PHYSIOLOGY 28: 199–209, 2013; doi:10.1152/physiol.00004.2013

cAMP and Mitochondria

Federica Valsecchi,[1]*
Lavoisier S. Ramos-Espiritu,[2]*
Jochen Buck,[2] Lonny R. Levin,[2] and
Giovanni Manfredi[1]

[1]Department of Neurology and Neuroscience, Weill Medical College of Cornell University, New York; and [2]Department of Pharmacology, Weill Medical College of Cornell University, New York
gim2004@med.cornell.edu
*F. Valsecchi and L. S. Ramos-Espiritu contributed equally to this work.

Phosphorylation of mitochondrial proteins has emerged as a major regulatory mechanism for metabolic adaptation. cAMP signaling and PKA phosphorylation of mitochondrial proteins have just started to be investigated, and the presence of cAMP-generating enzymes and PKA inside mitochondria is still controversial. Here, we discuss the role of cAMP in regulating mitochondrial bioenergetics through protein phosphorylation and the evidence for soluble adenylyl cyclase as the source of cAMP inside mitochondria.

Mitochondria are traditionally viewed as the major source of cellular ATP and a hub of intermediate metabolism. Over the years, we have come to appreciate that mitochondria also perform several additional crucial functions in cell signaling and regulation (23, 50, 54, 55, 92, 106, 128), and mitochondrial dysfunction has been implicated in numerous human diseases. Genetic disorders affecting mitochondrial proteins encoded either by the nuclear or the mitochondrial genomes (primary mitochondrial disorders) are implicated in cancer, diabetes, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), and cardiomyopathy (98, 118, 119, 126, 127, 141).

To be able to respond to changes in substrate availability and bioenergetic demands, mitochondria require rapid, short-term, metabolic adaptation mechanisms. Over the past decade, numerous studies have linked posttranslational modifications of mitochondrial enzymes to the regulation of energy metabolism in mammalian cells. The molecular mechanisms underlying these regulations (i.e., how they are linked to metabolic needs) and their physiological significance are still under active investigation. Specifically, reversible phosphorylation of mitochondrial proteins has emerged as an important player in the regulation of mitochondrial oxidative metabolism. Yet, many of the protein kinases, their regulatory elements, and their molecular targets remain to be identified. Recently, a quantitative map of phosphoproteins in liver mitochondria was generated. This broad analysis identified 811 phosphosites (100 of which had not been described before) on 295 different mitochondrial proteins (58), implicating reversible phosphorylation as a fundamental regulatory mechanism of mitochondrial metabolism.

The ubiquitous second messenger cyclic AMP (cAMP) and its cellular effector protein kinase A (PKA) constitute one of the most widely studied signaling cascades, yet the roles of cAMP signaling and PKA phosphorylation of mitochondrial proteins in the regulation of mitochondrial metabolism remain controversial issues. This is because the source of cAMP in mitochondria, the precise localization of PKA, and the distinction between the effects of PKA acting inside or on the outside of mitochondria are often difficult to resolve. In this review, we summarize current knowledge on the mechanisms for regulating cAMP levels in mitochondria and the role of cAMP effectors in regulating mitochondrial energy metabolism and other relevant aspects of mitochondrial signaling.

General cAMP Signaling System

Cyclic AMP is generated from ATP via adenylyl cyclases (ACs) and degraded via phosphodiesterases (PDEs). In mammals, there are two types of class III adenylyl cyclases: membrane bound and soluble. There are nine genes encoding transmembrane adenylyl cyclases (tmAC) and a single gene encoding multiple isoforms of soluble adenylyl cyclase (sAC). TmACs are regulated by heterotrimeric G proteins and the pharmacological activator forskolin, whereas sAC is directly regulated by calcium (70, 88), physiological changes in ATP (88), and bicarbonate (29), which means its activity also reflects local fluctuations in CO_2 and pH_i (136). TmACs are thought to be exclusively restricted to the plasma membrane, with the notable exception that they can also be found in internalized endosomes and may signal as they traffic from the plasma membrane (22, 53). There are reports of heterotrimeric G-protein alpha subunits found in the mitochondria (7, 89), but, to date, no one has demonstrated presence of the tmAC stimulatory Gs protein nor tmACs at mitochondria. In contrast, there are several reports demonstrating sAC at mitochondria (discussed below).

The cAMP generated by adenylyl cyclases is tightly regulated by phosphodiesterases (PDE), which catabolize cAMP into 5′ AMP (12, 14, 32, 65). There are 11 known PDE families; some are specific for cAMP (PDEs 4, 7, 8), others for cGMP (PDEs 5, 6, 9), whereas the remainder (PDEs 1–3, 10, 11) catabolize both cAMP and cGMP. Each of

the PDE families includes multiple isoforms with distinct enzymatic characteristics, modes of regulation, expression patterns, and distribution throughout the cell (14, 31, 76, 96).

Cyclic AMP effector molecules contribute to the complexity and specificity of cAMP signaling. PKA, a well studied cAMP downstream effector, is a tetrameric enzyme consisting of two catalytic domains (C) and two regulatory domains (R). In mammals, there are three known isoforms of catalytic subunits (Cα, Cβ, Cγ) and four isoforms of regulatory subunits (RIα, RIβ, RIIα, RIIβ). cAMP binding to R releases active C subunits, which phosphorylate key substrates (77, 130, 131). A structurally diverse group of proteins called A kinase anchoring proteins (AKAPs) direct PKA to distinct subcellular sites (46, 144, 147). In addition to tethering PKA to its particular subcellular location, AKAPs also act as scaffolds to PKA's substrates, cyclases, PDEs, and protein phosphatases, facilitating regulation of the cAMP cascade (10, 47, 48, 82, 129, 146). Cyclic AMP can also mediate its effects via other effectors; cAMP modulates two types of cyclic nucleotide-regulated channels plus a family of guanine-nucleotide exchange factors (GEFs) called exchange proteins activated by cAMP (EPAC) (17, 64, 124). Cyclic nucleotide regulated channels include cyclic nucleotide-gated channels (cNGC) (15, 18, 36, 72) and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels (16, 108, 140, 155); however, neither has been reported to be found at mitochondria (34). There are two EPAC isoforms, EPAC1 and EPAC2 (also called cAMP-GEF1 and cAMP-GEF2), which are expressed in different tissues (113). EPACs, when bound to cAMP, activate the small GTPases Rap1 and Rap2 proteins and function via PKA-independent mechanism to stimulate phosphoinositide-hydrolysing phospholipase C epsolon (PLCε)-generating inositol-1,4,5-trisphosphate that regulates calcium intracellular signaling pathways (40, 41, 73, 85, 99, 100, 121).

Compartmentalization of cAMP Signaling

Cyclic AMP had long been assumed to be a freely diffusible second messenger (11, 67). However, the idea that cAMP signaling was compartmentalized first emerged in the late 1970s, when it was appreciated that, in a single cell type, different hormones or neurotransmitters stimulated cAMP production but resulted in different physiological outcomes. In heart, both PGE_1 and isoproterenol elevated cAMP, but only isoproterenol caused activation of phosphorylase kinase and inhibition of glycogen synthase (60–62, 74, 75). Yet cAMP compartmentalization did not gain widespread acceptance until Forster resonance energy transfer (FRET based) (45, 107, 151, 152) and biophysical methods (71, 111) for measuring cAMP in situ in living cells revealed that cAMP levels are not uniform within cells. Now it is widely accepted that intracellular membranes and/or PDEs targeted to discrete cellular locations limit the diffusion of cAMP pools (31, 150). Compartmentalization of cAMP signaling into a variety of independently regulated functional domains explains how a single second messenger can mediate a wide variety of physiological processes within a cell (FIGURE 1). In this context, cAMP functional compartments can be defined as pools of cAMP, which are confined in a restricted region of the cell, such as an organelle, and which are locally generated and regulated from other distinct parts of the cell. Immunocytochemistry revealed sAC to be distributed throughout the cytoplasm, at the centrioles, inside nuclei and mitochondria, on the mitotic spindle, and in the mid-body (153), suggesting it could be a local source of cAMP at these sites (21, 153, 154). In fact, sAC inside mitochondria now represents one of the best-characterized cAMP signaling domains.

cAMP Signaling in Mitochondria

There appear to be at least two independent cAMP domains that signal in mitochondria, one associated with the cytosolic-mitochondrial interface and a second one wholly contained inside the mitochondrial matrix. In regard to the source of cAMP in the mitochondrial matrix, two contradicting theories have emerged. The first, based on results obtained with mitochondrially targeted cAMP reporters, postulates that cAMP is generated in the cytosol and is then translocated into the mitochondrial matrix through yet unidentified transport mechanisms (45). The second, based on functional results, but not on direct identification of cAMP, affirms that the inner mitochondrial membrane is impermeable to cAMP and that matrix cAMP is generated locally by resident adenylyl cyclases, such as sAC (1, 2, 4). The availability of more sensitive and specific cAMP reporters correctly and exclusively targeted to the mitochondrial matrix will likely help resolve this issue. The pools of cAMP in mitochondria are postulated to activate PKA that resides in the various mitochondrial compartments. Although the presence of PKA on the mitochondrial outer membrane was extensively demonstrated, PKA was also found in the intermembrane space (see *cAMP effectors in mitochondria*) and in the matrix (29). The mechanisms that allow PKA to enter mitochondria, however, are still unknown, since the protein lacks apparent targeting signals. The following sections will outline some of the key aspects of cAMP signaling in mitochondria.

Generation of cAMP in Mitochondria

The earliest studies using sAC-specific antisera demonstrated co-localization with mitochondria by immunocytochemistry and subcellular fractionation (153). Parallel studies also showed bicarbonate-regulated (i.e., sAC-like) adenylyl cyclase activity enriched in mitochondrial fractions. Ladilov and coworkers confirmed these findings in primary cells, demonstrating sAC localization at mitochondria of coronary endothelial cells (83) and cardiac myocytes (9). In these cell types, mitochondrially localized sAC plays a role in apoptosis; in fact, in coronary endothelial cells, acidotic stress caused a translocation of sAC from the cytosol to mitochondria in what appears to be an early step in ischemia-induced apoptosis (83). The precise localization in relationship with mitochondria was not addressed, but it is likely to involve association with the outer membrane surface. sAC-generated cAMP plays at least one other role within mitochondria. Studying regulation of oxidative phosphorylation (OXPHOS), we found a difference between extra-mitochondrial cAMP and membrane-permeable cAMP analogs. In whole cells, membrane-permeable analogs of cAMP stimulated oxygen consumption, whereas forskolin stimulation of tmACs in concert with the broadly specific PDE inhibitor 3-isobutyl-1-methylxanthine (IBMX) had no effect (4). In contrast, it must be noted that in a different report the strong activation of tmAC by cholera toxin promoted serine phosphorylation in a subunit of complex I (CI), leading to increased respiration and CI activity (104, 117, 133) but no effect on the activity of cytochrome oxidase. Despite the partial discrepancy in the findings on the effects of extramitochondrial cAMP on respiratory activity, taken together, the data suggest that cAMP plays a role on mitochondrial respiration. In the matrix space, we proposed that the source of cAMP could be sAC, based on Western blot results and by demonstrating that cAMP generation in intact mitochondria could be stimulated by a sAC-specific activator (i.e., bicarbonate) and inhibited by a sAC-specific inhibitor (i.e., KH7). Other experiments independently confirmed this sAC-defined pathway resided within a membrane-protected compartment inside mitochondria. The pathway was sensitive to exogenously added PDE or an anti-sAC inhibitory monoclonal antibody, but only in sonicated mitochondria; intact mitochondria remained insensitive (4). We subsequently identified an isoform of PDE2A as a component of this intramitochondrial, sAC-defined domain. The unique NH_2 terminus of isoform 2 of PDE2A targets the enzyme to the mitochondrial matrix, where it opposes the action of sAC in regulating OXPHOS (2).

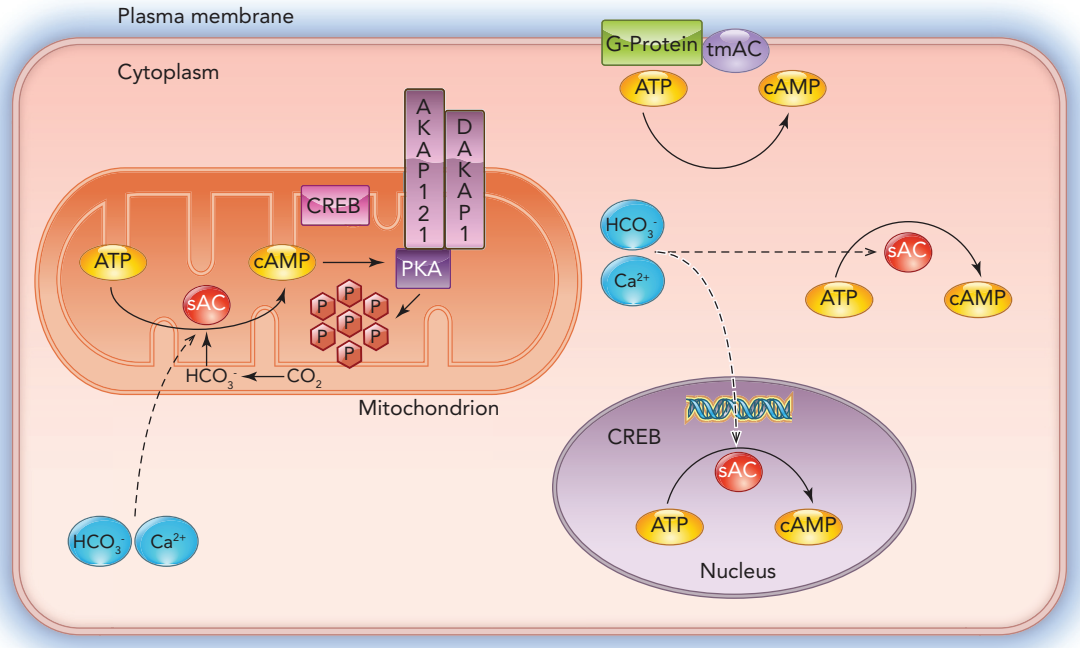

FIGURE 1. **cAMP-PKA-sAC compartmentalization**
In mammalian cells, cAMP is generated from ATP via the action of the transmembrane adenylyl cyclase (tmsAC) and G protein located in the plasma membrane or via the action of soluble adenylyl cyclase (sAC) located in the cytoplasm, in the nucleus, and in the mitochondrial matrix. sAC can be activated by Ca^{2+} and HCO_3^- derived from the extracellular space. In mitochondria, sAC can be activated by HCO_3^- generated from the CO_2 derived from the Krebs cycle via the action of carbonic anhydrase. CREB is not only localized in the nucleus but is also attached to the mitochondrial inner membrane. Specific AKAP mitochondrial proteins (AKAP121 and DAKAP1) interact with PKA to induce phosphorylation of mitochondrial substrates.

Future studies will have to determine the mechanisms of how sAC becomes localized in the mitochondrial matrix and of PKA import into mitochondria.

cAMP Effectors in Mitochondria

PKA has been proposed to be associated with, or in, mitochondria since the 1970s (43, 79, 123, 139). The sub-mitochondrial distribution of PKA appears to depend on the tissue, cell type, and method of detection utilized. Recently, Ma et al. showed that PKA is present in human placental mitochondria (91), and sucrose density gradient separation showed that the catalytic (C) subunit of PKA is predominantly found in the mitochondrial outer membrane. There is also considerable evidence that PKA is localized in the mitochondrial matrix (116, 123, 132). Schwoch et al. found a two-fold increase in PKA-specific activity in the mitochondrial matrix fraction relative to the whole mitochondrion, and they demonstrated that both forms of regulatory subunit (RI and RII) reside on the matrix side of the inner mitochondrial membrane in a variety of rat tissues, including liver, skeletal muscle, pancreas, and kidney (123). PKA was independently found in the inner mitochondrial membrane fraction from bovine heart (116, 132, 134). Although their abundances differed, both RI and RII subunits were found in the inner membrane and matrix of mitochondrial preparations, and the C subunit was found to be enriched in the matrix (116, 123, 132). These authors proposed the existence of a cAMP-PKA signaling cascade, stimulating the enzymatic activity of complex I, thereby increasing cellular respiration. In another study, in heart, hypoxia and ischemia increased mitochondrial PKA activity. The PKA-dependent phosphorylation of COX subunits (I, IVi1, and Vb) was postulated to reduce COX activity, leading to mitochondrial dysfunction in myocardial injury (109).

These studies, addressing PKA functions in different sub-compartments of mitochondria (outer, inner membrane, and matrix) utilized standard purification methods (i.e., density centrifugation, digitonin treatment), which suffer from specific drawbacks; for example, in studying intermembrane space, the use of digitonin could disrupt the inner membrane, resulting in matrix contamination of the intermembrane space. Recently, a fluorescent sensor of PKA actions has been developed to characterize cAMP-dependent PKA activity in intact mitochondria (5). The PKA sensor and quencher are small enough to move into and measure PKA activity in the intermembrane space. Addition of cAMP (together with the PKA sensor and quencher) to intact mitochondria measures PKA activity at both the outer membrane and the intermembrane space, whereas addition of cAMP to trypsin-treated mitochondria eliminates outer membrane activity and reveals PKA activity only within the intermembrane space. Finally, total PKA activity measured after sonication of untreated mitochondria includes matrix-localized PKA. Using these treatments, the relative distribution of PKA activity in the matrix, intermembrane space, and outer membrane was estimated to be 79%:8%:13%, respectively. These results were comparable with earlier reports, which found that 90% of PKA activity in mitochondria is located in the matrix/intermembrane space (115). A recent analysis of mitochondrial phosphopeptides identified over 100 potential PKA targets in mitochondrial proteins (58).

PKA localization can also be inferred from, or measured in conjunction with, mitochondrial AKAPs. AKAPs direct PKA to subcellular sites, and many AKAPs have been localized to mitochondria. There are numerous splice variants of AKAP1 (S-AKAP84, AKAP121, D-AKAP1, and AKAP149), which share an NH_2-terminal core but have distinct COOH termini, identified in mitochondria. S-AKAP84 binds RII and a known mitochondrial target sequence (87); AKAP121 binds and targets RIIα to the cytoplasmic surface of mitochondria (52, 115); D-AKAP1, which is a novel type of AKAP interacting with both type I and type II regulatory subunits of PKA, contains the same mitochondrial targeting signal sequence as S-AKAP84 (66); and AKAP79 was found in placental mitochondria (91). In addition, localization of a second dual-specific AKAP (D-AKAP2), recognizing both RI and RII forms of PKA, showed predominantly mitochondrial immunostaining (142).

A mitochondrial GTP-binding protein, Rab 32, was shown to function as an AKAP (6). Although it does not have a prototypical mitochondrial targeting sequence, Rab32 has a pair of COOH-terminal cysteines directing it to mitochondria. Rab32 has an important role in the regulation of mitochondrial fission.

Another mitochondrial protein, sphingosine kinase interacting protein (SKIP), was subsequently found to have AKAP activity specific for type I PKA (93). Immunoblots of mitochondrial subfractions revealed that SKIP is found in both the intermembrane space and the matrix of murine heart mitochondria, where it presumably tethers PKA.

Another cAMP downstream effector, EPAC, has been identified in mitochondria (27, 110, 143). EPAC1-based fluorescent reporters have been targeted to the mitochondria to study the dynamics of cAMP signaling in the organelle (45, 94). Moreover, the GTP-binding protein Rap1 is also found in mitochondria (78, 135). To date, the function of EPAC in mitochondria is still unknown, and its association with this organelle requires further investigation; but, if confirmed, EPAC presence in mitochondria could suggest the existence of a coordinated cAMP-GTPase signaling.

Mitochondrial Regulation by cAMP: Extra-Mitochondrial Effects

Control of Mitochondrial Morphology and Mitophagy

Mitochondria are highly dynamic organelles that undergo reshaping via fission and fusion events. In humans, dysfunction of these processes causes neurological disorders. The dynamin-related GTPase proteins regulating mitochondrial morphology include two mitofusin isoforms [mitofusin 1 (Mfn1), mitofusin 2 (Mfn2)], optic atrophy 1 (OPA1), dynamin-related protein 1 (Drp1), and fission protein 1 (Fis1) (13, 145). Mitochondrial morphology is directly linked with the activity of the electron transport chain (145), but the mechanisms correlating form with function remain poorly understood.

Cyclic AMP can regulate mitochondrial morphology. PKA phosphorylation of Ser637 on Drp1 (also referred to as Ser656 in different splice variants) blocks its translocation to the mitochondrial surface. Phosphorylated Drp1 promotes mitochondrial elongation by allowing fusion to proceed unopposed (25, 26, 35); dephosphorylated Drp1 promotes fission. Phosphomimetic mutants of Drp1 or overexpression of PKA on the outer mitochondrial membrane induces mitochondrial fusion, forming a neuroprotective reticulum and increasing neuronal survival (95). Furthermore, PKA located in the outer mitochondria and the phosphatase PP2A modulates neuronal development inhibiting or activating, respectively, mitochondrial division (35, 42). The process of mitophagy (the autophagy of mitochondria) depends on mitochondrial morphology, because mitochondria must fragment before removal via the autophagosome (57). Certain autophagy signals, such as amino acid or serum starvation, elevate cytoplasmic cAMP, leading to PKA-mediated Drp1 phosphorylation. Phosphorylated Drp1 promotes mitochondrial fusion that spares the mitochondria increasing cell survival (56).

Regulation of Mitochondrial Biogenesis: cAMP Response Element-Binding Protein

The cAMP response element-binding protein (CREB) is a ubiquitous transcription factor that regulates the transcription of cAMP response element-regulated genes (125, 148). It belongs to the CREB/ATF1 family of cAMP/Ca^{2+} responsive transcription factors. PKA phosphorylation activates CREB. Nuclear CREB regulates PGC-1α, the master regulator of mitochondrial biogenesis (30). Blocking CREB or PGC-1α in mouse hepatocytes inhibited mitochondrial biogenesis induced by pyrroloquinoline quinone (PQQ), a redox cofactor and antioxidant compound.

In addition to its nuclear roles, CREB is also found bound to the mitochondrial DNA (24, 86). In brain, mitochondrial CREB regulates mitochondrial gene expression and neuronal survival (86). CREB is thought to be transported to the mitochondria via chaperone molecules, such as Hsp70 (86). CREB uptake was promoted by the mitochondrial membrane potential and by TOM complex, responsible for the translocation of the proteins from the cytoplasm into the mitochondria (39). Once inside mitochondria, CREB interacts with cAMP response elements (CREs) in the mitochondrial DNA, thereby regulating expression of mitochondrial components of the electron transport chain. It appears to bind directly to the D-loop of mitochondrial DNA. Mitochondrial CREB phosphorylation was shown to regulate expression of mitochondrial DNA-encoded proteins, such as ND1, ND6, and COXIII/ATP6 (39). Based on experiments with either wild-type or Ser133 mutant mitochondrially targeted CREB, It was proposed that CREB can be phosphorylated by PKA within mitochondria (114). Nevertheless, these experiments did not exclude the possibility that CREB could be imported into mitochondria already in the phosphorylated state. Alteration in mitochondrial CREB activity affects the expression of mitochondrial genes ND2, ND4, and NDU5 (all subunits of CI), leading to impairment of related CI activity (86). CREB is also involved in redox state mechanisms, since phosphorylation of mitochondrial CREB at Ser133, in response to the antioxidant and the iron chelator deferoxamine, promoted survival in cortical neurons exposed to oxidative stress (114).

Regulation of Apoptosis

Apoptosis initiated at mitochondria involves a family of Bcl-related proteins, including both anti-apoptotic (Bcl-2, Bcl_{XL}) and pro-apoptotic (Bax, Bak, BAD, BIM, Bcl_{XS}) members. Pro-apoptotic Bcl-related proteins modulate the release of cytochrome c from mitochondria. The cAMP signaling pathway can promote pro-apoptotic pathways in response to different stressors (69). In endothelial cells (84) and cardiomyocytes (9), acidosis during ischemia was proposed to induce sAC association with mitochondria and activation of PKA, presumably via local production of cAMP at the outer membrane. PKA-mediated Bax phosphorylation resulted in its translocation to mitochondria and activation of the apoptotic pathway leading to cytochrome c release and caspase-9 cleavage. In these experimental conditions, inhibition of sAC or PKA prevented the mitochondrial translocation of Bax, whereas inhibition or stimulation of tmAC had no effect, suggesting the direct involvement of locally generated cAMP through sAC. On the contrary, it was proposed that BAD phosphorylation by PKA anchored to the mitochondrial outer membrane leads to blockage of BAD IL-3-dependent

apoptotic activity (59). However, in these earlier reports, the source of cAMP at the mitochondria was not investigated. Therefore, cAMP-regulated phosphorylation of apoptotic proteins may have different effects, depending on cell types and environmental conditions.

Regulation of Mitochondria by cAMP: Intra-Mitochondrial Effects

Modern methods enable identification of many phosphorylated proteins, including membrane transporters, metabolic enzymes, and OXPHOS complexes (8, 33). These, along with the known kinase-phosphatase functions within the mitochondrial matrix, have recently been extensively reviewed (33). Unfortunately, the function of the vast majority of these posttranslational modifications on mitochondrial metabolism remains poorly understood. Here, we summarize the current understanding of the effects of sAC activation on mitochondrial energy metabolism and the effects of protein phosphorylation on complexes I and IV of the respiratory chain, two major components of the OXPHOS system.

Effects of Intramitochondrial sAC Activation on Mitochondrial Energy Metabolism

As introduced above, sAC and PKA may reside inside mitochondria and may regulate OXPHOS via phosphorylation of multiple substrates including subunits of cytochrome c oxidase (COX or complex IV) (1, 4). Carbonic anhydrases (CA) are ubiquitous enzymes that equilibrate CO_2 levels with bicarbonate and a proton, and two isoforms of type V CA have been shown to reside inside mitochondria (49, 63). The coincident localization of sAC, carbonic anhydrases, and the Krebs cycle suggested that the CO_2 produced by the Krebs cycle could be converted to bicarbonate by mitochondrial carbonic anhydrase and sensed by sAC. We demonstrated that sAC-PKA regulation of OXPHOS was sensitive to physiologically relevant intramitochondrial bicarbonate concentrations, metabolically generated CO_2, and carbonic anhydrase inhibition (4). Thus the intramitochondrial sAC-PKA pathway senses metabolic activity providing a mechanism linking respiratory activities to nutritional availability. As nutrients become available to the cell, local CO_2 levels increase and bicarbonate matrix concentration rises, stimulating the mitochondrial cAMP-PKA pathway. This leads to increased OXPHOS activity while simultaneously limiting ROS production. Another important implication of this local mitochondrial signaling pathway is that the changes in cAMP level in the mitochondrial domain are not necessarily associated with the cAMP levels in the rest of the cell.

Mitochondrial Protein Phosphorylation: Complex I

The cAMP/PKA pathway plays an important role in the regulation of mitochondrial complex I (CI; NADH: ubiquinone oxidoreductase), the first entry point of the electron transport chain. CI is composed of 44 or 45 subunits, divided into core and accessory subunits, encoded by nuclear and mitochondrial DNA (80). Although several CI subunits are phosphorylated, the functional effect of phosphorylation is not completely clear yet. Phosphorylation has been postulated to play a role in CI assembly (19, 33, 101, 120), but further investigation is necessary to correlate protein phosphorylation with CI function. In particular, the relation between CI phosphorylation and function has been investigated in one of the accessory subunits, NDUFS4. This is a nuclear-encoded 18-kDa protein that regulates the assembly and activity of CI (81, 138). The NDUFS4 subunit is a hotspot of mutations in humans, associated with fatal neurological syndromes (20, 104, 105). As shown in whole fibroblasts and isolated mitochondria, NDUFS4 is phosphorylated by PKA in the mitochondrial matrix or in the cytosol before mitochondrial import, whereby phosphorylation could be regulating the protein import into mitochondria (28, 37, 102, 103). Although further experiments are needed to conclusively establish under which circumstances NDUFS4 phosphorylation takes place outside or inside mitochondria, intriguingly, fibroblasts from patients harboring mutant NDUFS4 displayed reduced levels of cAMP-dependent NDUFS4 phosphorylation and reduced activation of CI (104). In addition, cAMP-elevating agents, which led to NDUFS4 phosphorylation, were able to overcome CI inhibition in wild-type fibroblasts (38). Finally, the cAMP-PKA pathway and the 18-kDa accessory subunit of CI may play a role in the pathology of chromosome 21 trisomy (137). Human fibroblasts from patients with chromosome 21 trisomy display reduced cAMP levels and decreased cAMP-dependent phosphorylation of NDUFS4.

Mitochondrial Protein Phosphorylation: Complex IV

Our group identified a specific residue (S58) of COX subunit IV-1 (COXIV-1) as one of the targets of the intramitochondrial sAC-PKA pathway (1, 4). This modification regulates COX allosteric inhibition by matrix ATP; when COXIV-1 is dephosphorylated, COX activity is inhibited by ATP (1). ATP inhibition may play a role in the transition to an energy storage state by facilitating accumulation of unused substrates, such as fat and glycogen. Other

subunits of COX are also subject to cAMP regulation, but their relationship with the intramitochondrial sAC-PKA pathway is still unclear. In hypoxia and myocardial ischemia/reperfusion, hyperphosphorylation of COX subunit I (COXI), IV-1, and Vb inhibit COX activity, increasing ROS production. This effect was completely prevented by the addition of the PKA inhibitor H89 (51, 109). Phosphorylation of COX subunit II (COXII) by mitochondrial c-Src resulted in increased COX activity in osteoclasts, and this regulation appeared to be dependent on cAMP. On the other hand, calcitonin downregulates COX activity via cAMP (97, 149). Furthermore, heart mitochondria isolated after heart failure exhibit increased cAMP-dependent phosphorylation of COX at threonine residues (112).

Increased cAMP signaling in mitochondria, presumably via at least a subset of these phosphorylation events, can partially rescue COX deficiencies. Cybrids harboring a mutation in the COXI gene exhibit increased ROS production and elevated PGC1α and NRF1 expression, suggesting compensatory mitochondrial biogenesis. sAC targeted to the mitochondria (mt-sAC) of these cells improved the bioenergetic defects, including COX activity, respiration, and normalization of OXPHOS biogenesis (3).

Recently, Hüttemann and colleagues thoroughly reviewed the current evidence of COX subunit phosphorylation in multiple mammalian species and under physiological and pathological conditions such as inflammation and ischemia. A large number of phosphosites involved serine or threonine

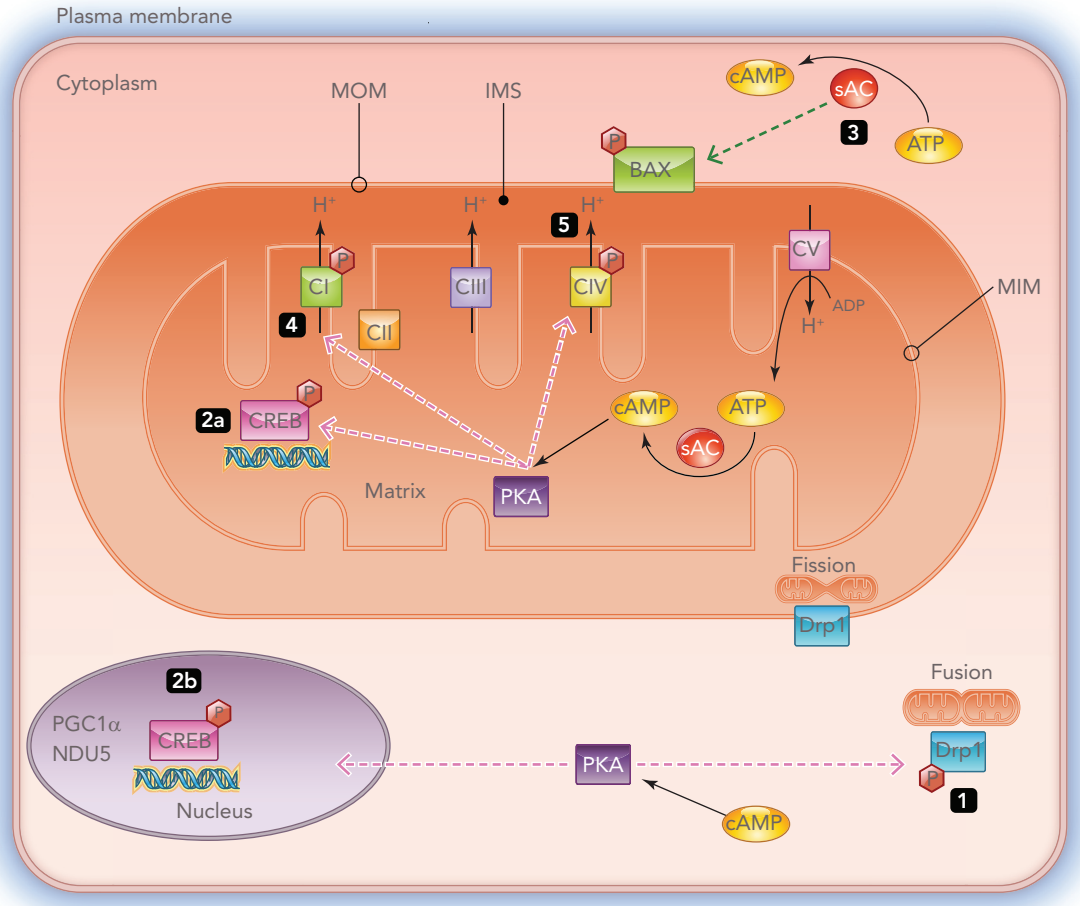

FIGURE 2. **Extra- and intra-mitochondrial regulation of cAMP pathway**
The cartoon shows the cAMP compartmentalization in cytoplasm and mitochondria and its effects on a variety of mitochondrial functions. *1*: cAMP derived from the action of sAC on ATP induces the activation of PKA and the phosphorylation of Drp1, which prevents its translocation to mitochondria and is retained in the cytoplasm, thereby promoting mitochondrial fusion; in contrast, dephosphorylated Drp1 translocates to mitochondria, inducing mitochondrial fission (see section *Control of Mitochondrial Morphology and Mitophagy*). *2*: CREB localized on mitochondrial DNA can be phosphorylated by PKA, residing in the mitochondrial matrix (*2a*) and induce mitochondrial biogenesis through the activation of PGC1α or NDU5 transcription in the nucleus (*2b*) (see *Regulation of Mitochondrial Biogenesis: CREB*). *3*: sAC in the cytosol leads to phosphorylation and traslocation of BAX into mitochondria and activation of the pro-apoptotic pathway (see *Regulation of Apoptosis*). *4*: PKA in the matrix activates complex I phosphorylation on the 18-kDa subunit (see *Mitochondrial Protein Phosphorylation: Complex I*). *5*: PKA in the matrix regulates COX activity via COXIV-1 phosphorylation (see *Mitochondrial Protein Phosphorylation: Complex IV*). MOM, mitochondrial outer membrane; IMS, intermembrane space; MIM, mitochondrial inner membrane.

residues, but the responsible kinase remains to be identified in most cases. Nevertheless, this strongly suggests the existence of multiple potential targets of cAMP-PKA phosphorylation on COX, which is likely to play a significant role in regulating mitochondrial bioenergetics and ROS production (68). The broader picture suggests that different phosphorylation sites and their localization relative to the CIV holocomplex (e.g., matrix side vs. intermembrane space or transmembrane domains) have very different effects on the enzyme kinetics, activating in some cases and inhibiting in others. This type of complex regulation may all be responsive to cAMP-PKA signaling, but the latter could have different function in the different mitochondrial compartments. This possibility is reinforced by the complete separation of the intramitochondrial cAMP pool from the extra-mitochondrial one (3).

Conclusions

The discovery of a compartmentalized source of cAMP in mitochondria generated by sAC has led to new ideas on how this ubiquitous signaling system is able to provide short-term metabolic regulation for a discrete subcellular organelle. The integration of this signaling system in the microenvironment of mitochondria, where it can sense changes in pH, CO_2, calcium, and ATP, participates in the modulation of enzymatic activities by protein phosphorylation. The phosphorylation of CI and COX subunits described above are examples of many such events that lead to reversible activation or inhibition of OXPHOS in response to metabolic demands and environmental cues (FIGURE 2). These examples suggest that cAMP-dependent regulation of OXPHOS is involved in maintaining a balance between energy storage and energy consumption as well as ROS production in respiring mitochondria. It is also necessary to remember that several subunits of the respiratory chain contain transmembrane domains, which means they are exposed to both the intermembrane space and the matrix; these may be sensitive to different pools of cAMP, with potentially competing, additive, or synergistic effects on enzymatic activity. Nevertheless, we predict that, with the rapid expansion of the mitochondrial phosphoproteome and the repertoire of mitochondrial phosphodiestarases, kinases, and phosphatase involved in regulating protein phosphorylation and enzymatic function, we will soon be able to draw a more precise map of this complex signaling network. We also expect that the increasing knowledge of the downstream targets of cAMP signaling will shed new light on the mechanism of communication between mitochondria and other organelles that regulate fundamental processes, such as mitochondrial biogenesis and turnover. ■

Funding was provided by National Institutes of Health Grants GM-088999 and Muscular Dystrophy Association, GM-62328, HD-059913, and NS-55255.

J. Buck and L. R. Levin own equity interest in CEP Biotech, which has licensed commercialization of a panel of monoclonal antibodies directed against sAC. All other authors declare no conflicts of interest, financial or otherwise.

Author contributions: F.V. and L.R.-E. prepared figures; F.V., L.R.-E., J.B., L.R.L., and G.M. drafted manuscript; F.V., L.R.-E., J.B., L.R.L., and G.M. edited and revised manuscript; F.V., L.R.-E., J.B., L.R.L., and G.M. approved final version of manuscript.

References

1. Acin-Perez R, Gatti DL, Bai Y, Manfredi G. Protein phosphorylation and prevention of cytochrome oxidase inhibition by ATP: coupled mechanisms of energy metabolism regulation. *Cell Metab* 13: 712–719, 2011.
2. Acin-Perez R, Russwurm M, Gunnewig K, Gertz M, Zoidl G, Ramos L, Buck J, Levin LR, Rassow J, Manfredi G, Steegborn C. A phosphodiesterase 2A isoform localized to mitochondria regulates respiration. *J Biol Chem* 286: 30423–30432, 2011.
3. Acin-Perez R, Salazar E, Brosel S, Yang H, Schon EA, Manfredi G. Modulation of mitochondrial protein phosphorylation by soluble adenylyl cyclase ameliorates cytochrome oxidase defects. *EMBO Mol Med* 1: 392–406, 2009.
4. Acin-Perez R, Salazar E, Kamenetsky M, Buck J, Levin LR, Manfredi G. Cyclic AMP produced inside mitochondria regulates oxidative phosphorylation. *Cell Metab* 9: 265–276, 2009.
5. Agnes RS, Jernigan F, Shell JR, Sharma V, Lawrence DS. Suborganelle sensing of mitochondrial cAMP-dependent protein kinase activity. *J Am Chem Soc* 132: 6075–6080, 2010.
6. Alto NM, Soderling J, Scott JD. Rab32 is an A-kinase anchoring protein and participates in mitochondrial dynamics. *J Cell Biol* 158: 659–668, 2002.
7. Andreeva AV, Kutuzov MA, Voyno-Yasenetskaya T. A G alpha12 is targeted to the mitochondria and affects mitochondrial morphology and motility. *FASEB J* 22: 2821–2831, 2008.
8. Aponte AM, Phillips D, Hopper RK, Johnson DT, Harris RA, Blinova K, Boja ES, French S, Balaban RS. Use of (32)P to study dynamics of the mitochondrial phosphoproteome. *J Proteome Res* 8: 2679–2695, 2009.
9. Appukuttan A, Kasseckert SA, Micoogullari M, Flacke JP, Kumar S, Woste A, Abdallah Y, Pott L, Reusch HP, Ladilov Y. Type 10 adenylyl cyclase mediates mitochondrial Bax translocation and apoptosis of adult rat cardiomyocytes under simulated ischaemia/reperfusion. *Cardiovasc Res* 93: 340–349, 2012.
10. Asirvatham AL, Galligan SG, Schillace RV, Davey MP, Vasta V, Beavo JA, Carr DW. A-kinase anchoring proteins interact with the phosphodiesterases in T lymphocyte cell lines. *J Immunol* 173: 4806–4814, 2004.
11. Bacskai BJ, Hochner B, Mahaut-Smith M, Adams SR, Kaang BK, Kandel ER, Tsien RY. Spatially resolved dynamics of cAMP and protein kinase A subunits in Aplysia sensory neurons. *Science* 260: 222–226, 1993.
12. Beavo JA, Brunton LL. Cyclic nucleotide research-still expanding after half a century. *Nat Rev Mol Cell Biol* 3: 710–718, 2002.
13. Benard G, Rossignol R. Ultrastructure of the mitochondrion and its bearing on function and bioenergetics. *Antioxid Redox Signal* 10: 1313–1342, 2008.
14. Bender AT, Beavo JA. Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol Rev* 58: 488–520, 2006.

15. Biel M. Cyclic nucleotide-regulated cation channels. *J Biol Chem* 284: 9017–9021, 2009.

16. Biel M, Wahl-Schott C, Michalakis S, Zong X. Hyperpolarization-activated cation channels: from genes to function. *Physiol Rev* 89: 847–885, 2009.

17. Bos JL. EPAC: a new cAMP target and new avenues in cAMP research. *Nature Rev Mol Cell Biol* 4: 733–738, 2003.

18. Bradley J, Reisert J, Frings S. Regulation of cyclic nucleotide-gated channels. *Curr Opin Neurobiol* 15: 343–349, 2005.

19. Brandt U. Energy converting NADH:quinone oxidoreductase (complex I). *Annu Rev Biochem* 75: 69–92, 2006.

20. Budde SM, van den Heuvel LP, Janssen AJ, Smeets RJ, Buskens CA, DeMeirleir L, Van Coster R, Baethmann M, Voit T, Trijbels JM, Smeitink JA. Combined enzymatic complex I and III deficiency associated with mutations in the nuclear encoded NDUFS4 gene. *Biochem Biophys Res Commun* 275: 63–68, 2000.

21. Bundey RA, Insel PA. Discrete intracellular signaling domains of soluble adenylyl cyclase: camps of cAMP? *Sci STKE* 2004: pe19, 2004.

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

23. Cali T, Ottolini D, Brini M. Mitochondrial Ca^{2+} neurodegeneration. *Cell Calcium* 52: 73–85, 2012.

24. Cammarota M, Paratcha G, Bevilaqua LR, Levi de Stein M, Lopez M, Pellegrino de Iraldi A, Izquierdo I, Medina JH. Cyclic AMP-responsive element binding protein in brain mitochondria. *J Neurochem* 72: 2272–2277, 1999.

25. Cereghetti GM, Stangherlin A, Martins de Brito O, Chang CR, Blackstone C, Bernardi P, Scorrano L. Dephosphorylation by calcineurin regulates translocation of Drp1 to mitochondria. *Proc Natl Acad Sci USA* 105: 15803–15808, 2008.

26. Chang CR, Blackstone C. Drp1 phosphorylation and mitochondrial regulation. *EMBO Rep* 8: 1088–1089; author reply 1089–1090, 2007.

27. Chen J, Shi X, Padmanabhan R, Wang Q, Wu Z, Stevenson SC, Hild M, Garza D, Li H. Identification of novel modulators of mitochondrial function by a genome-wide RNAi screen in *Drosophila melanogaster*. *Genome Res* 18: 123–136, 2008.

28. Chen R, Fearnley IM, Peak-Chew SY, Walker JE. The phosphorylation of subunits of complex I from bovine heart mitochondria. *J Biol Chem* 279: 26036–26045, 2004.

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

30. Chowanadisai W, Bauerly KA, Tchaparian E, Wong A, Cortopassi GA, Rucker RB. Pyrroloquinoline quinone stimulates mitochondrial biogenesis through cAMP response element-binding protein phosphorylation and increased PGC-1alpha expression. *J Biol Chem* 285: 142–152, 2010.

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

32. Conti M, Jin SL. The molecular biology of cyclic nucleotide phosphodiesterases. *Prog Nucleic Acid Res Mol Biol* 63: 1–38, 1999.

33. Covian R, Balaban RS. Cardiac mitochondrial matrix and respiratory complex protein phosphorylation. *Am J Physiol Heart Circ Physiol* 303: H940–H966, 2012.

34. Craven KB, Zagotta WN. CNG and HCN channels: two peas, one pod. *Annu Rev Physiol* 68: 375–401, 2006.

35. Cribbs JT, Strack S. Reversible phosphorylation of Drp1 by cyclic AMP-dependent protein kinase and calcineurin regulates mitochondrial fission and cell death. *EMBO Rep* 8: 939–944, 2007.

36. Cukkemane A, Seifert R, Kaupp UB. Cooperative and uncooperative cyclic-nucleotide-gated ion channels. *Trends Biochem Sci* 36: 55–64, 2011.

37. De Rasmo D, Palmisano G, Scacco S, Technikova-Dobrova Z, Panelli D, Cocco T, Sardanelli AM, Gnoni A, Micelli L, Trani A, Di Luccia A, Papa S. Phosphorylation pattern of the NDUFS4 subunit of complex I of the mammalian respiratory chain. *Mitochondrion* 10: 464–471, 2010.

38. De Rasmo D, Signorile A, Larizza M, Pacelli C, Cocco T, Papa S. Activation of the cAMP cascade in human fibroblast cultures rescues the activity of oxidatively damaged complex I. *Free Radic Biol Med* 52: 757–764, 2012.

39. De Rasmo D, Signorile A, Roca E, Papa S. cAMP response element-binding protein (CREB) is imported into mitochondria and promotes protein synthesis. *FEBS J* 276: 4325–4333, 2009.

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

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

42. Dickey AS, Strack S. PKA/AKAP1 and PP2A/Bbeta2 regulate neuronal morphogenesis via Drp1 phosphorylation and mitochondrial bioenergetics. *J Neurosci* 31: 15716–15726, 2011.

43. Dimino MJ, Bieszczad RR, Rowe MJ. Cyclic AMP-dependent protein kinase in mitochondria and cytosol from different-sized follicles and corpora lutea of porcine ovaries. *J Biol Chem* 256: 10876–10882, 1981.

45. DiPilato LM, Cheng X, Zhang J. 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, 2004.

46. Diviani D, Dodge-Kafka KL, Li J, Kapiloff MS. A-kinase anchoring proteins: scaffolding proteins in the heart. *Am J Physiol Heart Circ Physiol* 301: H1742–H1753, 2011.

47. Dodge-Kafka KL, Soughayer 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* 437: 574–578, 2005.

48. 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* 20: 1921–1930, 2001.

49. Dodgson SJ, Forster RE, Storey BT, Mela L. Mitochondrial carbonic anhydrase. *Proc Natl Acad Sci USA* 77: 5562–5566, 1980.

50. Duchen MR. Mitochondria and calcium: from cell signalling to cell death. *J Physiol* 529: 57–68, 2000.

51. Fang JK, Prabu SK, Sepuri NB, Raza H, Anandatheerthavarada HK, Galati D, Spear J, Avadhani NG. Site specific phosphorylation of cytochrome c oxidase subunits I, IVi1 and Vb in rabbit hearts subjected to ischemia/reperfusion. *FEBS Lett* 581: 1302–1310, 2007.

52. Feliciello A, Rubin CS, Avvedimento EV, Gottesman ME. Expression of A kinase anchor protein 121 is regulated by hormones in thyroid and testicular germ cells. *J Biol Chem* 273: 23361–23366, 1998.

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

54. Galluzzi L, Kepp O, Kroemer G. Mitochondria: master regulators of danger signalling. *Nat Rev Mol Cell Biol* 13: 780–788, 2012.

55. Goldenthal MJ, Marin-Garcia J. Mitochondrial signaling pathways: a receiver/integrator organelle. *Mol Cell Biochem* 262: 1–16, 2004.

56. Gomes LC, Di Benedetto G, Scorrano L. During autophagy mitochondria elongate, are spared from degradation and sustain cell viability. *Nat Cell Biol* 13: 589–598, 2011.

57. Gomes LC, Scorrano L. High levels of Fis1, a pro-fission mitochondrial protein, trigger autophagy. *Biochim Biophys Acta* 1777: 860–866, 2008.

58. Grimsrud PA, Carson JJ, Hebert AS, Hubler SL, Niemi NM, Bailey DJ, Jochem A, Stapleton DS, Keller MP, Westphall MS, Yandell BS, Attie AD, Coon JJ, Pagliarini DJ. A quantitative map of the liver mitochondrial phosphoproteome reveals posttranslational control of ketogenesis. *Cell Metab* 16: 672–683, 2012.

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

60. Hayes JS, Bowling N, King KL, Boder GB. Evidence for selective regulation of the phosphorylation of myocyte proteins by isoproterenol and prostaglandin E1. *Biochim Biophys Acta* 714: 136–142, 1982.

61. Hayes JS, Brunton LL, Brown JS, Reese JB, Mayer SE. Hormonally specific expression of cardiac protein kinase activity. *Proc Natl Acad Sci USA* 76: 1570–1574, 1979.

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

63. Henry RP. Multiple roles of carbonic anhydrase in cellular transport and metabolism. *Annu Rev Physiol* 58: 523–538, 1996.

64. Holz GG. Epac: A new cAMP-binding protein in support of glucagon-like peptide-1 receptor-mediated signal transduction in the pancreatic beta-cell. *Diabetes* 53: 5–13, 2004.

65. Houslay MD. Adaptation in cyclic AMP signalling processes: a central role for cyclic AMP phosphodiesterases. *Semin Cell Dev Biol* 9: 161–167, 1998.

66. Huang LJ, Durick K, Weiner JA, Chun J, Taylor SS. Identification of a novel protein kinase A anchoring protein that binds both type I and type II regulatory subunits. *J Biol Chem* 272: 8057–8064, 1997.

67. Hunter T. Signaling–2000 and beyond. *Cell* 100: 113–127, 2000.

68. Huttemann M, Helling S, Sanderson TH, Sinkler C, Samavati L, Mahapatra G, Varughese A, Lu G, Liu J, Ramzan R, Vogt S, Grossman LI, Doan JW, Marcus K, Lee I. Regulation of mitochondrial respiration and apoptosis through cell signaling: cytochrome c oxidase and cytochrome c in ischemia/reperfusion injury and inflammation. *Biochim Biophys Acta* 1817: 598–609, 2012.

69. Insel PA, Zhang L, Murray F, Yokouchi H, Zambon AC. Cyclic AMP is both a pro-apoptotic and anti-apoptotic second messenger. *Acta Physiol (Oxf)* 204: 277–287, 2012.

70. Jaiswal BS, Conti M. Calcium regulation of the soluble adenylyl cyclase expressed in mammalian spermatozoa. *Proc Natl Acad Sci USA* 100: 10676–10681, 2003.

71. Karpen JW, Rich TC. High-resolution measurements of cyclic adenosine monophosphate signals in 3D microdomains. *Methods Mol Biol* 307: 15–26, 2005.

72. Kaupp UB, Seifert R. Cyclic nucleotide-gated ion channels. *Physiol Rev* 82: 769–824, 2002.

73. Kawasaki H, Springett GM, Mochizuki N, Toki S, Nakaya M, Mastsuda M, Housman DE, Graybiel AM. A family of cAMP-binding porteins that directly activate Rap1. *Science* 282: 2275–2279, 1998.

74. Keely SL. Activation of cAMP-dependent protein kinase without a corresponding increase in phosphorylase activity. *Res Commun Chem Pathol Pharmacol* 18: 283–290, 1977.

75. Keely SL. Prostaglandin E1 activation of heart cAMP-dependent protein kinase: apparent dissociation of protein kinase activation from increases in phosphorylase activity and contractile force. *Mol Pharmacol* 15: 235–245, 1979.

76. Keravis T, Lugnier C. Cyclic nucleotide phosphodiesterase (PDE) isozymes as targets of the intracellular signalling network: benefits of PDE inhibitors in various diseases and perspectives for future therapeutic developments. *Br J Pharmacol* 165: 1288–1305, 2012.

77. Kim C, Xuong NH, STS. Crystal structure of a complex between the catalytic and regulatory (RIalpha) subunits of PKA. *Science* 307: 690–696, 2005.

78. Kim S, Mizoguchi A, Kikuchi A, Takai Y. Tissue and subcellular distributions of the smg-21/rap1/Krev-1 proteins which are partly distinct from those of c-ras p21s. *Mol Cell Biol* 10: 2645–2652, 1990.

79. Kleitke B, Sydow H, Wollenberger A. Evidence for cyclic AMP-dependent protein kinase activity in solated guinea pig and rat liver mitochondria. *Acta Biol Med Ger* 35: K9–K17, 1976.

80. Koopman WJ, Distelmaier F, Smeitink JA, Willems PH. OXPHOS mutations and neurodegeneration. *EMBO J* 32: 9–29, 2013.

81. Koopman WJ, Nijtmans LG, Dieteren CE, Roestenberg P, Valsecchi F, Smeitink JA, Willems PH. Mammalian mitochondrial complex I: biogenesis, regulation, and reactive oxygen species generation. *Antioxid Redox Signal* 12: 1431–1470, 2010.

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

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

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

85. Laurent AC, Breckler M, Berthouze M, Lezoualc'h F. Role of Epac in brain and heart. *Biochem Soc Trans* 40: 51–57, 2012.

86. Lee J, Kim CH, Simon DK, Aminova LR, Andreyev AY, Kushnareva YE, Murphy AN, Lonze BE, Kim KS, Ginty DD, Ferrante RJ, Ryu H, Ratan RR. Mitochondrial cyclic AMP responsive element-binding protein (CREB) mediates mitochondrial gene expression and neuronal survival. *J Biol Chem* 280: 40398–40401, 2005.

87. Lin RY, Moss SB, Rubin CS. Characterization of S-AKAP84, a novel developmentally regulated A kinase anchor protein of male germ cells. *J Biol Chem* 270: 27804, 1995.

88. Litvin TN, Kamenetsky M, Zarifyan A, Buck J, Levin LR. Kinetic properties of "soluble" adenylyl cyclase. Synergism between calcium and bicarbonate. *J Biol Chem* 278: 15922–15926, 2003.

89. Lyssand JS, Bajjalieh SM. The heterotrimeric [corrected] G protein subunit G alpha i is present on mitochondria. *FEBS Lett* 581: 5765–5768, 2007.

91. Ma MP, Thomson M. Protein kinase A subunit alpha catalytic and a kinase anchoring protein 79 in human placental mitochondria. *Open Biochem J* 6: 23–30, 2012.

92. Mcbride HM, Neuspiel M, Wasiak S. Mitochondria: more than just a powerhouse. *Curr Biol* 16: R551–R560, 2006.

93. Means CK, Lygren B, Langeberg LK, Jain A, Dixon RE, Vega AL, Gold MG, Petrosyan S, Taylor SS, Murphy AN, Ha T, Santana LF, Tasken K, Scott JD. 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: 1227–1235, 2011.

94. Mehta S, Zhang J. Reporting from the field: genetically encoded fluorescent reporters uncover signaling dynamics in living biological systems. *Annu Rev Biochem* 80: 375–401, 2011.

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

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

97. Miyazaki T, Neff L, Tanaka S, Horne WC, Baron R. Regulation of cytochrome c oxidase activity by c-Src in osteoclasts. *J Cell Biol* 160: 709–718, 2003.

98. Mootha VK, Bunkenborg J, Olsen JV, Hjerrild M, Wisniewski JR, Stahl E, Bolouri MS, Ray HN, Sihag S, Kamal M, Patterson N, Lander ES, Mann M. Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria. *Cell* 115: 629–640, 2003.

99. Oestreich EA, Malik S, Goonasekera SA, Blaxall BC, Kelley GG, Dirksen RT, Smrcka AV. Epac and phospholipase Cepsilon regulate Ca^{2+} release in the heart by activation of protein kinase Cepsilon and calcium-calmodulin kinase II. *J Biol Chem* 284: 1514–1522, 2009.

100. Oestreich EA, Wang H, Malik S, Kaproth-Joslin KA, Blaxall BC, Kelley GG, Dirksen RT, Smrcka AV. Epac-mediated activation of phospholipase C(epsilon) plays a critical role in beta-adrenergic receptor-dependent enhancement of Ca^{2+} mobilization in cardiac myocytes. *J Biol Chem* 282: 5488–5495, 2007.

101. Palmisano G, Sardanelli AM, Signorile A, Papa S, Larsen MR. The phosphorylation pattern of bovine heart complex I subunits. *Proteomics* 7: 1575–1583, 2007.

102. Papa S, De Rasmo D. Complex I deficiencies in neurological disorders. *Trends Mol Med* 19: 61–69, 2013.

103. Papa S, Sardanelli AM, Cocco T, Speranza F, Scacco SC, Technikova-Dobrova Z. The nuclear-encoded 18 kDa (IP) AQDQ subunit of bovine heart complex I is phosphorylated by the mitochondrial cAMP-dependent protein kinase. *FEBS Lett* 379: 299–301, 1996.

104. Papa S, Scacco S, Sardanelli AM, Vergari R, Papa F, Budde S, van den Heuvel L, Smeitink J. Mutation in the NDUFS4 gene of complex I abolishes cAMP-dependent activation of the complex in a child with fatal neurological syndrome. *FEBS Lett* 489: 259–262, 2001.

105. Petruzzella V, Vergari R, Puzziferri I, Boffoli D, Lamantea E, Zeviani M, Papa S. A nonsense mutation in the NDUFS4 gene encoding the 18 kDa (AQDQ) subunit of complex I abolishes assembly and activity of the complex in a patient with Leigh-like syndrome. *Hum Mol Genet* 10: 529–535, 2001.

106. Pizzo P, Drago I, Filadi R, Pozzan T. Mitochondrial Ca^{2+} homeostasis: mechanism, role, and tissue specificities. *Pflügers Arch* 464: 3–17, 2012.

107. 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* 5: 1176–1180, 2004.

108. Postea O, Biel M. Exploring HCN channels as novel drug targets. *Nat Rev Drug Discov* 10: 903–914, 2011.

109. Prabu SK, Anandatheerthavarada HK, Raza H, Srinivasan S, Spear JF, Avadhani NG. Protein kinase A-mediated phosphorylation modulates cytochrome c oxidase function and augments hypoxia and myocardial ischemia-related injury. *J Biol Chem* 281: 2061–2070, 2006.

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

111. 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* 116: 147–161, 2000.

112. Rosca M, Minkler P, Hoppel CL. Cardiac mitochondria in heart failure: normal cardiolipin profile and increased threonine phosphorylation of complex IV. *Biochim Biophys Acta* 1807: 1373–1382, 2011.

113. Roscioni SS, Elzinga CR, Schmidt M. Epac: effectors and biological functions. *Naunyn Schmiedebergs Arch Pharmacol* 377: 345–357, 2008.

114. Ryu H, Lee J, Impey S, Ratan RR, Ferrante RJ. Antioxidants modulate mitochondrial PKA and increase CREB binding to D-loop DNA of the mitochondrial genome in neurons. *Proc Natl Acad Sci USA* 102: 13915–13920, 2005.

115. Sardanelli AM, Signorile A, Nuzzi R, Rasmo DD, Technikova-Dobrova Z, Drahota Z, Occhiello A, Pica A, Papa S. Occurrence of A-kinase anchor protein and associated cAMP-dependent protein kinase in the inner compartment of mammalian mitochondria. *FEBS Lett* 580: 5690–5696, 2006.

116. Sardanelli AM, Technikova-Dobrova Z, Speranza F, Mazzocca A, Scacco S, Papa S. Topology of the mitochondrial cAMP-dependent protein kinase and its substrates. *FEBS Lett* 396: 276–278, 1996.

117. Scacco S, Vergari R, Scarpulla RC, Technikova-Dobrova Z, Sardanelli A, Lambo R, Lorusso V, Papa S. cAMP-dependent phosphorylation of the nuclear encoded 18-kDa (IP) subunit of respiratory complex I and activation of the complex in serum-starved mouse fibroblast cultures. *J Biol Chem* 275: 17578–17582, 2000.

118. Scatena R. Mitochondria and cancer: a growing role in apoptosis, cancer cell metabolism and dedifferentiation. *Adv Exp Med Biol* 942: 287–308, 2012.

119. Scheffler IE. *Mitochondria*. New York: Wiley-Liss, 2008.

120. Scheffler IE, Yadava N, Potluri P. Molecular genetics of complex I-deficient Chinese hamster cell lines. *Biochim Biophys Acta* 1659: 160–171, 2004.

121. Schmidt M, Evellin S, Weernink PA, von Dorp F, Rehmann H, Lomasney JW, Jakobs KH. A new phospholipase-C-calcium signalling pathway mediated by cyclic AMP and a Rap GTPase. *Nat Cell Biol* 3: 1020–1024, 2001.

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

124. Seino S, Shibasaki T. PKA-dependent and PKA-indenpendent pathways for cAMP-regulated exocytosis. *Physiol Rev* 85: 1303–1342, 2005.

125. Shaywitz AJ, Greenberg ME. CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu Rev Biochem* 68: 821–861, 1999.

126. Singh KK, Costello L. *Mitochondria and Cancer*. New York: Springer, 2009.

127. Szendroedi J, Phielix E, Roden M. The role of mitochondria in insulin resistance and Type 2 diabetes mellitus. *Nat Rev Endocrinol* 8: 92–103, 2012.

128. Tait SW, Green DR. Mitochondria and cell signalling. *J Cell Sci* 125: 807–815, 2012.

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

130. Taylor SS, Ilouz R, Zhang P, Kornev AP. Assembly of allosteric macromolecular switches: lessons from PKA. *Nat Rev Mol Cell Biol* 13: 646–658, 2012.

131. Taylor SS, Yang J, Wu J, Haste NM, Radzio-Andseim E, Anand G. PKA: a portrait of protein kinase dynamics. *Biochim Biophys Acta* 1697: 259–269, 2004.

132. Technikova-Dobrova Z, Sardanelli AM, Papa S. Phosphorylation of mitochondrial proteins in bovine heart: characterization of kinases and substrates. *FEBS Lett* 322: 51–55, 1993.

133. Technikova-Dobrova Z, Sardanelli AM, Speranza F, Scacco S, Signorile A, Lorusso V, Papa S. Cyclic adenosine monophosphate-dependent phosphorylation of mammalian mitochondrial proteins: enzyme and substrate characterization and functional role. *Biochemistry* 40: 13941–13947, 2001.

134. Technikova-Dobrova Z, Sardanelli AM, Stanca MR, Papa S. cAMP-dependent protein phosphorylation in mitochondria of bovine heart. *FEBS Lett* 350: 187–191, 1994.

135. Thomson M. What are guanosine triphosphate-binding proteins doing in mitochondria? *Biochim Biophys Acta* 1403: 211–218, 1998.

136. Tresguerres M, Levin LR, Buck J. Intracellular cAMP signaling by soluble adenylyl cyclase. *Kidney Int* 79: 1277–1288, 2011.

137. Valenti D, Manente GA, Moro L, Marra E, Vacca RA. Deficit of complex I activity in human skin fibroblasts with chromosome 21 trisomy and overproduction of reactive oxygen species by mitochondria: involvement of the cAMP/PKA signalling pathway. *Biochem J* 435: 679–688, 2011.

138. Valsecchi F, Koopman WJ, Manjeri GR, Rodenburg RJ, Smeitink JA, Willems PH. Complex I disorders: causes, mechanisms, and development of treatment strategies at the cellular level. *Dev Disabil Res Rev* 16: 175–182, 2010.

139. Verdanis A. Protein kinase activity at the inner membrane of mammalian mitochondria. *J Biol Chem* 252: 807–813, 1977.

140. Wahl-Schott C, Biel M. HCN channels: structure, cellular regulation and physiological function. *Cell Mol Life Sci* 66: 470–494, 2009.

141. Wallace DC. Mitochondria and cancer. *Nat Rev Cancer* 12: 685–698, 2012.

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

143. Wang Z, Dillon TJ, Pokala V, Mishra S, Labudda K, Hunter B, Stork PJ. Rap1-mediated activation of extracellular signal-regulated kinases by cyclic AMP is dependent on the mode of Rap1 activation. *Mol Cell Biol* 26: 2130–2145, 2006.

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

145. Westermann B. Bioenergetic role of mitochondrial fusion and fission. *Biochim Biophys Acta* 1817: 1833–1838, 2012.

146. Willoughby D, Wong W, Schaack J, Scott JD, Cooper DM. An anchored PKA and PDE4 complex regulates subplasmalemmal cAMP dynamics. *EMBO J* 25: 2051–2061, 2006.

147. Wong W, Scott JD. AKAP signalling complexes: focal points in space and time. *Nat Rev Mol Cell Biol* 5: 959–970, 2004.

148. Wu Z, Huang X, Feng Y, Handschin C, Gullicksen PS, Bare O, Labow M, Spiegelman B, Stevenson SC. Transducer of regulated CREB-binding proteins (TORCs) induce PGC-1alpha transcription and mitochondrial biogenesis in muscle cells. *Proc Natl Acad Sci USA* 103: 14379–14384, 2006.

149. Yang WL, Iacono L, Tang WM, Chin KV. Novel function of the regulatory subunit of protein kinase A: regulation of cytochrome c oxidase activity and cytochrome c release. *Biochemistry* 37: 14175–14180, 1998.

150. Zaccolo M. Phosphodiesterases and compartmentalized cAMP signalling in the heart. *Eur J Cell Biol* 85: 693–697, 2006.

151. Zaccolo M, Cesetti T, Di Benedetto G, Mongillo M, Lissandron V, Terrin A, Zamparo I. Imaging the cAMP-dependent signal transduction pathway. *Biochem Soc Trans* 33: 1323–1326, 2005.

152. 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* 2: 25–29, 2000.

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

154. Zippin JH, Levin LR, Buck J. CO_2/HCO_3^--responsive soluble adenylyl cyclase as a putative metabolic sensor. *Trends Endocrinol Metab* 12: 366–370, 2001.

155. Zong X, Krause S, Chen CC, Kruger J, Gruner C, Cao-Ehlker X, Fenske S, Wahl-Schott C, Biel M. Regulation of hyperpolarization-activated cyclic nucleotide-gated (HCN) channel activity by cCMP. *J Biol Chem* 287: 26506–26512, 2012.