A seminal study of soluble adenylyl cyclase

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In mammals, a soluble bicarbonate-regulated adenylyl cyclase is required for sperm fertility. Crystal structures of a cyanobacterial cousin provide new insights into the molecular mechanisms of activation and catalysis in soluble adenylyl cyclase and other class III nucleotidyl cyclases.

Although bicarbonate may be better known for putting the oomph in baking soda, it can also be thought of as a signal transducing 'first messenger' that controls the capacitation of sperm—a series of steps by which ejaculated spermatozoa become competent to fertilize eggs. At the concentrations of bicarbonate normally found in the ovarian ducts, this metabolite can directly stimulate the activity of a sperm-specific, soluble adenylyl cyclase (sAC)[1], an enzyme that converts ATP into 3′,5′-cyclic-adenosine-5′-monophosphate (cAMP). The resulting increase in cAMP activates protein kinase A, which in turn regulates proteins involved in sperm motility[2] and in morphological changes at the head of the sperm required for fusion with oocytes[3]. Human sAC could therefore be an important drug target for the purposes of contraception or, conversely, treatment of male infertility[2]. On page 32 of this issue of *Nature Structural & Molecular Biology*, Steegborn *et al.* present six crystal structures of a cyanobacterial sAC that is regulated by bicarbonate and Ca^{2+} ions in a manner similar to that of the human enzyme[4]. These structures reveal the molecular basis for Ca^{2+} regulation and demonstrate that bicarbonate induces a conformational change that helps coalesce the active site of the enzyme (**Fig. 1**).

The ten mammalian adenylyl cyclases[5] are categorized by sequence homology as class III adenylyl cyclases[6] and have an active site formed at the interface of two homologous domains (called C1 and C2). Despite the dimeric pseudosymmetry of their catalytic core, only one functional active site is created, although the pseudo two-fold related 'vestigial' active site could serve as a regulatory site (**Fig. 1**). Isoforms AC1–9 are integral membrane proteins (the transmembrane or 'tm'ACs), whereas the tenth isoform is soluble (sAC)[7]. The class III catalytic domains of mammalian tmACs[8–10] and several from *Trypanosoma brucei*[11] were the first to be described at the atomic level. The tmAC structures revealed the structure of the C1-C2 catalytic core and that production of cAMP from ATP occurs via two-metal ion catalysis, with metal A playing the role of an electrophilic catalyst and metal B stabilizing the pyrophosphate leaving group[10].

Mammalian ACs have low basal activity and require activation by external proteins or metabolites to produce physiologically relevant amounts of cAMP. The tmACs are activated by the heterotrimeric G protein $G\alpha_s$ and thus are responsive to endocrine hormones such as glucagon and epinephrine. All but AC9 are also activated by the hypotensive drug forskolin, which binds in the vestigial active site (**Fig. 1**). Interestingly, sAC is insensitive to G proteins and forskolin. Instead, it is synergistically activated by bicarbonate and Ca^{2+} (ref. 12), both of which are required for sperm capacitation[3]. On the basis of the tmAC structures, it was proposed that activators such as $G\alpha_s$ and forskolin function by altering or even driving formation of the interface between the C1 and C2 domains, where the active site is formed[13]. However, the lack of a reliable model for the 'basal' state of tmAC (that is, not bound to either $G\alpha_s$ or forskolin) makes it difficult to fully understand the molecular basis for how these regulators control activity. The structures of sAC reported in this issue provide additional insights into this fundamental question.

Streptomyces platensis sAC is a cyanobacterial class III AC that is synergistically activated by Ca^{2+} and bicarbonate[1]. It is therefore a convenient model system for crystallographic studies aimed at understanding the unique regulation of its mammalian counterpart. Despite the fact that the catalytic core of *S. platensis* sAC is formed by two identical domains and has two functional active sites, its tertiary structure and conformational flexibility are remarkably similar to those of tmACs (**Fig. 1**). When bound to the nonhydrolyzable ATP analog α,β-methylene-adenosine-5′-triphosphate (AMPcPP, also called α,β-Me-ATP), sAC adopts an 'open' conformation similar to that of $G\alpha_s$-bound tmAC. Only metal B is observed bound in both active sites of the enzyme. By soaking crystals with heavy metal analogs of Ca^{2+}, the authors demonstrate that Ca^{2+} can substitute for Mg^{2+} at the metal B site and thereby coordinate the triphosphate tail of the nucleotide. By virtue of its stronger interactions with ATP, Ca^{2+} enables nucleotides to bind more tightly. Notably, Ca^{2+} ions are inhibitory toward tmACs[5], and thus this mode of activation seems unique to sACs.

The molecular basis for regulation by bicarbonate is less straightforward. Because soaking crystals in bicarbonate destroyed them, the authors had to flash-soak and freeze crystals to catch a glimpse of the bicarbonate-induced conformational change. Although no electron density for bicarbonate is observed, bicarbonate treatment of the sAC–AMPcPP complex results in the collapse of active site loops around the substrate analog (**Fig. 1**). Interestingly, the binding of adenosine-5′-(Rp)-α-thio-triphosphate (Rp-ATPαS) induces a similar conformational change, leading the authors to speculate that the conformation of sAC in complex with Rp-ATPαS may be similar to that assumed during the transition state. While in its closed conformation, the active sites of sAC are better organized and bind both metal A and metal B. Interestingly, the bicarbonate (or Rp-ATPαS)-induced conformational change of sAC is similar to that which occurs in tmAC upon binding ATP analogs (**Fig. 1**), which also results in collapse of active site loops and promotes the binding of two metal ions[9]. However, unlike sAC, tmAC is considered to be in an active state regardless of whether it adopts the open or closed conformation because it is bound to its activator, $G\alpha_s$.

Is the crystal structure of the sAC–AMPcPP complex then representative of a basal, inactive class III nucleotidyl cyclase? If so, one could hypothesize that the basal conformation of tmAC (without $G\alpha_s$ and forskolin) would adopt a conformation that is only slightly more open than in $G\alpha_s$- and forskolin-bound tmAC. However, several lines of evidence argue against this. The *T. brucei* catalytic domains crystallized as inactive monomers[11], suggesting that, at least in trypanosomes, the basal catalytic domains are at best weakly associated, and thus ensured to be of low activity. Accordingly, the affinity of the tmAC C1 and C2 domains is known to be greatly enhanced by forskolin and $G\alpha_s$[14]. Finally, the *S. platensis* sAC structure has not yet been determined in the absence of ligands, suggesting that there may be a distinct confor-

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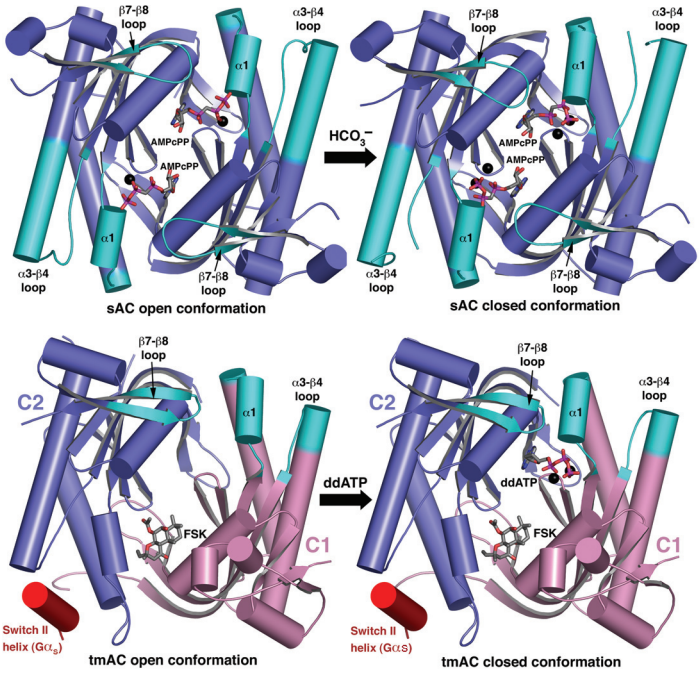

Figure 1 Atomic structures and conformational changes of *S. platensis* and $G\alpha_s$-bound transmembrane adenylyl cyclases. Structural regions that undergo ligand-induced conformational changes are cyan. *Streptomyces platensis* adenylyl cyclase (sAC) is a homodimer (both subunits colored blue), and has two active sites, demarked by the nonhydrolyzable substrate analog AMPcPP (top left). Upon treatment with bicarbonate, the α1 helices and β7-β8 loops collapse upon the active sites to create a 'closed' conformation (top right). The α3-β4 loops also change, although they move primarily into the plane of the figure. Note that the sAC active site binds two metal ions (black spheres) in its closed conformation. $G\alpha_s$-bound transmembrane adenylyl cyclase (tmAC, bottom left) is composed of two homologous domains (C1 and C2, pink and blue, respectively) and adopts an open conformation similar to that of sAC structure (top left), although the active site is slightly more open in sAC. Only the switch II helix of $G\alpha_s$ is shown (red). The hypotensive drug forskolin (FSK) binds in a vestigial active site adjacent to $G\alpha_s$. Binding of the substrate analog β-L-2′,3′-dideoxy-5′-ATP (ddATP) alters the same regions of the catalytic core as does bicarbonate treatment of sAC, leading to a similar closed conformation (bottom right). Helices are represented by cylinders and β-strands by arrows. Nitrogen atoms are blue, oxygens red, carbons gray and phosphates purple.

mation for the ligand-free enzyme. AMPcPP may simply trap the enzyme, as Rp-ATPαS is proposed to do, somewhere between the basal and activated states.

Regardless, it is now quite convincing that manipulation of the active site loops, whether in a pseudosymmetric setting (as in mammalian ACs) or in a homodimer (as in cyanobacterial sAC), is an ancient and conserved mechanism for allosteric control of class III AC activity (**Fig. 1**). The most notable feature of the bicarbonate-induced conformational change of sAC is the collapse of the α1 helix and β7-β8 loop toward the active site on both sides of the homodimeric enzyme[4]. In tmACs, $G\alpha_s$ binds between the α1-α2 and α3-β4 loops of the C2 domain, thereby facilitating formation of the active site on the opposite side of the enzyme. An insertion within the α3-β4 loop of the *T. brucei* AC domain is likewise responsible for external regulation of activity[11].

Future studies of sAC will undoubtedly focus on the mechanism of its unique regulation by bicarbonate, which remains an intriguing mystery despite its profound effects on the sAC structure. Additional studies are also required to fully understand the molecular basis for the enzymatic transformation of ATP to cAMP. The competitive ATP analogs used to trap sAC and tmAC in their 'active' conformations bind in ways that are not necessarily indicative of how ATP binds. For example, AMPcPP binds to the basal state of sAC in a conformation that seems appropriate for in-line attack by the ribose 3′ hydroxyl, but after bicarbonate treatment its α-phosphate no longer retains the required reaction geometry[4]. Two-metal ion catalysis also predicts that metal A directly coordinates the 3′ hydroxyl, as is strongly implied by atomic structures of T7 DNA polymerase[15]. This has yet to be observed in any of the class III crystal structures. However, the obvious conformational flexibility of structural elements within the sAC and tmAC active sites (**Fig. 1**) and the variety of ways in which nucleotide analogs bind to them bode well for the development of therapeutic or contraceptive drugs. The active site is flexible and roomy enough that it could accommodate drugs with substantial modifications—modifications that would undoubtedly be required to achieve isoform specificity[16].

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