The adenylyl and guanylyl cyclase superfamily

James H Hurley

New structures solved in 1997 revealed that the adenylyl cyclase core consists of a pair of catalytic domains arranged in a wreath. Homologous catalytic domains are arranged in diverse adenylyl and guanylyl cyclases as symmetric homodimers or pseudosymmetric heterodimers. The kinship of the adenylyl and guanylyl cyclases has been confirmed by the structure-based interconversion of their nucleotide specificities. Catalysis is activated when two metal-binding aspartate residues on one domain are juxtaposed with a key aspargine–arginine pair on the other. Allosteric activators of mammalian adenylyl cyclase, forskolin and the stimulatory G protein α subunit, promote the catalytically optimal juxtaposition of the two domains.

Addresses
Laboratory of Molecular Biology, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0580, USA; e-mail: jh8e@nih.gov

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Abbreviations

AC	adenylyl cyclase
cAMP	3′,5′ cyclic adenosine monophosphate
$G_{\beta\gamma}$	G protein β and γ subunits
GC	guanylyl cyclase
$G_{i\alpha}$	inhibitory G protein α subunit
$G_{s\alpha}$	stimulatory G protein α subunit
sGC	soluble GC

Introduction

3′,5′ cyclic adenosine monophosphate (cAMP) is the archetypal second messenger in species from bacteria to man. Its discovery in 1957 by Sutherland and co-workers led to the second messenger paradigm, a central concept in understanding how information is transmitted in cells. cAMP is synthesized from ATP following hormone stimulation in a wide variety of mammalian cells [1–6]. cAMP is produced in mammals by a family of at least nine adenylyl cyclase (AC) isozymes. The mammalian ACs differ from one another in their activation or inhibition by Ca^{2+}/calmodulin, phosphorylation by protein kinases A and C, the inhibitory G protein α subunit ($G_{i\alpha}$) and the G protein β and γ subunits ($G_{\beta\gamma}$). All mammalian ACs are activated by the GTP-bound stimulatory G protein α subunit ($G_{s\alpha}$) and all but AC9 are activated by the hypotensive drug forskolin.

The known mammalian ACs consist of 12 transmembrane helices and two cytoplasmic catalytic domains [7] (Figure 1). The two catalytic domains within the chain are referred to as C_1 and C_2. They are homologous, but are not identical to each other. The homologous portions of the cytoplasmic domains that are required for catalysis are referred to as C_{1a} and C_{2a}, and the remainder of the first and second cytoplasmic domains are C_{1b} and C_{2b}. Isolated AC catalytic domains reproduce many of the established properties of intact ACs [6,8–10].

cAMP plays a central role in regulating transcription in bacteria and in development in fungi and parasites. Most bacterial ACs are structurally unrelated to mammalian ACs and will not be discussed further here. A small number of bacterial ACs are homologous. These ACs are referred to as ‘class III’ in order to distinguish them from the nonhomologous bacterial ACs. Class III bacterial, yeast, slime mold and parasite ACs contain a single catalytic domain that is homologous to the mammalian C_1 and C_2 regions. This single domain contains all of the catalytic determinants from both the C_1 and C_2 regions of mammalian AC [11•]. These single-domain ACs are therefore believed to function naturally as homodimers with two active sites.

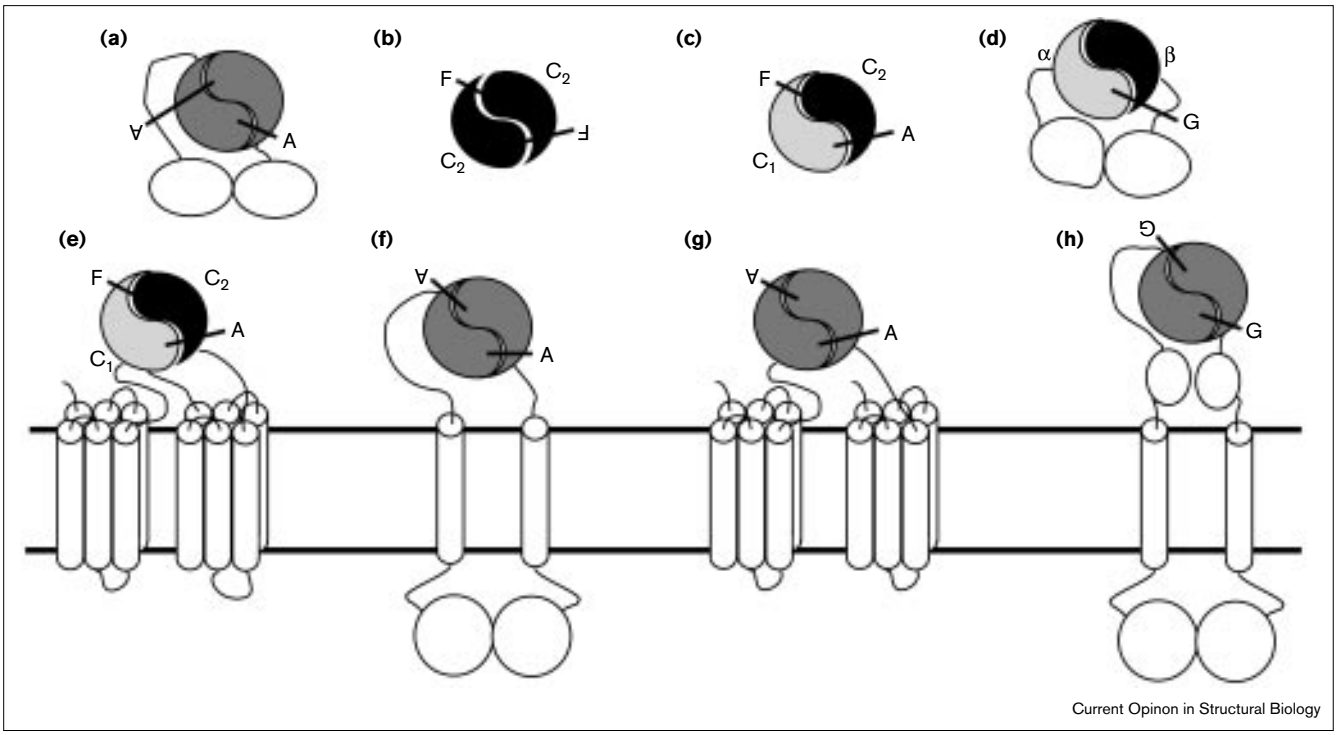
cGMP is a key messenger in phototransduction and in nitric oxide and atrial natriuretic peptide signaling [12]. The guanylyl cyclases (GCs) synthesize cGMP using a mechanism that is stereochemically analogous to that of the ACs. GCs are distributed throughout the animal kingdom and occur in both transmembrane and soluble forms. The transmembrane GCs contain a single transmembrane crossing and a single catalytic domain per polypeptide chain, and function as homodimers. Soluble GCs (sGCs) function as αβ heterodimers, with one catalytic domain contributed by each of the two subunits. All known GC catalytic domains are homologous to the mammalian AC C_1 and C_2 regions.

The past two years have seen the first crystal structures of AC catalytic domains. The structure determinations have been closely followed by a series of structurally inspired mutagenic analyses of both AC and GC catalytic domains. The soluble mammalian AC system has been exploited in order to answer some long-standing mechanistic questions. In this review, I attempt to address the implications of these new structures on the mechanism and regulation of both ACs and GCs. The emphasis is on mammalian ACs since both available crystal structures and much of the mutational and mechanistic data pertain to these enzymes.

Structure of adenylyl cyclase: a long time coming

Forty years elapsed between the discovery of AC and the structure determination of its catalytic unit. Mammalian ACs are polytopic membrane proteins that are daunting to crystallographers. Tang and Gilman [8] built on insights from homologies to construct the first soluble form of

Figure 1



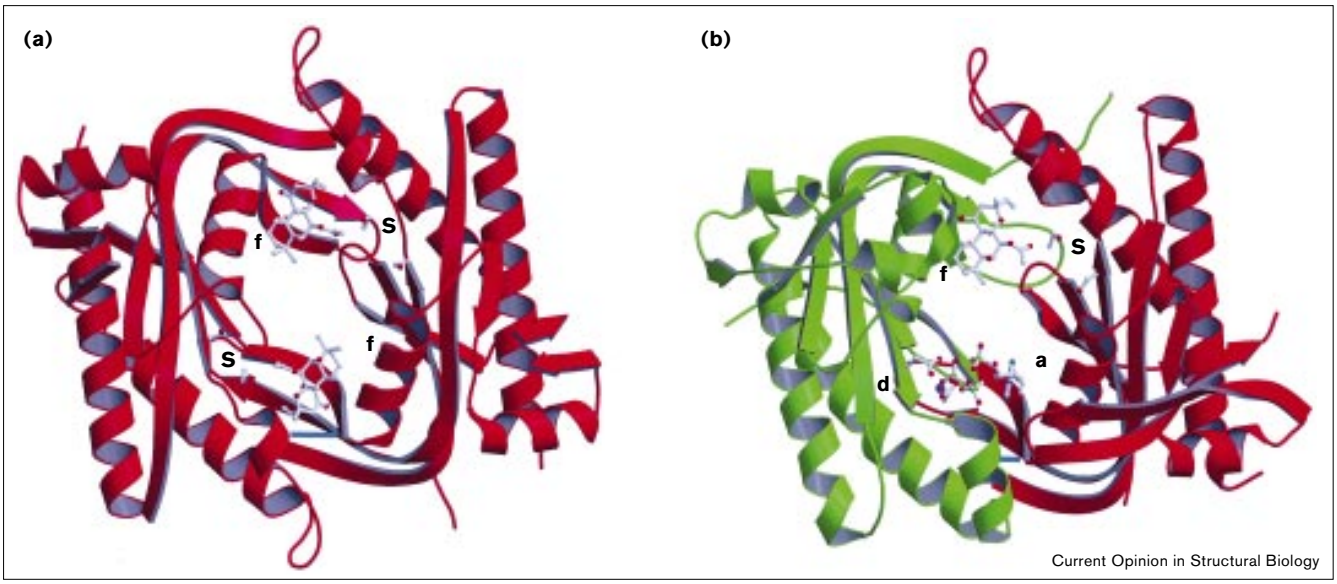
Topologies of natural and non-natural ACs and GCs [6]. **(a)** Yeast AC and soluble bacterial ACs. **(b)** Soluble mammalian C_2 homodimer. **(c)** C_1–C_2 heterodimer. **(d)** Soluble GC. **(e)** Mammalian AC. **(f)** *Dictyostelium* germination-specific AC (ACG) and parasite ACs. **(g)** A new membrane AC from the bacterium *Stigmatella aurantiaca* [41•]. **(h)** Membrane GCs. Letters A, F and G represent ATP, forskolin and GTP-binding sites, respectively. The twofold rotated ('upside down') letters represent twofold symmetry-related binding sites. C_1 and C_1-like domains are lightly shaded; C_2 and C_2-like domains are black. Homodimer domains are intermediately shaded in order to indicate that they have both C_1 and C_2-like properties and are thereby capable of complementing one another.

mammalian AC. This soluble AC chimaera consisted of the C_1 and C_2 domains of AC1 and AC2, respectively, connected by a soluble linker. It was perhaps not surprising that this soluble AC was enzymatically active, since soluble nonmammalian homologs were already known. What was most remarkable was the fact that this soluble AC, like the intact enzyme, was activated by both forskolin and $G_{s\alpha}$.

The linked C_1–C_2 construct was greatly encouraging to those seeking the structure of AC and was a major conceptual advance. Its utility was limited by its poor expression and quick degradation. The use of mixtures of the C_{1a} and C_2 regions that had been expressed and purified separately eliminated most of these problems [9,10]. These mixtures could be made in amounts that allowed a number of previously impractical biochemical experiments to be undertaken for the first time. In the course of experiments with isolated C_{1a} and C_2 proteins, it became clear that they could form homodimers as well as heterodimers. The homodimer of the C_2 domain, which is expressed as a recombinant protein far better than C_{1a} domains, displayed trace levels of enzyme activity. The activity of the C_2 homodimer is orders of magnitude below that of the fully active C_{1a}–C_2 heterodimer [13], but it is clearly discernible *in vitro* at very high enzyme concentrations and is also discernible in an *in vivo* complementation assay in an *Escherichia coli* strain lacking the *cya* gene that codes for AC [8]. My colleagues and I used limited proteolysis and mass spectrometry to refine the domain boundaries that had originally been defined by sequence homology and revealed an approximately 225 amino acid core that is responsible for the activity of the C_2 domain [13]. This shortened C_2 domain produced the crystals that led to the first structure determination of an AC catalytic core [14••].

The remaining obstacles to determining the structure of a soluble AC heterodimer were resolved. The AC5 C_{1a} domain proved to be more stable as a recombinant protein than other C_{1a} domains [15,16]. Mixtures of C_{1a} and C_2 domains contain homodimers as well as heterodimers, hence it is not surprising that the only reported crystal form of an AC heterodimer was grown in the presence of $G_{s\alpha}$. $G_{s\alpha}$ binds very tightly to the heterodimer and, presumably, stabilizes it relative to the homodimers. The only drawback to this approach is that it cannot be used to determine a basal-state structure of the AC heterodimer in the absence of an activator. The structure of

Figure 2



Symmetry and pseudosymmetry in cyclase homodimers and heterodimers. **(a)** Structure of the C_2 homodimer. **(b)** Structure of the C_1–C_2 heterodimer. C_1 and C_2 domains are green and red, respectively. Binding sites are indicated: f, forskolin; a, P-site inhibitor; s, the AC2 pair Ser891 and Ser942; and d, the AC5 pair Asp396 and Asp440, related by the pseudo-twofold axis to the serine pair. The crystallographic Mg^{2+} ion is shown in purple near the P-site inhibitor. The solid blue bar near the bottom of each wreath shows that the interdomain α1–β5′ distance is shorter in the $G_{s\alpha}$ and forskolin-activated heterodimer compared to the forskolin-bound homodimer, indicating the scale of active site closure.

the AC5 C_{1a}–AC2 C_2 heterodimer was determined by molecular replacement using the C_2 homodimer and $G_{i\alpha}$ structures [17••].

Symmetric and pseudosymmetric cyclase wreaths

The structure of the AC2 C_2 homodimer revealed two C_2 monomers intertwined like two boughs in a wreath [14••] (Figure 2). The catalytic site and the forskolin regulatory site are both formed at the dimer interface, within a single deep cleft between the boughs. Dimer interface residues are better conserved among AC and GC catalytic domains than other surface residues, suggesting that the wreath-like dimer would be conserved in mammalian AC heterodimers and in other members of the AC/GC superfamily. The structure of the AC5 C_1–AC2 C_2 heterodimer confirmed that the heterodimer has an essentially identical wreath-like arrangement [17••].

The catalytic ATP-binding site and the regulatory forskolin-binding site are intimately related. The C_2 homodimer binds two molecules of forskolin at two sites that are related in the crystal by local twofold symmetry. Only one of the forskolin sites is present in the fully active C_1–C_2 heterodimer [18]. The second forskolin binds to the homodimer in a site that overlaps with the active site in the C_1–C_2 heterodimer [11•,17••]. There are many similarities between the binding of forskolin and the binding of the adenine moiety of ATP. The hydrophobic purine and diterpene moieties of ATP and forskolin form van der Waals' contacts with corresponding residues. The adenine N_6 makes a hydrogen bond with a conserved aspartate (AC2 Asp1018), whereas the functionally essential O_1 hydroxyl of forskolin makes a hydrogen bond to the C_1 counterpart of the same aspartate (AC1 Asp419). The replacement of a catalytic site aspartate (AC1 Asp354, AC5 Asp440) by a forskolin site serine (AC2 Ser942) is the most important difference between these otherwise similar sites. The close similarity between the forskolin site and the active site probably explains why the C_2 homodimer has a very low level of residual activity, despite the replacement of its active site with a second forskolin-binding site.

Sources of specificity

There is now overwhelming evidence that the wreath architecture is a general property of the AC and GC superfamily, despite the absence of a crystal structure of a GC catalytic domain. Homology models for GCs were derived from the AC wreath using both the homodimer and the heterodimer structures as starting points. These models correctly predicted the determinants of GTP specificity in both homodimeric membrane GCs [19••] and heterodimeric sGCs [20••]. The success of the homology modeling and mutagenic analysis provides indirect but still compelling evidence that all GCs conform to the archetypal wreath.

Recent studies have clarified the origins of nucleotide specificity in the ACs and GCs. The two most important residues are situated on the C_2 domain and β subunit in heterodimeric ACs and GCs, respectively, and they interact directly with the outer edge of the six-membered purine ring of the

nucleotide [11•,17••,19••,20••]. In ACs, an aspartate and a lysine (AC2 Asp1018 and Lys938) recognize the exocyclic amine and the unprotonated N_1 of adenine, respectively. The aspartate and lysine discriminate against the O_6 and N_2, and the protonated N_1 of guanine, respectively. In GCs, the aspartate and lysine are replaced with a cysteine and a glutamate. The cysteine probably forms a weakly polar hydrogen bond to the O_6 of guanine and the glutamate probably accepts hydrogen bonds from hydrogens on the N_1 and N_2 of guanine. In AC heterodimers, a glutamine (AC5 Gln503) from the C_1 domain stabilizes the conformation of the lysine and contributes to specificity indirectly [17••,20••]. This glutamine is replaced with Arg592 from the α subunit in sGC. This arginine is thought to stabilize the specificity pocket glutamate [20••]. A mainchain carbonyl interaction with the adenine N_6 also contributes to the ATP specificity of AC. Since this interaction is hard to perturb by mutagenesis, it has not been possible to engineer a completely GTP-specific mutant AC [20••].

The equivalent Arg→Gln replacement in a homodimeric retina GC, retGC-1, resulted in a constitutively active enzyme [19••], although this was not seen in heterodimeric AC and sGC. The difference between the two studies is that homodimer mutants change both sides of the active site at once. The GC specificity pocket arginine (retGC-1 Arg995) is located adjacent to the homodimer twofold axis, so the arginines from each monomer approach each other very closely. Normal regulation of the specificity-reversed homodimeric GC is restored by replacing two hydrophobic residues, Leu998 and Phe999, with the AC counterparts, isoleucine and tryptophan. The phenylalanine is part of both the dimer interface and the purine pocket [19••]. These findings show there is interplay between the structure of the dimer interface and the ability to bind substrate. This idea is provocative because it suggests that the regulation of subunit interactions in the dimer could control activity, in part, by altering the structure of the specificity pocket.

P sites, pyrophosphates and polymerases

Several lines of evidence converge on a rough but clear picture of the AC reaction pathway. There are limitations to the picture in that no transition-state analogs are established and crystal structures of ground-state complexes have been elusive. The central ingredients in our current picture of the enzyme mechanism are kinetic and mutagenic analyses of catalytic determinants, the structure of AC bound to a 'P site' adenosine analog, pyrophosphate and Mg^{2+} ions, model building of the ATP complex, and analogies to the well-studied DNA polymerase I family.

P-site inhibitors, so called because they contain a purine ring, are noncompetitive inhibitors with respect to the forward reaction of AC [21]. P-site inhibitors are, however, competitive inhibitors with respect to cAMP in the reverse reaction [22•]. P-site inhibition is enhanced when AC is activated by forskolin and $G_{s\alpha}$. Inhibition is sensitive to mutations that affect the K_m for ATP, but the pattern of sensitivity is different. For example, the AC1 mutation K923A (AC2 residue 938) increases the K_m(ATP) threefold, but the K_i for adenosine 2′-deoxy 3′-monophosphate increases 200-fold.

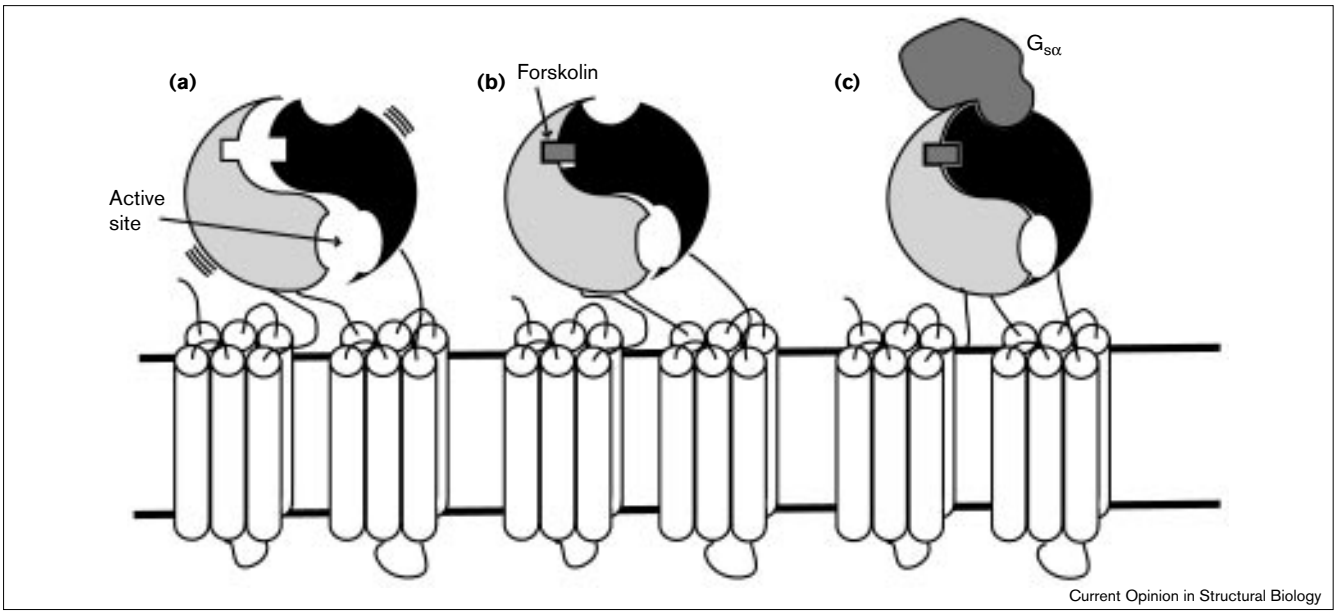
This odd class of inhibitors plays a key role in the structural biology and enzymology of AC for several reasons. Different mutational effects on ATP and P-site binding suggest that they bind different conformations of AC. The enhanced binding of P-site inhibitors to activated conformations of AC suggests that the product-bound conformation is close to the transition state-bound conformation, although other explanations have been offered [17••]. Both P-site inhibitors tested bound to the $G_{s\alpha}$-activated soluble AC heterodimer, whereas ATP and its competitive inhibitors fail to bind in this crystal. Although conformational changes are likely, the P-site complexes are the still the best starting point for modeling the structure of the ATP complex.

When the structure of the C_2 homodimer was determined, similarities were noted between a substructure comprising less than half of the C_2 domain and a variety of unrelated proteins containing a double-split βαββαβ motif [23,24]. This is one of the simplest and most abundant motifs in protein structure. The most extensive similarities were with the palm domains of various DNA and RNA polymerases of the DNA polymerase I family. Only two amino acids are conserved between the polymerases and the cyclases, but these two C_1 domain aspartates (AC1 310 and 354; AC5 396 and 440) are critical for enzyme function [11•,25]. These polymerases catalyze the Mg^{2+} ion-dependent attack of the 3′ hydroxyl of a growing primer on the α phosphate of a deoxynucleotide. The reaction is analogous to that of the ACs and GCs, which catalyze the intramolecular attack of a 3′ hydroxyl on a nucleotide α phosphate. Stryer and co-workers have noted the close stereochemical similarities between DNA polymerase I and photoreceptor GC [26]. The structure of the AC heterodimer–P-site inhibitor complex includes one Mg^{2+} ion, which is bound with precisely the same coordination as the 'B' ion in the two ion catalytic site of the polymerase palm domain. This finding conclusively confirms a functional similarity between the AC/GC superfamily and the DNA polymerases, although it does not resolve whether this similarity arose by convergent or divergent evolution.

A two metal mechanism

ACs were shown to require two Mg^{2+} ions for catalysis nearly a quarter of a century ago [27]. One ion binds together with ATP, while a second acts kinetically as a free Mg^{2+} ion. The crystallographically located ion in the AC heterodimer–P-site inhibitor complex almost certainly corresponds to the former [17••]. The putative second metal ion has so far eluded detection in crystals of AC. A recent structure of the T7

Figure 3



Scheme for the activation of intact AC by forskolin and $G_{s\alpha}$. **(a)** Hypothetical basal state, with conformational flexibility suggested by motion lines. **(b)** The forskolin, but not $G_{s\alpha}$-activated state, based on the C_2 homodimer structure. **(c)** The forskolin and $G_{s\alpha}$-activated state, based on the heterodimer structure. The 7° rotation is shown to scale. The C_1 and C_2 domains are lightly shaded and black, respectively.

DNA polymerase primer–template–nucleotide complex shows how the nucleotide probably binds to AC [28••]. The surprise is that in the T7 polymerase complex, the 'A' metal ion binds to the same acid pair as the 'B' ion. Moreover, the B ion interacts with all three phosphates and ATP is bound in an unusual tightly folded conformation, also seen in DNA polymerase β [29]. The A ion can be incorporated into the AC–ATP complex without violating known stereochemical constraints. The A ion is predicted to be coordinated directly by the 3′ hydroxyl of ATP and thereby activates it for attack on the α phosphate. Both the A and B ions directly interact with the α phosphate oxygens and are expected to stabilize the transition state.

Genetic screens have isolated Asn1025 and Arg1029 of AC2 as being essential for catalysis [30•]. The replacement of either one of these residues by alanine or serine reduces activity by about two orders of magnitude. Modeling suggests these two residues could play a role in stabilizing the negative charge on the pyrophosphate leaving group. Lys1067, located on the β7–β8 'lid', also contributes to pyrophosphate interactions. The acid pair on the C_1 domain cooperates with an asparagine–arginine pair on the C_2 domain to enable catalysis. The key conclusion is that two pairs of residues, one from each of the C_1 and C_2 domains, are juxtaposed in order to carry out catalysis. This concept is fundamental to thinking about the regulation of AC enzyme velocity. The close juxtaposition of the C_1 and C_2 domains in the proper orientation is clearly a prerequisite both for substrate binding, as described above, and for catalysis.

Allosteric activation: Elmer's glue plus a seven degree rotation

The complexes of the AC catalytic core with forskolin and $G_{s\alpha}$ suggest activation mechanisms. The AC2 C_2 homodimer revealed that forskolin acts as a direct intermolecular bridge that attaches the two boughs of the cyclase wreath to each other, much like Elmer's glue. AC9's unresponsiveness to forskolin can be traced to replacements of a serine and a leucine (AC2 Ser942 and Leu912) in the forskolin-binding site. Responsiveness is restored by mutating these two residues to their counterparts in forskolin-sensitive ACs [31•].

Since the discovery of forskolin as an AC activator, there has been speculation about the existence of its endogenous counterpart. The forskolin-binding site could be functionless detritus left over from the early evolution of an asymmetric core from a symmetric one. If so, it is hard to understand why such a destabilizing hydrophobic cleft would have been preserved in all mammalian AC structures, rather than being filled in or made more polar. The binding site is sterically closed and buries 90% of the solvent-accessible surface area of the forskolin molecule, which seems to rule out a macromolecular ligand. Attempts to identify the "endogenous forskolin" in the 1980s were unsuccessful [32]. There is new hope because soluble AC provides a far more sensitive read-out than was available in the past and the genetic manipulation of forskolin responsiveness may also be helpful in screening. sGC contains a hydrophobic pocket of unknown function, formed from a disabled catalytic site. sGC is not activated by forskolin, but it is activated by another nonphysiological hydrophobic

compound, YC-1, which might act using a forskolin-like mechanism [33].

Like forskolin, $G_{s\alpha}$ bridges C_1 and C_2 by forming direct interactions with each domain [17••,34••,35,36]. $G_{s\alpha}$ binds to a hydrophobic negatively charged groove formed by the α2 and α3 helices of the C_2 domain, and to the hydrophobic N-terminal portion of the C_1 domain. $G_{s\alpha}$ can function as Elmer's glue between the domains, although this cannot be its sole function. Mutation of the $G_{s\alpha}$ contact on the C_1 domain abolishes activation of soluble AC in the absence of forskolin, but $G_{s\alpha}$ activation can be partially rescued in the presence of forskolin [34••]. This shows that the 'gluing' together of the cyclase dimer is a prerequisite to activation by $G_{s\alpha}$. A 'nongluing' $G_{s\alpha}$ mutant is still capable of activating a preassembled dimer that was glued together by forskolin.

A comparison of the $G_{s\alpha}$-bound AC heterodimer and the unbound homodimer structures suggests that the 'nongluing' role of $G_{s\alpha}$ is to induce a 7° rotation of the C_1 domain relative to C_2. $G_{s\alpha}$ binds to the outside of the wreath, proximal to the forskolin side of the cleft and distal to the catalytic side of the cleft. $G_{s\alpha}$ pushes on the proximal portion of the C_1 domain indirectly, via the α1–α2 loop of the C_2 domain. This torque pushes the catalytic site closed, with the C_1 aspartate pair moving 2 Å closer to the C_2 asparagine–arginine pair. Less interesting explanations for the 7° rotation cannot be completely ruled out at this stage. The bulk of the second forskolin could act as a wedge for propping open the homodimer counterpart of the active site, for example.

This conformational change is undramatic compared to those seen in many other allosteric enzymes. On the other hand, the comparison can only be made between the forskolin and $G_{s\alpha}$-bound heterodimer and the forskolin-bound homodimer, because the forskolin-free structure is unavailable. The C_2 homodimer probably has a very low enzyme activity because it lacks the catalytic aspartate pair, not because its conformation matches that of the inactive AC heterodimer. Larger structural changes are anticipated between the true basal conformation and the dually activated conformation seen in the heterodimer.

What role, if any, does forskolin and $G_{s\alpha}$ "glue" play in the normal activation of an intact mammalian AC (Figure 3)? The C_1 and C_2 domains are probably preassociated in intact AC and therefore may not need an external dimerization agent. The two chains of the sGC heterodimer and the membrane GC homodimer are also associated in the basal state, as well as in the activated state. On the other hand, it seems an improbable coincidence that the soluble AC model would be activated so potently by this mechanism if it had no natural role. A working hypothesis is that the C_1 and C_2 domains of intact AC are preassociated, but in a loose arrangement that might have some conformational flexibility. Activator binding might pin the domains into a more tightly defined conformation through the same interactions that glue soluble AC. This hypothesis fits the observation that the most forskolin-sensitive soluble AC combinations are activated more than 10^3-fold, substantially more than observed for any intact AC.

Despite the spectacular headway made in understanding mammalian AC activation by $G_{s\alpha}$ and forskolin, much less is known about other AC and GC activation mechanisms. Mammalian AC inhibition by $G_{i\alpha}$ is thought to occur through a reciprocal mechanism to that observed for $G_{s\alpha}$ activation [17••,34••]. $G_{i\alpha}$ probably binds to the α2–α3 groove on C_1 and pushes the active site into a less favorable conformation. It is unclear as to whether AC activation by Ca^{2+}/calmodulin, $G_{\beta\gamma}$ binding and protein phosphorylation can be worked into the scheme of regulation by domain movements or whether there are entirely different mechanisms at work. The principles of mammalian AC regulation by G_α subunits seem to depend fundamentally on the asymmetry of these systems. This suggests that the regulation of homodimeric membrane ACs and GCs may differ markedly from the regulation of heterodimeric cyclases.

The noncatalytic regions of AC have received less attention than the catalytic domains over the past two years. Their structures have been elusive, but there is no question that they matter for regulation. Devreotes and co-workers isolated a puzzling point mutant in the juxtamembrane region preceding the C_1 domain of *Dictyostelium* aggregation-specific AC (ACA) that constitutively activates the enzyme [37]. Noncatalytic regions of AC may play a role in $G_{\beta\gamma}$ activation. Even though a flexible loop preceding α3 in the C_2 domain is critical for $G_{\beta\gamma}$ binding [38], this region alone is insufficient to make the soluble AC heterodimer $G_{\beta\gamma}$ sensitive. The function of the C_{1b} region, the locus for calmodulin binding to AC1 [39,40], needs to be further explored. Finally, there is the matter of the 12 transmembrane segments. The possible transporter role suggested a decade ago [7] has yet to be confirmed, despite considerable effort. No other clear-cut function, aside from a trivial role in membrane anchoring, has emerged. A bacterial AC that has six transmembrane segments was recently cloned [41•]. It probably forms homodimers with 12 transmembrane segments. Perhaps it will suggest a new perspective on this vexing question.

Conclusion

Forty years after AC was discovered, crystal structures of the mammalian AC catalytic core have revealed conserved mechanisms for catalysis and specificity. The catalytic mechanism is conserved among a larger superfamily of ACs and GCs. The regulation of the AC and GC superfamily members is, in contrast, quite diverse. Two of the most important regulatory mechanisms are now understood in neat atomic detail — activation of mammalian AC by forskolin and $G_{s\alpha}$. A picture of $G_{i\alpha}$ inhibition of AC5 is emerging as well. There are many other regulators of ACs and GCs, in addition to forskolin and G_α subunits. Nitric oxide, the activator of sGC, is perhaps the most timely example. We have yet to understand in a mechanistic sense how these regulators control AC or GC activity. A major

challenge for future structural studies will be to understand how such a wide range of regulators modulates the activity of an equally diverse set of enzymes. Will all of them act through the dimer interface, as do forskolin and the G_α subunits? Or will entirely different mechanisms emerge?

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- • of special interest
- •• of outstanding interest

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