G protein regulation of adenylate cyclase

William F. Simonds

Adenylate cyclase integrates positive and negative signals that act through G protein-coupled cell-surface receptors with other extracellular stimuli to finely regulate levels of cAMP within the cell. Recently, the structures of the cyclase catalytic core complexed with the plant diterpene forskolin, and a cyclase–forskolin complex bound to an activated form of the stimulatory G protein subunit $G_s\alpha$ have been solved by X-ray crystallography. These structures provide a wealth of detail about how different signals could converge at the core cyclase domains to regulate catalysis. In this article, **William Simonds** reviews recent advances in the molecular and structural biology of this key regulatory enzyme, which provide new insight into its ability to integrate multiple signals in diverse cellular contexts.

Never have we been so close to understanding the molecular mechanisms that govern the generation of cAMP, the ubiquitous and archetypal intracellular second messenger. Two advances in structural biology in 1997 have catapulted forward our understanding of adenylate cyclase (AC): determination of the three-dimensional crystal structure of the AC catalytic core by Hurley and co-workers[1], and the subsequent structural determination by Tesmer *et al*.[2] of a complex containing the cyclase catalytic domains bound to an active conformation of the cyclase-stimulating G protein α-subunit, $G_s\alpha$. For the first time, it is possible to visualize in exquisite detail how the cyclase core domain might translate various regulatory inputs into altered enzymatic function. These stunning crystallographic achievements give hope that the next few years could provide glimpses of the catalytic core of various AC isoforms locked in functional embrace with other protein regulators, including other G protein subunits that impart inhibitory and stimulatory signals to the AC core.

These exciting structural advances are merely the latest milestones on a scientific journey begun in earnest some 40 years ago by Earl Sutherland and colleagues. They first recognized the importance of cAMP and its catalyst AC in the hyperglycaemic response of the liver to 'first messenger' hormonal signals such as adrenaline and glucagon[3,4]. It is recognized that AC activity is ubiquitous and inherent to multiple polypeptide isoforms targeted to the cell membranes by clusters of transmembrane-spanning protein helices (Fig. 1a). There, AC can respond to the actions of extracellular hormones either by the direct interaction with subunits of membrane-anchored G proteins such as $G_s\alpha$, or indirectly as a consequence of hormonal alteration of intracellular ionic composition and kinase activity, or both. The resulting alterations in intracellular cAMP levels directly govern the activity of protein kinase A (PKA) and indirectly govern metabolic, cytomechanical and transcriptional events within cells of various types. Molecular cloning has identified at least nine isoforms of AC with different expression patterns and that respond positively or negatively to distinct sets of regulatory inputs (Fig. 1; Tables 1 and 2, Refs 5–36). The diversity of isoforms and distinct patterns of regulation allow AC to serve as an integrator of relevant stimulatory and inhibitory signals in cells specialized for different functions throughout the body. This review summarizes recent advances in

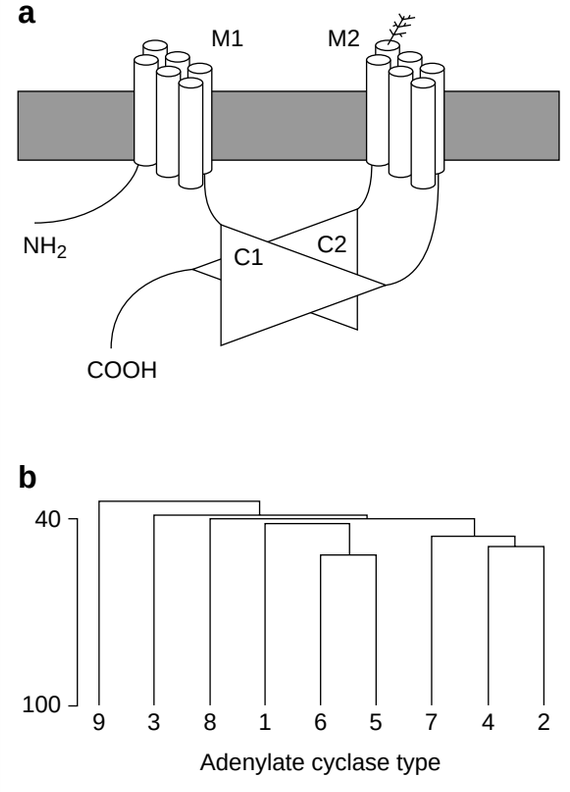

Fig. 1. a: Schematic diagram of the proposed membrane topology of the adenylate cyclases based on hydropathy analysis[5] and the terminology of Gao and Gilman[11]. The grey rectangle represents the plasma membrane into which the clusters M1 and M2 of six transmembrane spanning α-helical segments anchor the enzyme. The cytosolic domains include the N-terminal region, the homologous ~25 kDa catalytic domains C1 and C2 interacting in a head-to-tail manner[1,2], and the C-terminus. The cDNAs for all nine principal cyclase isoforms predict sites of N-linked glycosylation in M2 (branching tuft) and the glycoprotein nature of several AC isoforms has been proven by glycohydrolytic analysis. **b:** Sequence relationships among the nine AC isoforms are represented in a dendrogram generated by the program PILEUP in which the vertical distance is proportional to the similarity between sequences. A scale approximating the percent sequence similarity is provided on the left, in which the similarity between AC9 and AC3 determined by the program GAP is indicated as 40%.

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understanding the regulation of AC with particular emphasis on its regulation by G proteins. In addition to the view presented here, the reader is referred to more detailed recent reviews by experts in the biochemical, physiological and integrative aspects of AC (Refs 37–40).

Adenylate cyclase: a family of G_s-regulated isoenzymes

Isolation of the cDNA for the first AC (AC1) by Krupinski *et al*.[5] was accomplished using sequence information from proteolytic peptides derived from affinity-purified brain AC. The cDNAs for AC1, along with the eight other AC isoforms subsequently isolated by probe cross-hybridization or PCR, encode large integral membrane proteins (Table 1). All are predicted by hydropathy analysis to share a common topology with five domains in sequence: a cytoplasmic N-terminal region, a membrane-anchoring hydrophobic domain (M1) consisting of six transmembrane helices, a large cytoplasmic domain (C1), a second transmembrane helical cluster (M2) and a second cytoplasmic domain (C2), homologous to the first, at the C-terminus (Fig. 1a). The predicted topology of AC1–AC9 resembles that of the ATP-binding cassette (ABC) membrane transporters such as the P-glycoprotein[5], although evidence that any mammalian AC functions as a channel or pump is lacking. All nine AC isoforms contain at least one site predicted to undergo *N*-linked glycosylation in M2. Following the demonstration by Tang and Gilman[41] of a recombinant soluble AC entirely lacking M1 and M2, a large body of biochemical and structural evidence has made it clear that the interaction of the homologous C1 and C2 domains lies at the heart of the cyclase catalytic mechanism (see below). C1 and C2 share homology not only with each other but with adenylate cyclases from *Dictyostelium* and yeast, and with soluble and transmembrane guanylate cyclases. It is likely that all share a common catalytic mechanism that requires dimerization of identical or homologous domains[42].

All of the cloned mammalian AC are stimulated by G_s and all but AC9 (Ref. 20) are also potently stimulated by the plant diterpene, forskolin. G_s and the related G_{olf} are AC-stimulating heterotrimeric G proteins[3]. Like all of these GTPase switch proteins, the G protein α-subunits bind GTP and adopt an active conformation in which they modulate effector proteins until signalling is terminated by the action of an intrinsic GTPase activity and reassociation with the Gβγ complex[43]. The GTPase 'off' reaction is accelerated by proteins of the RGS (regulator of G-protein signalling) family for some G proteins, but not apparently for G_s (Ref. 44). The active conformation of Gα subunits can be stabilized by the binding of non-hydrolysable GTP analogues such as GTPγ–S or transition-state analogue such as the combination of GDP and $AlF4^-$ (Ref. 43). The structure of G_sα–GTPγ–S and its AC contact sites have recently been determined using crystallography[2,45] (Fig. 2). The structure of G_sα, like that of G_iα and G_tα, consists of an α-helical domain bound through two linking peptides to a p21 Ras-like domain containing a six-stranded β-sheet[43]. The AC contact sites on G_sα involve two regions within its Ras-like domain (Fig. 2 inset). The identity of both sites was anticipated by site-directed mutagenesis of G_sα (Refs 46, 47).

Table 1. Mammalian isforms of adenylate cyclase

Adenylate cyclase (AC) type	Size (no. of amino acids)	mRNA expression	Refs
AC1	1134	Brain, retina, adrenal medulla	5, 6
AC2	1090	Brain, olfactory bulb > lung	6, 7
AC3	1144	Olfactory neurones, brain, retina, aorta, lung, testis	8–10
AC4	1064	Kidney, brain, heart, liver, lung	11
AC5	1184	Heart > brain > kidney	12, 13
AC6	1165	Heart, brain > kidney, testis, spleen, liver	13–16
AC7	1099	Lung, heart, spleen, kidney, brain	17, 18
AC8	1248	Brain[a]	14, 19
AC9	1353	Skeletal muscle, brain > kidney lung, liver, heart	20, 21

[a]Northern blotting[19], demonstrated the expression of AC8 message in brain, while reverse transcriptase (RT)–PCR identified AC8 mRNA in brain, but not in heart, liver, kidney, testis or skeletal muscle[14].

Although uniformly regulated by G_sα, the expression pattern and other regulatory properties of the nine principal AC isoforms vary widely, accounting for distinctive cell- and tissue-specific patterns of AC responsiveness. Although mRNA for certain isoforms such as AC4, AC7 and AC9 is expressed in a wide variety of tissues, other AC subtypes exhibit more restricted expression patterns (Table 1). For example, AC1 (Ref. 6) and AC8 (Refs 14, 19) are expressed only in neural tissue while AC5 is expressed predominantly in heart and brain[12]. In the brain, transcripts corresponding to all AC isoforms can be demonstrated, although the distribution of particular isoforms among brain regions is distinctive[17,19,20]. The profiles of regulatory sensitivity of the AC subtypes also vary significantly. Biochemical analysis of purified or transfected recombinant AC isoforms reveals variable sensitivity to regulators such as protein kinases, Ca^{2+} and calmodulin (CaM) (Table 2). The pronounced effects of Ca^{2+} and protein kinase C (PKC) on certain AC isoforms provides a means of integrating signals not only from different G protein-coupled receptors but from tyrosine kinases linked to phospholipase C and ligand-gated ion channels permeant to Ca^{2+} such as the NMDA receptor. AC2 is markedly stimulated upon activation of PKC by phorbol esters[30,32,35] as are other certain other AC isoforms (Table 2). G_s-stimulated but not basal activity of AC4 is inhibited by PKC (Ref. 33). AC subtypes 1, 3 and 8 with significant expression in the CNS, but not other AC isoforms, demonstrate robust stimulation by Ca^{2+}/CaM, whereas AC5 and AC6 are inhibited by submicromolar Ca^{2+} concentrations in a CaM-independent

Table 2. Adenylate cyclase: type-specific patterns of regulation

Adenylate cyclase type	Regulatory signal											
	$G_i\alpha$		$G\beta\gamma$		PKA		PKC[d]		Ca^{2+}/CaM		Other Ca^{2+}-mediated effects	
	Effect	Refs	Effect	Refs	Effect	Refs	Effect	Refs	Effect	Refs	Effect	Refs
AC1	↓	[a]	↓	25, 26			↑	[e], 30, 31	↑	35	↓	[g]
AC2	–	[b]	↑	25–27			↑	27, 32–34	–	7		
AC3	↓	22	–	25			↑	30, 31	↑	36	↓	[h]
AC4			↑	11			↓	[f]	–	11		
AC5	↓	23			↓	28			–	12	↓	[i]
AC6	↓	22, 24	–	13	↓	29	–	30	–	16	↓	[j]
AC7			(↑)	[c]			↑	17, 18	–	17		
AC8									↑	19	–	[g]
AC9			–	20					–	20	↓	[l]

[a]Inhibition of adenylate cyclase type 1 (AC1) by $G_i\alpha$ evident with forskolin or calcium/calmodulin (Ca^{2+}/CaM) stimulation but less effective on $G_s\alpha$-stimulated activity[23]. [b]Inhibition of AC2 by $G_i\alpha$ not evident using purified components[24], although experiments using COS7 cells co-transfected with AC2 and mutationally activated $G_i\alpha$ suggest an inhibitory effect[22]. [c]Indirect evidence in transfected HEK293 cells suggest that AC7 might be positively regulated by $G_{\beta\gamma}$ (Ref. 56). [d]Note that in many of the experiments cited under this heading the effect of protein kinase C (PKC) is inferred from treatment of intact cells with phorbol esters. [e]Phorbol ester potentiation of AC1 in transfected cells seen only to Ca^{2+}/CaM stimulation[30]. [f]Inhibition of AC4 by PKCα not of basal activity, but of $G_s\alpha$-stimulated component[33]. [g]AC1 but not AC8 is inhibited by CaM kinase IV (Ref. 74). [h]AC3 is inhibited by constitutively active CaM kinase II (Ref. 75). [i]AC5 is inhibited by calcium in a CaM-independent fashion[12]. [j]In membrane preparations AC6 is inhibited by submicromolar calcium concentrations in a CaM-independent fashion[15] and can be inhibited in intact cells by calcium ionophores[14]. [k]Enhanced cAMP production in response to immunosuppressant blockers of calcineurin suggest negative regulation of AC9 by this Ca/CaM-activated phosphatase[21].

manner[38]. AC5 (Ref. 28) and AC6 (Ref. 29) can also be inhibited by protein kinase A (PKA) providing a means of desensitization at the effector level in tissues such as heart. A further level of regulatory complexity is achieved by the differential response of the various AC isoforms to subunits of the $G_i\alpha$ family and to $G\beta\gamma$ complexes.

Inhibition of adenylate cyclase by Gα subunits of the G_i family

The complexity of hormonal control of adenylate cyclase was first evinced by Rodbell and co-workers, who demonstrated dual stimulatory and inhibitory G protein regulatory pathways[4]. In most settings, the inhibitory action of G protein-coupled receptors on AC activity can be blocked by treatment of cells with pertussis toxin (PTX), an exotoxin from *Bordetella pertussis* with ADP-ribosyltransferase activity. The recognition that a G protein was the major 41 kDa pertussis toxin substrate in many tissues led to the purification of G_i and, ultimately, to the cloning of $G_{i1}\alpha$ (Ref. 3). With the identification and cloning of several related G_{i1}-related proteins, it has become clear that the ability of a Gα to serve as a substrate for PTX-mediated ADP-ribosylation results from the presence of a required cysteine four residues from the Gα–C-terminus. This C-terminal modification 'uncouples' the G protein from the receptor and accounts for the block of G protein-coupled receptor-mediated AC inhibition by PTX seen in many systems[48]. Within the family of G_i-related proteins, three G_i isoforms, G_{i1}, G_{i2} and G_{i3}, as well as G_o and G_z, have been implicated in AC inhibition – all but G_z are PTX substrates (see below).

The sensitivity of only certain AC isoforms to inhibition by G_i proteins provides yet another opportunity for integration of signalling at the effector level (Table 2). In reconstitution and transfection studies, for example, AC3, AC5 and AC6 have been shown to be sensitive to inhibition by G_{i1} (Refs 22–24) whereas AC2 was not[24]. As AC2 (Refs 25–27) but not AC3 (Ref. 25) or AC6 (Ref. 13) can be stimulated by Gβγ subunits, which would be released upon G_i activation (see below), the profile of isoform sensitivity allows for an unambiguous response to signals from G_i-coupled receptors. The CaM (as well as forskolin)-stimulated activity of AC1, more so than its G_s-stimulated activity, can also be inhibited (Ref. 23). Another PTX-sensitive G protein abundant in the brain, G_o, can inhibit AC1 (Ref. 24) but not AC2 (Ref. 22) or AC5 (Ref. 23). As AC1, which is restricted in expression to neural tissues, is also inhibited by Gβγ subunits[25], this allows the subunits of G protein-coupled receptor-activated G_o to work in concert. The pertussis toxin-insensitive G_z mediates inhibition of AC (Ref. 49) through actions on certain AC isoforms such as AC1 and AC5 (Ref. 50).

The inhibition of certain AC subtypes by $G_i\alpha$ involves a direct interaction of the proteins[23], and although reconstitution studies suggest that it acts at a site distinct from G_s (Ref. 24) the molecular mechanism remains unclear. Post-translational modification of G_i by N-terminal fatty acylation is essential for its AC-inhibitory properties[23], and is likely also to be a requirement for G_o- and G_z-mediated AC-inhibition. Myristoylated forms of G_{i1}, G_{i2} and G_{i3} are all competent to inhibit AC in reconstituted systems[24]. Residues of G_{i2} that might be involved in its

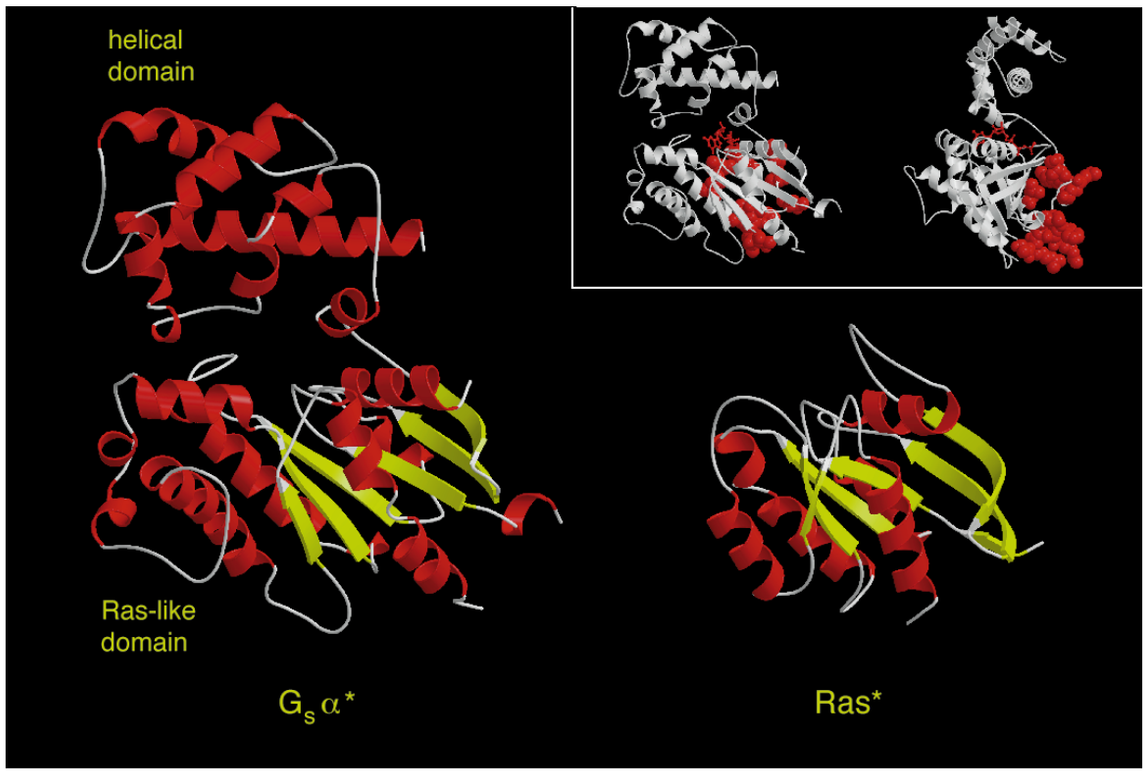

Fig. 2. The structure of $G_s\alpha$ compared to $p21^{ras}$. Ribbon diagrams of the structures of a $G_s\alpha$–GTPγ–S complex[45] ($G_s\alpha^*$) (left) and the Gly12 → Val mutant of Ha-Ras (Ref. 76) (Ras*) (right) determined by X-ray crystallography are shown to approximate scale. The helical and Ras-like domains of $G_s\alpha^*$ are indicated. α-Helices are shown in red, β-strands in yellow, and segments involved in coils and turns in white. The inset shows the adenylate cyclase contact sites of $G_s\alpha^*$ in red spacefill and the bound guanine nucleotide in red ball-and-stick, with the remainder of the structure in grey. The view on the left is the same as in the main figure, while the orientation on the right results from a 90° rotation toward the viewer about a vertical axis. The Brookhaven Protein Data Base (PDB) coordinate files (1azt for $G_s\alpha^*$, and 521p for the Ha-Ras mutant) were oriented in RASMOL (Ref. 77), compiled in MOLSCRIPT (Ref. 78) and rendered in RASTER3D (Ref. 79).

AC inhibitory function have been identified by chimeragenesis and alanine-scanning mutagenesis[51,52]. There is a recent report of a recombinant soluble form of AC5 that retains its sensitivity to G_i-inhibition[53], a model system that might further facilitate functional and structural analysis of the G_i inhibitory mechanism.

Gβγ regulation of adenylate cyclase isoforms

The β and γ subunits of G proteins form a tight, irreversible heterodimer which functions as a single entity in the G protein cycle. It is now recognized that the Gβγ complex, though originally conceptualized as the passive, stabilizing binding partner for Gα subunits at the completion of a signalling cycle, carries an independent signal to many types of cellular effectors upon G protein activation[54]. Their direct role in AC regulation was unsuspected until the cloning of the first AC isoforms and the incisive reconstitution experiments of Tang and Gilman[25,35]. Recombinant AC provided the first example of a purified effector molecule directly regulated by Gβγ (Ref. 26).

Not all AC isoforms respond to Gβγ (Table 2). The isoform-specific nature of AC regulation by Gβγ was evident from the initial demonstration of differential effects on AC1, AC2 and AC3 (Ref. 25). In the presence of activated $G_s\alpha$, AC1 was inhibited by Gβγ, whereas AC2 was potently stimulated and AC3 showed no effect. The conditional nature of the Gβγ regulation suggested a mechanism for integration of signals normally insufficient to affect AC activity, but which might, in the presence of activated G_s, exert profound auxiliary effects[25]. This hypothesis was substantiated by cotransfection experiments employing AC2 (insensitive to $G_i\alpha$ inhibition as noted above) which demonstrated a conditional stimulation of AC activity by Gβγ released from G_i-coupled receptors[55]. AC4, a close homologue of AC2, is also stimulated by Gβγ (Ref. 11), and there is indirect evidence to suggest that AC7, which belongs to the same AC subfamily, might be similarly regulated[56].

At least five Gβ isoforms and 11 Gγ isoforms have been cloned, presenting a large possible number of signalling heterodimers with potentially different regulatory properties[54]. When the AC1-inhibitory and AC2-stimulatory properties of native and recombinant Gβγ heterodimers containing β1, β 2,γ1,γ2 and γ3 were studied by reconstitution, few differences were found[57]. The exception was γ1-containing heterodimers which were found to be much less potent in both assays[57]. Post-translational modification including C-terminal isoprenylation of Gγ is essential for Gβγ modulation of AC, and it is possible that the reduced potency of Gγ1-containing

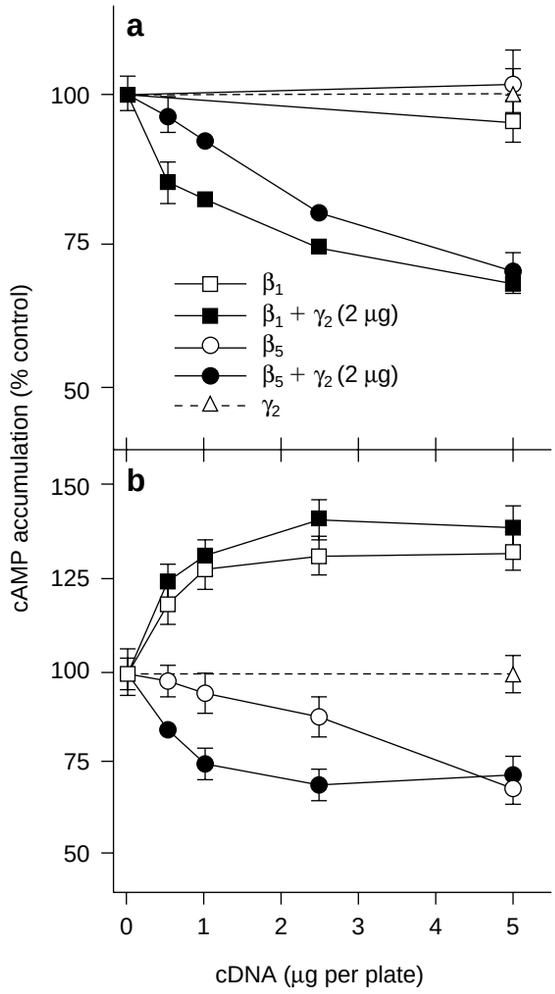

Fig. 3. Differential modulation of AC1 and AC2 by Gβγ subunits. The accumulation of cAMP was determined in COS cells cotransfected with cDNAs for AC1 **(a)** or AC2 in combination with mutationally-activated $G_s\alpha$ **(b)**, and the indicated amounts of $G\gamma_2$, $G\beta_1$ or $G\beta_5$. cAMP accumulation was measured 10 min after (a) the addition of 1 μM forskolin and 1 μM ionomycin, or (b) the addition of 0.5 mM each isobutylmethylxanthine and RO201724. Data are expressed as percent of controls and are the mean ± S.E. of three experiments performed in triplicate. Reproduced, with permission, from Ref. 60.

βγ heterodimers was a consequence of its differential lipid modification[57]. More recently, evidence for Gβ-specific differences in AC modulation have come from studies of Gβ (Ref. 58), a structurally distinct Gβ subunit expressed in brain and retina and which has been found to exhibit effector specialization[59]. Studies in cotransfected COS cells revealed that, while sharing with Gβ1 the ability to inhibit AC1, Gβ5 (unlike Gβ1) inhibited AC2 (Ref. 60) (Fig. 3). This finding raises the possibility of further complexities in G protein regulation of AC in the nervous system, and seems to suggest that the mechanisms of cyclase inhibition and stimulation might involve different sets of residues on the βγ complex.

Additional insight into the mechanism of Gβγ regulation of AC has come from several lines of experiments. Because Gβ contains a CaM binding site[61], and is inhibitory to CaM-stimulated AC, including AC1, one possible mechanism would be competition for CaM. This was not supported by studies with CaM-stimulated brain AC and recombinant AC1 (Refs 35, 62). N-terminal truncation of $G_s\alpha$ did not affect its ability to bind guanine nucleotides and stimulate AC, but neither did it affect the inhibitory or stimulatory effects of Gβγ on AC1 and AC2, respectively[24]. Because the Gα N-terminal helix forms an essential Gβγ interaction domain in the heterotrimer[63], this result excludes the possibility that Gβγ exerts its conditional effects on AC by binding to $G_s\alpha$ in the canonical fashion. Iyengar and co-workers identified a peptide derived from the AC2 sequence which was capable not only of blocking Gβγ stimulatory effects on AC2, but of interfering with multiple other effector interactions of Gβγ (Ref. 64). A control peptide from the corresponding region of AC3 was without effect. The AC2 peptide could be cross-linked to free Gβγ, but not the heterotrimer and a model of this region of AC2 docking to Gβγ was promulgated[65]. The validity of this model awaits experimental verification.

Insights from adenylate cyclase crystal structures

The dramatic elucidation by Hurley and co-workers of a crystal structure of an AC2 C2 homodimer[1] and the subsequent structural determination by Tesmer *et al*. of $G_s\alpha$–GTPγ-S in complex with an AC5 C1–AC2 C2 heterodimer[2] offer the first glimpses into the heart of the cyclase catalyst (Fig. 4). The Hurley group described an overall wreath-like structure consisting of two C2 domains packed head-to-tail into a dimer with extensive intermolecular interactions. On one side of the dimer (the 'ventral' side) lay a deep cleft lined with highly conserved hydrophilic residues, at either end of which was a pocket containing a bound forskolin molecule. The overall quaternary structure of the AC5 C1–AC2 C2 heterodimer reported by Sprang and colleagues[2] (Fig. 4) resembled that of the AC2 C2 homodimer, although important differences in the structures were noted. By soaking their crystals in pyrophosphate and adenosine-derived P-site inhibitors, they were able to determine that the likely substrate binding site was in a pocket on one side of the ventral cleft opposite a single molecule of bound forskolin on the other side of the cleft. The forskolin molecule interacts nearly equally with the two AC cytoplasmic domains, and by filling in a hydrophobic pocket serves as a stabilizing interdomain bridge[1]. The presence of one molecule of bound substrate and forskolin molecule per C1/C2 heterodimer[2] was supported by the results of equilibrium dialysis binding experiments with reconstituted $G_s\alpha$ and soluble AC5 C1 and AC2 C2 domains[66]. Modelling and analysis of conserved residues among forskolin-sensitive and -insensitive AC homologues led Hurley to speculate that the regulatory forskolin binding site has evolved from an ancestral second active site[42].

The GTPγS-activated $G_s\alpha$ bound predominantly to elements of AC2 C2, with fewer AC5 C1 contacts in the

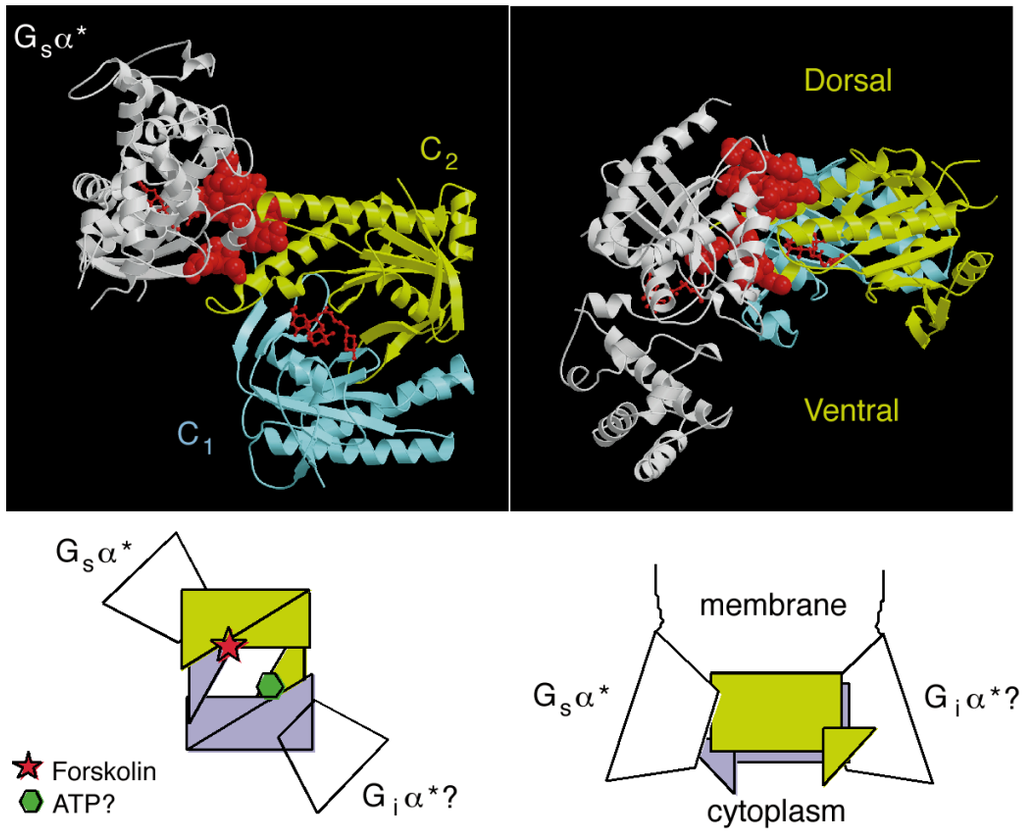

Fig. 4. The structure of the core catalytic domains of adenylate cyclase complexed with forskolin and $G_s\alpha$–GTPγS. (Upper) Ribbon diagram of the structure of a recombinant heterodimer of AC5 C1 (C1, blue) and AC2 C2 (C2, yellow) bound to $G_s\alpha$–GTPγS ($G_s\alpha^*$, coloured as in the inset to Fig. 2) and a piperazino derivative of forskolin (red ball-and-stick)[2]. The view is into the ventral cleft. On the right the structure has been rotated toward the viewer 90° around a horizontal axis to illustrate the dorsal and ventral surfaces of the cyclase catalytic core[1]. The ribbon diagrams were generated as in Fig. 2 using the PDB coordinate file 1azs. (Lower) Schematic diagrams of the complex in the same orientation as the ribbon diagrams above, with C1 in blue, C2 in yellow and Gα–subunits as open trapezoids. On the left the likely position of the cyclase catalytic site (ATP, green hexagon) in the ventral cleft opposite the site of forskolin binding (red star) is shown, based on the localization of P-site inhibitors soaked into the crystal by Tesmer *et al.*[2]. The hypothesized position of $G_i\alpha^*$ relative to the cyclase complex is indicated based on its homology to $G_s\alpha$, biochemical data and symmetry arguments[2]. On the right the most probable orientation of the complex in relation to the plasma membrane is shown, based in part on the direction of the N-terminal projections of the C1 and C2 structures in the complex[2] (cf. Fig. 1, upper). In this model, substrate would have ready access to ventral cleft of the catalytic core because of its cytoplasmic orientation. The fatty acid groups that are known to modify the N-termini of $G_s\alpha$ and $G_i\alpha$ and known to be involved in their membrane targeting are shown as squiggly lines.

Sprang heterodimeric cyclase structure proposed by Tesmer and colleagues[2]. The G_s–GTPγ-S complex binds near the forskolin binding site and opposite to the proposed active site (Fig. 4). The binding of $G_s\alpha$ in this way suggests an orientation for the AC heterodimer such that the dorsal surface was toward the plasma membrane and the ventral surface toward the cytoplasm for substrate access. Although such an orientation of the $G_s\alpha$–cyclase complex is consistent with the membrane orientation of the G protein heterotrimer proposed from crystallographic studies[67], it would be difficult to accommodate the model proposed for AC2 docking to Gβγ (Ref. 65). Comparison of the structures of activated $G_{i1}\alpha$ with that of $G_s\alpha$ suggests that it is differences in the conformation of the polypeptide backbone, and not in specific side-chains which impart the specificity for G_s–AC interaction[45]. Even though it binds at a distance from the active site, G_s might be able to impart its stimulatory effect on AC by reorientation of the C1/C2 heterodimer[2]. Because of the homology between G_s and G_i and biochemical evidence that G_i interacts at a site distinct from G_s (Ref. 24), and to accommodate a similar orientation to the membrane by the $G_i\alpha$-acylated N-terminus, Tesmer *et al.* propose that activated $G_i\alpha$ likely binds to AC in a position pseudosymmetric and opposite to G_s and near the substrate binding site[2] (Fig. 4, lower left). Such a position would surely offer opportunity to interfere with catalysis.

Concluding remarks and future directions

The heterogeneity of AC isoforms allows for tissue and cell-specific responsiveness to particular extracellular signals, with integration of Gα and Gβγ subunits, or both, with signals from other sources affecting intracellular Ca^{2+} levels and PKC activity. A great step forward in understanding the mechanism of catalysis and regulation of AC has come with the recent three-dimensional structural determination of the cyclase catalytic core,

and a complex of activated $G_s\alpha$ bound to a C1/C2 heterodimer.

Future efforts to extend the success in cyclase core crystallization described above will be rewarded with a greater understanding of how AC is regulated. Missing currently is a true 'basal' heterodimeric structure with which to compare the $G_s\alpha$-complexed activated structure, and allow further insight into the G_s-regulated cyclase catalytic mechanism. Structural determination of additional complexes including $G\beta\gamma$, activated $G_i\alpha$ and Ca^{2+}/CaM, or both, bound to recombinant domains of appropriate AC isoforms would expand our understanding of how cyclase catalysis is regulated. Targeted disruption in mice of AC1 has suggested a role for this neurally expressed isoform in spatial memory and synaptic plasticity[68], and knockout models of other AC isoforms would be expected to shed light on the importance of individual cyclases in different tissues and the degree of functional redundancy. Insight into the phenomena of tolerance and dependence may result from analysis of the mechanism of AC superactivation by chronic exposure to opioids and other agonists working through G_i-coupled receptors[69–71]. As several human diseases have been linked to genes encoding G_s and receptors coupled to AC stimulation[72], it is likely that loss- or gain-of-function mutations of particular AC isoforms could produce distinctive disease phenotypes. Lastly the AC structural advances reviewed above may facilitate efforts already underway to develop AC isoform-selective therapeutics[73] and might well revitalize the search for a possible endogenous forskolin-like compound, a biomolecule of great potential pharmacological interest.

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Chemokines and chemokine receptors in the CNS: a possible role in neuroinflammation and patterning

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Chemokines constitute a growing family of structurally and functionally related small (8–10 kDa) proteins associated with inflammatory-cell recruitment in host defence. In addition to their well-established role in the immune system, recent data suggest their involvement in the maintenance of CNS homeostasis, in neuronal patterning during ontogeny and as potential mediators of neuroinflammation, playing an essential role in leukocyte infiltration into the brain. Chemokines and their G protein-coupled receptors are constitutively expressed at low-to-negligible levels in various cell types in the brain. Their expression is rapidly induced by various neuroinflammatory stimuli, implicating them in various neurological disorders such as trauma, stroke and Alzheimer's disease, in tumour induction and in neuroimmune diseases such as multiple sclerosis or acquired immunodeficiency syndrome (AIDS). Here, **F. Mennicken, R. Maki, E. B. De Souza and R. Quirion** briefly summarize recent exciting findings in the field.

Chemokines regulate leukocyte/lymphocyte traffic and play a major role in homeostasis, inflammation and development of the immune system[1]. As in the immune system, chemokines and chemokine receptors in the CNS are constitutively expressed at low levels in astrocytes, microglia and neurones, or both, in the developing and adult brain and are induced by inflammatory mediators. Furthermore, chemokines and their receptors are upregulated in various neuroinflammatory diseases such as multiple sclerosis and acquired immune deficiency syndrome (AIDS), in brain tumours as well as in neurological disorders such as stroke and head trauma[2].

An overview of chemokines and chemokine receptors

The chemokines constitute a growing family of more than 40 distinct members divided into four distinct families on the basis of their structural conservation of specific cysteine residues[1–3] (Table 1). In addition to their chemotactic effects in the immune system, chemokines modulate a number of biological responses, including enzyme secretion, cellular adhesion, cytotoxicity, tumour cell growth, degranulation and T-cell activation[2,4].

The chemokines mediate their effects via G protein-coupled receptors of the seven transmembrane domain, rhodopsin-type superfamily[3,5] and have two main sites of interaction with their receptors. The low-affinity site appears to be responsible for the establishment of chemokine gradients on the surface of endothelial cells and within the extracellular matrix and facilitates by its interaction with the receptor the correct presentation of the second site, the high-affinity site essential for triggering signal transduction and receptor function[6].

The chemokines also interact with two types of non-signalling molecules. First, the Duffy antigen receptor for chemokines (DARC) is expressed on erythrocytes and endothelial cells and binds the different chemokines in order to limit chemokine blood levels[7]. Second, chemokines, which are highly basic molecules, interact physically with acidic extracellular components of endothelial cells. During this association, the chemokines are still active and can serve to establish a local concentration gradient from the source of chemokine secretion[2].

Localization and roles of the chemokines and their receptors in the brain

Chemokines and their receptors in the brain

Although numerous chemokines have been isolated from various neuronal cell lines[4], only a few are detected

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