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Role of soluble adenylyl cyclase in cell death and growth ☆

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ABSTRACT

cAMP signaling is an evolutionarily conserved intracellular communication system controlling numerous cellular functions. Until recently, transmembrane adenylyl cyclase (tmAC) was considered the major source for cAMP in the cell, and the role of cAMP signaling was therefore attributed exclusively to the activity of this family of enzymes. However, increasing evidence demonstrates the role of an alternative, intracellular source of cAMP produced by type 10 soluble adenylyl cyclase (sAC). In contrast to tmAC, sAC produces cAMP in various intracellular microdomains close to specific cAMP targets, e.g., in nucleus and mitochondria. Ongoing research demonstrates involvement of sAC in diverse physiological and pathological processes. The present review is focused on the role of cAMP signaling, particularly that of sAC, in cell death and growth. Although the contributions of sAC to the regulation of these cellular functions have only recently been discovered, current data suggest that sAC plays key roles in mitochondrial bioenergetics and the mitochondrial apoptosis pathway, as well as cell proliferation and development. Furthermore, recent reports suggest the importance of sAC in several pathologies associated with apoptosis as well as in oncogenesis. This article is part of a Special Issue entitled: The role of soluble adenylyl cyclase in health and disease.

1. Introduction

The discovery of the second messenger cAMP by Earl Wilbur Sutherland in 1958 [1] opened new perspectives in understanding the underlying mechanisms of the signaling processes that occur within and between cells. Intense research during the ensuing decades demonstrated that cAMP signaling is evolutionarily conserved and can be found in all species, from microorganisms to mammals. In animals, cAMP plays a fundamental role in numerous physiological processes, including development, organ and tissue homeostasis, aging and death. Furthermore, extensive and ongoing research established that cAMP signaling is involved in several pathogenesis, e.g., cancer, neurodegeneration, heart failure, and diabetes [2]. Although cAMP contributes to the regulation of hundreds cellular functions, two key classes of proteins control its cellular concentration. These classes are adenylyl cyclases, which synthetize cAMP, and cyclic nucleotide phosphodiesterases (PDEs), which degrade cAMP. To properly regulate diverse cellular functions, precision and target specificity of cAMP signaling is very important. Such precise signaling is predominantly defined by intracellular compartmentalization of three main downstream effectors for cAMP: protein kinase A (PKA), exchange protein activated by cAMP (EPAC) and cyclic nucleotide-gated ion channels. Additionally, selectivity and specificity of cAMP signaling rely on the compartmentalization of adenylyl cyclases, i.e., (i) transmembrane adenylyl cyclase (tmAC) within the plasmalemma, and (ii) soluble adenylyl cyclase (sAC) in the cytosol and within distinct organelles. In the present review, we have focused on sAC-dependent cAMP-signaling. Research in this field has revealed a contribution of sAC in numerous physiological and pathological processes. Here we described recent data about the contribution of sAC in cell death and growth.

2. cAMP signaling

2.1. Adenylyl cyclase

The conversion of ATP to cAMP is catalyzed by adenylyl cyclases, which represent a large family of enzymes consisting of six classes. In mammalian cells, cAMP is generated by class III adenylyl cyclases, a group consisting of ten members. Nine members of this class of cyclases belong to the tmAC subfamily. They share a common structural organization: 12 transmembrane helices and 2 cytoplasmic domains that form a (pseudo) heterodimer [3]. The tmAC contains two cytoplasmic domains forming the catalytic core of the enzyme with the active site at their interface [4,5]. The mechanisms controlling the activation and inhibition of tmAC by G_s and G_i proteins, respectively, are shared by all 9 types of tmACs, thereby allowing tmAC activity to be controlled by hormones or neurotransmitters. In contrast, tmACs differ in their

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regulation by protein kinases and various small molecules. Indeed, some types of tmACs may be activated through phosphorylation by Ca^{2+}/calmodulin-dependent protein kinase (types 1 and 8), by PKC (types 2,3,5,7), or by binding of calmodulin (types 1 and 8) [6]. Furthermore, tmACs type 4 and 5 can be inhibited by PKA-dependent phosphorylation or by Ca^{2+} binding [6]. This type-dependent regulation of tmAC activity, combined with differences in their tissue distribution/expression, facilitates the selectivity and specificity of cAMP signaling in different cell types and tissues.

According to the traditional model of cAMP-signaling, cAMP generated by tmAC freely diffuses throughout the cytosol and leads to the activation of three known downstream targets: PKA, EPAC and cyclic nucleotide-gated ion channels. However, the diffusion of cAMP throughout the cytosol makes it difficult to selectively activate distally localized targets without also activating more proximal targets, i.e., localized at the plasmalemma. Therefore, such cAMP diffusion would likely diminish specificity, selectivity and signal strength, and this model of cAMP signaling has changed over the years. In fact, cAMP diffusion is restricted within defined compartments due to PDE activity [7]. And it is now known that tmAC can continue to signal within the cell during internalization/endocytosis along with G-protein-coupled receptors, thereby defining endocytic cAMP microdomains [8,9]. Apart from tmAC, a second intracellular source of cAMP, type 10 sAC, was identified in mammalian cells [10]. Unlike tmAC, sAC possesses no transmembrane domains and is localized throughout the cell, e.g., in the cytosol, nucleus, mitochondria, and centriole [11]. Therefore, in contrast to tmAC, sAC produces cAMP in different intracellular microdomains close to specific cAMP targets. The activity of sAC, along with the actions of PDEs limiting cAMP diffusion [7] and preventing non-specific effector activation, enables both specificity and selectivity towards intracellular targets. In addition, sAC can be activated by divalent cations (e.g., Ca^{2+}, Mg^{2+}, Mn^{2+}) and is therefore involved in Ca^{2+} signaling. sAC also represents a unique intracellular bicarbonate sensor with enzymatic activity. Finally, a recent study by Zippin et al. [12] demonstrated that sAC-generated cAMP in β-cells reflects alterations in intracellular ATP, suggesting that sAC also serves as an intracellular ATP sensor.

sAC is expressed in all tissues and cells examined thus far. Although encoded by a single gene, multiple isoforms are generated by alternative splicing. The longest mammalian isoform represents full-length sAC (187 kDa) and is found predominantly in sperm [13]. Several shorter-length sAC proteins have also been identified, with predominant expression of a ~50 kDa protein that possesses about tenfold higher activity compared with full-length sAC. This isoform contains two catalytic domains. Additionally, several splicing variants of sAC, which have an altered or missing first catalytic domain (C1), have been identified in mouse and human cells [14–16]. However, it remains unclear whether these sAC variants with altered C1 domain have nucleotidyl cyclase activity [16,17]. Altogether, the presence of two sources of cAMP, namely, tmAC and sAC, offers the possibility for the selective regulation of diverse cellular functions.

2.2. Phosphodiesterases

cAMP synthesized by tmAC leads to the rapid activation of numerous effector proteins localized within the plasmalemma. Although cAMP is an easily diffusible molecule, its rapid diffusion throughout the cytosol would lead to the uncontrolled activation of diverse intracellular targets, thereby limiting the specificity of cAMP signaling. Cells have therefore developed mechanisms to limit the diffusion of cAMP and produce a membrane-to-cytosol cAMP concentration gradient [7,18]. PDEs play a key role in maintaining the gradient by hydrolyzing cAMP to 5-AMP and providing diffusional barriers [19,20]. In turn, PDEs co-localized with sAC in the cytosol or within diverse intracellular compartments may also prevent the uncontrolled diffusion of cAMP synthesized by sAC. Recent data also demonstrated that PDEs are targeted to discrete signaling complexes together with other cAMP effectors (PKA, EPAC) and their target proteins [21]. These complexes therefore permit the sculpting of local cAMP gradients and provide for the efficient activation of spatially localized targets. Furthermore, the co-localization of PDEs and PKA within one microdomain plays an important role in terminating the PKA-mediated phosphorylation of target proteins [19].

The existence of more than 100 PDE isoforms resulting from the differential expression and splicing of 11 PDE gene families [22] likely contributes to the specialized function and localization of PDEs. Localization of PDEs at the membrane or in the cytosol depends on the hydrophobicity of the NH2-terminal domains. For example, the hydrophobicity of the NH2-termini of PDE2A3, PDE2A3 and PDE3 provides for their membrane association [23]. Aside from PDE isoforms that selectively hydrolyze cAMP (PDE4, 7 and 8), some PDEs are cGMP-selective (PDE5, 6, and 9), whereas others (PDE1, 2, 3, 10, and 11) can hydrolyze both cAMP and cGMP. Interestingly, a recent study by Kim et al. [24] suggests a role for PDE4D as a molecular transducer of cAMP signaling independent of its classical enzymatic function. In summary, PDEs enable the specificity and selectivity of cAMP signaling by limiting the cytosolic cAMP diffusion and restricting the cAMP pool within distinct cellular compartments.

2.3. cAMP effectors

Three groups of direct cAMP effector molecules have been described, which play a key role in the complexity and specificity of cAMP signaling. Of these, PKA is the best characterized. This serine/threonine kinase is a tetrameric enzyme composed of two regulatory (R) and two catalytic (C) subunits. In unstimulated cells, with low cAMP concentration, the C subunits are inhibited via binding by the R subunits. Upon the binding of cAMP to each R subunit, the C subunits dissociate and become free to phosphorylate their substrates [25]. A structurally diverse group of proteins called A-kinase anchoring proteins (AKAP) bind R subunits and promote the localization of the PKA holoenzyme within distinct intracellular microdomains, e.g., the mitochondria, nucleus or plasmalemma. Furthermore, AKAP also act as scaffolds for PKA substrates, PDEs and protein phosphatases, facilitating the regulation of cAMP signaling [19,26–30].

The other important effectors of cAMP are EPAC, a guanine-nucleotide exchange factor, and two groups of cyclic nucleotide regulated channels, namely, cyclic nucleotide-gated channels [31–34] and hyperpolarization-activated cyclic nucleotide-gated channels [35–38]. Because these channels are mainly expressed in the plasmalemma, they are primarily under the control of tmAC activity. Although they play a role in diverse cellular functions, little is known about their contribution to the regulation of cell death and growth. In contrast, EPAC has been shown to localize to the cytosol, nucleus or mitochondria [39,40], and it significantly contributes to the control of apoptosis and proliferation. The concentration of cAMP required to activate purified EPAC in vitro was initially determined to be approximately 10-fold higher than the concentration which activates PKA [41]. However, recent data indicate that EPAC and PKA have similar affinities for cAMP in cells, suggesting that both effectors respond to physiologically relevant cAMP concentrations [42]. There are two EPAC isoforms, EPAC1 and EPAC2, which are expressed in various tissues [43]. When bound to cAMP, EPAC activates the small GTPases Rap1 and Rap2, which are upstream of several signaling cascades. Similar to PKA, EPAC isoforms seem to be spatially and temporally regulated and exert their biological functions through interactions with various scaffold proteins such as AKAPs, ezrin–radixin–moesin proteins, nuclear distribution element-like protein, and the small G protein Ran and Ran binding protein 2 [40].

3. Role of cAMP signaling in cell death

In adult organisms, tissue homeostasis depends on the balance between cell proliferation and the removal of cells by programmed