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MINIREVIEW

Catalytic Mechanism and Regulation of Mammalian Adenylyl Cyclases

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cAMP is a key player in the intracellular signaling pathways of hormones, neurotransmitters, odorants, and chemokines. By activating PKA and cyclic nucleotide-gated ion channels, this protean second messenger can change cellular attributes as diverse as the membrane potential and the rate of cell division. The key step in regulating intracellular cAMP is modulation of adenylyl cyclase activity. Adenylyl cyclase, the enzyme that synthesizes cAMP, is subject to coincident regulation by both extracellular and intracellular stimuli. After the original cloning of the mammalian adenylyl cyclase gene by Krupinski *et al*. (1989), at least nine isoforms of mammalian adenylyl cyclases have been cloned and analyzed, revealing diverse regulation by G proteins, Ca^{2+}, and phosphorylation (reviewed in Sunahara *et al*., 1996, and a monograph edited by Cooper, 1997). Historically, extensive biochemical analysis has been hampered by the inability to express and purify cyclase in its native, membrane-bound form. Fortunately, the catalytic core domains of adenylyl cyclase can be used to construct a soluble enzyme that maintains sensitivity to its physiological regulators and can be expressed in useful levels in *Escherichia coli* (Tang and Gilman, 1995). This soluble enzyme has a turnover number (ranging from 4 to 100 sec^{-1}) comparable to or greater than the maximal activity of the membrane-bound enzyme (Dessauer and Gilman, 1996; Whisnant *et al*., 1996; Yan *et al*., 1996; Scholich *et al*., 1997; Sunahara *et al*., 1997). It has emerged as an extraordinarily useful model for studying the biochemical mechanisms of catalysis and regulation of full-length mammalian adenylyl cyclases. In this review, we focus on how adenylyl cyclase catalyzes the formation of cAMP from ATP in response to its regulators. For the first time, this can be addressed in molecular detail using the recently solved structures of adenylyl cyclase associated with or without its stimulatory G protein and product analogs (Zhang *et al*., 1997b; Tesmer *et al*., 1997).

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Catalytic Core of Adenylyl Cyclase

Extracellular signals (i.e., hormones, neurotransmitters, odorants, and autocrines) require at least three membrane components to modulate intracellular cAMP: a heptahelical receptor, a heterotrimeric G protein, and adenylyl cyclase. The main stimulatory effect of adenylyl cyclase by hormones and neurotransmitters is mediated by $G_{s\alpha}$, the α subunit of G_s protein that stimulates adenylyl cyclase. Although each of the nine isoforms (types I–IX) of mammalian adenylyl cyclases has its unique and diverse regulation (Table 1), they share a common structure: two cytoplasmic domains (C_1 and C_2), each following a transmembrane stretch that hypothetically has six α-helices (M_1 and M_2) (Fig. 1). The C_1 and C_2 domains have ~230 amino acid regions (C_{1a} and C_{2a}) that share 50% or high similarity and form the catalytic core (Fig. 2). Systematic mutational analysis has shown that the amino acid residues from both C_{1a} and C_{2a} domains contribute to ATP binding and catalysis (Tang *et al*., 1995; Yan *et al*., 1997b). Truncation analysis has permitted expression and high yield purification of $G_{s\alpha}$-regulated, soluble enzymes using only C_{1a} and C_{2a} domains, from either the same or different mammalian adenylyl cyclases (Tang *et al*., 1995; Tang and Gilman, 1995; Dessauer and Gilman, 1996; Whisnant *et al*., 1996; Yan *et al*., 1996, 1998; Scholich *et al*., 1997; Sunahara *et al*., 1997). Such soluble enzymes also can be activated by the diterpene forskolin, derived from the root of the Indian plant *Coleus forskolhii* (Seamon and Daly, 1986).

The tertiary structure of the cyclase domain has been elucidated by the structure of a nearly inactive C_{2a} homodimer of type II adenylyl cyclase (IIC_2) (Zhang *et al*., 1997a, 1997b) and the fully active, heterodimeric combination of type V C_{1a} and type II C_{2a} (Tesmer *et al*., 1997). As expected from the high homology between C_{1a} and C_{2a}, their tertiary structures

ABBREVIATIONS: PKA, protein kinase A; PKC, protein kinase C.

are almost identical, consisting of a three-layer α/β sandwich with the C_{1a} and C_{2a} domains arranged in head-to-tail fashion as a wreath (Fig. 3, A and B). The cyclase domain contains a $\beta\alpha\beta\beta\alpha\beta$ substructure that resembles the palm domains of the polymerase I family of prokaryotic DNA polymerase, including *E. coli* DNA polymerase I and *Thermus aquaticus* (*Taq*) polymerase. The interface of C_{1a} and C_{2a} domains can accommodate two potential ATP binding sites (Fig. 3, A and B). Only one of the sites consists of crucial residues for catalysis and binds substrate, whereas the other is the site for the binding of forskolin (Dessauer *et al.*, 1997; Liu *et al.*, 1997; Tesmer *et al.*, 1997; Yan *et al.*, 1997b, 1998).

The wreathlike dimer arrangement is likely to exist in other enzymes that perform the same or similar reaction (Fig. 1; reviewed in Tang *et al.*, 1997). These include many homologous adenylyl cyclases and guanylyl cyclases (enzymes that convert GTP to cGMP). An integral membrane adenylyl cyclase that has six putative transmembrane-helices is found in *Mycobacteria* (MtAC). One transmembrane-helix form of adenylyl cyclases occurs in parasites (TbESAG and LdRAC) and in slime mold (DdACG). The family of membrane-bound receptor guanylyl cyclase from mammals (GC A-G), fruit fly (*Dm*GC), sea urchin (*Sp*GC), and nematode (*Ce*GC) is growing rapidly (Yu *et al.*, 1997, and references therein). There are peripheral membrane adenylyl cyclases from yeast and soluble adenylyl cyclases from bacteria and plant (BlAC, RmAC, Tobacco axi). Although not determined experimentally, cyclases with the homodimeric cyclase domain are predicted to have two NTP binding sites, consistent with positive cooperativity of membrane-bound guanylyl cyclase with respect to GTP (Wedel *et al.*, 1997; Liu *et al.*, 1997). NO-activated soluble guanylyl cyclases consist of α and β subunits that are homologous to C_{1a} and C_{2a} of mammalian adenylyl cyclase (Hobbs, 1997). The C_{1a}/C_{2a} structure is unlikely to apply to the energy state-regulated adenylyl cyclases from *Enterobacteria* and the calmodulin-sensitive adenylyl cyclase/toxins from *Bacillus anthracis* and *Bordetella pertussis* because no sequence similarity can be detected among them (Tang *et al.*, 1997, and references therein).

Structure of the $G_{s\alpha}$ and Forskolin Binding Sites of Mammalian Adenylyl Cyclases

Many neurotransmitters and hormones (i.e., epinephrine, dopamine, and luteinizing hormone) activate G_s-coupled receptors, leading to stimulation of mammalian adenylyl cyclase by GTP-$G_{s\alpha}$ at a picomolar concentration. Although $G_{s\alpha}$ is palmitoylated, the high affinity of $G_{s\alpha}$ for adenylyl cyclase is likely to depend on another unidentified lipid modification (Linder *et al.*, 1993; Kleuss and Gilman, 1997). The $G_{s\alpha}$ binding site of adenylyl cyclase has been localized to a small region of C_{1a} (amino terminus) and a much larger negatively charged and hydrophobic groove on C_{2a} (α2 and α3/β4) by mutagenic mapping (Yan *et al.*, 1997a). The $G_{s\alpha}$ binding site is ~30 Å away from the catalytic site (Fig. 3C) (Yan *et al.*, 1997a; Tesmer *et al.*, 1997). The adenylyl cyclase binding site of $G_{s\alpha}$ also has been mapped to switch 2 (α2) and the loop regions of α3/β5 and α4/β6; only switch 2 region changes conformation on the binding of GTP (Berlot and Bourne, 1992; Lambright *et al.*, 1994).

TABLE 1
Regulators of adenylyl cyclases

Class	Regulators	Cyclases	Effects	Conditions	Sites	References
G_s/G_i	$G_{s\alpha}$	All	+		High affinity: C_{1a} (amino terminus), C_{2a} (α2, α3/β4)	Yan *et al.*, 1997a; Tesmer *et al.*, 1997)
					Low affinity: ACVI-C_{1b}	Chen *et al.*, 1997
	$G_{\beta\gamma}$	I	−	CaM > $G_{s\alpha}$ >> forskolin	Unknown	Tang and Gilman, 1991
		II, IV, VII	+	$G_{s\alpha}$ >> forskolin	C_{2a} (α3)	Tang and Gilman, 1991; Gao and Gilman, 1991; Yoshimura *et al.*, 1996; Chen *et al.* 1995
	$G_{i\alpha}$	I, V, VI	−		Unknown	Taussig *et al*, 1994
	$G_{o\alpha}$	I	−	CaM >> forskolin > $G_{s\alpha}$	Unknown	Taussig *et al.*, 1994
	$G_{z\alpha}$	I, V	−		Unknown	Kozasa and Gilman, 1995
Small chemicals	Forskolin	All except IX	+	$G_{s\alpha}$ synergy	C_1/C_2 interface	Zhang *et al.*, 1997b; Tesmer *et al.* 1997; Yan *et al.*, 1998
	Adenosine	All	−	Enhanced by PPi	C_1/C_2 interface, cAMP competitor	Tesmer *et al.* 1997; Liu *et al.*, 1997
Feedback inhibition	PKA	V, VI	−	$G_{s\alpha}$, forskolin	Ser674 (ACVI)	Iwami *et al.*, 1995; Chen *et al.*, 1997
Ca^{2+}/G_q	Ca^{2+}/CaM	I, VIII	+		ACI-C_{1b} (495–522), ACIII, VIII–unknown	Tang *et al.*; Cali *et al.*, 1994; Vorherr *et al.*, 1993; Wu *et al.*, 1993
	PKC	II, V, VII	+		ACII (1034-1068, Thr 1057)	Kawabe *et al.*, 1994; Jacobowitz and Iyengar, 1994; Yoshimura *et al.* 1993; Zimmermann and Taussig, 1996; Levin and Reed, 1995; Bol *et al.* 1997
		VI	−		Unknown	Lai *et al.* 1997
	Ca^{2+a}	V, VI	−		C_{1b}[b]	Yoshimura and Cooper, 1992; Scholich *et al.* 1997
	CaM kinase[a]	III	−			Wei *et al.*, 1996
	PP2B[a]	IX	−			Antoni *et al.*, 1995

[a] Direct modulation of adenylyl cyclases remains to be determined.
[b] There are high and low affinity sites and C_{1b} is likely to be the low affinity site, which is common among adenylyl cyclases.

The crystal structure of $VC_1/IIC_2/G_{s\alpha}$ reveals the molecular nature of the $G_{s\alpha}$/adenylyl cyclase interaction (Tesmer *et al*., 1997). The interface between adenylyl cyclase and $G_{s\alpha}$ is substantial (1800 Å^2), and switch 2 is a major component of the adenylyl cyclase binding site of $G_{s\alpha}$ (Tesmer *et al*., 1997). The main contact between C_1 and $G_{s\alpha}$ is the hydrophobic interaction between AC1 F293 and $G_{s\alpha}$ W281 (Tesmer *et al*., 1997; Yan *et al*., 1997a). The $C_2/G_{s\alpha}$ interaction is both polar (negative in C_2 and positive in $G_{s\alpha}$) and hydrophobic (Tesmer *et al*., 1997; Yan *et al*., 1997a). Kinetic analysis has suggested more than one $G_{s\alpha}$ binding sites based on the biphasic activation of type VI enzyme by $G_{s\alpha}$, and the low affinity site of type VI enzyme seems to be blocked by a peptide from the C_{1b} region (Chen *et al*., 1997). This putative $G_{s\alpha}$ binding site at the C_{1b} region may play a role in interacting with the region that is not involved in the contact between C_{1a}/C_{2a} and $G_{s\alpha}$ in the $VC_1/IIC_2/G_{s\alpha}$ structure (i.e., $\alpha 4/\beta 6$, which is 10 Å away from C_{1a}/C_{2a}; Tesmer *et al*., 1997).

Forskolin, a hypotensive diterpene, has been profoundly useful as a pharmacological probe for adenylyl cyclase function *in vivo* and *in vitro* (Seamon and Daly, 1986). Forskolin binds to only one site on the C_1/C_2 heterodimer, which is virtually identical to the two nearly symmetric sites on the IIC_2 homodimer (Dessauer *et al*., 1997a; Tesmer *et al*., 1997; Zhang *et al*., 1997b). Interactions between forskolin and adenylyl cyclase are predominantly hydrophobic, but specificity is enhanced by hydrogen bonds between O1 and O11 and C_1 and between the 7-acetyl and a serine at the $\beta 2'$-$\beta 3'$ turn on C_2 (927 and 942 on type I and II, respectively) (Fig. 3D). This is supported by structure-activity studies showing that 1-OH and 9-OH groups of forskolin and interaction between 7-acetyl groups of forskolin and S942 of IIC_2 are required for optimal activation of adenylyl cyclase by forskolin (Robbins *et al*., 1996; Yan *et al*., 1998).

One major surprise in the structure of adenylyl cyclase is the existence of a highly conserved hydrophobic pocket at the interface of C_{1a}/C_{2a}. Judged by primary sequence, this pocket in mammalian type IX enzyme is different from the other

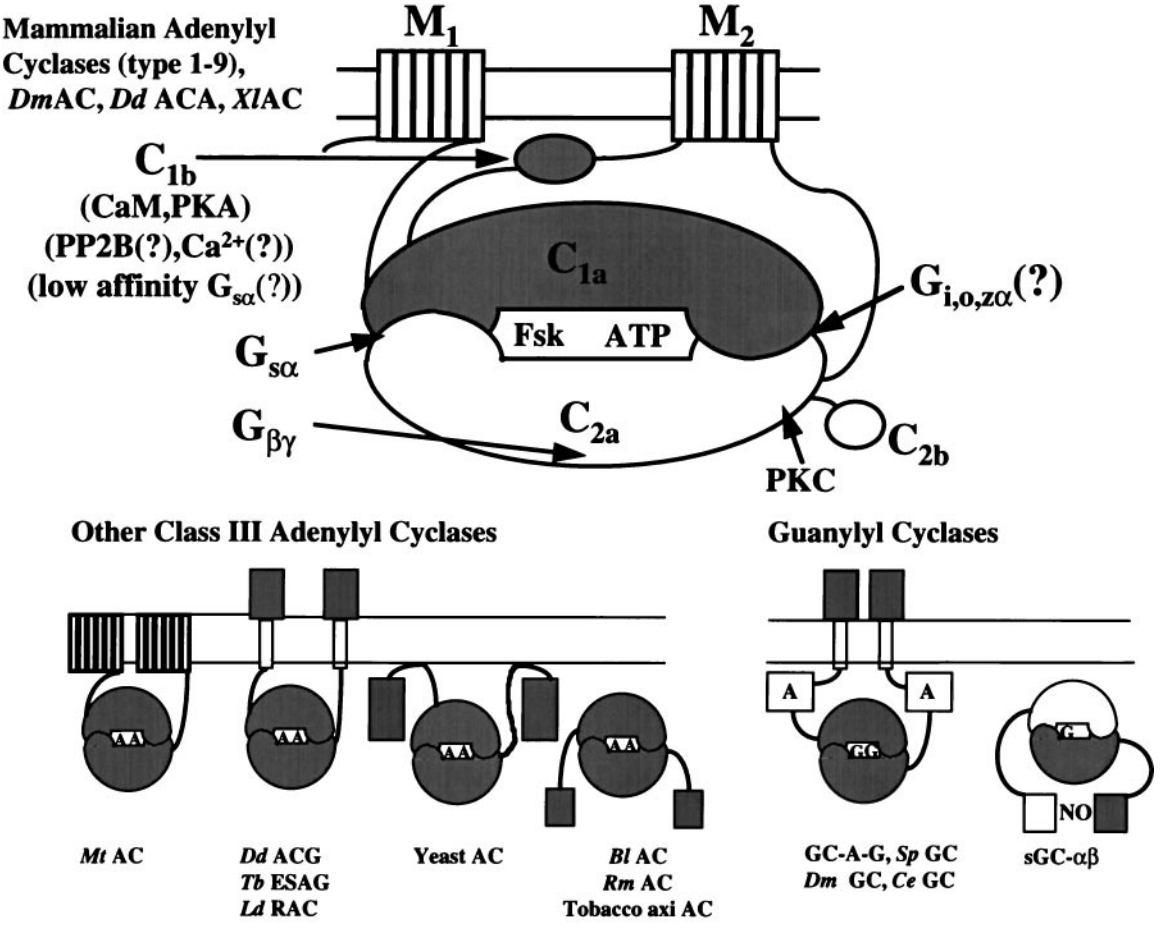

Fig. 1. Models of adenylyl cyclases. Mammalian adenylyl cyclase with indicated sites for catalysis and binding of the regulators (*top*) and the related adenylyl and guanylyl cyclases (*bottom*). Adenylyl cyclases (types I–IX): type I (Genbank accession numbers M25579, L05500), type II (M80550), type III (M55075), type IV (M80633), type V (M88649, M83533, Z29371, M96159), type VI (M94968, M96160, L01115, M93422), type VII (U12919, Z49806), type VIII (L26986, Z35309), type IX (Z50190), *Xl*AC: *Xenopus laevis* adenylyl cyclase (Z46958), *Dm*AC: *Drosophila melanogaster* adenylyl cyclase (rutabaga: M81887, DAC9:AF005630), *Dd*ACA: *Dictyostelium discoideum* adenylyl cyclase involved in aggregation (M87279), MtAC: *Mycobacteria tuberculosis* adenylyl cyclase (AF017731), TbESAG: *Trypanosoma brucei* expression site-associated gene (X52118, X52120, X52121), LdRAC: *Leishmania donovani* receptor-adenylyl cyclase (U17042, U17043), DdACG: *Dictyostelium discoideum* adenylyl cyclase that is involved in germination (M87278), yeast AC from *Schizosaccharomyces pombe* (M24942) and *Saccharomyces cerevisiae* (M12057); BlAC: *Brevibacterium liquefaciens* adenylyl cyclase (X57541), RmAC: *Rhizobium meliloti* adenylyl cyclase (M35096), Tobacco axi AC: adenylyl cyclase from Tobacco axi (AF026389), GC-A-G: mammalian guanylyl cyclases GC-A (J05677), GC-B (M26896), GC-C (M55636), GC-D (L37203), GC-E (L36029), GC-F (L36030), and GC-G (AF024622), *Dm*GC: *Drosophila melanogaster* guanylyl cyclase (X72800, L35598), *Sp*GC: sea urchin guanylyl cyclase (M22444), *Ce*GC: *Caenorhabditis elegans* guanylyl cyclase (Yu *et al*., 1997), sGC-$\alpha\beta$: soluble guanylyl cyclase α and β subunits (M57405, X63282, U27117 for α and M22562, M57507, U27123 for β).

eight types of mammalian adenylyl cyclases, and its homolog in fruit fly is such that it cannot be activated by forskolin (Premont, 1992; Iourgenko *et al*., 1997). A single mutation (tyrosine to leucine) of mammalian type IX enzyme can confer both binding and activation by forskolin (Yan *et al*., 1998). The sterically occluded nature of the binding site suggests that bulky molecules, such as proteins, are unlikely to bind to this site. Therefore, if a physiologically relevant activator of adenylyl cyclase exists that is directed to the forskolin binding site, it is likely to be a small molecule rather than a protein. Seamon and coworkers tested candidate molecules, but as yet no such small molecule has been identified (Laurenza *et al*., 1989). Perhaps newly available genetic tools will facilitate a renewed search (Yan *et al*., 1998). An analogous compound could modulate the soluble guanylyl cyclase that is predicted to have only one GTP binding site and a deep hydrophobic pocket similar to the forskolin binding site of C_{1a}/C_{2a}. There is no report of forskolin activation of soluble

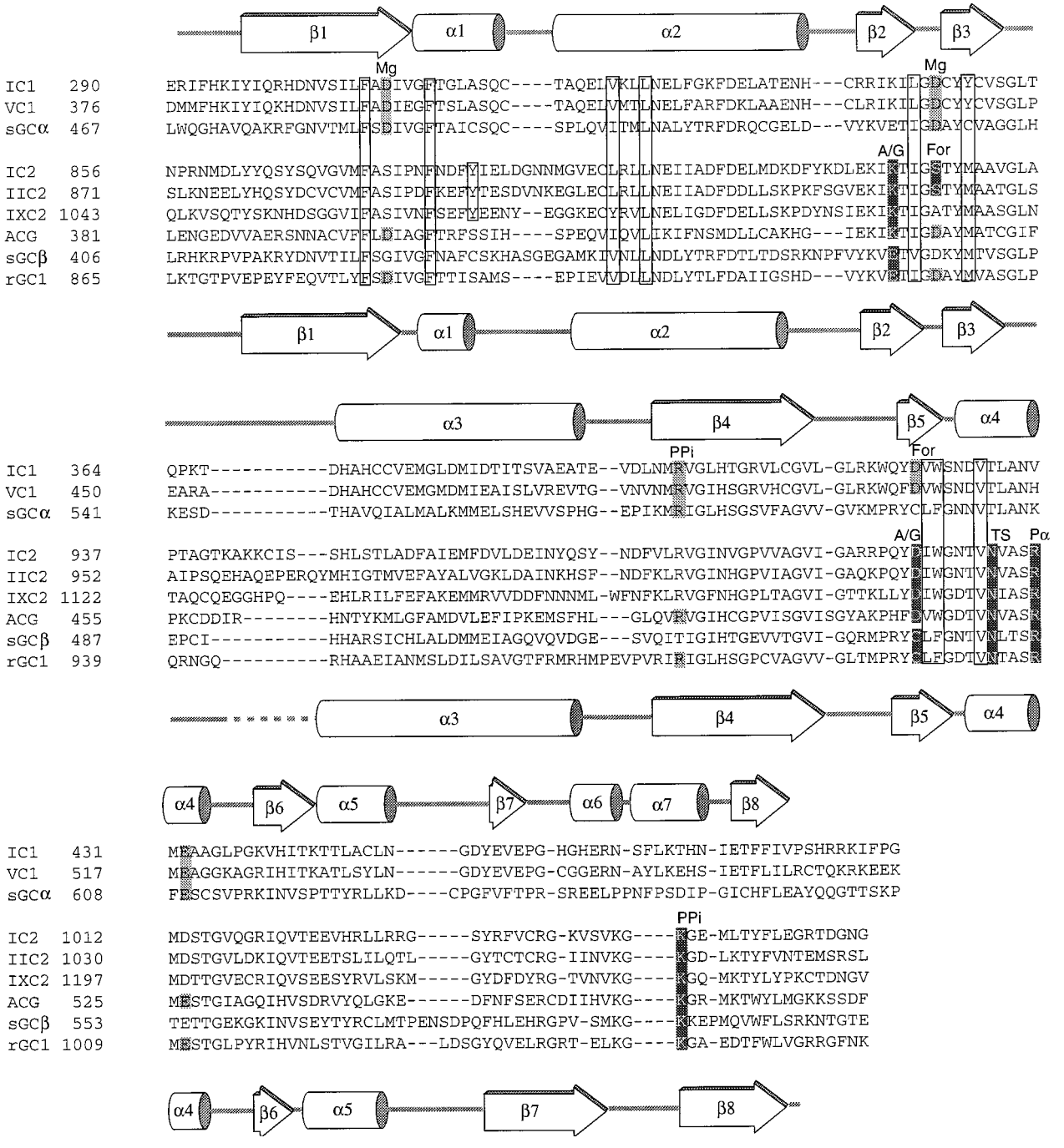

Fig. 2. Alignment of representative adenylyl and guanylyl cyclase sequences. *Roman numerals*, mammalian adenylyl cyclase types I, II, V, and IX from cow, rat, dog, and mouse, respectively, with accession numbers M25579, M80550, M88649, and U30602. sGCα and sGCβ are bovine soluble guanylyl cyclase α1 and β1 subunits, accession numbers X54014 and Y00770. ACG and rGC1 are the germination-specific adenylyl cyclase of *D. discoideum*, and the photoreceptor specific membrane guanylyl cyclase-1, accession numbers M87278 and S74247. Secondary structures are shown for VC_1 (*top*) and IIC_2 (*bottom*). *Unshaded boxes*, residues that form hydrophobic pockets. *Dark letters in lightly shaded boxes*, residues with C_1-like roles. *White letters in darkly shaded boxes*, residues with C_2-like roles.

guanylyl cyclase, and the key forskolin binding residues are absent in the pocket of soluble guanylyl cyclase.

The C_1/C_2 heterodimer counterpart of the second C_2 homodimer forskolin site seems to be incapable of binding forskolin because of a replacement of the critical serine by an aspartic acid (Fig. 3E, *ACI D354*). The aspartic acid precludes forskolin binding because it overlaps sterically with the 7-acetyl group. This aspartic acid in the C1 domain is critical for catalysis; therefore, the residue at this position probably is the most important difference between C_1 and C_2 domain sequences. Rotating the C_1–C_2 heterodimer about the pseudo-2-fold axis relating the domains reveals that ATP and forskolin make many analogous interactions (Fig. 3, D and E). The forskolin O1 and adenine N6 both donate hydrogen bonds to an aspartic acid at the same position on $\beta5$ or $\beta5'$, respectively. This suggests that the forskolin binding

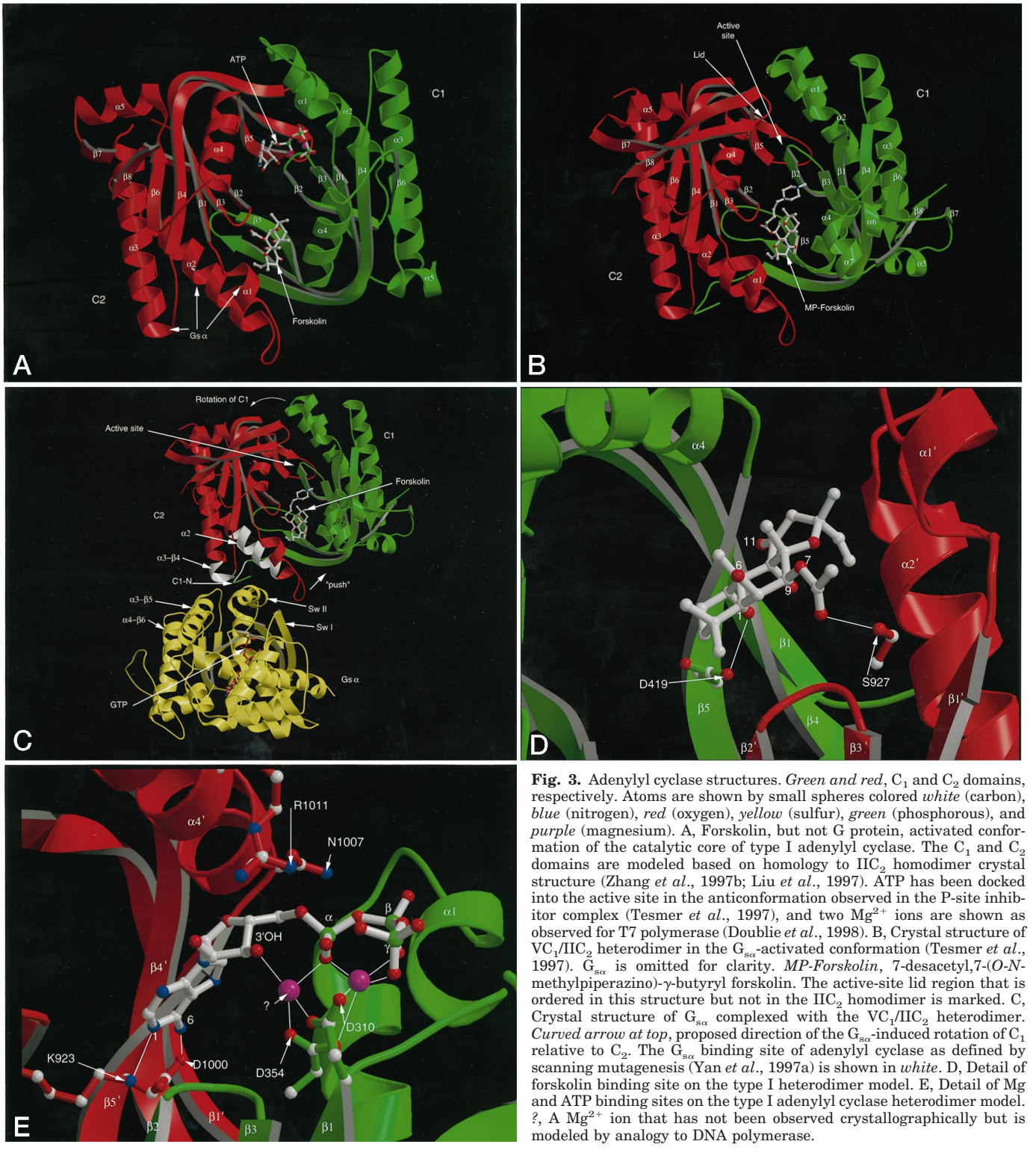

Fig. 3. Adenylyl cyclase structures. *Green and red*, C_1 and C_2 domains, respectively. Atoms are shown by small spheres colored *white* (carbon), *blue* (nitrogen), *red* (oxygen), *yellow* (sulfur), *green* (phosphorous), and *purple* (magnesium). A, Forskolin, but not G protein, activated conformation of the catalytic core of type I adenylyl cyclase. The C_1 and C_2 domains are modeled based on homology to IIC_2 homodimer crystal structure (Zhang *et al.*, 1997b; Liu *et al.*, 1997). ATP has been docked into the active site in the anticonformation observed in the P-site inhibitor complex (Tesmer *et al.*, 1997), and two Mg^{2+} ions are shown as observed for T7 polymerase (Doublie *et al.*, 1998). B, Crystal structure of VC_1/IIC_2 heterodimer in the $G_{s\alpha}$-activated conformation (Tesmer *et al.*, 1997). $G_{s\alpha}$ is omitted for clarity. *MP-Forskolin*, 7-desacetyl,7-(*O-N*-methylpiperazino)-γ-butyryl forskolin. The active-site lid region that is ordered in this structure but not in the IIC_2 homodimer is marked. C, Crystal structure of $G_{s\alpha}$ complexed with the VC_1/IIC_2 heterodimer. *Curved arrow at top*, proposed direction of the $G_{s\alpha}$-induced rotation of C_1 relative to C_2. The $G_{s\alpha}$ binding site of adenylyl cyclase as defined by scanning mutagenesis (Yan *et al.*, 1997a) is shown in *white*. D, Detail of forskolin binding site on the type I heterodimer model. E, Detail of Mg and ATP binding sites on the type I adenylyl cyclase heterodimer model. *?*, A Mg^{2+} ion that has not been observed crystallographically but is modeled by analogy to DNA polymerase.

site evolved from one of two active sites in an ancestral adenylyl cyclase. Although the hypothetical endogenous counterpart of forskolin is, in a physiological sense, unknown, it seems that in a structural and evolutionary sense, its closest counterpart is ATP.

Regulation of Mammalian Adenylyl Cyclases by G Proteins, Ca^{2+} Signals, and Phosphorylation

In addition to their regulation by $G_{s\alpha}$ and forskolin, mammalian adenylyl cyclases are subjected to complex regulation by other G proteins, Ca^{2+} signals, and phosphorylation (Table 1). One of the major advances in understanding cAMP-mediated signal transduction is in redefining the action of the members of the G_i family (G_i, G_o, G_z) that can be activated by diverse hormones and neurotransmitters (i.e., adenosine, epinephrine, and cannabinoid). On activation, the G_i family of proteins releases two potential regulators, G_α and $G_{\beta\gamma}$; this process can be blocked by treatment with pertussis toxin. Traditionally, the activation by the G_i family is viewed as producing only inhibition of adenylyl cyclase activity. Such inhibition does occur and can be mediated through the α subunit of G_i, G_o, or G_z or through $G_{\beta\gamma}$ (Tang and Gilman, 1991; Taussig *et al*., 1994; Kozasa and Gilman, 1995). For certain subtypes of adenylyl cyclases, such as type V and VI enzymes (predominantly expressed in heart), enzyme activity is inhibited by $G_{i\alpha}$ and not altered by $G_{\beta\gamma}$ (Taussig *et al*., 1994). For type I enzyme, which is predominantly expressed in brain, where G_o makes up to 1% of total membrane proteins, the activity of type I enzyme on stimulation of G_o-coupled receptor is inhibited predominantly by $G_{\beta\gamma}$, and the α subunit of G_o facilitates this inhibition (Tang and Gilman, 1991; Taussig *et al*., 1994). Characterizing the response from different isoform of adenylyl cyclase has led to the conclusion that the activation of G_i/G_o-coupled receptors also can lead to the activation, rather than inhibition, of adenylyl cyclase (Gao and Gilman, 1991; Tang and Gilman, 1991; Yoshimura *et al*., 1996). In brain and other tissues that express type II, IV, and VII enzymes, the activation of G_i/G_o releases $G_{\beta\gamma}$, which potentiates the enzymatic activity when the enzymes are simultaneously activated by $G_{s\alpha}$.

Where and how do $G\beta\gamma$ and $G_{i\alpha}$ bind and regulate adenylyl cyclase activity? The $G_{\beta\gamma}$ binding site of type II adenylyl cyclase has been mapped to the C_2-α3 region (Chen *et al*., 1995). $G_{\beta\gamma}$ is likely to modulate type II enzyme by binding on only one domain (C_{2a}) to affect its conformation, indirectly promoting optimal alignment at the catalytic site (Zhang *et al*., 1997b). $G_{\beta\gamma}$ must bind a different site of type I enzyme because the α3 region of type I enzyme is not conserved with that of type II enzyme and peptides from this region do not block a $G_{\beta\gamma}$-mediated response (Chen *et al*., 1995). The action of $G_{i\alpha}$ is not competitive with the binding of $G_{s\alpha}$ (Taussig *et al*., 1994). Because the major contacts between $G_{s\alpha}$ and adenylyl cyclases (switch 2) also are conserved in $G_{i\alpha}$-based $VC_1/2C_2/G_{s\alpha}$ structure, other regions, such as α3/β5 of $G_{s\alpha}$, may play an crucial role in how $G_{s\alpha}$ achieves the specificity over $G_{i\alpha}$ (Tesmer *et al*., 1997; Skiba and Hamm, 1998). The binding site for α subunit of G_i, G_o, or G_z has not been determined, but it is speculated to bind the α2/α3 region of C_{1a} on the opposite site to $G_{s\alpha}$ binding site (Yan *et al*., 1997a; Tesmer *et al*., 1997). If this is the case, the binding site is close to the catalytic site and the binding of G_α proteins could disturb the optimal alignment, perhaps by blocking the "counterclockwise" rotation of C_1 (Fig. 3C).

Bioactive agonists also can activate G_q-coupled receptors, leading to PKC activation, which in turn modulate adenylyl cyclases in an isotype-specific manner. In cells that have an overexpressed individual isoform of adenylyl cyclase, phorbol esters activate most of the adenylyl cyclases, including type II, III, V, and VII adenylyl cyclases (Yoshimura and Cooper, 1993; Jacobowitz and Iyengar, 1994; Kawabe *et al*., 1994). Both type II and V adenylyl cyclases are phosphorylated by PKCα (Kawabe *et al*., 1994; Zimmermann and Taussig, 1996). *In vivo* analysis using chimeric type I/II enzymes and mutational analysis has mapped a region on C_{2a} (1034–1068) for PKC response, likely Thr1057 (Levin and Reed, 1995; Bol *et al*., 1997). Thr1057 is close to the carboxyl-terminal lid that is disordered in IIC_2, but it forms a catalytic lid over the active site in the heterodimer. The ability of this C_2 segment to form the catalytic lid could be altered by PKC phosphorylation of adenylyl cyclase (Fig. 3B).

Intracellular Ca^{2+} modulates diverse physiological responses; thus, it is not surprising that a Ca^{2+} signal regulates the enzyme activity of adenylyl cyclases and that cAMP also modulates Ca^{2+} release (Cooper *et al*., 1995). Activation of calmodulin by Ca^{2+} can activate type I and VIII enzymes directly and inhibit type III and type IX enzymes via calcium-dependent calmodulin kinase II and calcineurin/PP2B, respectively (Tang *et al*., 1991; Cali *et al*., 1994; Wei *et al*., 1996; Antoni *et al*., 1995). An amphipathic region at the C_{1b} region of type I enzyme has been demonstrated to be involved in calmodulin binding and activation (Vorherr *et al*., 1993; Wu *et al*., 1993). Currently, the precise mechanism of calmodulin activation is unknown. At physiological submicromolar levels, elevated intracellular Ca^{2+} concentration inhibits type V and VI enzyme activity. This inhibition is more profound with entry from extracellular Ca^{2+} than the release of Ca^{2+} from the internal stores (Boyajian *et al*., 1991; Yoshimura and Cooper, 1992; Cooper *et al*., 1994).Whether Ca^{2+} modulates type V and VI enzymes directly or via a Ca^{2+} binding protein remains elusive.

cAMP-mediated signaling is subject to desensitization after receptor activation. Substantial advances have been made in understanding desensitization at the receptor level, which involves G protein-coupled receptor kinase/arrestin, PKA, and receptor sequestration (Freedman, 1996, and references therein). Such desensitization also occurs at the level of adenylyl cyclase exemplified by the study of type VI enzyme. Type VI enzyme can be desensitized via direct phosphorylation by PKA or PKC. PKA phosphorylation of type VI enzyme results in a 50% reduction in $G_{s\alpha}$-stimulated activity (Chen *et al*., 1997). The phosphorylation site probably is Ser674 of the C_{1b} region because mutation of this site blocks the sensitivity of type VI enzyme to PKA (Chen *et al*., 1997). This mechanism could explain the cAMP-dependent desensitization of glucagon stimulation in hepatocytes, but it does not occur in desensitization of adenosine receptor 2a in PC12 cells (Premont *et al*., 1992; Lai *et al*., 1997). In PC12 cells, the predominant adenylyl cyclase is Ca^{2+}-inhibited type VI enzyme, and adenylyl cyclase activity can be desensitized on the stimulation of A2a receptor, a G_s-coupled receptor (Chern *et al*., 1995). Interestingly, the desensitization of type VI enzyme in PC12 cells is cAMP independent and can be blocked by treatment with pertussis toxin but not by a selec-

tive PKA inhibitor, H89 (Lai *et al.*, 1997). The desensitization in PC12 cells is mediated by a novel PKC (Ca^{2+} independent) that phosphorylates and inhibits type VI enzyme (Lai *et al.*, 1997).

Catalytic Mechanism

Adenylyl cyclase converts ATP to cAMP and pyrophosphate without a covalently enzyme-bound intermediate (Eckstein *et al.*, 1981; Dessauer and Gilman, 1996). The turnover number of purified membrane-bound and soluble adenylyl cyclases ranges from 1 to 100 sec^{-1} and K_m value for ATP of 30–400 μM. The forward reaction is sequential and bireactant in Mg^{2+}-ATP and free Mg^{2+} (Garbers and Johnson, 1975; Somkuti *et al.*, 1982). The mammalian adenylyl cyclase reaction proceeds by the inversion of configuration at the α-phosphate, consistent with a direct in-line displacement of pyrophosphate by attack of the 3′-OH on the α-phosphate (Eckstein *et al.*, 1981). The same inversion of configuration has been found for the homologous guanylyl cyclases (Koch *et al.*, 1990). Product release is random, with a preference for cAMP to dissociate first (Dessauer *et al.*, 1997).

The three available molecular structures of adenylyl cyclase indicate that cyclase exists in at least three conformational states: (1) catalytically inactive IIC_2, (2) $G_{s\alpha}$ and forskolin bound IIC_2 complexed with VC_1, and (3) $G_{s\alpha}$ and forskolin bound IIC_2 complexed with VC_1, adenosine analog, and pyrophosphate. These represent at least approximately ground state (E), substrate-free activated state (E*), and substrate/product bound state (E**) (Tesmer *et al.*, 1997; Zhang *et al.*, 1997b). The enzyme cycle is likely to proceed as follows (Fig. 4): the catalytic region of adenylyl cyclase (C_1/C_2 interface) undergoes a conformational transition (E to E*) that is promoted by activators [e.g., $G_{s\alpha}$, forskolin, G protein $\beta\gamma$ (type II adenylyl cyclase) and Ca^{2+}-calmodulin] or is blocked by inhibitors [e.g., $G_{i\alpha}$, G protein $\beta\gamma$ (type I adenylyl cyclase), free Ca^{2+}]. Although with ~10-fold lower affinity than for ATP, adenylyl cyclase does bind GTP, but it does not use GTP as a substrate. Thus, binding of substrate (ATP) must induce a further conformational change (E* to E**), which enables the enzyme to confirm its substrate (proofreading) and proceed through catalysis. After catalysis, the E* state could reform either before or after release of the product. Further structural characterization of the conformational changes on activation and during the enzyme reaction cycle will be critically important.

A class of adenosine analogs known as P-site inhibitors (due to the importance of an intact purine ring) has been used to probe the kinetic mechanism and active site structure of adenylyl cyclase (Johnson *et al.*, 1989). The potency of P-site inhibitors increases additively with 2′- or 5′-deoxy and 3′-phosphoryl (PPP > PP > P) moieties (Desaubry *et al.*, 1996). A point mutation at Lys923 of type I enzyme, a residue that is crucial in discrimination of ATP over GTP, affects the affinity for both ATP and P-site inhibitors (Tang *et al.*, 1995). P-site inhibitors compete with the binding of nonhydrolyzable ATP and cAMP; the reaction product, PPi, enhances the binding of P-site inhibitors (Dessauer *et al.*, 1997a). Thus, P-site inhibitors seem to bind the cAMP site with high affinity. In steady state kinetic analyses, P-site inhibitors behave noncompetitively or noncompetitively with respect to MgATP. This apparent paradox can be resolved if P-site inhibitors and reaction products (cAMP and PPi) bind to a different adenylyl cyclase conformation (E**) than does ATP (E*) (Fig. 4) (Tesmer *et al.*, 1997).

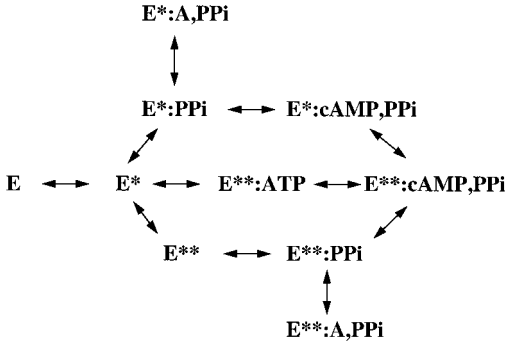

Fig. 4. Model of the enzyme cycle of adenylyl cyclase. See text for description. *A*, Adenosine analogs (P-site inhibitors). Less favored pathways for the release of PPi before the release of cAMP of E** to E* exist and are not shown for simplicity.

Structure of the Active Site and Implication for Regulation

Catalysis requires at least three steps. ATP must bind in a conformation in which the 3′-hydroxyl closely approaches the α-phosphorous. The 3′ OH of the ATP ribose must be activated for the attack on the 3′ oxygen atom of the α-phosphate. The transition state for the displacement of pyrophosphate by the 3′ oxygen atom must somehow be stabilized. Although the structure of a complex between adenylyl cyclase and ATP analog is not yet available, the C_{1a}/C_{2a} structure with P-site inhibitor/pyrophosphate complex and mutational data have provided an illuminating starting point for understanding the active site (Tang *et al.*, 1995; Liu *et al.*, 1997; Tesmer *et al.*, 1997; Yan *et al.*, 1997b) (Fig. 3D).

The active site of mammalian adenylyl cyclase is located in a deep cleft formed at the domain interface. It is composed primarily of β1, α1, the β2-β3 turn of the C_{1a} domain, and α4′ and β5′ of the C_{2a} domain. Adenine binds in a hydrophobic pocket corresponding to part of one of the two forskolin sites on the IIC_2 homodimer (Liu *et al.*, 1997; Tesmer *et al.*, 1997). Phosphate groups interact with an arginine side chain from each side of the cleft (Arg398 and Arg1011, AC1). The invariant Asn1025 (AC2) that is crucial for catalysis forms a water-mediated interaction with the adenine in the structure of $VC_1/IIC_2/G_{s\alpha}$/P-site inhibitor complex. The carboxyl-terminal region (AC2 1058–1071) forms a lid that is disordered in the IIC_2 homodimer but becomes ordered in $VC_1/IIC_2/G_{s\alpha}$ complex (Tesmer *et al.*, 1997; Zhang *et al.*, 1997b) (Fig. 3, A and B). Lys1067 (AC2) from this carboxyl-terminal "lid" is involved in binding ATP based on photoaffinity labeling and mutagenesis (Droste, 1996; Tang *et al.*, 1995). Hydrogen bonds between the adenine N6 and Asp1018 (AC2 numbering); and N1 and Lys938 (IIC_2) seem to confer specificity for adenine (Liu *et al.*, 1997; Tesmer *et al.*, 1997). In guanylyl cyclases, these residues are replaced by a cysteine and a glutamic acid, respectively. Mutations of these two residues at retinal-specific guanylyl cyclase (ret GC-1) converts this enzyme into a highly active and fully regulated adenylyl cyclase (Tucker *et al.*, 1998).

A single Mg^{2+} ion binds to pair of aspartates on C_1 (Tesmer *et al.*, 1997). The pair of aspartates is present in the overlaid structure of the palm domain of DNA polymerase I. The common Mg^{2+} binding site substantiates a biochemically relevant similarity between the two. There are almost cer-

tainly two, not one, Mg^{2+} ions in the ATP complex based on kinetics (Garbers and Johnson, 1975). Recent high-resolution structural analyses of DNA polymerase β and DNA polymerases from T7 phage and *Bacillus stearothermophilus* reveal that the aspartic acid pair on polymerases is capable of binding two Mg^{2+} ions (Sawaya *et al*., 1997; Doublie *et al*., 1998; Kiefer *et al*., 1998; Steitz, 1998). Polymerase Mg^{2+} ion B corresponds to the site observed in the P-site complex, except that it binds all three phosphate groups in a tridentate arrangement (Sawaya *et al*., 1997; Doublie *et al*., 1998; Steitz, 1998). Polymerase metal ion A is thought to be responsible for catalysis because it interacts with the α-phosphate and the model-built 3′-OH (Doublie *et al*., 1998, Kiefer *et al*., 1998). The site corresponding to metal ion A on adenylyl cyclase is sterically compatible with Mg^{2+} ion occupancy and seems to be the most likely site for the catalytic metal ion.

The four residues most clearly implicated in catalysis by mutational studies are closely juxtaposed in the vicinity of the metal ion and the reactive ribosyl and α-phosphate groups. Asn1025 and Arg1029 (Fig. 3D, *AC1 1007* and *1011*, respectively) are directly involved in catalysis based on mutagenesis and kinetic analysis (Yan *et al*., 1997b). The Arg1029 guanidino group is poised in both $VC_1/IIC_2/G_{s\alpha}$/forskolin/P-site inhibitor crystal structure and IC_1/IC_2/ATP models to interact with the α-phosphate (Liu *et al*., 1997; Tesmer *et al*., 1997). The best model for the ATP/cyclase complex shows the arginine interacting primarily with the bridging rather than nonbridging oxygen atoms, although the details remain to be established (Tesmer *et al*., 1997). Asn1025 is close to the α-phosphate bridging oxygen atoms in current ATP complex models and may assist Arg1029 in stabilizing the transition state or the leaving group.

The identity of the catalytic base in the reaction is unresolved. Base catalysis could involve either substrate-assisted catalysis (Schweins *et al*., 1995) or a protein side chain. If any protein side chain acts as a catalytic base, it is most likely Asp354 (Liu *et al*., 1997; Tesmer *et al*., 1997). Mutation of Asp354 in AC1 (Tang *et al*., 1995) and its counterparts in guanylyl cyclases (Yuen *et al*., 1994; Thompson and Garbers, 1995) leads to enzymes that are completely inactive within the detection limits of the assays used, typically $\sim 10^{-3}$ of wild-type enzyme. The counterpart of this aspartic acid in B. Stearothermophilus DNA polymerase forms a hydrogen bond to the model-built 3′-end of the primer (Kiefer *et al*., 1998), consistent with a role in deprotonating the 3′-OH. Mutation of the adjacent Asp310 reduces forskolin-stimulated activity by 900-fold (Liu *et al*., 1997). The mutant phenotypes are consistent with a polymerase-like two-metal mechanism in which one metal activates the 3′-OH as well as coordinating the α-phosphate (Sawaya *et al*., 1997; Doublie *et al*., 1998; Steitz, 1998).

The finding that the C_1 and C_2 domains juxtapose four critical catalytic residues, two each from C_1 and C_2, provides the first opportunity to understand regulation of adenylyl cyclase in atomic detail. The precise alignment of C_1 and C_2 seems to be crucial to simultaneously enable metal and nucleotide binding and transition state stabilization. The physical association of C_1 and C_2 is an essential precondition for formation of the catalytic site, although it is not sufficient for activated catalysis by itself. In the absence of activators, soluble C_1 and C_2 domains have approximately micromolar affinity for one another. Their affinity for each other is greatly increased by the presence of forskolin, $G_{s\alpha}$, or both (Whisnant *et al*., 1996; Yan *et al*., 1996). Forskolin acts as hydrophobic glue to attach the two domains, stabilizing the dimer by plugging a hydrophobic crevice (Zhang *et al*., 1997b). $G_{s\alpha}$ also bridges the two domains by binding to both C_1 and C_2, explaining its ability to promote dimerization (Tesmer *et al*., 1997; Yan *et al*., 1997a). Because C_1 and C_2 are covalently attached to each other in intact adenylyl cyclase, the dimerization-promoting effects of forskolin and $G_{s\alpha}$ are probably less important in intact adenylyl cyclase than in the soluble system. Comparison of the crystal structure of the active $VC_1/IIC_2/G_{s\alpha}$ with the IIC_2 homodimer suggests a possible structural mechanism that could underlie the allosteric activation of the preassembled dimer (Tesmer *et al*., 1997; Zhang *et al*., 1997b). If the position the C_2 domain is fixed, the C_1 domain in the heterodimer is found to rotate ~7° "counterclockwise" (Tesmer *et al*., 1997). This leads to small structural changes, typically <2 Å, in the positions of the main chain of active site residues. Although these changes may seem small, catalysis is so sensitive to precise stereochemical details that it is not unreasonable to imagine these small structural changes can lead to large changes in catalytic rate. We do not yet know how much of the small conformational change is due to $G_{s\alpha}$ binding and how much is due to intrinsic differences between the heterodimeric and homodimeric AC structures. There still is no structure of a low activity conformation of adenylyl cyclase bound to ATP. The α1-α2 loop of C_2 plays a critical role in this mechanism by communicating the structural change in the $G_{s\alpha}$ binding groove outward, yet this loop is poorly conserved among different mammalian adenylyl cyclase isoforms. Mutational studies of this region will be important to dissect the mechanism of allosteric linkage. The activation mechanism must be considered tentative until this information becomes available.

Future Directions

Might intact mammalian adenylyl cyclase exist as a dimer? Target-size analysis suggested that the activated adenylyl cyclase is a dimer, whereas higher order complexes among receptor, G protein, and adenylyl cyclase exist at the resting state (Rodbell *et al*., 1981). Hydrodynamic analysis of detergent solubilized or purified adenylyl cyclase also shows a possible monomer/dimer transition (Neer *et al*., 1984; Yeager *et al*., 1985). A functional oligomer of type I enzyme has been observed using immunoprecipitation of recombinant type I enzyme and its mutant (Tang *et al*., 1995). Although the dimerization is unlikely to be involved in catalysis directly, based on the mutational and structural analysis, it could be crucial for the regulation or localization of the enzyme (Tang *et al*., 1995). Each isoform has its distinct pattern of expression, but significant overlap in the tissue distribution of different isotypes has been observed (i.e., in hippocampus and cerebral cortex). If certain heterodimers between two different isotypes of enzyme are allowed, dimerization of adenylyl cyclase would add a new dimension to the already complex regulation of adenylyl cyclase.

The molecular structure of adenylyl cyclase has provided a framework for understanding the mechanisms of catalysis and regulation in atomic detail for the first time. The structure and biochemical analysis also have led to several intriguing questions. For example, what is the physiological

role of the hydrophobic pocket of adenylyl cyclase that binds forskolin? How does the binding of the regulators or phosphorylation outside the catalytic core (C_{1a}/C_{2a}) (i.e., C_{1b} region) regulate adenylyl cyclase? What are the roles of transmembrane domains? The latter question has become more puzzling now that we know the soluble regions alone are capable of $G_{s\alpha}$- and forskolin-regulated catalysis. Membrane localization and coordination of C_{1a}/C_{2a} interaction are the clearcut roles for transmembrane domains, but why are there 12 when 1 or 2 would have sufficed? A role in transmembrane transport was suggested by the topological similarity to other transporters (Krupinski *et al*., 1989), but efforts by several workers have thus far failed to demonstrate such a function. If the accumulation of data concerning adenylyl cyclase continues with the same exponential rise seen over the past 10 years, we can hope that answers to these questions should be available in the near future.

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References

Antoni FA, Barnard RJO, Shipston MJ, Smith SM, Simpson J, and Paterson JM (1995) Calcineurin feedback inhibition of agonist-evoked cAMP formation. *J Biol Chem* **270:**28055–28061.
Berlot CH and Bourne HR (1992) Identification of effector-activating residues of $G_{s\alpha}$. *Cell* **68:**911–922.
Bol GF, Gros C, Hulster A, Bosel A, and Pfeuffer T (1997) Phorbol ester-induced sensitisation of adenylyl cyclase type II is related to phosphorylation of threonine 1057. *Biochem Biophys Res Commun* **237:**251–256.
Boyajian CL, Garritsen A, and Cooper DM (1991) Bradykinin stimulates Ca^{2+} mobilization in NCB-20 cells leading to direct inhibition of adenylylcyclase: a novel mechanism for inhibition of cAMP production. *J Biol Chem* **266:**4995–5003.
Cali JJ, Zwaagstra JC, Mons N, Cooper DM, and Krupinski J (1994) Type VIII adenylyl cyclase A Ca^{2+}/calmodulin-stimulated enzyme expressed in discrete regions of rat brain. *J Biol Chem* **269:**12190–12195.
Chen J, Devivo M, Dingus J, Harry A, Li J, Sui J, Carty DJ, Blank JL, Exton JH, Stoffel RH, Inglese J, Lefkowitz RJ, Logothetis DE, Hildebrandt JD, and Iyengar R (1995) A region of adenylyl cyclase 2 critical for regulation of G protein $\beta\gamma$subunits. *Science (Washington D C)* **268:**1166–1169.
Chen Y, Harry A, Li J, Smit MJ, Bai X, Magnusson R, Pieroni JP, Weng G, and Iyengar R (1997) Adenylyl cyclase 6 is selectively regulated by protein kinase A phosphorylation in a region involved in $G_{s\alpha}$ stimulation. *Proc Natl Acad Sci USA* **94:**14100–14104.
Chern Y, Chiou JY, Lai HL, and Tsai MH (1995) Regulation of adenylyl cyclase type VI activity during desensitization of the A2a adenosine receptor-mediated cyclic AMP response: role for protein phosphatase 2A. *Mol Pharmacol* **48:**1–8.
Cooper DM, Yoshimura M, Zhang Y, Chiono M, and Mahey R (1994) Capacitative Ca^{2+} entry regulates Ca^{2+}-sensitive adenylyl cyclases. *Biochem J* **297:**437–440.
Cooper DM, Mons N, and Karpen JW (1995) Adenylyl cyclases and the interaction between calcium and cAMP signalling. *Nature (Lond)* **374:**421–424.
Cooper DMF (1997) Adenylyl cyclases. *Adv Second Messenger Phosphoprot Res* **32**.
Desaubry L, Shoshani I, and Johnson RA (1996) Inhibition of adenylyl cyclase by a family of newly synthesized adenine nucleoside 3′-polyphosphates. *J Biol Chem* **271:**14028–14034.
Dessauer CW and Gilman AG (1996) Purification and characterization of a soluble form of mammalian adenylyl cyclase. *J Biol Chem* **271:**16967–16974.
Dessauer CW and Gilman AG (1997) The catalytic mechanism of mammalian adenylyl cyclase-equilibrium binding and kinetic analysis of P-site inhibition. *J Biol Chem* **272:**27787–27795.
Dessauer CW, Scully TT, and Gilman AG (1997) Interactions of forskolin and ATP with the cytosolic domains of mammalian adenylyl cyclase. *J Biol Chem* **272:**22272–22277.
Doublie S, Tabor S, Long AM, Richardson CC, and Ellengerger T (1998) Crystal structure of bacteriophage T7 DNA polymerase complexed to a primer-template, a nucleoside triphosphate, and its processivity factor thioredoxin. *Nature (Lond)* **391:**251–258.
Droste M, Mollner S, and Pfeuffer T (1996) Localisation of an ATP-binding site on adenylyl cyclase type I after chemical and enzymatic fragmentation. *FEBS Lett* **391:**209–214.
Eckstein F, Romaniuk PJ, Heideman W, and Storm DR (1981) Stereochemistry of the mammalian adenylyl cyclase reaction. *J Biol Chem* **256:**9118–9120.
Freedman NJ and Lefkowitz RJ (1996) Desensitization of G protein-coupled receptors. *Recent Progr Horm Res* **51:**319–51.
Gao B and Gilman AG (1991) Cloning and expression of a widely distributed (type IV) adenylyl cyclase. *Proc Natl Acad Sci USA* **88:**10178–10182.
Garbers DL and Johnson RA (1975) Metal and metal-ATP interactions with brain and cardiac adenylate cyclase. *J Biol Chem* **250:**8449–8456.
Hobbs AJ (1997) Soluble guanylyl cyclase: the forgotten sibling. *Trends Pharmacol Sci* **18:**484–491.
Iourgenko V, Kliot B, Cann MJ, and Levin LR (1997) Cloning and characterization of a *Drosophila* adenylyl cyclase homologous to mammalian type IX. *FEBS Lett* **413:**104–108.
Iwami G, Kawabe J-I, Ebina T, Cannon PJ, Homcy CJ, and Ishikawa Y (1995) Regulation of adenylyl cyclase by protein kinase A. *J Biol Chem* **270:**12481–12484.
Jacobowitz O and Iyengar R (1994) Phorbol ester-induced stimulation and phosphorylation of adenylyl cyclase 2. *Proc Natl Acad Sci USA* **91:**10630–10634.
Johnson RA, Yeung SM, Stubner D, Bushfield M, and Shoshani I (1989) Cation and structural requirements for P site-mediated inhibition of adenylate cyclase. *Mol Pharmacol* **35:**681–688.
Kawabe J, Iwami G, Ebina T, Ohno S, Katada T, Ueda Y, Homcy CJ, and Ishikawa Y (1994) Differential activation of adenylyl cyclase by protein kinase C isoenzymes. *J Biol Chem* **269:**16554–16558.
Kiefer JR, Mao C, Braman JC, and Beese LS (1998) Visualizing DNA replication in a catalytically active *Bacillus* DNA polymerase crystal. *Nature (Lond)* **391:**304–307.
Kleuss C and Gilman AG (1997) $G_{s\alpha}$ contains an unidentified covalent modification that increases its affinity for adenylyl cyclase. *Proc Natl Acad Sci USA* **94:**6116–6120.
Koch KW, Eckstein F, and Stryer L (1990) Stereochemical course of the reaction catalyzed by guanylate cyclase from bovine retinal rod outer segments. *J Biol Chem* **265:**9659–9663.
Kozasa T and Gilman AG (1995) Purification of recombinant G proteins from Sf9 cells by hexahistidine tagging of associated subunits: characterization of α12 and inhibition of adenylyl cyclase by αz. *J Biol Chem* **270:**1734–1741.
Krupinski J, Coussen F, Bakalyar HA, Tang WJ, Feinstein PG, Orth K, Reed RR, and Gilman AG (1989) Adenylyl cyclase amino acid sequence: possible channel- or transporter-like structure. *Science (Washington DC)* **244:**1558–1564.
Lai HL, Yang TH, Messing RO, Ching YH, Lin SC, and Chern Y (1997) Protein kinase C inhibits adenylyl cyclase type VI activity during desensitization of the A2a-adenosine receptor-mediated cAMP response. *J Biol Chem* **272:**4970–4977.
Lambright DG, Noel JP, Hamm HE, and Sigler PB (1994) Structural determinants for activation of the α-subunit of a heterotrimeric G protein. *Nature (Lond)* **369:**621–628.
Laurenza A, Sutkowski EM, and Seamon KB (1989) Forskolin: a specific stimulator of adenylyl cyclase or a diterpene with multiple sites of action? *Trends Pharmacol Sci* **10:**442–447.
Levin LR and Reed RR (1995) Identification of functional domains of adenylyl cyclase using in vivo chimeras. *J Biol Chem* **270:**7573–7579.
Linder ME, Middleton P, Hepler JR, Taussig R, Gilman AG, and Mumby SM (1993) Lipid modifications of G proteins: α subunits are palmitoylated. *Proc Natl Acad Sci USA* **90:**3675–3679.
Liu Y, Ruoho AE, Rao VD, and Hurley JH (1997) Catalytic mechanism of the adenylyl and guanylyl cyclases: modeling and mutational analysis. *Proc Natl Acad Sci USA* **94:**13414–13419.
Neer EJ, Lok JM, and Wolf LG (1984) Purification and properties of the inhibitory guanine nucleotide regulatory unit of brain adenylate cyclase. *J Biol Chem* **259:**14222–14229.
Premont RT, Jacobowitz O, and Iyengar R (1992) Lowered responsiveness of the catalyst of adenylyl cyclase to stimulation by G_s in heterologous desensitization: a role for adenosine 3′,5′-monophosphate-dependent phosphorylation. *Endocrinology* **131:**2774–2784.
Robbins JD, Boring DL, Tang WJ, Shank R, and Seamon KB (1996) Forskolin carbamates: binding and activation studies with type I adenylyl cyclase. *J Med Chem* **39:**2745–2752.
Rodbell M, Lad PM, Nielsen TB, Cooper DM, Schlegel W, Preston MS, and Kempner ES (1981) The structure of adenylate cyclase systems. *Adv Cyclic Nucleotide Res* **14:**3–14.
Sawaya MR, Prasad R, Wilson SH, Kraut J, and Pelletier H (1997) Crystal structures of human DNA polymerase β complexed with gapped and nicked DNA: evidence for an induced fit mechanism. *Biochemistry* **36:**11205–11215.
Scholich K, Barbier AJ, Mullenix JB, and Patel TB (1997) Characterization of soluble forms of nonchimeric type V adenylyl cyclases. *Proc Natl Acad Sci USA* **97:**2915–2920.
Schweins T, Geyer M, Scheffzek K, Warshel A, and Kalbitzer HR (1995) Substrate-assisted catalysis as a mechanism for GTP hydrolysis of p21ras and other GTP-binding proteins. *Nat Struct Biol* **2:**36–44.
Seamon KB and Daly JW (1986) Forskolin: its biological and chemical properties. *Adv Cyclic Nucleotide Protein Phosphorylation Res* **20:**1–150.
Skiba NP and Hamm HE (1998) How $G_{s\alpha}$ activates adenylyl cyclase. *Nat Struct Biol* **5:**88–92.
Somkuti SG, Hildebrandt JD, Herberg JT, and Iyengar R (1982) Divalent cation regulation of adenylyl cyclase: an allosteric site on the catalytic component. *J Biol Chem* **257:**6387–6393.
Steitz TA (1998) A mechanism for all polymerases. *Nature (Lond)* **391:**231–232.
Sunahara RK, Dessauer CW, and Gilman AG (1996) Complexity and diversity of mammalian adenylyl cyclase. *Annu Rev Pharmacol Toxicol* **36:**461–480.
Sunahara RK, Dessauer CW, Whisnant RE, Kleuss C, and Gilman AG (1997) Interaction of $G_{s\alpha}$ with the cytosolic domains of mammalian adenylyl cyclase. *J Biol Chem* **272:**22265–22271.
Tang W-J and Gilman AG (1995) Construction of a soluble adenylyl cyclase activated by $G_{s\alpha}$ and forskolin. *Science (Washington DC)* **268:**1769–1772.
Tang W-J and Gilman AG (1991) Type-specific regulation of adenylyl cyclase by G protein $\beta\gamma$ subunits. *Science (Washington DC)* **254:**1500–1503.
Tang W-J, Krupinski J, and Gilman AG (1991) Expression and characterization of calmodulin-activated (type I) adenylylcyclase. *J Biol Chem* **266:**8595–8603.
Tang W-J, Stanzel M, and Gilman AG (1995) Truncation and alanine-scanning mutants of type I adenylyl cyclase. *Biochemistry* **34:**14563–14572.

Tang W-J, Yan S-Z, and Drum CL (1997) Class III adenylyl cyclase-regulation and underlying mechanisms. *Adv Second Messenger Phosphoprotein Res* **32:**137–151.
Taussig R, Tang WJ, Hepler JR, and Gilman AG (1994) Distinct patterns of bidirectional regulation of mammalian adenylyl cyclases. *J Biol Chem* **269:**6093–6100.
Tesmer JJG, Sunahara RK, Gilman AG, and Sprang SR (1997) Crystal structure of the catalytic domains of adenylyl cyclase in a complex with $G_{s\alpha}$-GTPγS. *Science (Washington DC)* **278:**1907–1916.
Thompson DK and Garbers DL (1995) Dominant negative mutations of the guanylyl cyclase-A receptor: extracellular domain deletion and catalytic domain point mutations. *J Biol Chem* **270:**425–430.
Tucker CL, Hurley JH, Miller JR, and Hurley JB (1998) Two amino acid substitutions convert a guanylyl cyclase -ret GC-1 into an adenylyl cyclase. *Proc Natl Acad Sci USA* **95:**5993–5997.
Vorherr T, Knopfel L, Hofmann F, Mollner S, Pfeuffer T, and Carafoli E (1993) The calmodulin binding domain of nitric oxide synthase and adenylyl cyclase. *Biochemistry* **32:**6081–6088.
Wedel BJ, Foster DC, Miller DE, and Garbers DL (1997) A mutation of the atrial natriuretic peptide (guanylyl cyclase-A) receptor results in a constitutively hyperactive enzyme. *Proc Natl Acad Sci USA* **94:**459–462.
Wei J, Wayman G, and Storm DR (1996) Phosphorylation and inhibition of type III adenylyl cyclase by calmodulin-dependent protein kinase II in vivo. *J Biol Chem* **271:**24231–24235.
Whisnant RE, Gilman AG, and Dessauer CW (1996) Interaction of the two cytoplasmic domains of mammalian adenylyl cyclase. *Proc Natl Acad Sci USA* **93:**6621–6625.
Wu Z, Wong ST, and Storm DR (1993) Modification of the calcium and calmodulin sensitivity of the type I adenylyl cyclase by mutagenesis of its calmodulin binding domain. *J Biol Chem* **268:**23766–23768.
Yan S-Z, Hahn D, Huang Z-H, and Tang W-J (1996) Two cytoplasmic domains of mammalian adenylyl cyclase form a $G_{s\alpha}$- and forskolin-activated enzymes in vitro. *J Biol Chem* **271:**10941–10945.
Yan S-Z, Huang Z-H, Rao VD, Hurley JH, and Tang W-J (1997a) Three discrete regions of mammalian adenylyl cyclase form a site for $G_{s\alpha}$ activation. *J Biol Chem* **272:**18849–18854.
Yan S-Z, Huang Z-H, Shaw RS, and Tang W-J (1997b) The conserved asparagine and arginine are essential for catalysis of mammalian adenylyl cyclase. *J Biol Chem* **272:**12342–12349.
Yan S-Z, Huang Z-H, Andrews RK, and Tang W-J (1998) Conversion of forskolin-insensitive to forskolin-sensitive (mouse-type IX) adenylyl cyclase. *Mol Pharmacol* **53:**182–187.
Yeager RE, Heideman W, Rosenberg GB, and Storm DR (1985) Purification of the calmodulin-sensitive adenylate cyclase from bovine cerebral cortex. *Biochemistry* **24:**3776–3783.
Yoshimura M and Cooper DM (1992) Cloning and expression of a Ca^{2+}-inhibitable adenylyl cyclase from NCB-20 cells. *Proc Natl Acad Sci USA* **89:**6716–6720.
Yoshimura M and Cooper DM (1993) Type-specific stimulation of adenylylcyclase by protein kinase C. *J Biol Chem* **268:**4604–4607.
Yoshimura M, Ikeda H, and Tabakoff B (1996) μ-Opioid receptors inhibit dopamine stimulated activity of type V adenylyl cyclase but enhance the dopamine stimulated activity of type VII adenylyl cyclase. *Mol Pharmacol* **50:**43–51.
Yu S, Avery L, Baude E, and Garbers DL (1997) Guanylyl cyclase expression in specific sensory neurons: a new family of chemosensory receptors. *Proc Natl Acad Sci USA* **94:**3384–3387.
Yuen PS, Doolittle LK, and Garbers DL (1994) Dominant negative mutants of nitric oxide-sensitive guanylyl cyclase. *J Biol Chem* **269:**791–793.
Zhang G, Liu Y, Qin J, Vo B, Tang W-J, Ruoho AE, and Hurley JH (1997a) Characterization and crystallization of a minimal catalytic core domain from mammalian type II adenylyl cyclase. *Protein Sci* **6:**903–908.
Zhang G, Liu Y, Ruoho AE, and Hurley JH (1997b) Structure of the adenylyl cyclase catalytic core. *Nature (Lond)* **386:**2477–253.
Zimmermann G and Taussig R (1996) Protein kinase C alters the responsiveness of adenylyl cyclases to G protein α and βγ subunits. *J Biol Chem* **271:**27161–27166.

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