Cyclic GMP: Cellular Regulatory Agent?

The biological regulatory processes that control the growth, differentiation, and hormonal responses of cells are poorly understood, although it is known that many regulatory events are affected by the intracellular concentrations of the cyclic nucleotide adenosine 3′,5′-monophosphate (cyclic AMP). A second cyclic nucleotide, guanosine 3′,5′-monophosphate (cyclic GMP), is also present in all living systems, but until recently few investigators have studied the relation of this compound to regulatory processes. It has now been proposed that cellular regulations may be influenced by the interaction of cyclic AMP and cyclic GMP—a proposal that has generated considerable interest among biologists.

One of the first persons to study cyclic GMP was Nelson Goldberg of the University of Minnesota. He believes that the intracellular concentrations of the two nucleotides in certain regulatory events are correlated and that these concentrations vary inversely in those events, a relationship that he likens to that embodied in the ancient Oriental concept of Yin and Yang. Although the hypothesis does not apply to all regulatory systems, it unifies many diverse events and is now being used by numerous investigators as a model for their experiments.

According to this hypothesis of interaction, the opposing effects of cyclic AMP and cyclic GMP are restricted to cellular systems that are composed of antithetical events, such as contraction-relaxation, glycogen synthesis–glycogen breakdown, and proliferation-contact inhibition—systems Goldberg describes as bidirectionally controlled. He distinguishes these systems from others that are susceptible to only one type of influence, such as the production of steroids by the adrenal cortex. This secretory system is susceptible to only one type of influence since there is no known antagonist of adrenocorticotrophic hormone on the production of steroids.

The proposed regulatory role of cyclic GMP can best be understood in the context of what is known about cyclic AMP. Changes in the intracellular concentrations of cyclic AMP are associated with such diverse processes as the regulation of enzyme biosynthesis in bacteria, the transmission of nerve impulses, the responses of various target cells to their respective hormones, and the inhibition of cell proliferation. By artificially increasing intracellular concentrations of cyclic AMP, it has been possible to show a direct cause and effect relationship between concentrations of the nucleotide and various regulatory events. However, many researchers believe that it is unlikely that all bidirectional processes are controlled solely by changes in the concentration of cyclic AMP. For example, tissue responses to the hormones glucagon or epinephrine are associated with increased cyclic AMP concentrations; but Goldberg says that the responses to insulin, which are antagonistic to those of glucagon and epinephrine, take place without, or prior to, any change in the intracellular concentrations of cyclic AMP. Since it appears that cyclic AMP cannot by itself mediate such regulatory events, Goldberg looked for a second regulatory compound. He proposes that cyclic GMP is an antagonist of cyclic AMP in bidirectionally controlled systems.

Cyclic GMP, like cyclic AMP, is always present in living organisms, but its intracellular concentrations are generally only one-tenth to one-fiftieth those of cyclic AMP. It is therefore very difficult to measure the concentration of this compound. Recently, however, researchers have developed analytical techniques for the measurement of cyclic GMP. One such technique is a radioimmunoassay that was developed by Alton Steiner of the University of North Carolina at Chapel Hill and is widely used. Accordingly, it is feasible to investigate the role of cyclic GMP in regulatory processes and its relationship to cyclic AMP. In particular, cyclic GMP assays enable investigators to monitor changes in the concentrations of the two cyclic nucleotides.

Hormones Affect Cyclic GMP

In 1970, Goldberg was able to associate a hormone-induced response of rat myocardium with an increase in intracellular cyclic GMP concentrations. Rat myocardium decreases in contractility in response to acetylcholine. The decrease occurs concurrently with a two- to threefold increase in the intracellular cyclic GMP concentrations and no change or a small delayed decrease in cyclic AMP concentrations. On the other hand, isoproterenol (which chemically and biologically is similar to norepinephrine) affects rat myocardial tissue by increasing its contractility, and the increase occurs concurrently with a 50 percent decrease in the concentration of cyclic GMP and an increase in the concentration of cyclic AMP.

Thus, Goldberg demonstrated that an increase in cyclic GMP was associated with a response to a hormonal action. He and others have subsequently shown similar hormone-associated changes in cyclic GMP concentrations in other cells. For example, they found that cholinergic stimulation is associated with the rapid accumulation of cyclic GMP in the rat uterus, intestines, ductus deferens, and submaxillary gland, and in rabbit lung, human peripheral blood lymphocytes, and mouse cerebellum. Moreover, Goldberg and his associate M. Haddox have shown that the effects of a number of other agents including oxytocin, insulin, serotonin, and histamine can be associated with a rapid increase in the intracellular cyclic GMP concentrations in systems in which these agents promote responses that are opposite to those produced by cyclic AMP or by agents that increase intracellular cyclic AMP concentrations.

The growth and growth inhibition of cells may also be associated with relative changes in the concentrations of the cyclic nucleotides. Researchers have shown that at least cyclic AMP is involved. Jacques Otten and his colleagues in Ira Pastan's Laboratory of Molecular Biology at the National Institutes of Health in Bethesda, Maryland, have shown that increased concentrations of cyclic AMP are correlated with contact inhibition of the growth of several varieties of fibroblast cells. Researchers in Pastan's laboratory have also shown that high con-

centrations of cyclic AMP in mouse fibroblasts inhibit proliferation and promote differentiation. For the mouse fibroblasts this effect of cyclic AMP may be opposed by cyclic GMP.

Mouse fibroblasts will grow in culture until they reach confluency and are contact-inhibited. The confluent cells will divide, however, if they are exposed to insulin. Goldberg found that, when confluent mouse fibroblasts of the 3T3 line were stimulated with insulin, their intracellular cyclic GMP concentrations increased in a manner dependent upon the concentration of insulin used. The cyclic GMP concentrations increased from 10- to 40-fold with insulin concentrations ranging from 0.1 to 1000 milliunits per milliliter.

The studies of the relative concentrations of the cyclic nucleotides during regulatory events show only correlations between concentrations of cyclic AMP and cyclic GMP and bidirectionally controlled events. They do not establish that relative changes in cyclic GMP concentrations cause these events. The difficulty in establishing causative relationships stems from the fact that the cyclic nucleotides do not easily enter cells. This difficulty has been circumvented in the case of cyclic AMP. There are derivatives of cyclic AMP which can enter cells and induce the responses attributed to this nucleotide. In addition, there are compounds that are known to specifically raise intracellular cyclic AMP concentrations by acting directly on the enzyme that catalyzes cyclic AMP formation. Similar phenomena have not yet been demonstrated for cyclic GMP.

Cyclic GMP has proved difficult to study for a number of reasons. The intracellular concentrations of the nucleotide are extremely low (10^{-8} to 10^{-7} mole per kilogram of wet tissue), and, in addition, the effects that are associated with changes in cyclic GMP concentrations appear to be associated with calcium influxes. All effects that are proposed to be cyclic GMP mediated are also calcium-dependent. Moreover, Gunther Schultz and Joel Hardman of E. W. Sutherland's laboratory at Vanderbilt University, Nashville, Tennessee, have shown that the enzyme that catalyzes the formation of cyclic GMP is stimulated by calcium. This dependency complicates attempts to determine the relationship between cyclic GMP and regulatory events.

There are several approaches to the further study of cyclic GMP actions. Hardman's group is investigating the enzyme (guanylate cyclase) that catalyzes the formation of cyclic GMP. If they could determine the regulators of that enzyme then they could specifically control the endogenous production of the nucleotide. Thus, it seems likely that hormones may activate or inactivate the enzyme, but Hardman and his colleagues have been unable to demonstrate any hormone-induced effects. They found that the enzyme is partly soluble and partly associated with particulate matter. Some investigators believe that the enzyme is loosely bound to the cell membrane and that purification disrupts the membrane association. It may be necessary to preserve this association in order to maintain the enzyme's response to hormones.

Another approach to the study of interactions between cyclic AMP and cyclic GMP is to investigate the effect of one nucleotide on the production and degradation of the other. In his work along these lines, Joseph Beavo from Hardman's laboratory looked for effects of cyclic GMP on the enzyme that degrades cyclic AMP. He used cell-free systems and was able to show that cyclic GMP stimulates the hydrolysis of cyclic AMP in homogenates of rat liver and other tissues. Whether this holds true in vivo, however, is unknown.

Direct Evidence of Interactions

Although cyclic GMP does not easily enter cells, it may be possible to increase intracellular concentrations of the nucleotide by the addition of large amounts of cyclic GMP to the extracellular medium. Gordon Tompkins and his colleagues at the University of California in San Francisco have attempted to produce cyclic GMP–associated effects in this way, and the results have been promising. In previous studies they had shown that high intracellular concentrations of cyclic AMP in cultured mouse fibroblasts inhibit the uptake of uridine, leucine, and 2-deoxyglucose, decrease the rates of RNA and protein synthesis, and stimulate protein degradation in these cells. They called these effects pleiotypic effects. By adding very high exogenous concentrations of cyclic GMP to mouse fibroblasts, they were able to overcome these pleiotypic effects of cyclic AMP.

A few groups of researchers have been able to add cyclic GMP derivatives to cells that participate in the immune response and to subsequently observe cyclic GMP–associated effects. Frank Austen and his colleagues at the Robert B. Brigham Hospital and Harvard Medical School in Boston are among those who have used this technique. In their studies of the immune response, they had noted that cyclic AMP can suppress the release of chemical mediators by cells of the immune system. Mast cells (which are involved in immediate hypersensitivity) can be stimulated by the antigen-antibody reaction on their surface to release one of three types of chemical mediators. These mediators are said to be responsible for the symptoms of allergic reactions. Their release is enhanced by cholinergic agents.

Since increased concentrations of cyclic GMP are correlated with responses to cholinergic agents in other systems, Austen and his colleagues attempted to add cyclic GMP derivatives to mast cells and thus duplicate the effects of these agents. They found that if they treated tissues with cyclic 8-bromo-GMP they could enhance the release of chemical mediators. They also obtained this effect when they added cyclic 8-bromo-GMP to lymphocytes (which are involved in delayed hypersensitivity). Austen and his associates were thus able to demonstrate bidirectional effects of cyclic AMP and cyclic GMP in two different immunological effector systems.

Other groups of investigators have been able to obtain similar results in their studies of the release of lysosomal enzymes by polymorphonuclear leukocytes of the immune system. The release is stimulated by the addition of cyclic GMP derivatives and inhibited by increased intracellular concentrations of cyclic AMP. Other researchers have, so far, been unable to obtain such results with other systems. Austen and his colleagues believe that they were successful because their system is tremendously sensitive to cholinergic agents. Their system responds to acetylcholine at concentrations of $10^{-10}M$, for example. There may also be technological problems with some of the other systems because of the relation between cyclic GMP and calcium, because the derivatives of cyclic GMP may not enter the cells, or because cyclic GMP may exert its effect only within a very narrow range of concentrations. The results of Tompkins, and those of the researchers who studied immunological responses, are en-

couraging but by no means conclusive.

This Yin-Yang hypothesis, then, is still a hypothesis. It is interesting in that it unifies diverse results and permits investigators to make predictions that can be experimentally tested. It is becoming increasingly clear that cyclic AMP cannot, by itself, regulate all bidirectional systems. The Yin-Yang hypothesis can give direction to new research in cellular regulation.

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Additional Readings

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Optical Communications: Specialized Applications Appear First

Telephone traffic is increasing at a rate of 15 percent per year (measured in voice circuit miles). Data transmission and video transmission are increasing at 25 and 35 percent per year, respectively (measured in binary bits transmitted). These are the projections for continued growth in telecommunications traffic contained in a report recently released by the National Academy of Engineering. One of the current approaches to meeting this rapidly increasing demand in communications capacity is the development of systems in which light waves transmit information. Optical systems can accommodate large volumes of information, because of the much greater magnitude of optical frequencies compared to those of the information being transmitted. Although they are still largely in a research and development stage, a few optical communications systems for specialized applications are being tested.

Among the components of such a system are a light source to generate the optical carrier wave, a modulator to impress the signal to be transmitted onto the light wave, and a photodetector to receive the modulated light wave at the end of the transmission path. The light is transmitted either through a glass optical fiber or through the air. In addition, electronic devices are required to convert the information signal into a form suitable for driving the modulator and to recover the signal from the photodetector.

For example, an experimental telephone system is being operated aboard the Navy cruiser U.S.S. *Little Rock*. This system, put together by the Naval Electronics Laboratory Center, San Diego, California, consists of a gallium arsenide light-emitting diode (LED) source, a silicon detector made from a p-n junction with an intrinsic layer between the p- and n-type regions (PIN photodiode), and glass optical fiber transmission lines. Although the loss in light intensity as the light travels through the fiber (that is, the optical attenuation) is not particularly low, this is not a problem for the short distances that messages travel on board a ship. Modulation is achieved directly by controlling the current through the gallium arsenide LED. According to Donald Albares of the Naval Electronic Laboratory Center, the telephone system operates at 3 to 4 kilohertz (audio bandwidth), and therefore does not tax the performance limits of any of its components. Optical fiber telephone systems are of special interest to the Navy because of security considerations. Fibers do not radiate signals, and hence are safe channels of communication; fibers also are not subject to interference from neighboring signals and therefore are not subject to cross talk. An operational demonstration of a closed circuit television that can transmit visual information aboard a ship is planned for the near future.

Low Attenuation Glass Fibers

Terrestrial optical communications systems, like the Navy's shipboard telephone system, will most likely use glass optical fibers as the transmission medium. However, long-distance transmission through fibers does not become practical unless a very low optical attenuation level is reached. The first breakthrough in the development of optical fibers was reported by researchers at Corning Glass Works, Corning, New York, nearly 3 years ago, when fibers with optical attenuations of 20 decibels per kilometer (compared to previous attenuations of more than 100 db/km) were produced. Since then, Corning has made, on a laboratory scale, fibers with attenuations as low as 2 db/km (at some wavelengths), according to Robert D. Maurer, manager of applied physics research at Corning. Corning's fibers, made from two different glasses, have an inner core with a higher index of refraction and an outer cladding with a lower index of refraction. The difference between the indices of refraction of the core and of the cladding causes the light to be confined to the fiber. The small diameter fiber is made by drawing down in a furnace a rod within a tube; in this way core diameters as small as 3 micrometers have been obtained. Before optical systems that depend on fibers are practical, researchers must learn how to make long lengths of fibers, how to make fiber bundles and cables, and how to join fibers together. Corning has developed some prototype cables and coupling devices.

Bell Telephone Laboratories, Holmdel, New Jersey, is also pursuing research in fiber development and has recently produced fibers with attenuations almost as low as those of the best Corning fibers. The Bell fibers, unlike those of Corning, use the same glass in the core and cladding, but the core and cladding are separated by an air space (Fig. 1). Since the air has a lower index of refraction than the glass, light is still confined to the fiber. Bell is also studying other kinds of optical fibers, including one similar to the Corning design. A third kind of fiber, developed at the Nippon Electric Company in Japan, has an index of refraction that varies continuously from the center to the surface of the fiber. This graded index of refraction also results in the confinement of the light.

An optical communications system having fibers with lower attenuations than those in the Navy shipboard experiment is being developed by GTE Laboratories, Waltham, Massachusetts. They are currently studying fibers that have a graded index of refraction (obtained from Japan), although other types of fibers are under consideration. Coupling devices to join fibers are also being developed. In other respects, the GTE system resembles those in which optical fibers are currently used. An aluminum gallium arsenide LED (emit-