A special section of 17 papers in the latest issue of *Paleoceanography*, entitled "Milankovitch Cycles Through Geologic Time," includes discussion of all sorts of sedimentary cycles. Many of the papers fail to mention Milankovitch cycles or consider them only in passing. Such hesitation is understandable. Accurate dating of sediments much older than 100 million years is still a horrendous problem, and the very nature of some cycles, whether they are a true record of conditions when the sediment formed or are later alterations, remains controversial.

Lawrence Hardie of Johns Hopkins University, Alfonso Bosellini of the University of Ferrara, and their colleagues do report in the special section that cyclic bedding in northern Italy is created by repeated flooding and exposure of shallow-water sediments and has the same pattern as created by small sea level changes of the past million years. Five cycles of sea level rise and fall, each on the order of tens of thousands of years long, are superimposed on longer sea level cycles on the order of 100,000 years long. The precession and eccentricity cycles would seem to be the only likely means of generating such a pattern, Hardie says. John Grotzinger of Lamont reports in the same issue that similar cycles appear in sediments of northwest Canada that are up to 2.2 billion years old.

Although the steady operation of orbital variations over hundreds of millions of years is not too surprising, the reliable response of Earth's climate system is. Most theories link orbital variations and climate through the behavior of polar ice caps. But according to widely held views of past climate, there were no large ice caps between 40 and 240 million years ago. Alternative linkages might include an influence on the strength of monsoons and their rainfall, as recently demonstrated in the case of the Indian monsoon of the past few hundred thousand years.

Aside from any insights into the workings of climate, the record of Milankovitch cycles in ancient sediments may allow dating of metronomic precision. Dating errors in such old sediments now amount to millions of years. Although exact ages could not be derived from Milankovitch cycles, as has been done for the past million years, the durations of geologic zones that are now often guessed at could be precisely determined. ■ **RICHARD A. KERR**

ADDITIONAL READING

P. E. Olsen, "A 40-million-year lake record of early Mesozoic orbital climatic forcing," *Science* **234**, 842 (1986).

M.A. Arthur and R.E. Garrison, Eds., special section on "Milankovitch cycles through geologic time," *Paleoceanography* **1**, 369–586 (1986).

Polyphosphoinositide Research Updated

Recent research clarifies the biochemistry of the polyphosphoinositide receptor system and gives new insights into its actions in cells

THE polyphosphoinositide lipids have moved rapidly in the past few years from relative obscurity to the center of the research stage. This increased interest is the result of research showing that the lipids are intermediaries that transmit the signals of a variety of hormones, neurotransmitters, and growth factors from the cell surface to the cell interior. With this key role now well established, researchers are turning more attention toward understanding the biochemistry of the polyphosphoinositide (PI) system and the physiological events caused by its activation.

The PI system has been linked to some half-dozen oncogenes, a development that has also helped to foster interest in the system. Oncogenes cause the cancerous transformation of cells.

During the past year or so researchers have obtained evidence indicating that the protein encoded by the cellular counterpart of the *ras* oncogene may be an integral component of the PI system. The first step in the activity of any of the numerous agents that work through the PI system is the binding of the agent to specific receptors located on the membranes of responding cells. As a result, the enzyme phospholipase C becomes activated and splits the membrane lipid polyphosphatidyl-4,5-bisphosphate (PIP_2) to release inositol trisphosphate and diacylglycerol, both of which act as "second messengers" for converting the signal at the receptor to an internal cellular response.

Indications are, however, that the phospholipase C is not directly connected to the various receptors, but interacts with them through a third membrane protein, one of the G proteins, which are so called because they bind the high energy compound guanosine triphosphate (GTP). The organization of PI-linked receptor complexes therefore parallels that of the well-studied β-adrenergic receptor complex. This receptor, which binds the neurotransmitter norepinephrine, is linked to the enzyme adenylate cyclase by means of a G protein. Adenylate cyclase converts adenosine triphosphate to the second messenger cyclic AMP.

The *ras* protein has certain properties in common with known G proteins, including the ability to bind GTP. A few years ago, there were indications that the *ras* protein might be a G protein that activates adenylate cyclase. Further investigation failed to support this hypothesis and the evidence now points to the possibility that the *ras* gene instead encodes a G protein for the PI system.

For example, Laurie Fleischman, Suresh Chahwala, and Lewis Cantley of Tufts University School of Medicine have found that introduction of either normal or oncogenic forms of the *ras* gene into cultured mouse cells results in increased conversion of PIP_2 to inositol phosphate and diacylglycerol. The simplest explanation of this result is that the *ras* protein stimulates phospholipase C activity.

Gene transfer experiments by Michael Wakelam of the University of Glasgow and his colleagues provide additional evidence for the possibility that *ras* gene encodes a G protein for the PI system. These investigators have found that cells in which the synthesis of the normal *ras* protein is increased show much higher production of inositol phosphates in response to growth factors that act through the PI system than do control cells. According to Wakelam, the results indicate that the *ras* protein is acting like a G protein in that it increases the coupling between the various growth factor receptors and phospholipase C.

Introduction of one of the oncogenic mutants of the *ras* gene into mouse cells results in greatly increased production of inositol phosphate even in the absence of added growth factor, the Glasgow workers find. The mutant *ras* product apparently acts on its own to stimulate phospholipase C, thereby causing the cells containing the gene to behave as if they are undergoing growth factor stimulation when they are not.

Other oncogenes have also been linked to the PI system. The connections of two of these, the *src* and *ros* genes, have become somewhat weaker during the past year. These oncogenes encode tyrosine kinases, enzymes that add phosphate groups to pro-

teins on the amino acid tyrosine. Early evidence indicated that the *src* and *ros* tyrosine kinases might also phosphorylate phosphoinositide lipids, thereby fostering the operation of the system by increasing PIP_2 synthesis. More recent findings by Cantley's group, among others, indicate that the enzymes do not do this, although they may increase PIP_2 formation indirectly by phosphorylating and consequently stimulating another enzyme in the synthetic pathway.

Oncogene connections that remain firm include that of the *sis* gene, which is derived from the gene for platelet-derived growth factor, one of the many agents that acts through a PI-linked receptor. In addition, the activation of the PI system results in increased expression of the cellular counterparts of the *fos* and *myc* oncogenes. The products of these genes may regulate the expression of other genes, thereby providing a means of achieving long-lasting alterations in cells responding to growth factors and other agents.

In neurons, changes in gene expression may be needed for memory formation. For example, Michael Greenberg of Harvard Medical School, and Edward Ziff and Lloyd Greene of New York University Medical Center in New York City find that agents that mimic the activity of the neurotransmitter acetylcholine can stimulate the expression of the cellular *fos* gene and also of one or more actin genes.

The findings raise the possibility that growth factors and neurotransmitters use similar methods to effect long-term cellular responses. "The evidence suggests that *fos* gene activation may be a central mechanism by which cells sense changes in the external environment," Greenberg says. Greenberg and his colleagues have not demonstrated that the acetylcholine analogs work through any component of the PI system, but he says that is one possibility.

In any event, the characteristics of the PI system are consistent with its involvement in memory and learning. The components of the system are found in high concentrations in many brain areas. "A large number of neurotransmitters use this receptor mechanism," says Michael Berridge of Cambridge University, "and they are widely distributed in brain."

Among the PI-linked neurotransmitters are norepinephrine, when it acts through the α-adrenergic receptor, and acetylcholine, when it works through the muscarinic receptor. According to Fulton Crews and Rueben Gonzales of the University of Florida College of Medicine in Gainesville, these receptors account for the largest releases of inositol phosphates in the hippocampus and cerebral cortex, two brain regions that are likely to be very important in memory formation—and in abnormal memory loss. Deterioration of the acetylcholine-producing neurons in the cortex and hippocampus is one of the hallmarks of Alzheimer's disease.

In many cases, degeneration of incoming nerves results in an increased sensitivity of the responding cells to the neurotransmitter released by the neurons. This helps to compensate for the lost nerve signals and maintain the function of the responding cells. However, research by Crews, Gonzales, and their colleagues suggests that the PI-linked α-adrenergic and muscarinic receptors in the brain may be less capable of compensating for the loss of innervating neurons by developing this kind of supersensitivity. This may help to explain why attempts to treat Alzheimer's patients by increasing acetylcholine production in the brain have not produced significant improvements in their condition.

"A large number of neurotransmitters use this receptor mechanism, and they are widely distributed in brain."

Another characteristic of the PI system that is consistent with a role in memory formation is its ability to produce effects that persist even after the initiating stimulus is no longer present. "The phosphatidylinositol system changes neuronal responsiveness over a period of minutes to hours," Crews notes. This time frame is reminiscent of that for the acquisition of short-term memory, which appears to be an intermediate stage in the development of long-term memory that has the capacity of persisting for years.

One of the major thrusts of current research on the PI system concerns how its activation produces specific neuronal responses, including those that might contribute to memory formation. Several groups of investigators have shown that PI-linked neurotransmitters influence nerve cell activity by opening or closing ion channels. Somewhat surprisingly, the most recent evidence indicates that the inositol trisphosphate branch of the system may be at least as important in this regard as the diacylglycerol branch.

The inositol trisphosphate that is split from PIP_2 by phospholipase C acts to increase the internal calcium ion concentration of cells by releasing the ions from internal storage sites. The calcium ions can then effect cellular responses in a variety of ways.

In particular, calcium ions act in conjunction with diacylglycerol to stimulate protein kinase C, an enzyme that attaches phosphate groups to proteins. Kinase enzymes are known to be important regulators of the activities of enzymes and other proteins. Although the physiological targets of protein kinase have not been definitively identified, possible candidates include the proteins forming the membrane channels that allow ions to move in or out of cells. Such ion movements alter the electrical properties of cells, thereby mediating cellular responses to stimulation.

Calcium ions can also contribute indirectly to protein kinase C activation by making more diacylglycerol available. According to Philip Majerus and his colleagues at Washington University School of Medicine, the ions alter the specificity of phospholipase C so that it can split phosphatidylinositol, as well as PIP_2. Phosphatidylinositol yields diacylglycerol and inositol monophosphate, but not the trisphosphate.

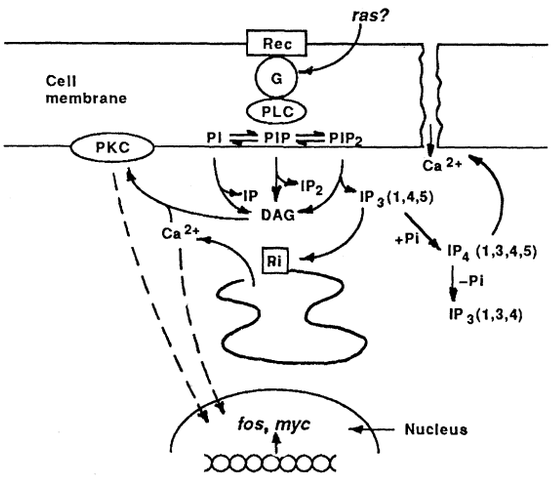
Protein kinase C has therefore received the lion's share of attention as an effector of the cellular responses in the PI system, with inositol trisphosphate considered primarily as a provider of the calcium ions or diacylglycerol needed for activation of the enzyme. Researchers have, as expected, linked protein kinase C to the opening or closing of ion channels, but at least three groups have now found that inositol trisphosphate is also important in this regard.

Moreover, in a particularly surprising result, two groups have produced evidence indicating that inositol trisphosphate mediates neurotransmitter or hormone secretion by at least some responding cells. One group includes Jeffery Barker of the National Institute of Neurological and Communicative Diseases and Stroke (NINCDS), Bernard Dufy of the University of Bordeaux, France, and their colleagues, and the other includes Haruhiro Higashida of the National Heart, Lung, and Blood Institute, David Brown of the University of London School of Pharmacy, and their collegues.

Although the two groups are using different cells for their investigations, they have obtained similar results. The Barker group has been studying the stimulation of cultured pituitary cells by thyrotropin-releasing hormone (TRH), which causes the cells to secrete the hormone prolactin. Higashida and Brown use a line of hybrid neuroblastoma-glioma cells that have neuronal properties. The hybrid cells respond to the pain-producing peptide bradykinin by secreting the neurotransmitter acetylcholine.

Both cell types respond to appropriate stimulation with a brief initial period of hyperpolarization; that is, the cell interiors become more negatively charged than in the normal resting state and therefore less prone

A simplified view of the PI system



An agent that works through the PI system first binds to a specific receptor (Rec) on the cell surface. This signal is transmitted through a G protein (G), which may be the cellular ras *gene product, to the enzyme phospholipase C (PLC). This enzyme splits PIP_2 to diacylglycerol (DAG) and inositol trisphosphate [$IP_3(1,4,5)$], and may also split the other phosphatidylinositide lipids (PI and PIP). The DAG and the calcium ions that are released by inositol trisphosphate from the cell's internal stores activate the enzyme protein kinase C. The inositol trisphosphate is also converted to inositol-1,3,4,5-tetrakisphosphate (IP_4), which apparently stimulates calcium ion entry from the cell exterior. The IP_4 is broken down to the 1,3,4-isomer of inositol trisphosphate. The effects of protein kinase C activity and elevated calcium ion concentrations include the activation of the* fos *and* myc *oncogenes.*

to respond positively to stimulation. The hyperpolarization is caused by the inositol trisphosphate-mediated increase in calcium ions and the consequent opening of an ion channel that allows positively charged potassium ions to flow out of the cells.

The brief hyperpolarization is then followed by a much longer period of action potentials, electrical impulses indicating that the cells are firing. Both groups of investigators find that the action potentials are the result of protein kinase C activity influencing the operation of ion channels.

The surprise came when both groups obtained evidence suggesting that the cells they are studying begin to secrete prolactin or acetylcholine during the period of hyperpolarization. Barker, Dufy, and Thomas Smith of NINCDS did this indirectly by measuring the capacitance of the pituitary cells, which is an indication of membrane area. They found that the capacitance began to increase early in the period of hyperpolarization. The most likely cause of an increase in membrane area is fusion of the prolactin-containing secretory vesicles with the cell membrane.

Higashida and Brown had a more direct measure of acetylcholine secretion by the neuroblastoma-glioma cells. They cultured the cells with embryonic muscle cells that respond to acetylcholine stimulation with characteristic electrical changes. The investigators found that the changes again began during the period of hyperpolarization. "The results support the idea that inositol trisphosphate is responsible for transmitter release," Higashida says.

Another indication of the importance of inositol trisphosphate in mediating neuronal responses comes from Solomon Snyder's laboratory at The Johns Hopkins University Medical Institutions. The Snyder group has demonstrated that brain neurons contain specific, high-affinity binding sites for the agent. The concentrations of the receptors are 100 to 300 times higher in brain than in peripheral cells. "In most places in the brain," Snyder says, "the receptors are distributed identically with protein kinase C." He notes, however, that a few regions that contain the enzyme do not contain the receptors.

The bag cell of the marine snail *Aplysia*, an organism that is frequently used as model for studying learning and behavior, is a third cell type in which both inositol trisphosphate and protein kinase C have been linked to specific cellular responses, in this case by Leonard Kaczmarek and his colleagues at Yale University School of Medicine. When bag cells are stimulated, their responses resemble those of the pituitary and neuroblastoma-glioma cells described previously.

The bag cells first undergo a transient period of hyperpolarization, which the Kaczmarek group has linked to inositol trisphosphate. They then fire repetitively for some 30 minutes during which they release a series of peptide hormones that bring about egg-laying behavior in *Aplysia*. According to Kaczmarek, secretion of the bag cell hormones appears to occur during the period of neuronal firing, although the possibility that it begins at an undetectable level during the brief hyperpolarization cannot be ruled out at present.

Kaczmarek says that the prolonged firing can be attributed to the effects of protein kinase C, which acts to open a special calcium channel that is about twice as large as the ordinary calcium channel of bag cells. "The neurons have covert calcium channels that are never used until you activate protein kinase C," he says. The channels may be a special adaptation of the bag cell, which has an unusual activity pattern. The cells are quiescent most of the time. They become active only once a day, or once every other day, but then fire for 30 minutes.

The origin of the large calcium channels is unknown. Kaczmarek speculates that they may be located in vesicles located beneath the bag cell membrane that fuse to the membrane under the influence of protein kinase C. Alternatively, the channels may be present in the membrane, but in a closed form that does not admit calcium ions until the channels open as a result of protein kinase C activity.

The biochemistry of the PI system is now known to be more complicated than it appeared at first, although recent results have cleared up a mystery that arose about 18 months ago when Robin Irvine and Peter Downes of Cambridge University in England detected an unexpected isomer of inositol trisphosphate after activation of the PI system. The inositol trisphosphate isomer that is released by the splitting of PIP_2 has the phosphates on carbons 1, 4, and 5 of the inositol ring. The isomer identified by Irvine and Downes has its three phosphates on carbons 1, 3, and 4. This discovery raised two questions: How is the 1,3,4-isomer synthesized and does it have a physiological role in signal transmission?

The first question has been answered. Research by the Irvine group, some of which was performed in collaboration with Berridge, has shown that inositol-1,4,5-trisphosphate is first phosphorylated on carbon 3 to produce inositol-1,3,4,5-tetrakisphosphate. Then the phosphate on carbon 5 of this compound is removed, thus producing inositol-1,3,4-trisphosphate.

Although the function, if any, of the 1,3,4-trisphosphate isomer is still unclear, Irvine and R. Moor, also of Cambridge, have evidence indicating that the 1,3,4,5-tetrakisphosphate is another second messenger in the PI system. It apparently acts to increase cellular calcium ion concentrations, but by admitting the ions from the cell exterior, not by releasing them from internal stores as inositol-1,4,5-trisphosphate does.

Meanwhile, the Majerus group has identified a number of inositol cyclic phosphates that may also be second messengers in the PI system. For example, some of these have calcium ion-releasing effects similar to those of inositol-1,4,5-trisphosphate. "The scope of the biochemistry [of the PI system] is far from worked out," Majerus maintains. Research on the biochemistry and physiology of the PI system seems certain to hold its appeal for some time to come. ■

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