A New View of Receptor Action

Research on the calcium ion–linked receptors features the identification of a new second messenger and a possible connection to oncogene action

Within the past year or two, cell biologists have been taking a new look at a quantitatively minor group of membrane phospholipids, namely, the polyphosphoinositides. It now appears that these compounds play a central role in signal transmission for a wide variety of neurotransmitters, hormones, and growth factors.

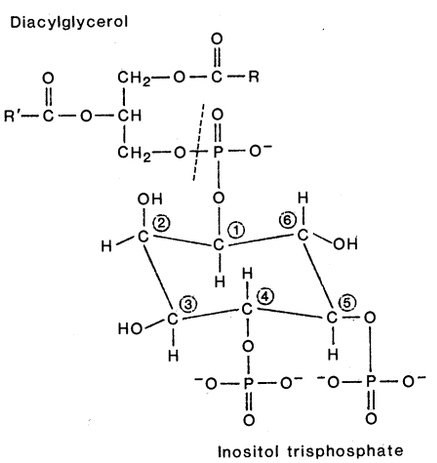
The polyphosphoinositide system is unusual because its activation by one or another of these agents releases two products, both of which act as second messengers that evoke the cell's responses. One of these products, inositol trisphosphate, has only recently been recognized as a second messenger. "It is one of the hottest things that has come up in hormone function," says John Williamson of the University of Pennsylvania School of Medicine. "It is equivalent to the discovery of cyclic AMP as a second messenger."

Moreover, the system may be the site of action of the drug lithium, which is often used to treat simple manias and also the bipolar form of the disease in which mania alternates with depression. Finally, because both normally occurring growth factors and tumor promoters (chemicals that are not carcinogenic by themselves but which foster the cancer-causing effects of true carcinogens) act through the system, it is a potential target of oncogenes in eliciting the malignant transformation of cells. In fact, two groups of investigators now have evidence that the products of the *ros* and *src* oncogenes may participate in polyphosphoinositide synthesis (see box on p. 272.) This suggests that the oncogenes may contribute to uncontrolled cell division by increasing the availability of the polyphosphoinositides.

The work on the polyphosphoinositides has already introduced a fundamental new view of the function of a widely distributed class of receptors, the ones in which activation causes an increase in calcium ion concentrations within responsive cells. These receptors include the muscarinic receptor for the neurotransmitter acetylcholine and the α_1-adrenergic receptor for norepinephrine, which are found on nerve, muscle, and many other types of cells, the thrombin receptor on platelets, and one type of receptor for the hormone vasopressin.

For many years, the increase in intracellular calcium ions was thought to be a direct result of the binding by the receptors of their specific activating agents. The calcium ions were supposed to serve as the second messenger that transmitted the hormonal signal from the outer membrane to the interior of the cell, evoking such responses as muscle contraction and the stimulation of specific enzymes. This would make calcium ions the functional equivalent of cyclic AMP, which is synthesized in response to activation of another class of receptors, of which the β-adrenergic receptor for norepinephrine is a classic example.

According to the new view, calcium ions have been replaced as second messenger by inositol trisphosphate and diacylglycerol, the two products released from a polyphosphoinositide as a consequence of receptor activation. The inositol trisphosphate itself causes the increase in calcium ions, which can still modulate further reactions in the cell, but are effectively demoted to "third messenger." The diacylglycerol acts independently, bringing about its effects by stimulating a kinase, an enzyme that attaches phosphate groups to proteins.



Structure of phosphatidylinositol 4,5-bisphosphate (PIP_2). Activation of the calcium ion–linked receptors stimulates hydrolysis of PIP_2 by a phospholipase C, which splits the compound at the position indicated by the dotted line to form diacylglycerol and inositol trisphosphate, both of which act as second messengers. Phosphatidylinositol (PI) has hydroxyl groups, not phosphates, on positions 4 and 5, and phosphatidylinositol 4-phosphate (PIP) has a phosphate on position 4 and a hydroxyl on position 5.

A consensus about this new scheme, especially regarding the role of inositol trisphosphate, has emerged only within about the past 6 months, but the inositol-containing phospholipids were implicated in receptor function some 30 years ago. In the 1950's, Mabel Hokin and Lowell Hokin, first at McGill University in Montreal and then at the University of Wisconsin–Madison, found that acetylcholine increases the turnover of phosphatidylinositol. The compound is broken down to diacylglycerol and inositol 1-phosphate and then is resynthesized much more quickly than in cells not exposed to acetylcholine. The Hokins proposed that these phospholipid changes might be intimately involved in the actions of the neurotransmitter, although the exact role of the changes was not clear.

Most investigators credit Robert Michell of the University of Birmingham, England, with initiating what might be called the modern era of research on the inositol-containing phospholipids. In a 1975 publication, based on both his own work and that of others, he proposed that increased phosphatidylinositol turnover generally accompanies the activation of calcium-linked receptors, and is the cause, not the effect, of the increased intracellular calcium ion concentrations.

Shortly after that came the first indications that the polyphosphoinositides, which differ from phosphatidylinositol by having either one or two additional phosphate groups on the inositol ring, might be more important in mediating the receptor effects. (Phosphatidylinositol 4-phosphate, the compound with one additional phosphate, is abbreviated as PIP and phosphatidylinositol 4,5-bisphosphate is PIP_2.) Ata Abdel-Latif and his colleagues at the Medical College of Georgia showed that acetylcholine stimulation of iris smooth muscle enhances the breakdown of the polyphosphoinositides. The investigators also showed that the breakdown releases inositol trisphosphate, an indication that the enzyme phospholipase C catalyzes the hydrolysis.

However, calcium ions appeared to be needed for this effect, which implies that polyphosphoinositide breakdown could not be bringing about the increases in intracellular calcium ions in response to receptor activation. If the phospholipid breakdown behaved as Michell suggested, then it should precede the accumulation of calcium ions and be independent of it. Of course, even if polyphosphoinositide hydrolysis was dependent on calcium mobilization, it could still play some other role in receptor activity.

That left two major issues to be resolved concerning the role of the inositol-containing phospholipids in receptor activation and signal transmission. Investigators had to determine whether phosphatidylinositol or polyphosphoinositide breakdown was primary. And they had to determine whether it depended on calcium mobilization.

Polyphosphoinositide breakdown does appear to be a general consequence of

New Oncogene Targets?

Two groups of investigators* have recently suggested a novel way in which oncogenes might contribute to the malignant transformation of cells. The products of the genes in question, *ros* and *src*, are both tyrosine kinases, enzymes that can attach phosphate groups to tyrosine residues in proteins. The new work shows that the enzymes can also phosphorylate phosphatidylinositol, thus increasing the formation of a polyphosphoinositide that mediates signal transmission for several hormones, neurotransmitters, and—most pertinent in this regard—growth factors (see story on p. 271).

The results of the two groups are similar. Ian Macara, Guido Marinetti, and Piero Balduzzi of the University of Rochester School of Medicine and Dentistry have found that the *ros* kinase phosphorylates phosphatidylinositol in the test tube to produce phosphatidylinositol 4-phosphate (PIP), the immediate precursor of phosphatidylinositol 4,5-bisphosphate (PIP_2). The purified *src* kinase phosphorylates both phosphatidylinositol and PIP, according to Lew Cantley, Raymond Erikson, and their colleagues at Harvard University.

When the growth factor and other receptors are activated, PIP is hydrolyzed, releasing diacylglycerol and inositol trisphosphate, which serve as second messengers to evoke the cell's responses. By increasing the concentration of PIP_2 the *ros* and *src* products may mimic what happens during activation of the receptors. According to the Harvard group, the *src* product also forms phosphatidic acid by attaching phosphate to diacylglycerol, a finding that suggests that the kinase may be involved in removing, as well as producing, this second messenger.

Although the Rochester workers did not detect PIP_2 formation in their test tube assay, they found that transformation of cells with UR2, the virus carrying the *ros* gene, results in increased incorporation of radioactively labeled phosphate into PIP and PIP_2. This indicates that both compounds are being broken down and resynthesized more rapidly than in normal cells. The concentrations of their hydrolysis products (inositol bis- and trisphosphates) also were increased in the transformed cells.

Using a temperature-sensitive mutant of Rous sarcoma virus, which is the virus that contains the *src* gene, the Harvard group showed that increased turnover of PIP, PIP_2, and phosphatidic acid correlates with transformation. Cells infected by the mutant are transformed at 35°C, but not at 41°C. Within 20 minutes after the induction of transformation by lowering the temperature from 41° to 35°C, the incorporation of radioactive phosphate into the three compounds increases. Uninfected cells did not show these changes. "The result is very suggestive that the *src* kinase does this directly, or stimulates the activity of other enzymes that do," Cantley explains. "The simplest explanation, because of the in vitro results, is that it does it directly."

The phosphorylation of phosphatidylinositol appears to be a specific effect of tyrosine kinases. For example, both groups find that another kinase, which phosphorylates proteins on serine and threonine residues, does not catalyze the reaction.

Moreover, both groups of investigators find that PIP_2 inhibits the ability of the oncogene products to phosphorylate phosphatidylinositol and also blocks their tyrosine kinase activity. The finding is consistent with the idea that PIP_2 may help to regulate its own formation, a common occurrence in synthetic pathways.

Even though the tyrosine kinase activity of the *src* gene product was discovered more than 5 years ago by Erikson and his colleagues, when they were at the University of Colorado Medical Center in Denver, investigators have had little luck in determining which of the many proteins phosphorylated by the enzyme might produce the characteristic changes of transformation. The current work suggests that the *ros* and *src* kinases may instead function by phosphorylating phosphatidylinositols, thus increasing the availability of PIP_2 and the second messengers released from it, especially diacylglycerol. This compound activates protein kinase C, an effect mimicked by the tumor-promoting phorbol esters, which cause, among other things, a stimulation of cell division. The *src* and *ros* products are located at the inner cell membrane, the correct site for their proposed effects.

Two oncogenes have already been linked to growth factors. Part of the *sis* gene codes for a protein in platelet-derived growth factor and the *erbB* gene codes for a segment of the receptor for epidermal growth factor. If *ros* and *src* transform by virtue of their actions on the polyphosphoinositide system, then the effect could be a third example of an intersection with growth factor activity. Much more work will be needed to determine whether this is the case.

It will also be interesting to determine whether any of the other tyrosine kinases, which include the products of several additional oncogenes, the receptor for epidermal growth factor, and possibly the receptors for platelet-derived growth factor and for insulin, can phosphorylate phosphatidylinositols. Meanwhile, as Macara notes, "At this point the evidence that the phosphatidylinositol kinase is involved is probably no worse than the evidence for the involvement of tyrosine kinase."—**J.L.M.**

*Y. Sugimoto, M. Whitman, L. C. Cantley, R. L. Erikson, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2117 (1984); I. G. Macara, G. V. Marinetti, P. C. Balduzzi, *ibid.*, in press.

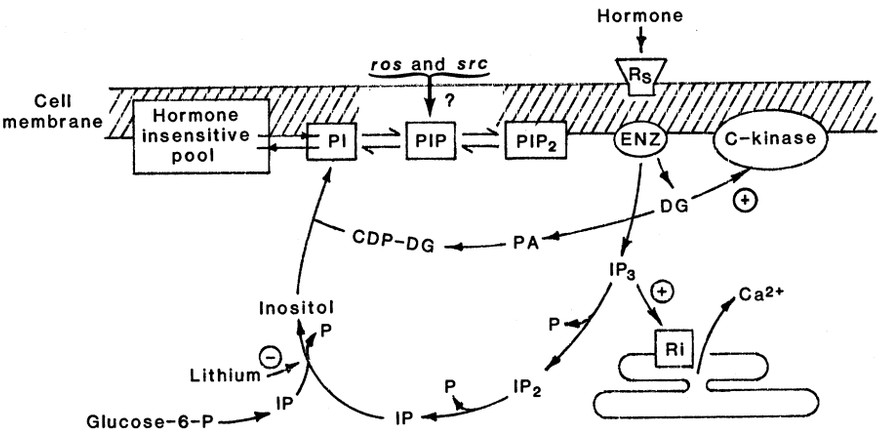
stimulation of calcium-linked receptors. Especially within the past year or so, numerous investigators have shown that the breakdown increases in many different types of cells in response to a wide variety of agents.

Evidence that the change may be physiologically important comes from studies of the speed of the reaction. "You have to look at the early time periods to see if the changes are fast enough to account for calcium mobilization," notes Michael Berridge of the University of Cambridge, England. For example, Michell and his colleagues found that polyphosphoinositide breakdown is faster than that of phosphatidylinositol when liver cells are treated with vasopressin, a finding that suggests that the phosphatidylinositol breakdown may be a secondary event.

In addition, Berridge finds that when the insect salivary gland is stimulated with the neurotransmitter 5-hydroxytryptamine, the earliest measurable change is an increase in the concentrations of inositol bis- and trisphosphates, which would be released by polyphosphoinositide hydrolysis. Both of these effects are calcium independent, although some investigators have found that in different systems the stimulation of phospholipid breakdown requires calcium ions.

Some of the most convincing evidence for the proposed role of the polyphosphoinositides comes from demonstrations that inositol trisphosphate can mobilize calcium ions. When a cell is stimulated by the binding of a hormone or neurotransmitter to the appropriate receptor, the increase in calcium ion concentrations is initially caused by the release of the ions from internal stores, and later by transport from outside the cell. The discovery that the calcium ions are released first from internal stores suggested that there had to be a way of transmitting the signal from the outer cell membrane to the interior structures.

Last fall, Hanspeter Streb and Irene Schulz of the Max-Planck-Institute for Biophysics in Frankfurt, Germany, in collaboration with Berridge and Robin Irvine, also at Cambridge, produced direct evidence that inositol trisphosphate could serve as that signal. They showed that the compound releases calcium ions from intracellular stores in pancreatic cells that had been treated to make them permeable to the highly charged molecule. Neither inositol 1-phosphate, which is released from phosphatidylinositol, nor inositol bisphosphate, which is released from PIP, produces the effects. This indicates that the PIP_2 hydrolysis is important for calcium ion release and that the changes in the concentrations of the other molecules are secondary. The trisphosphate releases the calcium ions from the same stores as acetylcholine, Berridge says, and the mitochondria are apparently not the source.



Proposed model for the role of the polyphosphoinositides in receptor activation. Binding of a hormone, neurotransmitter, or growth factor to its receptor activates the enzyme (ENZ) that splits the polyphosphoinositide (PIP_2) to inositol trisphosphate (IP_3), which releases calcium ions from internal stores, and diacylglycerol (DG), which activates protein kinase C. Resynthesis of PIP_2 involves the conversion of IP_3 to inositol by stepwise removal of the three phosphate groups to form first inositol bisphosphate (IP_2), then inositol phosphate (IP), and finally inositol (I). The last step is inhibited by the drug lithium. The diglyceride is converted to phosphatidic acid by the addition of a phosphate group and then to the cytosine nucleotide derivative (CDP-DG). Inositol and CDP-DG combine to produce phosphatidylinositol (PI), which is phosphorylated to PIP. Phosphorylation of PIP regenerates PIP_2.

Similar results with liver cells have been obtained by James Putney and his colleagues at the Medical College of Virginia, in collaboration with Berridge, and by Williamson's group. The endoplasmic reticulum appears to be the source of the ions.

Moreover, the confusion regarding the calcium ion requirements of PIP_2 breakdown is clearing. John Exton of Vanderbilt University, who originally doubted that the compound could be providing a second messenger for the calcium ion increase, now says, "I was skeptical of this hypothesis initially, but our data generally agree with what everyone else is finding." Phospholipase C does require calcium ions for its activity, he notes, but apparently the levels in the unstimulated cell are already adequate.

In addition, Abdel-Latif has taken another look at the apparent calcium dependency of the acetylcholine stimulation of PIP_2 breakdown in iris smooth muscle. The original experiments showed that the effect could be duplicated by a calcium ionophore that transports calcium ions across cell membranes. This result suggested that the calcium ions are stimulating hydrolysis of the phospholipid.

In more recent work, Abdel-Latif finds that the effects of the ionophore are prevented by agents that prevent binding of acetylcholine and norepinephrine to their receptors. "This means that the ionophore was releasing the neurotransmitters from cells and these in turn caused the breakdown of the polyphosphoinositides," he explains.

The polyphosphoinositide system may be the site of action of the drug lithium. Several years ago, the late James H. Allison of Washington University School of Medicine observed that lithium causes a large decrease in inositol concentrations in nerve cells. Allison and William Sherman, also of Washington University School of Medicine, then showed that there is a concomitant increase in the concentration of inositol 1-phosphate. These effects have been found by Sherman and his colleagues to be caused by lithium inhibition of the enzyme that catalyzes the removal of the phosphate from inositol 1-phosphate.

In brain virtually all of the inositol required for resynthesis of the polyphosphoinositides is produced either by splitting the three phosphates from inositol trisphosphate or by synthesis from glucose. Both paths require removal of phosphate from inositol 1-phosphate and are blocked by lithium. "It amounts to a back-up of water behind a dam so that downstream inositol is not available for resynthesizing the phospholipids," Sherman says. As a result, the responses of cells to appropriate neurotransmitters will eventually be diminished.

There are indications, Berridge says, that the cells most sensitive to lithium's effects are those that are being most actively, even excessively, stimulated, as may happen in a condition such as

mania. "I don't think that you could design a better drug," he points out. "It has little effect in the normal operational range, but becomes increasingly effective when the receptors are hyperactive."

The other product released by hydrolysis of PIP_2 is diacylglycerol. A few years ago, Yasutomi Nishizuka and his colleagues at Kobe University School of Medicine in Japan showed that diacylglycerol activates a protein kinase, which has been given the designation protein kinase C because it requires calcium ions for its activity. Phosphorylation by kinases is a common method for controlling the activities of enzymes. Cyclic AMP produces its effects as second messenger by activating protein kinase A, which can in turn influence enzyme activities by phosphorylating the proteins. Kinase C presumably plays an analogous role, although less is known about its important physiological targets and actions.

One of the more intriguing results with protein kinase C is its implication in the control of cell division and differentiation. About a year and a half ago, investigators learned that the tumor-promoting phorbol esters can activate the enzyme (*Science*, 15 April 1983, p. 291). Apparently, the tumor promoters, which are hydrophobic, can penetrate the cell membrane and effectively substitute for diacylglycerol in activating the kinase.

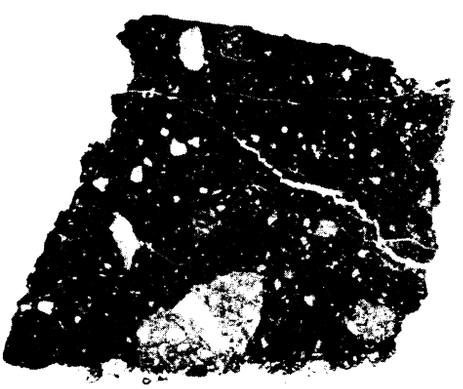
In addition, investigators from several laboratories have shown that growth factors, including epidermal growth and platelet-derived growth factors, stimulate the turnover of inositol phospholipids and the breakdown of PIP_2. "It seems that there is, irrespective of the stimulus you look at, a correlation between cell division and inositol lipid turnover," Michell points out. The possibility that some oncogene products might act by increasing the availability of the polyphosphoinositides has not exactly diminished interest in the research.

Even before the discovery of the suggested oncogene connection, polyphosphoinositide research was generating a great deal of interest and attracting new investigators to the fold because of what it is revealing about hormone and neurotransmitter action.—**JEAN L. MARX**

Second Lunar Meteorite Identified

Japanese researchers have announced that a search of their collection of meteorites found in Antarctica has uncovered a rock that must have been blasted off the face of the moon. It is only the second such lunar meteorite ever found and offers the possibility that a second new site on the moon never visited by man or machine has been sampled.

The analyses in hand, although few in number, seem convincing. Keizo Yanai and Hideyasu Kojima of the National Institute of Polar Research in Tokyo presented their mineralogical and chemical evidence for another lunar meteorite last month at the Ninth Symposium on Antarctic



Meteorites, held in Tokyo 22 to 24 March. When the 25-gram, dusty-gray meteorite designated Yamato 791197 was cut into thin, translucent sections, small fragments or clasts of lighter minerals stood out against a dark brown background. The larger clasts contained mostly the mineral plagioclase and minor amounts of pyroxene and olivine. The smaller clasts were individual mineral fragments. Most of the clasts showed signs of having been shocked as by an impact. The Japanese researchers also found a few small glass spherules.

This appearance alone was enough to convince two Americans who were given an opportunity to inspect a thin section of the meteorite. Jeffrey Taylor and Klaus Keil of the University of New Mexico, who have worked extensively with both meteorites and Apollo moon rocks, could see immediately that this meteorite closely resembled rocks from the lunar highlands called regolith breccias, rocks formed from lunar soil and rock fragments under the pressure of a meteorite impact.

The evidence goes beyond the meteorite's appearance. Yanai and Hideyasu found that the ratio of manganese to iron in pyroxene and olivine minerals of their Yamato meteorite is about half that of the most similar type of meteorite but about the same as that of lunar rocks. In addition, Robert Clayton of the University of Chicago has determined the oxygen isotope composition of a sample supplied by the Japanese researchers. The meteorite value is "bang on the lunar value. There's not much data in yet, but it's all consistent. I'm convinced," says Clayton. He and others have not waited for the extensive analyses accorded the American find of last year (*Science*, 15 April 1983, p. 288) because these results by themselves form a strong argument for a lunar origin. Only one class of meteorites has an oxygen isotope composition that is close to the lunar composition, but the mineralogy of these meteorites is entirely different from that of lunar rocks and the Yamato meteorite.

Perhaps the most exciting possibility is that the new discovery is the product of a second impact at a different site on the moon from the first. Lunar specialists were pleased to find chemical indications that the lunar meteorite in the U.S. Antarctic collection is not from the vicinity of the sampling by Apollo astronauts or Soviet landers. A second impact site, possibly from near the visible edge or even the far side of the moon, would be a real find.

Researchers are anxious that any new lunar meteorites be opened to the kind of consortium study that quickly produced such a variety of analyses of the first specimen. Since the ultimate number of lunar meteorites may not exceed two or three in the present combined Antarctic collections of more than 6000, the need for close cooperation is obvious.—**RICHARD A. KERR**