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Classics

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100 Years of Biochemistry and Molecular Biology

The Regulation of Adenyl Cyclase by G-protein: the Work of Alfred G. Gilman

Resolution of Some Components of Adenylate Cyclase Necessary for Catalytic Activity
(Ross, E. M., and Gilman, A. G. (1977) *J. Biol. Chem.* 252, 6966–6969)

Reconstitution of Hormone-sensitive Adenylate Cyclase Activity with Resolved Components of the Enzyme
(Ross, E. M., Howlett, A. C., Ferguson, K. M., and Gilman, A. G. (1978) *J. Biol. Chem.* 253, 6401–6412)

This is the third and final *Journal of Biological Chemistry* (JBC) Classic in a series of Nobel pieces of work on epinephrine and cAMP. The first Classic described Earl Sutherland's discovery of cyclic AMP and its formation by adenyl cyclase (1). These findings led Sutherland to speculate that epinephrine binds to a cell surface receptor, which stimulates adenyl cyclase, causing the formation of cyclic AMP, which then exerts its effect in the cell by activating phosphorylase. The next two Classics fill in the gaps in Sutherland's scheme. The second Classic told the story of Edwin Krebs' discovery of cyclic AMP-dependent protein kinase, the enzyme that is stimulated by cyclic AMP to activate phosphorylase (2). This third Classic explains how Alfred Gilman discovered that hormone receptors stimulate adenyl cyclase via G-protein. Together, these three Classics provide an excellent overview of how hormone signaling cascades were first elucidated.

Alfred Goodman Gilman was born in 1941 in New Haven, CT. At the time, his father, Alfred A. Gilman, was on the faculty of the Department of Pharmacology at the Yale Medical School. The bulk of Gilman's childhood was spent in White Plains, a suburb of New York City, while his father was first on the faculty of The College of Physicians and Surgeons of Columbia University and then was the founding Chairman of Pharmacology at the new Albert Einstein College of Medicine. Due to his father's influence, Gilman majored in biochemistry at Yale University. He graduated in 1962.

The summer after college Gilman worked in Allan Conney's lab at Burroughs Wellcome in New York and published his first two papers. This experience thoroughly convinced him to do research, and he headed off to Case Western Reserve University in Cleveland in the fall of 1962 enrolled in a novel M D.-Ph. D. program. He initially intended to work with his father's friend, Earl Sutherland, on cyclic AMP. However, shortly after his arrival, Sutherland left for Vanderbilt University. Instead, Gilman worked with Sutherland's collaborator, Theodore Rall, who played a pivotal role in Sutherland's discovery of cyclic AMP and adenyl cyclase, as related in a previous JBC Classic (1). In Rall's lab, Gilman worked on cyclic AMP in the thyroid gland, marking the beginning of his long research career in cyclic nucleotide research.

In 1969 Gilman moved to Bethesda, MD to do a 3-year postdoctoral fellowship with Marshall Nirenberg at the National Institute of General Medical Sciences. There, he developed a simple and sensitive assay for cyclic AMP, which helped make second messengers accessible to everyone. Gilman then became an Assistant Professor of Pharmacology at the University of Virginia in Charlottesville in 1971 and continued to work on cyclic AMP and adenyl cyclase.

According to Sutherland's experiments, the binding of epinephrine and other hormones to cell surface receptors stimulated adenyl cyclase, causing the formation of cyclic AMP, which then exerted its effects in the cell through additional signaling cascades. This gave rise to the

Alfred Gilman at the 1994 Nobel Prize awards.

question of how the cell surface receptors interacted with adenyl cyclase. Martin Rodbell proposed that a transducer acted as an intermediary between receptors and adenyl cyclase, and he also showed that guanosine triphosphate (GTP) needed to be present in order for hormones to activate the enzyme. In Gilman's lab, they were working on solubilizing and purifying the components of hormone-sensitive adenyl cyclase systems. Unfortunately, hormonal responsiveness was quickly lost when detergents were used for solubilization, and adenyl cyclase itself seemed to be very labile.

The turning point for Gilman came when Gordon Tomkins developed S49 cells, which were killed by cyclic AMP, and Henry Bourne subsequently produced a variant (cyc^-) of these cells that appeared to lack adenyl cyclase. Elliot Ross, a new postdoctoral fellow in Gilman's lab, was able to reconstitute the cyc^- mutant *in vitro* by adding adenyl cyclase extracts to the cyc^- membranes. However, as reported in the first JBC Classic reprinted here, Gilman and Ross discovered that when they inactivated the adenyl cyclase in the detergent extract, they were still able to use it to restore complete activity in the cyc^- membranes. Treatment with proteases revealed that both the detergent extract and the cyc^- membranes contained proteins necessary for adenyl cyclase activity. These observations led to their proposal that two proteins were required for adenyl cyclase activity, one contained in the detergent extract and one in the cyc^- membranes.

In the second JBC Classic Gilman and Ross, along with Allyn Howlett and Kenneth Ferguson, show that the cyc^- membranes contain adenyl cyclase (their adenyl cyclase-deficient phenotype reflects their loss of the other protein components of the system) and that the detergent extract contains a regulatory protein. Gilman and his co-workers also note that the regulatory protein contains two functional components. They proposed that the role of the hormone receptor was to regulate the interaction between adenyl cyclase and the regulatory protein.

This regulatory protein became the central focus of the Gilman laboratory. They soon discovered that the protein was a homogenous guanine nucleotide-binding protein, capable of activating adenyl cyclase in its Gpp(NH)p or fluoride-activated forms. The signal-coupling protein was named G-protein. Eventually, it was determined that a hormone-activated receptor triggers the exchange of GTP for bound GDP in the G-protein, causing a conformational change, which induces the dissociation of its α subunit bearing GTP from its $\beta\gamma$ subunit.

Adenyl cyclase is then activated by Gα-GTP. The GTP bound to the α subunit is eventually hydrolyzed to GDP, and the subunits reassociate.

After Gilman's initial discoveries, it became abundantly clear that the G-protein family plays an essential transducing role in linking hundreds of cell surface receptors to effector proteins at the plasma membrane. The systems are widely used in nature, controlling processes ranging from mating in yeast to cognition in humans. Because of the profound significance of his work, Gilman was awarded the 1994 Nobel Prize in Physiology or Medicine with Rodbell "for their discovery of G-proteins and the role of these proteins in signal transduction in cells."

In 1981, Gilman moved to Dallas to chair the Department of Pharmacology at the University of Texas Southwestern Medical Center. Today, he still chairs this department. He also holds the Raymond and Ellen Willie Distinguished Chair of Molecular Neuropharmacology.

In addition to his research endeavors, Gilman was the primary editor (in 1980, 1985, and 1990) of a well known pharmacology textbook, *The Pharmacological Basis of Therapeutics*, that was started by his father and Louis S. Goodman. Gilman also served on the JBC Editorial Board. He has received a number of honors and awards for his work including the Richard Lounsbery Award (The National Academy of Sciences, 1987), the American Association of Medical Colleges Award for Distinguished Research in the Biomedical Sciences (1988), the Albert Lasker Basic Medical Research Award (1989), the Passano Foundation Award (1990), the American Heart Association Basic Science Research Prize (1990), and the Louis S. Goodman and Alfred Gilman Award in Drug Receptor Pharmacology (American Society of Pharmacology & Experimental Therapeutics, 1990). Additionally, Gilman was elected to the National Academy of Sciences (1986), The American Academy of Arts & Sciences (1988), and the Institute of Medicine of the National Academy of Sciences (1989).[1]

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REFERENCES

1. JBC Classics: Rall, T. W., and Sutherland, E. W. (1958) *J. Biol. Chem.* **232,** 1065–1076; Sutherland, E. W., and Rall, T. W. (1958) *J. Biol. Chem.* **232,** 1077–1092 (http://www.jbc.org/cgi/content/full/280/42/e39)
2. JBC Classics: Walsh, D. A., Perkins, J. P., and Krebs, E. G. (1968) *J. Biol. Chem.* **243,** 3763–3765; Ahn, N. G., Seger, R., Bratlien, R. L., Diltz, C. D., Tonks, N. K., and Krebs, E. G. (1991) *J. Biol. Chem.* **266,** 4220–4227 (http://www.jbc.org/cgi/content/full/280/43/e40)
3. Gilman, A. G. (1995) Alfred G. Gilman—autobiography. *Les Prix Nobel. The Nobel Prizes 1994* (Frängsmyr, T., ed) Stockholm
4. Gilman, A. G. (1997) G proteins and regulation of adenylyl cyclase. *Nobel Lectures, Physiology or Medicine 1991–1995* (Ringertz, N., ed) World Scientific Publishing Co., Singapore

[1] All biographical information on Alfred G. Gilman was taken from Refs. 3 and 4.