How Cells Respond to Signals

Cells have many behaviors—they contract, secrete, divide, differentiate, and signal other cells. Although biologists have long recognized many of the stimuli for these events, they are only now beginning to understand the details of signal transduction—namely, how a cell converts a stimulus into a response. Participants at a recent meeting, "Signal Transduction in Biological Systems," held in Bethesda, Maryland, discussed some of the molecular interactions in cells that support signal transduction.*

Much of the information centered around certain components of cell membranes that regulate cell behavior. For instance, G proteins, inositol phospholipid metabolites, and factors that alter ion channels all play active roles in determining cellular responses to stimuli. In addition to these major topics, researchers presented new information about a possible novel mechanism for insulin action.

Two themes dominated the meeting. First, any given cellular response is subject to multiple molecular controls. And second, stimulation of the same pathway of signal transduction can result in very different cellular responses, depending on interactions with other molecules and the type of cell involved.

Expanding Roles for G Proteins

When light stimulates photoreceptors in the retina or when certain chemical factors bind to their receptors, G proteins in the membrane become active. G proteins, so-called because they bind guanine nucleotides, "act as transducers of information across cell membranes," says Alfred Gilman of the University of Texas Health Science Center in Dallas. "They couple the activity of receptors with effector molecules inside the cell." The effectors, in turn, mediate cellular responses to the original stimulus. The entire chain of events comprises signal transduction, in which G proteins are increasingly seen to play important roles.

There are several different types of G proteins, which are described in terms of their function as G_s (stimulatory for adenylate cyclase), G_i (inhibitory for adenylate cyclase), G_o (other, unknown function), and G_t (transducin, in retinal photoreceptors).

All of the G proteins characterized so far are made of three subunits, but it is the α subunit that scientists see as playing the most important functional role. "The α subunit seems to carry out the effector function of the G proteins," says Martin Rodbell of the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina. "In my view, these are the real intracellular messengers. We have good evidence that α subunits are released from membranes in response to hormones or guanine nucleotides; therefore, they are mobile and make good candidates as messenger molecules. Alpha subunits can regulate glucose and magnesium ion transport as well as activating or inhibiting adenylate cyclase."

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Alfred Gilman *of the University of Texas Health Science Center says that G proteins "act as transducers of information across cell membranes."*

When a receptor is inactive, the G protein coupled to it binds GDP (guanosine diphosphate). Light or binding of a hormone or neurotransmitter activates the receptor, causing the alpha subunit of the coupled G protein to bind GTP (guanosine triphosphate). According to Rodbell's theory, the GTP/Gα subunit complex dissociates from the membrane and activates adenylate cyclase, which catalyzes cAMP synthesis (if a G_s protein is involved). Then, cAMP stimulates the phosphorylation of proteins, either in the membrane or in the cytoplasm, which leads more directly to cellular responses.

To turn off the cell's response, the complex of G protein and GTP must be inactivated. This occurs when an enzyme, GTPase, removes a phosphate group from GTP to make GDP. "The catalytic rate of GTPase is very slow," says Gilman. "It takes about 15 seconds to hydrolyze GTP to GDP, which allows the system to persist in an active state for a long time."

Gilman has new evidence from complementary DNA (cDNA) clones that "there are two molecular forms of the α subunit of G_s— one is short (45,000 daltons), and the other is long (52,000 daltons)." Two forms of the subunit were known to exist, but their molecular differences have been unclear. Gilman, Janet Robishaw, and Michael Graziano, also at the Health Science Center, do not yet know how the two forms of the $G_s\alpha$ subunit differ in function, but they plan to study how the two forms are differentially regulated.

Although much information about G proteins comes from studies of how they regulate adenylate cyclase, G proteins do not couple all receptors with adenylate cyclase. Certain growth factor receptors are an exception, according to Rodbell. And controversy exists about the possible role for G proteins in another signaling system, that involving inositol phospholipid metabolism.

Ion Channels: Multiple Forms of Regulation

Ions flow through the membranes of all cells, but much information about ion channels comes from cells that have a high density of channels, particularly nerve and muscle cells. Depending on their state of activation, membrane ion channels conduct sodium, potassium, calcium, or chloride ions in and out of cells. For many years, researchers spoke in terms of two kinds of ion channels—those controlled by the electrical potential across the membrane, and those activated by ligand binding to a receptor. Recently, scientists have discovered new levels of complexity in terms of channel structure and have also found that second messengers may help to regulate channel function.

Robert Stroud of the University of California at San Francisco and his colleagues describe certain common elements of ion channel structure using the acetylcholine receptor with its sodium channel as an example. Stroud notes that, to form a channel, membrane proteins contain alpha helices

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that sequester charges away from surrounding lipids. This, he thinks, lowers the energy required to maintain the structure of the channel with its charged portions facing the pore and its outer regions facing noncharged lipids in the membrane.

Stroud and his collaborators have recently crystallized the acetylcholine receptor (AChR), the protein that binds the neurotransmitter acetylcholine. "The acetylcholine receptor is an ion channel found between the membranes of a nerve and a muscle cell, or between two nerve cells, or in fish electric organs," says Stroud. "It changes a chemical signal [acetylcholine] into an electrical signal, allowing the influx of about 10,000 sodium ions per millisecond per molecule. The five subunits of the protein, 2 α, 1 β, 1 γ, and 1 δ, collaborate to generate the ion channel."

AChR ion channels are filled with water and all five subunits of the receptor have regions that span the membrane many times. Stroud has evidence that each subunit has one transmembrane alpha helical region with a charged "stripe" that forms part of the channel pore. He proposes that the binding of acetylcholine induces a conformational change in the structure of the protein and lowers the activation energy of the channel to allow hydrated sodium ions to pass through.

Other researchers focus on mechanisms that regulate ion channel function. Recent information indicates that some channels previously known to be activated by threshold changes in the electrical potential across a membrane are also regulated by second messenger systems.

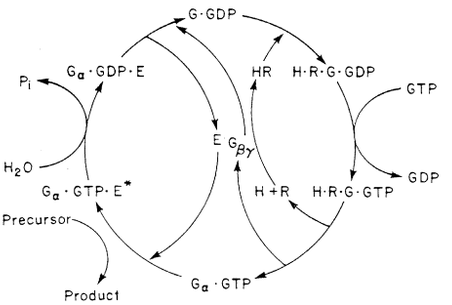
"Two kinds of experiments have been important in understanding how ion channel function is regulated," says William Catterall of the University of Washington in Seattle. "First are electrophysiology experiments in which people have shown that the activity of these channels can be regulated by cAMP-dependent kinase or by protein kinase C. Second are biochemistry experiments in which subunits of purified channels are shown to be substrates for phosphorylation by a kinase."

Working independently, Catterall and Franz Hofmann of the University of Saarlands in West Germany, have new evidence that the kinase stimulated by cAMP regulates a population of calcium channels in skeletal muscle. The species of calcium channel affected and its mode of regulation are similar to calcium channels already described in neurons and heart muscle cells.

The calcium channel Catterall and his coworkers study is sometimes referred to as an L channel, because it takes a long time to be activated and requires a high level of membrane depolarization to open. "These calcium channels are regulated by second messengers as well by membrane voltage," says Catterall. "Cyclic AMP increases their activity and protein kinase C decreases it."

Second messengers also regulate the activity of some sodium and potassium channels in addition to certain calcium channels. For example, Catterall and his group have evidence that rat brain cells in primary culture have sodium channels that are phosphorylated by cAMP-dependent kinase. To date, there is no clear effect on sodium channel function due to phosphorylation.

Other researchers have shown that channel function varies with second messenger production, but have not yet demonstrated that the channel proteins themselves are the biochemical substrates for kinases.



Molecular interactions of G proteins during signal transduction. Hormone (H) binding to its receptor (R) causes GTP to bind to the Gα subunit. This activates effector (E) molecules inside the cell to make a product. The Gα · GTP · E complex is inactivated by removing a phosphate group (P_i) and reforming the unstimulated G · GDP complex. [*From A. Gilman,* Annu. Rev. Biochem., *in press*]

For example, Haruhiro Higashida of National Heart, Lung, and Blood Institute and David Brown of the University of London have just reported that two metabolites of inositol phospholipid breakdown, inositol trisphosphate (IP_3) and diacylglycerol (DG), control two different potassium currents in cultured neuroblastoma cells. One is an outward calcium-dependent potassium current. The other is a secondary inward potassium current resulting primarily from inhibition of a different outward (M) current. Bradykinin, a neuroactive peptide, stimulates inositol phospholipid turnover in these cells and produces the two second messengers and the two potassium currents studied by Higashida and Brown. They think that IP_3 and calcium stimulate the outward potassium current and that DG ultimately regulates the secondary inward current.

Thus, evidence increasingly points to multiple controls of ion channels and much of the new information indicates regulatory roles for cAMP-dependent protein kinase as well as for protein kinase C.

Inositol Phospholipid Metabolism and Calcium Regulation

In recent years, convincing evidence has accumulated indicating that membrane lipids do more than form a water-repelling environment in which proteins function. Some lipids and their metabolites also play an active role in controlling what goes on inside cells.

For example, the metabolites of a specific group of membrane lipids, the inositol phospholipids, help to regulate intracellular calcium levels and trigger enzyme activity. Phosphatidylinositol (PI), and its derivatives PIP and PIP_2, which carry additional phosphate groups, are normal constituents of cell membranes. When a hormone or other stimulus outside the cell activates a receptor that is coupled to phospholipase C, the enzyme degrades at least PIP_2 to produce two intracellular signals, inositol trisphosphate (IP_3) and diacylglycerol (DG).

IP_3 causes the release of calcium ions from a compartment of the endoplasmic reticulum and thus raises the concentration of calcium inside the cell. Diacylglycerol activates an enzyme, protein kinase C, to phosphorylate certain proteins. Degradation of DG yields arachidonic acid, yet another intracellular signal.

Cellular responses to these events vary, depending on the type of cell involved. Brain cells release neurotransmitters, salivary glands secrete saliva, smooth muscle cells contract, and certain normal cells transform into cancer cells. Current topics in receptor-mediated changes in PI metabolism include multiple forms of protein kinase C, the debate about G protein regulation, and the origin and physiological significance of various isomers of the inositol phosphates.

Recently, several groups of scientists have predicted multiple forms of protein kinase C from complementary DNA clones. For example, Yasutomi Nishizuka of Kobe University School of Medicine in Kobe, Japan, and his co-workers identify two different kinds of protein kinase C in rat brain tissue and find high concentrations of the enzyme in certain regions of the brain, including the hippocampus, amygdala, and cerebellum. One form of the enzyme has 673 amino acids more than the other, probably because of different ways of splicing the same gene, not because different genes code for the two kinases, Nishizuka proposes. But "the biological significance of this heterogeneity is not yet known," he says.

Researchers generally agree that G proteins appear to mediate phospholipase C activity and PIP_2 breakdown, but they ques-

tion what kind of G protein is involved. In some cells the G protein may be similar to G_i, which inhibits adenylate cyclase activity, but not all cells appear to be the same in this respect.

Robert Michell of the University of Birmingham in Birmingham, England, says "I don't think there is much doubt now that the signal [a chemical stimulus or light] gets to a receptor and goes through a G protein. And most of the evidence suggests that the G proteins regulating phosphoinositidase are not the same as those that regulate adenylate cyclase activity."

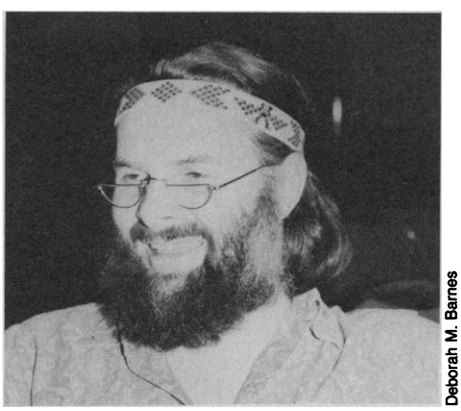
New information from Leonard Kohn and Daniela Corda of the National Institute of Diabetes and Digestive and Kidney Diseases indicates that several regulatory mechanisms control thyroid hormone production, one of which involves PIP_2 breakdown and a regulatory G protein. Two hormones, thyroid-stimulating hormone (TSH) and norepinephrine (NE), trigger hormone release from the thyroid gland in vivo. Both stimulate PIP_2 breakdown, and TSH also stimulates cAMP production.

Kohn and Corda's new work shows that NE, acting at α_1 receptors, triggers PIP_2 breakdown via a regulatory protein that is like an inhibitory G protein (G_i) and can be blocked with pertussis toxin. The TSH receptors coupled to PIP_2 breakdown are not coupled to this kind of G protein. Thus, two signals that trigger hormone release from the thyroid gland in vivo, stimulate the same PIP_2 transduction pathway, but only one receptor type seems to be coupled to a regulatory G_i-like protein.

Other researchers use cell-free systems, containing either membrane-bound phospholipase C or solubilized enzyme, and find different requirements for proteins that mediate PIP_2 breakdown. For example, John Fain and his colleagues, of the University of Tennessee School of Medicine in Memphis, have new evidence that both adenine and guanine nucleotides stimulate phospholipase C activity in rat liver cell membranes and in soluble preparations of the enzyme.

Because both kinds of nucleotides regulate phospholipase C activity, Fain proposes that G proteins help to regulate the enzyme in his system, but also points out that "there may be a nucleotide regulatory site that is separate from a G binding protein." Whether this ambiguity of regulatory proteins is caused by the characteristics of his preparation or whether it represents multiple regulatory processes in vivo, Fain cannot yet be certain. But, he says, it is important to realize that "there is a lot of indirect evidence for G protein regulation of PIP_2 breakdown, but no one has isolated the G protein responsible."

Another issue in studies of phosphatidylinositide metabolism stems from the existence of multiple inositol phosphate isomers and their potential significance. Until recently, researchers assumed that PIP_2 breakdown yielded inositol trisphosphate having phosphate groups in the 1, 4, and 5 positions of the inositol ring. But Robin Irvine of the AFRC Institute of Animal Physiology in Cambridge, England, and his colleagues identified another isomer with phosphate groups in the 1, 3, and 4 positions, a compound which could not arise directly from PIP_2 breakdown.



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Robert Michell *of the University of Birmingham discusses the regulation of phosphatidylinositide metabolism.*

At about the same time, Philip Majerus of Washington University in St. Louis described a cyclic form of the compound, 1,2 cyclic-4,5 inositol trisphosphate. Although the 1,3,4 form has no known biological activity, the cyclic form, the 1,4,5 isomer, and (1,4) IP_2 cause the release of calcium from internal stores.

Researchers have now identified additional inositol phosphates with four and five phosphate groups, calling into question their origins as well as their functional significance.

Novel Role Proposed For Insulin Action

Alan Saltiel, of Rockefeller University in New York, and his colleagues have just shown that insulin may regulate the breakdown of a novel membrane glycolipid to produce intracellular second messengers that mediate some of the overall actions of insulin. Saltiel reports that insulin binding to its receptors in a line of cultured muscle cells stimulates an enzyme, a specific phospholipase C, to cleave a carbohydrate-containing membrane phospholipid. He proposes that this glycolipid is similar to the one that anchors certain proteins to membranes.

"People have been working for several years on a chemically undefined molecule that mediates the actions of insulin," says Saltiel. "There has been a controversy about whether a second messenger regulates insulin action. We have identified two possible second messengers and their precursor."

The second messengers are diacylglycerol and an inositol phosphate–containing carbohydrate, which Saltiel calls an inositol phosphate glycan. Although the Rockefeller group has no specific information about the physiological role of the water-soluble glycan, Saltiel proposes that it may activate the phosphodiesterase that stimulates the degradation of cAMP, thus decreasing the amount of intracellular cAMP.

"The Saltiel work is certainly interesting," says Ora Rosen of the Memorial Sloan-Kettering Cancer Center in New York. "There are two research directions that now need to be pursued. The first is to show if there is a link between the tyrosine kinase activity of the insulin receptor and the activation of phospholipase C which then generates the two second messengers. The second is to demonstrate how many of the actions of insulin can be explained by this mechanism."

To date, many of the molecular mechanisms of how insulin stimulates the influx of glucose into muscle cells and increases glycogen synthesis are still speculations. Insulin is known to cause a decrease in cyclic AMP levels in muscle cells, a response that may be mediated, at least in part, by the new inositol phosphate glycan reported by Saltiel.

But insulin is not known to stimulate the breakdown of phosphatidylinositol, a well-documented function of phospholipase C. Thus, Saltiel and his colleagues are also left with the tasks of characterizing their glycolipid-specific phospholipase C and to identify more precisely the structure and function of the membrane glycolipid it cleaves. ■

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ADDITIONAL READING

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