity can become limiting for certain therapies as PDE4D-dependent desensitization of β -AR leads to biased agonism of β -AR, which implies that treatment for COPD and asthma involving the use of β -AR agonists to relieve bronchoconstriction becomes refractory in the long-term [22]. Association of some alleles of PDE4B with schizophrenia has also been reported [17].

Mutations in PDE6 have been shown to cause impaired cGMP-gated channel regulation in rod cells, with autosomal recessive mutation in alpha subunits of PDE6 being associated with retinitis pigmentosa in males [35]. PDE11-associated mutations have been linked to endocrinopathy in humans but evidence of equivalence in mice is lacking in these studies [17].

In the vascular system, PDE5 represents the major metabolic pathway for cGMP [12]. PDE5A silencing has been connected to cardiac hypertrophy [103]. It is also known that the PDE5-NO-cGMP signal cascade regulates acute and chronic cardiac stress. Deficiencies of natriuretic peptide receptor or GC-A PKG-I and certain NOSs are all associated with cardiac hypertrophy and arterial hypertension [60,74]. The absence of a functional NO-cGMP pathway, pGC, and compromised dual-specificity PDE activities have been shown to cause defective neovascularization and angiogenesis, and leaky endothelium as well [8,13,37,71,86,98]. PDE5 inhibitors have been tested in patients with vasculoproliferative disorders such as pulmonary hypertension, and the PDE5 inhibitor derivative Viagra® has been commercialized for treatment of erectile dysfunction in males, which indicates the important role of compartmentalized regulation of cGMP in reproductive biology [12,85].

Recent studies suggest that silencing MRP4 negatively regulates proliferation of smooth muscle cells and therefore can be beneficial for the treatment of pulmonary arterial hypertension [42,87]. On the other hand, stimulation of MRP4 can attenuate diarrhea, as validated by the exacerbated CTX-induced secretory diarrhea seen in *Mrp4*^{-/-} mice [58]. The findings may be therapeutically extended to other forms of diarrhea in irritable bowel syndrome, diverticulitis and inflammatory bowel diseases. Overexpression of MRP4 was shown to delay wound repair by decreasing fibroblast migration rate, which implies that a certain threshold of local cyclic nucleotide is important for maintaining tissue integrity [91].

There have been accumulating evidences correlating the perturbations at the level of AKAP-PKA signaling to cardiac diseases. Overstimulated calcineurin/NFAT activity in the absence of AKAP79 leads to cardiac hypertrophy [65,96]. Heart-failure has been correlated with the upregulated expression of the AKAPs: AKAP-Lbc, AKAP18, AKAP2, and SPHKAP which results in increased number of PKA activity foci and Ca²⁺ reabsorption altering myocardial contractility[79]. With the involvement of AKAP-mediated complexes in synaptic plasticity, loss of these complexes leads to defects in hippocampal learning and memory[4].

We recently demonstrated that inflammatory bowel disorders (IBDs) are characterized by specialized inducible NOS (iNOS)-governed cGMP microdomains at the plasma membrane (unpublished data). The CFTR was found in a macromolecular signaling complex with iNOS within these microdomains, which potentiates CFTR function in a dysregulated manner and causes diarrhea in IBD. With a linear correlation between iNOS function and

IBD activity and numerous cellular functions dependent upon cGMP, it is imperative that such cGMP microdomains would have a predominant role in IBD pathophysiology.

Concluding remarks

A cell possesses distinct set of cellular messengers and their dependent pathways often overlap. Therefore, it is important to address which factors segregate and allow cross-communication of these pathways. The determinants would be first, composition of the signal node whether it involves progenitors and effectors of multiple and overlapping sensitivities capable of unique biological roles, second, how fast the signaling process is neutralized, whether it is transient or stable and how it fits in the frame of temporal and spatial existence of transducers and effectors. Intuitively, signal cross-talk pertains to permutation and combination of signaling components that further augments the signal diversity. A simple scheme of a compartmentalized cyclic nucleotide response is depicted in Figure 1.

The concept of compartmentalized signaling as of today is astonishingly advanced and appreciated and truly, the crude cytosolic assessments of the changes in cellular messengers are leveling out. This can be primarily attributed to the need for us to understand how compartmentalized signaling is fundamentally important to cellular behavior in health and disease and the availability of sophisticated tools/probes that allow very efficient monitoring of cellular processes. With the FRET based cyclic nucleotide sensors we are now able to monitor the compartmentalized cyclic nucleotide dynamics in live cells. The generation of target specific sensors allows us to understand plasma membrane microdomains- and organelle-specific signal compartmentalization. A recent microscopy technique, two-photon excitation integrated to FRET, has advanced the analysis of microdomain signaling by preventing photodamage of the sample, increasing signal to background ratio and high-quality imaging of the highly scattering tissue like brain slices. Total internal reflection microscopy allows plasma membrane restricted visualization of the signaling processes. Single-particle tracking microscopy module to visualize quantum-dot labeled molecules can add more information about how the surface molecules are arranged spatially to effect compartmentalized signaling. Other than these technologies, compartmentalized signaling surveillance can be studied with photosensitive caged compounds (modified cAMP, cGMP, calcium, and other biomolecules containing photoremovable protective group) which allow the resolution of global and localized signaling events by spatial and temporal manipulation of the uncaging beam [32]. Specific filters that allow isolating solely cellular extensions (e.g. pseudopods) can be used to differentiate the cyclic-nucleotide response in these extensions from the cell body [48].

It is now known that many scaffold-associated proteins are implicated in cancer and neurodegenerative disorders [24]. It prompts us to consider that targeting a protein complex rather than a single protein is a better therapeutic approach, as it allows the simultaneous control of multiple signaling cascades in complex diseases by hitting a single protein rather than multiple disease-related proteins.

Acknowledgments

We thank Ms. Jin Emerson-Cobb for editing the manuscript. This work was supported by National Institute of Health (NIH) grants DK080834 and DK093045 and Cystic Fibrosis Foundation NAREN12PO.

References

1. Abrahamsen H, Baillie G, Ngai J, Vang T, Nika K, Ruppelt A, Mustelin T, Zaccolo M, Houslay M, Tasken K. TCR- and CD28-mediated recruitment of phosphodiesterase 4 to lipid rafts potentiates TCR signaling. *J Immunol.* 2004; 173:4847–4858. [PubMed: 15470025]
2. Ashby MC, Tepikin AV. Polarized calcium and calmodulin signaling in secretory epithelia. *Physiol Rev.* 2002; 82:701–734. [PubMed: 12087133]
3. Baillie GS, Sood A, McPhee I, Gall I, Perry SJ, Lefkowitz RJ, Houslay MD. beta-Arrestin-mediated PDE4 cAMP phosphodiesterase recruitment regulates beta-adrenoceptor switching from Gs to Gi. *Proc Natl Acad Sci U S A.* 2003; 100:940–945. [PubMed: 12552097]
4. Bauman AL, Goehring AS, Scott JD. Orchestration of synaptic plasticity through AKAP signaling complexes. *Neuropharmacology.* 2004; 46:299–310. [PubMed: 14975685]
5. Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. *Nat Rev Mol Cell Biol.* 2000; 1:11–21. [PubMed: 11413485]
6. Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature.* 1993; 361:31–39. [PubMed: 8421494]
7. Bulenger S, Marullo S, Bouvier M. Emerging role of homo- and heterodimerization in G-protein-coupled receptor biosynthesis and maturation. *Trends Pharmacol Sci.* 2005; 26:131–137. [PubMed: 15749158]
8. Bussolati B, Dunk C, Grohman M, Kontos CD, Mason J, Ahmed A. Vascular endothelial growth factor receptor-1 modulates vascular endothelial growth factor-mediated angiogenesis via nitric oxide. *Am J Pathol.* 2001; 159:993–1008. [PubMed: 11549592]
9. Buxton IL, Brunton LL. Compartments of cyclic AMP and protein kinase in mammalian cardiomyocytes. *J Biol Chem.* 1983; 258:10233–10239. [PubMed: 6309796]
10. Carnegie GK, Smith FD, McConnachie G, Langeberg LK, Scott JD. AKAP-Lbc nucleates a protein kinase D activation scaffold. *Mol Cell.* 2004; 15:889–899. [PubMed: 15383279]
11. Cha B, Kim JH, Hut H, Hogema BM, Nadarja J, Zizak M, Cavet M, Lee-Kwon W, Lohmann SM, Smolenski A, Tse CM, Yun C, de Jonge HR, Donowitz M. cGMP inhibition of Na⁺/H⁺ antiporter 3 (NHE3) requires PDZ domain adapter NHERF2, a broad specificity protein kinase G-anchoring protein. *J Biol Chem.* 2005; 280:16642–16650. [PubMed: 15722341]
12. Cheepala S, Hulot JS, Morgan JA, Sassi Y, Zhang W, Naren AP, Schuetz JD. Cyclic Nucleotide Compartmentalization: Contributions of Phosphodiesterases and ATP-Binding Cassette Transporters. *Annu Rev Pharmacol Toxicol.* 2013; 53:231–253. [PubMed: 23072381]
13. Chen H, Levine YC, Golan DE, Michel T, Lin AJ. Atrial natriuretic peptide-initiated cGMP pathways regulate vasodilator-stimulated phosphoprotein phosphorylation and angiogenesis in vascular endothelium. *J Biol Chem.* 2008; 283:4439–4447. [PubMed: 18079117]
14. Chen Y, Cann MJ, Litvin TN, Iourgenko V, Sinclair ML, Levin LR, Buck J. Soluble adenylyl cyclase as an evolutionarily conserved bicarbonate sensor. *Science.* 2000; 289:625–628. [PubMed: 10915626]
15. Coghlan VM, Perrino BA, Howard M, Langeberg LK, Hicks JB, Gallatin WM, Scott JD. Association of protein kinase A and protein phosphatase 2B with a common anchoring protein. *Science.* 1995; 267:108–111. [PubMed: 7528941]
16. Colledge M, Dean RA, Scott GK, Langeberg LK, Haganir RL, Scott JD. Targeting of PKA to glutamate receptors through a MAGUK-AKAP complex. *Neuron.* 2000; 27:107–119. [PubMed: 10939335]
17. Conti M, Beavo J. Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling. *Annu Rev Biochem.* 2007; 76:481–511. [PubMed: 17376027]
18. Cooper DM, Crossthwaite AJ. Higher-order organization and regulation of adenylyl cyclases. *Trends Pharmacol Sci.* 2006; 27:426–431. [PubMed: 16820220]
19. Cullen PJ, Lockyer PJ. Integration of calcium and Ras signalling. *Nat Rev Mol Cell Biol.* 2002; 3:339–348. [PubMed: 11988768]
20. Dao KK, Teigen K, Kopperud R, Hodneland E, Schwede F, Christensen AE, Martinez A, Doskeland SO. Epac1 and cAMP-dependent protein kinase holoenzyme have similar cAMP

- affinity, but their cAMP domains have distinct structural features and cyclic nucleotide recognition. *J Biol Chem.* 2006; 281:21500–21511. [PubMed: 16728394]
21. Davare MA, Avdonin V, Hall DD, Peden EM, Burette A, Weinberg RJ, Horne MC, Hoshi T, Hell JW. A beta2 adrenergic receptor signaling complex assembled with the Ca²⁺ channel Cav1.2. *Science.* 2001; 293:98–101. [PubMed: 11441182]
 22. Dekkers BG, Racke K, Schmidt M. Distinct PKA and Epac compartmentalization in airway function and plasticity. *Pharmacol Ther.* 2013; 137:248–265. [PubMed: 23089371]
 23. Dessauer CW. Adenylyl cyclase--A-kinase anchoring protein complexes: the next dimension in cAMP signaling. *Mol Pharmacol.* 2009; 76:935–941. [PubMed: 19684092]
 24. Dev KK. Making protein interactions druggable: targeting PDZ domains. *Nat Rev Drug Discov.* 2004; 3:1047–1056. [PubMed: 15573103]
 25. Diviani D, Abuin L, Cotecchia S, Pansier L. Anchoring of both PKA and 14-3-3 inhibits the Rho-GEF activity of the AKAP-Lbc signaling complex. *EMBO J.* 2004; 23:2811–2820. [PubMed: 15229649]
 26. Diviani D, Soderling J, Scott JD. AKAP-Lbc anchors protein kinase A and nucleates Galpha 12-selective Rho-mediated stress fiber formation. *J Biol Chem.* 2001; 276:44247–44257. [PubMed: 11546812]
 27. Dodge-Kafka KL, Souhayer J, Pare GC, Carlisle Michel JJ, Langeberg LK, Kapiloff MS, Scott JD. The protein kinase A anchoring protein mAKAP coordinates two integrated cAMP effector pathways. *Nature.* 2005; 437:574–578. [PubMed: 16177794]
 28. Dodge KL, Khouangsathiene S, Kapiloff MS, Mouton R, Hill EV, Houslay MD, Langeberg LK, Scott JD. mAKAP assembles a protein kinase A/PDE4 phosphodiesterase cAMP signaling module. *EMBO J.* 2001; 20:1921–1930. [PubMed: 11296225]
 29. Dougherty MK, Morrison DK. Unlocking the code of 14-3-3. *J Cell Sci.* 2004; 117:1875–1884. [PubMed: 15090593]
 30. Dransfield DT, Bradford AJ, Smith J, Martin M, Roy C, Mangeat PH, Goldenring JR. Ezrin is a cyclic AMP-dependent protein kinase anchoring protein. *EMBO J.* 1997; 16:35–43. [PubMed: 9009265]
 31. Dyachok O, Isakov Y, Sagetorp J, Tengholm A. Oscillations of cyclic AMP in hormone-stimulated insulin-secreting beta-cells. *Nature.* 2006; 439:349–352. [PubMed: 16421574]
 32. Ellis-Davies GC. Caged compounds: photorelease technology for control of cellular chemistry and physiology. *Nat Methods.* 2007; 4:619–628. [PubMed: 17664946]
 33. Fagan KA, Rich TC, Tolman S, Schaack J, Karpen JW, Cooper DM. Adenovirus-mediated expression of an olfactory cyclic nucleotide-gated channel regulates the endogenous Ca²⁺-inhibitable adenylyl cyclase in C6-2B glioma cells. *J Biol Chem.* 1999; 274:12445–12453. [PubMed: 10212219]
 34. Fagan KA, Smith KE, Cooper DM. Regulation of the Ca²⁺-inhibitable adenylyl cyclase type VI by capacitative Ca²⁺ entry requires localization in cholesterol-rich domains. *J Biol Chem.* 2000; 275:26530–26537. [PubMed: 10843990]
 35. Farber DB, Danciger M. Identification of genes causing photoreceptor degenerations leading to blindness. *Curr Opin Neurobiol.* 1997; 7:666–673. [PubMed: 9384551]
 36. Fredriksson R, Schioth HB. The repertoire of G-protein-coupled receptors in fully sequenced genomes. *Mol Pharmacol.* 2005; 67:1414–1425. [PubMed: 15687224]
 37. Furst R, Bubik MF, Bihari P, Mayer BA, Khandoga AG, Hoffmann F, Rehberg M, Krombach F, Zahler S, Vollmar AM. Atrial natriuretic peptide protects against histamine-induced endothelial barrier dysfunction in vivo. *Mol Pharmacol.* 2008; 74:1–8. [PubMed: 18413663]
 38. Gabriel SE, Brigman KN, Koller BH, Boucher RC, Stutts MJ. Cystic fibrosis heterozygote resistance to cholera toxin in the cystic fibrosis mouse model. *Science.* 1994; 266:107–109. [PubMed: 7524148]
 39. Gorbunova YV, Spitzer NC. Dynamic interactions of cyclic AMP transients and spontaneous Ca(2+) spikes. *Nature.* 2002; 418:93–96. [PubMed: 12097913]
 40. Gretarsdottir S, Sveinbjornsdottir S, Jonsson HH, Jakobsson F, Einarsson U, Agnarsson U, Shkolny D, Einarsson G, Gudjonsdottir HM, Valdimarsson EM, Einarsson OB, Thorgeirsson G, Hadzic R, Jonsdottir S, Reynisdottir ST, Bjarnadottir SM, Gudmundsdottir T, Gudlaugsdottir GJ,

- Gill R, Lindpaintner K, Sainz J, Hannesson HH, Sigurdsson GT, Frigge ML, Kong A, Gudnason V, Stefansson K, Gulcher JR. Localization of a susceptibility gene for common forms of stroke to 5q12. *Am J Hum Genet.* 2002; 70:593–603. [PubMed: 11833004]
41. Guo D, Tan YC, Wang D, Madhusoodanan KS, Zheng Y, Maack T, Zhang JJ, Huang XY. A Rac-cGMP signaling pathway. *Cell.* 2007; 128:341–355. [PubMed: 17254971]
 42. Hara Y, Sassi Y, Guibert C, Gambaryan N, Dorfmueller P, Eddahibi S, Lompre AM, Humbert M, Hulot JS. Inhibition of MRP4 prevents and reverses pulmonary hypertension in mice. *J Clin Invest.* 2011; 121:2888–2897. [PubMed: 21670499]
 43. Haugh JM. Localization of receptor-mediated signal transduction pathways: the inside story. *Mol Interv.* 2002; 2:292–307. [PubMed: 14993384]
 44. Hendricks M, Ha H, Maffey N, Zhang Y. Compartmentalized calcium dynamics in a *C. elegans* interneuron encode head movement. *Nature.* 2012; 487:99–103. [PubMed: 22722842]
 45. Honda A, Adams SR, Sawyer CL, Lev-Ram V, Tsien RY, Dostmann WR. Spatiotemporal dynamics of guanosine 3',5'-cyclic monophosphate revealed by a genetically encoded, fluorescent indicator. *Proc Natl Acad Sci U S A.* 2001; 98:2437–2442. [PubMed: 11226257]
 46. Hong D, Jaron D, Buerk DG, Barbee KA. Transport-dependent calcium signaling in spatially segregated cellular caveolar domains. *Am J Physiol Cell Physiol.* 2008; 294:C856–866. [PubMed: 18160488]
 47. Houslay MD, Adams DR. PDE4 cAMP phosphodiesterases: modular enzymes that orchestrate signalling cross-talk, desensitization and compartmentalization. *Biochem J.* 2003; 370:1–18. [PubMed: 12444918]
 48. Howe AK, Baldor LC, Hogan BP. Spatial regulation of the cAMP-dependent protein kinase during chemotactic cell migration. *Proc Natl Acad Sci U S A.* 2005; 102:14320–14325. [PubMed: 16176981]
 49. Huang C, Hepler JR, Chen LT, Gilman AG, Anderson RG, Mumby SM. Organization of G proteins and adenylyl cyclase at the plasma membrane. *Mol Biol Cell.* 1997; 8:2365–2378. [PubMed: 9398661]
 50. Huang P, Lazarowski ER, Tarran R, Milgram SL, Boucher RC, Stutts MJ. Compartmentalized autocrine signaling to cystic fibrosis transmembrane conductance regulator at the apical membrane of airway epithelial cells. *Proc Natl Acad Sci U S A.* 2001; 98:14120–14125. [PubMed: 11707576]
 51. Jackson EK, Raghvendra DK. The extracellular cyclic AMP-adenosine pathway in renal physiology. *Annu Rev Physiol.* 2004; 66:571–599. [PubMed: 14977414]
 52. Jackson EK, Ren J, Cheng D, Mi Z. Extracellular cAMP-adenosine pathways in the mouse kidney. *Am J Physiol Renal Physiol.* 2011; 301:F565–573. [PubMed: 21653635]
 53. Kass DA, Takimoto E, Nagayama T, Champion HC. Phosphodiesterase regulation of nitric oxide signaling. *Cardiovasc Res.* 2007; 75:303–314. [PubMed: 17467673]
 54. Klauck TM, Faux MC, Labudda K, Langeberg LK, Jaken S, Scott JD. Coordination of three signaling enzymes by AKAP79, a mammalian scaffold protein. *Science.* 1996; 271:1589–1592. [PubMed: 8599116]
 55. Landa LR Jr, Harbeck M, Kaihara K, Chepurny O, Kitiphongspattana K, Graf O, Nikolaev VO, Lohse MJ, Holz GG, Roe MW. Interplay of Ca²⁺ and cAMP signaling in the insulin-secreting MIN6 beta-cell line. *J Biol Chem.* 2005; 280:31294–31302. [PubMed: 15987680]
 56. Lehnart SE, Wehrens XH, Reiken S, Warrier S, Belevych AE, Harvey RD, Richter W, Jin SL, Conti M, Marks AR. Phosphodiesterase 4D deficiency in the ryanodine-receptor complex promotes heart failure and arrhythmias. *Cell.* 2005; 123:25–35. [PubMed: 16213210]
 57. Li C, Dandridge KS, Di A, Marrs KL, Harris EL, Roy K, Jackson JS, Makarova NV, Fujiwara Y, Farrar PL, Nelson DJ, Tigyi GJ, Naren AP. Lysophosphatidic acid inhibits cholera toxin-induced secretory diarrhea through CFTR-dependent protein interactions. *J Exp Med.* 2005; 202:975–986. [PubMed: 16203867]
 58. Li C, Krishnamurthy PC, Penmatsa H, Marrs KL, Wang XQ, Zaccolo M, Jalink K, Li M, Nelson DJ, Schuetz JD, Naren AP. Spatiotemporal coupling of cAMP transporter to CFTR chloride channel function in the gut epithelia. *Cell.* 2007; 131:940–951. [PubMed: 18045536]

59. Linder AE, McCluskey LP, Cole KR 3rd, Lanning KM, Webb RC. Dynamic association of nitric oxide downstream signaling molecules with endothelial caveolin-1 in rat aorta. *J Pharmacol Exp Ther.* 2005; 314:9–15. [PubMed: 15778264]
60. Lopez MJ, Wong SK, Kishimoto I, Dubois S, Mach V, Friesen J, Garbers DL, Beuve A. Salt-resistant hypertension in mice lacking the guanylyl cyclase-A receptor for atrial natriuretic peptide. *Nature.* 1995; 378:65–68. [PubMed: 7477288]
61. Lucas KA, Pitari GM, Kazerounian S, Ruiz-Stewart I, Park J, Schulz S, Chepenik KP, Waldman SA. Guanylyl cyclases and signaling by cyclic GMP. *Pharmacol Rev.* 2000; 52:375–414. [PubMed: 10977868]
62. Lynch MJ, Baillie GS, Houslay MD. 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.* 2007; 35:938–941. [PubMed: 17956250]
63. Madhusoodanan KS, Murad F. NO-cGMP signaling and regenerative medicine involving stem cells. *Neurochem Res.* 2007; 32:681–694. [PubMed: 17043768]
64. McConnachie G, Langeberg LK, Scott JD. AKAP signaling complexes: getting to the heart of the matter. *Trends Mol Med.* 2006; 12:317–323. [PubMed: 16809066]
65. Molkenstein JD, Lu JR, Antos CL, Markham B, Richardson J, Robbins J, Grant SR, Olson EN. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell.* 1998; 93:215–228. [PubMed: 9568714]
66. Mongillo M, McSorley T, Evellin S, Sood A, Lissandron V, Terrin A, Huston E, Hannawacker A, Lohse MJ, Pozzan T, Houslay MD, Zaccolo M. Fluorescence resonance energy transfer-based analysis of cAMP dynamics in live neonatal rat cardiac myocytes reveals distinct functions of compartmentalized phosphodiesterases. *Circ Res.* 2004; 95:67–75. [PubMed: 15178638]
67. Mongillo M, Tocchetti CG, Terrin A, Lissandron V, Cheung YF, Dostmann WR, Pozzan T, Kass DA, Paolucci N, Houslay MD, Zaccolo M. Compartmentalized phosphodiesterase-2 activity blunts beta-adrenergic cardiac inotropy via an NO/cGMP-dependent pathway. *Circ Res.* 2006; 98:226–234. [PubMed: 16357307]
68. Mor A, Philips MR. Compartmentalized Ras/MAPK signaling. *Annu Rev Immunol.* 2006; 24:771–800. [PubMed: 16551266]
69. Nagayama T, Zhang M, Hsu S, Takimoto E, Kass DA. Sustained soluble guanylate cyclase stimulation offsets nitric-oxide synthase inhibition to restore acute cardiac modulation by sildenafil. *J Pharmacol Exp Ther.* 2008; 326:380–387. [PubMed: 18456872]
70. Naren AP, Cobb B, Li C, Roy K, Nelson D, Heda GD, Liao J, Kirk KL, Sorscher EJ, Hanrahan J, Clancy JP. A macromolecular complex of beta 2 adrenergic receptor, CFTR, and ezrin/radixin/moesin-binding phosphoprotein 50 is regulated by PKA. *Proc Natl Acad Sci U S A.* 2003; 100:342–346. [PubMed: 12502786]
71. Netherton SJ, Maurice DH. Vascular endothelial cell cyclic nucleotide phosphodiesterases and regulated cell migration: implications in angiogenesis. *Mol Pharmacol.* 2005; 67:263–272. [PubMed: 15475573]
72. Nikolaev VO, Moshkov A, Lyon AR, Miragoli M, Novak P, Paur H, Lohse MJ, Korchev YE, Harding SE, Gorelik J. Beta2-adrenergic receptor redistribution in heart failure changes cAMP compartmentation. *Science.* 2010; 327:1653–1657. [PubMed: 20185685]
73. Okamoto T, Schlegel A, Scherer PE, Lisanti MP. Caveolins, a family of scaffolding proteins for organizing “preassembled signaling complexes” at the plasma membrane. *J Biol Chem.* 1998; 273:5419–5422. [PubMed: 9488658]
74. Oliver PM, Fox JE, Kim R, Rockman HA, Kim HS, Reddick RL, Pandey KN, Milgram SL, Smithies O, Maeda N. Hypertension, cardiac hypertrophy, and sudden death in mice lacking natriuretic peptide receptor A. *Proc Natl Acad Sci U S A.* 1997; 94:14730–14735. [PubMed: 9405681]
75. Ostrom RS, Gregorian C, Drenan RM, Xiang Y, Regan JW, Insel PA. Receptor number and caveolar co-localization determine receptor coupling efficiency to adenylyl cyclase. *J Biol Chem.* 2001; 276:42063–42069. [PubMed: 11533056]

76. Ostrom RS, Insel PA. The evolving role of lipid rafts and caveolae in G protein-coupled receptor signaling: implications for molecular pharmacology. *Br J Pharmacol.* 2004; 143:235–245. [PubMed: 15289291]
77. Patterson RL, van Rossum DB, Gill DL. Store-operated Ca²⁺ entry: evidence for a secretion-like coupling model. *Cell.* 1999; 98:487–499. [PubMed: 10481913]
78. Penmatsa H, Zhang W, Yarlagadda S, Li C, Conoley VG, Yue J, Bahouth SW, Buddington RK, Zhang G, Nelson DJ, Sonecha MD, Manganiello V, Wine JJ, Naren AP. Compartmentalized cyclic adenosine 3',5'-monophosphate at the plasma membrane clusters PDE3A and cystic fibrosis transmembrane conductance regulator into microdomains. *Mol Biol Cell.* 2010; 21:1097–1110. [PubMed: 20089840]
79. Perino A, Ghigo A, Scott JD, Hirsch E. Anchoring proteins as regulators of signaling pathways. *Circ Res.* 2012; 111:482–492. [PubMed: 22859670]
80. Piggott LA, Hassell KA, Berkova Z, Morris AP, Silberbach M, Rich TC. Natriuretic peptides and nitric oxide stimulate cGMP synthesis in different cellular compartments. *J Gen Physiol.* 2006; 128:3–14. [PubMed: 16769793]
81. Ponsioen B, Zhao J, Riedl J, Zwartkruis F, van der Krogt G, Zaccolo M, Moolenaar WH, Bos JL, Jalink K. Detecting cAMP-induced Epac activation by fluorescence resonance energy transfer: Epac as a novel cAMP indicator. *EMBO Rep.* 2004; 5:1176–1180. [PubMed: 15550931]
82. Rehmann H, Das J, Knipscheer P, Wittinghofer A, Bos JL. Structure of the cyclic-AMP-responsive exchange factor Epac2 in its auto-inhibited state. *Nature.* 2006; 439:625–628. [PubMed: 16452984]
83. Reiersen GW, Guo S, Mastronardi C, Licinio J, Wong ML. cGMP Signaling, Phosphodiesterases and Major Depressive Disorder. *Curr Neuropharmacol.* 2011; 9:715–727. [PubMed: 22654729]
84. Rich TC, Fagan KA, Nakata H, Schaack J, Cooper DM, Karpen JW. Cyclic nucleotide-gated channels colocalize with adenylyl cyclase in regions of restricted cAMP diffusion. *J Gen Physiol.* 2000; 116:147–161. [PubMed: 10919863]
85. Rosen RC, Kostis JB. Overview of phosphodiesterase 5 inhibition in erectile dysfunction. *Am J Cardiol.* 2003; 92:9M–18M.
86. Sabrane K, Kruse MN, Fabritz L, Zetsche B, Mitko D, Skryabin BV, Zwiener M, Baba HA, Yanagisawa M, Kuhn M. Vascular endothelium is critically involved in the hypotensive and hypovolemic actions of atrial natriuretic peptide. *J Clin Invest.* 2005; 115:1666–1674. [PubMed: 15931395]
87. Sassi Y, Lipskaia L, Vandecasteele G, Nikolaev VO, Hatem SN, Cohen Aubart F, Russel FG, Mougnot N, Vrignaud C, Lechat P, Lompre AM, Hulot JS. Multidrug resistance-associated protein 4 regulates cAMP-dependent signaling pathways and controls human and rat SMC proliferation. *J Clin Invest.* 2008; 118:2747–2757. [PubMed: 18636120]
88. Schwartz JH. The many dimensions of cAMP signaling. *Proc Natl Acad Sci U S A.* 2001; 98:13482–13484. [PubMed: 11717418]
89. Sears CL, Kaper JB. Enteric bacterial toxins: mechanisms of action and linkage to intestinal secretion. *Microbiol Rev.* 1996; 60:167–215. [PubMed: 8852900]
90. Sinha C, Ren A, Arora K, Moon CS, Yarlagadda S, Zhang W, Cheepala SB, Schuetz JD, Naren AP. Multi-drug resistance protein 4 (MRP4)-mediated regulation of fibroblast cell migration reflects a dichotomous role of intracellular cyclic nucleotides. *J Biol Chem.* 2012
91. Sinha C, Ren A, Arora K, Moon CS, Yarlagadda S, Zhang W, Cheepala SB, Schuetz JD, Naren AP. Multi-drug Resistance Protein 4 (MRP4)-mediated Regulation of Fibroblast Cell Migration Reflects a Dichotomous Role of Intracellular Cyclic Nucleotides. *J Biol Chem.* 2013; 288:3786–3794. [PubMed: 23264633]
92. Smith FD, Langeberg LK, Scott JD. The where's and when's of kinase anchoring. *Trends Biochem Sci.* 2006; 31:316–323. [PubMed: 16690317]
93. Smith FD, Scott JD. Anchored cAMP signaling: onward and upward - a short history of compartmentalized cAMP signal transduction. *Eur J Cell Biol.* 2006; 85:585–592. [PubMed: 16524641]

94. Smith KE, Gu C, Fagan KA, Hu B, Cooper DM. Residence of adenylyl cyclase type 8 in caveolae is necessary but not sufficient for regulation by capacitative Ca(2+) entry. *J Biol Chem.* 2002; 277:6025–6031. [PubMed: 11744699]
95. Stork PJ, Schmitt JM. Crosstalk between cAMP and MAP kinase signaling in the regulation of cell proliferation. *Trends Cell Biol.* 2002; 12:258–266. [PubMed: 12074885]
96. Sussman MA, Lim HW, Gude N, Taigen T, Olson EN, Robbins J, Colbert MC, Gualberto A, Wiecek DF, Molkentin JD. Prevention of cardiac hypertrophy in mice by calcineurin inhibition. *Science.* 1998; 281:1690–1693. [PubMed: 9733519]
97. Takimoto E, Belardi D, Tocchetti CG, Vahebi S, Cormaci G, Ketner EA, Moens AL, Champion HC, Kass DA. Compartmentalization of cardiac beta-adrenergic inotropy modulation by phosphodiesterase type 5. *Circulation.* 2007; 115:2159–2167. [PubMed: 17420342]
98. Tanabe M, Ueda M, Endo M, Kitajima M. The effect of atrial natriuretic peptide on pulmonary acid injury in a pig model. *Am J Respir Crit Care Med.* 1996; 154:1351–1356. [PubMed: 8912747]
99. Tasken K, Aandahl EM. Localized effects of cAMP mediated by distinct routes of protein kinase A. *Physiol Rev.* 2004; 84:137–167. [PubMed: 14715913]
100. Tavalin SJ, Colledge M, Hell JW, Langeberg LK, Huganir RL, Scott JD. Regulation of GluR1 by the A-kinase anchoring protein 79 (AKAP79) signaling complex shares properties with long-term depression. *J Neurosci.* 2002; 22:3044–3051. [PubMed: 11943807]
101. Tresguerres M, Levin LR, Buck J. Intracellular cAMP signaling by soluble adenylyl cyclase. *Kidney Int.* 2011; 79:1277–1288. [PubMed: 21490586]
102. Trewavas AJ, Malho R. Signal Perception and Transduction: The Origin of the Phenotype. *Plant Cell.* 1997; 9:1181–1195. [PubMed: 12237382]
103. Tsai EJ, Kass DA. Cyclic GMP signaling in cardiovascular pathophysiology and therapeutics. *Pharmacol Ther.* 2009; 122:216–238. [PubMed: 19306895]
104. Wei C, Wang X, Chen M, Ouyang K, Song LS, Cheng H. Calcium flickers steer cell migration. *Nature.* 2009; 457:901–905. [PubMed: 19118385]
105. Willoughby D, Cooper DM. Ca²⁺ stimulation of adenylyl cyclase generates dynamic oscillations in cyclic AMP. *J Cell Sci.* 2006; 119:828–836. [PubMed: 16478784]
106. Wong W, Goehring AS, Kapiloff MS, Langeberg LK, Scott JD. mAKAP compartmentalizes oxygen-dependent control of HIF-1α. *Sci Signal.* 2008; 1:ra18. [PubMed: 19109240]
107. Xiang Y, Naro F, Zoudilova M, Jin SL, Conti M, Kobilka B. Phosphodiesterase 4D is required for beta2 adrenoceptor subtype-specific signaling in cardiac myocytes. *Proc Natl Acad Sci U S A.* 2005; 102:909–914. [PubMed: 15644445]
108. Yuan J, Slice LW, Rozengurt E. Activation of protein kinase D by signaling through Rho and the α subunit of the heterotrimeric G protein G13. *J Biol Chem.* 2001; 276:38619–38627. [PubMed: 11507098]
109. Yuste R, Majewska A, Holthoff K. From form to function: calcium compartmentalization in dendritic spines. *Nat Neurosci.* 2000; 3:653–659. [PubMed: 10862697]
110. Zabel U, Kleinschnitz C, Oh P, Nedvetsky P, Smolenski A, Muller H, Kronich P, Kugler P, Walter U, Schnitzer JE, Schmidt HH. Calcium-dependent membrane association sensitizes soluble guanylyl cyclase to nitric oxide. *Nat Cell Biol.* 2002; 4:307–311. [PubMed: 11887187]
111. Zaccolo M, De Giorgi F, Cho CY, Feng L, Knapp T, Negulescu PA, Taylor SS, Tsien RY, Pozzan T. A genetically encoded, fluorescent indicator for cyclic AMP in living cells. *Nat Cell Biol.* 2000; 2:25–29. [PubMed: 10620803]
112. Zaccolo M, Movsesian MA. cAMP and cGMP signaling cross-talk: role of phosphodiesterases and implications for cardiac pathophysiology. *Circ Res.* 2007; 100:1569–1578. [PubMed: 17556670]
113. Zhang J, Ma Y, Taylor SS, Tsien RY. Genetically encoded reporters of protein kinase A activity reveal impact of substrate tethering. *Proc Natl Acad Sci U S A.* 2001; 98:14997–15002. [PubMed: 11752448]
114. Zippin JH, Farrell J, Huron D, Kamenetsky M, Hess KC, Fischman DA, Levin LR, Buck J. Bicarbonate-responsive “soluble” adenylyl cyclase defines a nuclear cAMP microdomain. *J Cell Biol.* 2004; 164:527–534. [PubMed: 14769862]

Figure 1.

An activated G protein coupled receptor (GPCR) triggers cAMP synthesis from membrane-bound adenylyl cyclase (AC) and subsequently, a cAMP-regulated microdomain is constituted in which a cAMP-responsive target (here CFTR) gets instantaneously regulated by controlled flux of cAMP reflected by a precise balance of synthesis by AC, extrusion by cAMP-efflux transporters (here MRP4) and catabolism by phosphodiesterases (PDEs). Formation of a macromolecular complex assembled onto specialized scaffolds that communicate with the cytoskeletal elements can be a theme of the cyclic-nucleotide microdomain. MRP4 and cGMP-specific PDE can also regulate the flow of cGMP synthesized by guanylate cyclase (GC) in the vicinity of cGMP-responsive proteins.

★ Indicates activated GPCR.

● cAMP

● cGMP