SIGNAL TRANSDUCTION

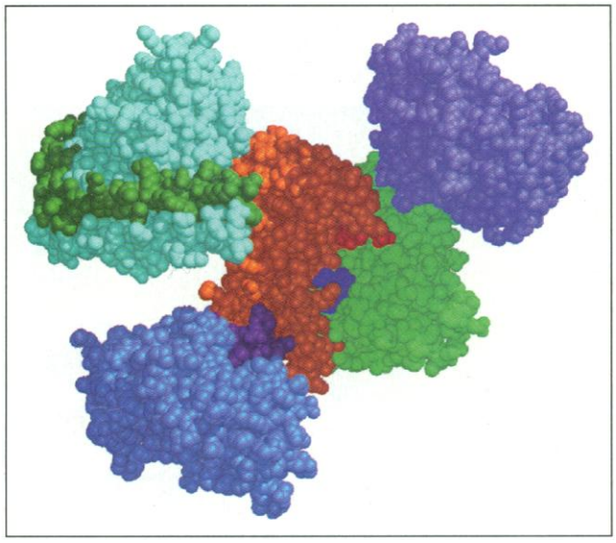
Pieces of the True Grail: A G Protein Finds Its Target

Henry R. Bourne

Like knights following the Holy Grail, biochemists have long sought to understand how hormones stimulate adenylyl cyclase, the membrane-bound enzyme that makes the intracellular "second messenger," adenosine 3′,5′-monophosphate (cyclic AMP). Their 40-year pilgrimage has identified three key pieces of the true Grail—serpentine receptors, adenylyl cyclase (the enzyme that makes cyclic AMP), and G_s, a trimeric guanosine triphosphate (GTP)–binding protein that relays the stimulating signal from receptors to the enzyme. Now a dazzling crystal structure fits together two pieces of the Grail: Tesmer *et al.* report (*1*), on page 1907 of this issue, the structure of the α subunit of G_s ($G_{s\alpha}$) in a complex with its downstream effector, adenylyl cyclase.

The new structure dazzles because it provides the first three-dimensional (3D) picture of an intimate embrace between two signaling proteins that "talk" to one another at the surface of the plasma membrane and also because it suggests how protein regulators other than $G_{s\alpha}$ may interact with adenylyl cyclase. Moreover, $G_{s\alpha}$ binds at a site on the enzyme distant from the substrate-binding pocket, furnishing the investigators the rare opportunity of proposing a testable hypothesis that explains the allosteric mechanism by which one signaling molecule regulates another, at the atomic level.

Discarding the biochemically intractable transmembrane domain of adenylyl cyclase, Tesmer *et al.* chose to crystallize the enzyme's two water-soluble cytoplasmic domains, C_{1a} and C_2 (viewed from the cytoplasm in the figure, and colored green and orange, respectively). The catalytic activities of the C_{1a}-$C_2\gamma$ heterodimer and that of all adenylyl cyclase isozymes (nine to date, and still counting) respond identically to the two molecules crystallized with C_{1a} and C_2: Forskolin (dark blue), a diterpene stimulator of cyclic AMP synthesis (and the active ingredient of a traditional medicine in India), is cradled in a groove between C_{1a} and C_2. The GTP-bound activating protein, $G_{s\alpha}$ (blue and purple), binds to a cleft at one corner of the C_2 domain. The cleft clasps the α2 helix of $G_{s\alpha}$ (purple), which is cognate to the "switch 2" regions that change conformation when GTP binds to other G protein α subunits. The surface of $G_{s\alpha}$·GTP that contacts adenylyl cyclase overlaps with the surface used by guanosine diphosphate (GDP)–bound α subunits to bind βγ subunits (*2*, *3*). Binding to adenylyl cyclase does not alter the shape of $G_{s\alpha}$, as documented by a structure (*4*) of $G_{s\alpha}$·GTP crystallized by itself, reported on page 1943 of this issue.



Catalysis illuminated. Adenylyl cyclase and its many regulators. As viewed from the cytoplasm, two domains (orange and green) of the enzyme's catalytic core cradle an activator, forskolin (dark blue), and ATP (red, modeled into the catalytic pocket). α_s (blue and purple, lower left) was crystallized with adenylyl cyclase. The locations of βγ (turquoise and dark green, upper left) and of α_i (violet, upper right) are postulated, as described in the text.

The figure shows C_{1a} and C_2 in hypothetical association with two other proteins whose structures are known (*2*, *5*, *6*), although neither has been crystallized with the enzyme. These are a G protein βγ subunit (turquoise and dark green) and the α subunit (violet) of a trimeric G protein, G_i, that mediates inhibition of cyclic AMP synthesis by certain hormones. Tesmer *et al.* propose that the negative regulator, $G_{i\alpha}$·GTP, binds to a cleft in the C_{1a} domain located at the opposite corner of the enzyme from the homologous $G_{s\alpha}$-binding cleft of C_2. βγ may inhibit or stimulate cyclic AMP synthesis, depending on the isozyme of adenylyl cyclase to which it binds (*7*, *8*). The βγ dimer is thought to activate the type II isozyme by binding near a third corner of the heterodimer, as suggested by the observation (*9*) that a C_2 peptide representing this region of the type II isozyme prevents βγ from stimulating cyclic AMP synthesis.

The crystal structure specifies the probable orientations of C_{1a} and C_2 with respect to the cytoplasmic surface of the plasma membrane, even though it lacks the enzyme's transmembrane domain. Tesmer *et al.* infer this orientation from the locations of two parts of $G_{s\alpha}$—its NH_2-terminus, which is anchored in the membrane by covalently attached palmitate, and its COOH-terminal tail, which interacts with cytoplasmic loops of serpentine receptors (*10*, *11*). In the figure, both termini of $G_{s\alpha}$ are on the membrane side of the complex, away from the viewer. If correct, this orientation specifies likely orientations of other membrane-bound regulators, such as $G_{i\alpha}$·GTP and βγ. Thus, Tesmer *et al.* suggest that a recent hypothetical model (*12*) docks βγ to type II adenylyl cyclase in an upside down orientation, which would point the prenyl group of the γ chain toward the cytoplasm rather than toward the membrane.

Deducing a molecular mechanism for stimulation of adenylyl cyclase by $G_{s\alpha}$·GTP required clever stratagems to handle two difficulties—the unknown location of the enzyme's active site and the lack of an unstimulated C_{1a}-C_2 structure to compare with that of the activated heterodimer. Because substrate analogs did not form crystals with adenylyl cyclase, Tesmer *et al.* instead used a chemical mimic of the reaction products to find the active site. Painstaking kinetic analysis of adenylyl cyclase activity (*13*) had shown that an adenosine analog and pyrophosphate together act as a dead-end inhibitor, binding more tightly to the catalytic site than do the natural products of the enzyme reaction, cyclic AMP and pyrophosphate. Soaking the two compounds into the crystal revealed the location of a putative catalytic site, allowing the investigators to model adenosine triphosphate (ATP; red in the figure) in a hy-

The author is at the University of California Medical Center, San Francisco, CA 94143, USA. E-mail: h_bourne@quickmail.ucsf.edu

An enhanced version of this Perspective with links to additional resources is available for *Science* Online subscribers at www.sciencemag.org

pothetical catalytic transition state. The location of this active site, nestled in the central groove between C_{1a} and C_2 (like forskolin but at a site farther from $G_{s\alpha}$·GTP) would allow ATP or cyclic AMP to diffuse easily from or into the cytoplasm. Site-directed mutations had suggested catalytic roles for several amino acids close to the site. Mutation of one of these, arginine-1029 of the C_2 domain, sharply reduces catalytic activity but does not alter the Michaelis constant (K_m) for ATP (*14*, *15*). In the transition state model, arginine-1029 stabilizes the pentacoordinate 5′-α-phosphate intermediate of the cyclase reaction. This arginine's role strongly resembles that of the "arginine fingers" that stabilize transition states of GTP hydrolysis in Ras-like guanosine triphosphatases (GTPases) bound to GTPase-activating proteins and in G protein α subunits (*16*).

So, how does the catalytic site increase cyclic AMP synthesis in response to a $G_{s\alpha}$·GTP molecule bound at a rather distant location (30 Å away)? Because the C_{1a}-C_2 dimer could not be crystallized in the absence of activators, the enzyme's unstimulated conformation was modeled on the previously reported (*17*) 3D structure of a recombinant C_{1a}-C_2 homodimer, a complex that shows negligible catalytic activity even in the presence of forskolin and $G_{s\alpha}$·GTP. Comparison of the putative inactive structure with that of the $G_{s\alpha}$·GTP–C_{1a}-C_2 complex suggests an intriguing scenario for activation [for details, see figure 6 in (*1*)]: The α2 helix of $G_{s\alpha}$·GTP, occupying a cleft between loops at the ends of two helices of C_2, widens the cleft by 3 Å and pushes a loop that contacts the C_{1a} domain. As a result, the domains rotate subtly (by ~7°) in relation to one another, around an axis (vertical in the figure) that passes through the substrate-binding pocket. The rotation puts a gentle squeeze on the catalytic site, persuading key residues of both domains to click into a configuration that promotes the cyclase reaction. Thus, a gossamer web of inference supports a plausible model, which will be rigorously tested with additional mutations, biochemical experiments, and crystal structures.

Gallant knights in other laboratories toil to fit one remaining piece of the Grail into its proper place. Their quest will eventually reveal, in atomic detail, how hormone receptors embrace trimeric G proteins and turn them on.

References

1. J. J. G. Tesmer, R. K. Sunahara, A. G. Gilman, S. R. Sprang, *Science* **278**, 1907 (1997).
2. M. A. Wall *et al.*, *Cell* **83**, 1047 (1995).
3. D. G. Lambright *et al.*, *Nature* **379**, 311 (1996).
4. R. K. Sunahara, J. J. G. Tesmer, A. G. Gilman, S. R. Sprang, *Science* **278**, 1943 (1997).
5. D. E. Coleman *et al.*, *ibid.* **265**, 1405 (1994).
6. J. Sondek, A. Bohm, D. G. Lambright, H. E. Hamm, P. B. Sigler, *Nature* **379**, 369 (1996).
7. R. Iyengar, *FASEB J.* **7**, 768 (1993).
8. R. Taussig and A. G. Gilman, *J. Biol. Chem.* **270**, 1 (1995).
9. J. Chen *et al.*, *Science* **268**, 1166 (1995).
10. Reviewed in P. B. Wedegaertner, P. T. Wilson, H. R. Bourne, *J. Biol. Chem.* **270**, 503 (1995).
11. Reviewed in H. R. Bourne, *Curr. Opin. Cell Biol.* **9**, 134 (1997).
12. Y. Chen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 2711 (1997).
13. C. Dessauer and A. G. Gilman, *J. Biol. Chem.* **272**, 27787 (1997).
14. W. J. Tang, M. Stanzel, A. G. Gilman, *Biochemistry* **34**, 14563 (1995).
15. S. Z. Yan, Z. H. Huang, R. S. Shaw, W. J. Tang, *J. Biol. Chem.* **272**, 12342 (1997).
16. Reviewed in H. R. Bourne, *Nature* **389**, 673 (1997).
17. G. Zhang, Y. Liu, A. E. Ruoho, J. H. Hurley, *ibid.* **386**, 247 (1997).

TRANSCRIPTION

Getting Around the Nucleosomes

Jonathan Widom

The 2 meters of DNA inside each eukaryotic cell is wrapped locally around tiny spools called nucleosomes, each made of eight proteins called histones, which are in turn packed together to form chromatin. This packaging method efficiently organizes these lengthy molecules, but when it comes time to make another copy of the DNA for a daughter cell or messenger RNA from a gene, they can get in the way. Each strand of DNA in a nucleosome is inaccessible along most of its length in every helical turn (*1*), the higher orders of chromatin folding further restricting accessibility of the DNA (*2*). Nevertheless, the cell somehow solves this problem: Each gene is wrapped in many nucleosomes, yet is successfully transcribed; classic electron micrographs reveal transcriptional elongation occurring predominantly on DNA packaged in nucleosomes in vivo; and in vitro, RNA polymerases can transcribe right through nucleosomal DNA. A new study, published in this week's issue on page 1960 (*3*), shows how the very large polymerases of eukaryotic cells perform this feat: Studitsky *et al.* carefully analyze the mechanism of transcription through nucleosomes by eukaryotic RNA polymerase III (Pol III).

How RNA polymerases are able to transcribe their chromatin substrates has been the subject of intensive study (*4*). Early experiments analyzed the ability of bacteriophage and eukaryotic RNA polymerases to transcribe through reconstituted nucleosomal DNA and showed that, if the promoter is occluded by histone octamer, transcription initiation is essentially blocked. However, if the promoter extends sufficiently far off the nucleosome, polymerases initiate efficiently and readily extend through one or more nucleosomes.

Important new insights have come from recent methodological advances. In one, resolution of nucleosome isomers with distinct electrophoretic mobilities (which reflect different positions of the histone octamer on the DNA) reveals changes in position before and after transcription (*5*, *6*). Thus, transcription can cause repositioning of the histone octamer on DNA or even complete release of free DNA. The balance between these outcomes depends on the nature and concentration of competitor DNA (or nucleosomes) available to trap histones displaced from the transcription templates, and on the rate of transcription. When transcription is slow and sufficient naked DNA is available upstream of the nucleosome, repositioning can occur more frequently than release and is biased such that the histone octamer moves backward along the template, toward the promoter. Under these conditions, the histone octamer appears to step around the elongating polymerase without fully dissociating from the DNA (*3*, *7*).

Another technical innovation allows real-time analysis of the first passages of synchronous polymerases over nucleosomal templates (*3*, *7*–*9*). When transcription is slowed so that a gene is transcribed over the

The author is in the Department of Biochemistry, Molecular Biology, and Cell Biology, and Department of Chemistry, Northwestern University, Evanston, IL 60208–3500, USA. E-mail: j-widom@nwu.edu