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Bicarbonate-Regulated Soluble Adenylyl Cyclase

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Summary

'Soluble adenylyl cyclase' (sAC) represents a novel form of mammalian adenylyl cyclase structurally, molecularly, and biochemically distinct from the G protein-regulated, transmembrane adenylyl cyclases (tmACs). sAC possesses no transmembrane domains and is insensitive to classic modulators of tmACs, such as heterotrimeric G proteins and P site ligands. Thus, sAC defines an independently regulated cAMP signaling system within mammalian cells.

sAC is directly stimulated by bicarbonate ion both *in vivo* in heterologously expressing cells and *in vitro* using purified protein. sAC appears to be the predominant form of adenylyl cyclase (AC) in mammalian sperm, and its direct activation by bicarbonate provides a mechanism for generating the cAMP required to complete the bicarbonate-induced processes necessary for fertilization, including hyperactivated motility, capacitation, and the acrosome reaction. Immunolocalization studies reveal sAC is also abundantly expressed in other tissues which respond to bicarbonate or carbon dioxide levels suggesting it may function as a general bicarbonate/CO_2 sensor throughout the body.

Cyclic adenosine monophosphate (cAMP) is a nearly ubiquitous second messenger that transduces signals to intracellular effectors including cAMP-dependent protein kinase (PKA), guanine exchange factors (i.e., exchange proteins activated by cAMP, EPACs) [1, 2] and cyclic nucleotide gated channels [3]. Historically, the only known source of cAMP in mammals was a family of hormone-responsive, transmembrane adenylyl cyclases (tmACs). These cyclases are characterized by 12 putative membrane spanning domains and sensitivity to heterotrimeric G proteins as well as the non-physiological regulator forskolin [4, 5].

A distinct adenylyl cyclase (AC) activity was detected in the cytosolic fraction from the testis of adult rats [6]. This activity was insensitive to forskolin, fluoride, FSH, LH, and other known stimulators of tmACs [7, 8, 9]. A biochemically-related cyclase activity also appeared associated with the particulate fraction from epididymal or ejaculated sperm [6].

Recently, our laboratory purified a 48-kDa soluble protein with adenylyl cyclase activity from the cytosol of 1000 rat testis using classical protein purification methods [10, 11]. Degenerate nucleotide primers corresponding to peptides derived from this protein were used to clone the corresponding cDNA from rat testis [10]. The sAC cDNA predicts a 187 kDa protein and the amino acid sequences obtained from the purified 48 kDa protein suggest that it is proteolytically processed from the amino terminus of the full-length 187 kDa precursor molecule. This N-terminal portion of sAC contains two putative catalytic domains (Figure

Figure 1. Isoforms of soluble adenylyl cyclase
In addition to its two catalytic domains (C1 and C2), full length sAC (sAC_{fl}) contains consensus P-loop (P) and Leucine Zipper (L-Zip) sequences [10]. The truncated form of sAC (sAC_t), which consists of just the catalytic domains, is 20 times more active than sAC_{fl}.

1), which are more similar to the catalytic domains of cyanobacterial adenylyl cyclases than to the catalytic domains of other mammalian cyclases [10].

cDNAs encoding full length sAC (sAC_{fl}) or a protein engineered to approximate the truncated version purified from rat testis cytosol (sAC_t) were expressed in HEK293 cells. Each exhibited AC activity that was insensitive to forskolin activation and G-protein modulation [10]. Additionally, the truncated form exhibited 10-20 fold higher activity than the full-length protein (Figure 1) indicating a possible auto-regulatory mechanism present in the full length protein [10]. Northern analysis and in situ hybridization studies indicate high level expression in testis, specifically in male germ cells [10, 12]. However, more sensitive RT-PCR analysis [12] and Western blotting using sAC-specific antisera [13] indicate sAC is expressed in virtually all tissues.

Several lines of evidence suggested that sAC could be bicarbonate-sensitive. Adenylyl cyclase activities from spermatozoa of several mammalian species were shown to be stimulated by bicarbonate [14, 15, 16]. This activation was postulated to be responsible for the bicarbonate-dependent, cAMP mediated increase in sperm motility [14, 15] and induction of post-ejaculatory maturation, termed capacitation, required for mammalian sperm to be competent to fertilize the egg [17, 18]. Bicarbonate activated adenylyl cyclase activity had also been reported in fluid-transporting tissues, including cortex and medulla of the kidney, ocular ciliary processes, corneal endothelium, and choroid plexus [19].

We demonstrated that sAC is directly responsible for bicarbonate sensitive AC activity. Heterologously expressed sAC protein was stimulated by bicarbonate in both *in vivo* cellular cAMP accumulation assays and *in vitro* cyclase assays using purified protein. The EC_{50} of this stimulation was approximately 25 mM [13], equivalent to the serum bicarbonate concentration. Furthermore, sAC_t which is comprised almost exclusively of catalytic domains, was stimulated by bicarbonate, suggesting modulation occurs directly on the catalytic center of the enzyme. Bicarbonate did not appear to be stimulating the enzymatic activity by altering the assay pH or by liberating carbon dioxide [13].

Among mammalian adenylyl cyclases, bicarbonate regulation appears to be unique to sAC; however, sensitivity to bicarbonate is conserved in related prokaryotic adenylyl cyclases. As mentioned above, the catalytic domains of sAC are homologous to the catalytic domains of cyanobacterial cyclases, and we found that a cyanobacterial AC, Spirulina platensis CyaC, was also bicarbonate regulated [13] demonstrating that sAC is an evolutionarily conserved bicarbonate sensor. This bicarbonate sensing AC provides a mechanism for cyanobacteria to regulate metabolic processes in response to nutritional availability (Figure 2).

Western [13] and immunohistochemical data (data not shown) confirm the presence of sAC protein in a number of bicarbonate-responsive tissues and cells. sAC protein accumulates to high levels in developing germ cells (Wuttke MS, *et al.* Unpublished data) as predicted by *in situ* analysis of its mRNA [12], and it is abundantly expressed in the head, midpiece, and tail of mature spermatozoa (Wuttke MS, *et al.* Unpublished data). sAC protein also localizes to the apical membrane in colon (data not shown) and is present in kidney [13] where it appears to be in regions associated with

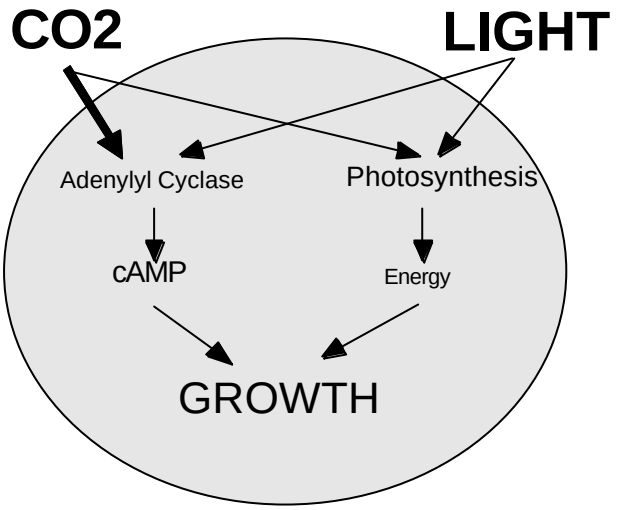

Figure 2. The role of bicarbonate-sensitive adenylyl cyclase in cyanobacterial metabolism and growth.
Cyanobacteria are thought to be responsible for the photosynthesis that transformed the early pre-Cambrian carbon dioxide-rich atmosphere into an oxygen atmosphere [21]. Photosynthetic cyanobacteria require both light and carbon dioxide to grow. In the presence of these nutritional requirements, cAMP is thought to be a positive signal for metabolic activity. The cyanobacterial adenylyl cyclase CyaC was shown to be responsive to the light sensitive [22] two-component system [23], and we demonstrated that it is also responsive to carbon dioxide/bicarbonate [13].

bicarbonate homeostasis (Pastor-Soler NM, Breton S, Brown D. Unpublished data).
While the targets of cAMP include EPACs and cyclic nucleotide gated channels, PKA is the best studied effector of cAMP signaling. A growing family of proteins called A kinase anchoring proteins (AKAPs) tether PKA holoenzyme to distinct and varied subcellular locations including mitochondria, nuclear membrane and nuclear matrix [20]. Previous models describing cellular cAMP signaling depended on cyclases localized at the plasma membrane as the sole source of cAMP to activate these anchored PKA holoenzymes. In these models, cAMP would have to diffuse from the plasma membrane to distant, intracellular sites (Figure 3a) making it difficult to selectively activate distally localized PKA without also activating more proximal targets. Signaling by diffusion would also result in a lack of specificity because PKA targets would be independent of the origin of the cAMP signal. Early methods for measuring cAMP generated in vivo based on fluorescence resonance energy transfer between subunits of PKA suggested that the second messenger would readily diffuse from tmACs throughout the cytosol [24, 25]. However, more recent quantification of intracellular cAMP signals based on stimulation of cyclic nucleotide gated

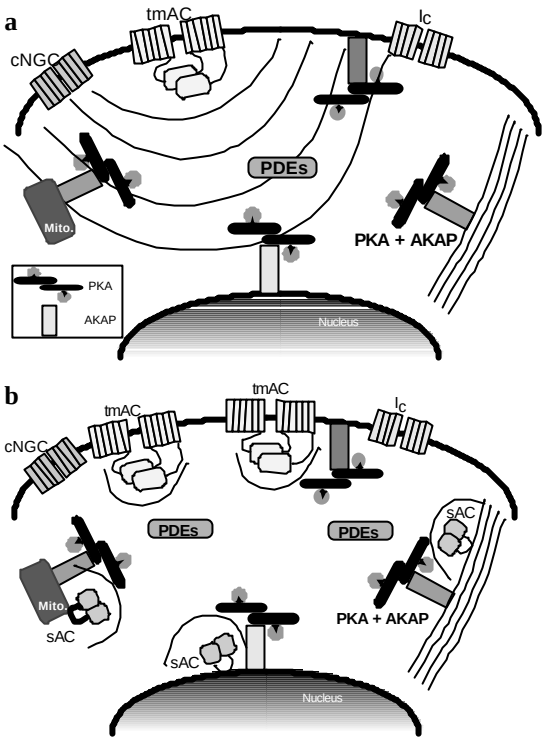

Figure 3. Models of cAMP signal transduction.
a) Previous models of cAMP signaling require the diffusion of cAMP from plasma membrane-localized tmACs to targets localized throughout the cell. These targets include effectors on the plasma membrane [cyclic nucleotide gated channels (cNGC) and channels activated by PKA (I_C)] and tethered to a variety of intracellular sites (PKA bound to AKAPs). Diffusion of cAMP throughout the cytosol would likely diminish specificity, selectivity, and signal strength. This model is further complicated by the presence of phosphodiesterases (PDEs) which degrade cAMP preventing its diffusion.
b) Cytosolic localization of sAC provides both specificity and selectivity by permitting generation of cAMP proximal to intracellular targets. Furthermore, this model for cAMP action incorporates PDEs, which would act to limit diffusion and prevent non-specific effector activation.

Table 1. sAC and tmACs define distinct cAMP signaling pathways.

	sAC	tmACs
Subcellular localization	Soluble and particulate	Plasma membrane
Evolutionary relatedness	Cyanobacteria	'First' appearance: multicellular organisms
Modulation	Bicarbonate and proteolytic activation	G proteins and other second messengers
Physiological signals	Metabolic activity ? pH ?	Hormones Olfactants

channels (cNGC) indicate that local elevations are far greater than the increases measured in whole cells [26] suggesting that cAMP had to be signaling in microdomains. sAC represents a possible alternative source of cAMP that is not limited to the plasma membrane; therefore, it could form discrete signaling microdomains with intracellular effectors (Figure 3b).

Soluble adenylyl cyclase represents a novel, bicarbonate-stimulated source of cAMP. sAC differs from classical tmACs in its cellular localization and modulation, thus making it an excellent candidate for the regulation of physiological systems not under the control of classic hormone signaling. For example, carbon dioxide generation during metabolic processes or pH changes would alter intracellular bicarbonate concentration thereby modulating sAC activity (Table 1). sAC is present in virtually all tissues examined and its bicarbonate sensitivity has been conserved between mammals and cyanobacteria, one of the earliest forms of life on earth. Such conservation suggests that a bicarbonate sensitive adenylyl cyclase plays a fundamental physiological role common to most, if not all, organisms.

Key words Acrosome Reaction; Cyanobacteria; Cyclic AMP; Cytosol; Energy Metabolism; Hydrogen-Ion Concentration; Metabolism; Second Messenger Systems; Signal Transduction; Sperm Capacitation; Sperm Motility

Abbreviations AC: adenylyl cyclase; AKAP: A kinase anchoring protein; cAMP: cyclic adenosine monophosphate; cNGC: cyclic nucleotide gated channels; EPACs: exchange proteins activated by cAMP; PDE: phosphodiesterase; PKA: cAMP-dependent protein kinase; sAC: soluble adenylyl cyclase; sAC_{fl}: full-length isoform of sAC protein; sAC_t: truncated isoform of sAC protein; tmAC: transmembrane adenylyl cyclase

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