The Polyphosphoinositides Revisited

The membrane phospholipids called the polyphosphoinositides help to mediate many cellular responses, possibly including oncogene effects

The membrane phospholipids called the polyphosphoinositides have within the past 2 years been found to play a leading role in signal transmission for a major group of hormones, neurotransmitters, and growth factors. As a result, interest in the polyphosphoinositide system has surged, as was amply demonstrated at a recent symposium on "New Insights into Cell and Membrane Transport Processes," which was sponsored by Smith Kline & French Laboratories and held in Philadelphia on 11 to 14 March.

Whereas last year's Smith Kline Symposium (on "Mechanisms of Receptor Regulation") featured two presentations on the system, this year's included a full session devoted to it. In addition, the polyphosphoinositides turned up in the talks of several speakers from the other sessions. Not only is the system involved in mediating a wide range of normal cellular responses but, the new work shows, there is growing evidence connecting it with the uncontrolled growth and other aberrant effects of oncogenes—genes that cause the malignant transformation of cells.

The hormones and other agents whose effects are mediated by the polyphosphoinositides are the ones that increase calcium ion concentrations within the cell when they bind to their specific receptors on the outer membrane. In fact, for years this increase was thought to be the first step in transmitting the signals into the cell. However, especially during the past 2 to 3 years, that view has undergone a dramatic change as several lines of evidence have shown that the increased calcium ion concentrations are themselves secondary to the production of inositol 1,4,5-trisphosphate (*Science*, 20 April 1984, p. 271).

When an appropriate agent binds to its receptor, the first event is the splitting of the polyphosphoinositide called PIP_2 (for polyphosphatidylinositol 4,5-bisphosphate), thus forming the trisphosphate and diacylglycerol. Both of these substances are active in signal transmission. The trisphosphate increases calcium ion concentrations, and the ions, working through the protein calmodulin, activate appropriate target enzymes. Meanwhile the diacylglycerol, in conjunction with the calcium ions, turns on the enzyme protein kinase C. Protein kinases regulate the activities of other cellular enzymes by attaching phosphate groups to them. The physiological targets of protein kinase C have not yet been identified.

One of the more intriguing aspects of this system is its involvement in the regulation of cell division. Normal growth factors, including platelet-derived growth factor (PDGF), act through it. In addition, tumor promoters, chemicals which potentiate the effects of true carcinogens although they do not cause cells to become cancerous by themselves, also feed into the polyphosphoinositide system. Promoters directly activate protein kinase C.

The polyphosphoinositide system, because it participates in the regulation of cell division and is complex, provides several potential sites for oncogene action. As Lewis Cantley of Harvard University points out, "If phosphatidylinositol turnover is important in cell proliferation, then the components of the pathway are candidates to be oncogene targets."

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These targets may include the growth factors themselves, their receptors, and the pathway enzymes. For example, the *sis* oncogene is derived from the cellular PDGF gene. The supposition is that malignant transformation by the *sis* oncogene leads to overproduction of a PDGF-like material.

Other oncogene products may act more directly on the phosphatidylinositols. About a year ago, two groups, one including investigators from Cantley's laboratory and that of Raymond Erikson, also at Harvard, and another from Ian Macara's laboratory at the University of Rochester School of Medicine and Dentistry, reported that the products of the *src* and *ros* oncogenes stimulate the attachment of phosphate groups to phosphatidylinositols, thus producing both PIP_2 and PIP (for phosphatidylinositol 4-phosphate), which is the compound from which PIP_2 is synthesized.

This result implies that the uncontrolled division of cells transformed by *src* or *ros* might be caused by the increased availability of the phospholipids. However, the *src* and *ros* products are much better known for their ability to phosphorylate proteins on the amino acid tyrosine. The question then is which effect—tyrosine phosphorylation or phosphatidylinositol phosphorylation—is important for transformation. Conceivable answers include both or neither, although data presented by Cantley at the Smith Kline meeting do suggest a role for phosphatidylinositol phosphorylation.

The original work with *src* was done with the gene from Rous sarcoma virus, which causes cancer in chickens. This gene, like the other oncogenes that have been identified in cancer viruses, is of cellular origin. It was picked up by the virus during the course of infection. More recently, Cantley, David Kaplan of Harvard Medical School, Brian Schaffhausen of Tufts University School of Medicine, and their colleagues have been investigating the activities of the cellular *src* gene, which has a structure slightly different from viral *src* and does not transform cells. They could detect little or no phosphatidylinositol kinase activity associated with the product of the cellular gene, which also has a greatly reduced tyrosine kinase activity compared to the viral *src* product.

The Harvard and Tufts workers went on to show that phosphatidylinositol kinase activity could be found to be associated with the cellular *src* product when the "middle T antigen" gene of polyoma virus is introduced into cells. This gene is the transforming gene of polyoma virus and its product associates with the *src* product in the cell membrane. A mutant middle T antigen gene that produces a product that does not bind to the *src* product neither increased the enzyme activity nor transformed cells.

Studies of polyoma virus mutants generally showed a correlation between transformation and increased phosphatidylinositol kinase activity in immune precipitates of the cellular *src* product with the middle T antigen, according to

Cantley. The problem with this, he points out, is that the middle T antigen can also increase tyrosine phosphorylation by the cellular *src* gene product. Studies by other investigators have shown a correlation between tyrosine kinase activity and transformation. However, the Harvard and Tufts workers identified one polyoma mutant in which the middle T antigen retained its capacity to activate the tyrosine kinase but had a greatly reduced ability to increase the phosphatidylinositol kinase. This mutant transformed cells very poorly. The concurrent reductions in transforming and phosphatidylinositol kinase capabilities, even while tyrosine kinase activity remains high, favors the hypothesis that it is the phosphatidylinositol effect that is important, but more work will be needed to establish this.

Still another oncogene product may interact with the phosphoinositide system, according to data presented by Cantley at the Philadelphia meeting. In cells that have been transformed by the *ras* gene, PIP_2 concentrations are reduced while those of diacylglycerol are increased. This finding suggests that the *ras* product might stimulate the enzyme that catalyzes the release of trisphosphate and diacylglycerol from PIP_2, although it is not yet known whether this is a direct or indirect effect.

Recent work has also shown that the *ras* gene product increases in yeast the synthesis of cyclic AMP (cyclic adenosine monophosphate), another important mediator of hormonal and neurotransmitter effects (*Science*, 2 November 1984, p. 527). This observation is not necessarily incompatible with that of the Cantley group. The polyphosphoinositide and cyclic AMP–dependent systems interact in ways that are complex and not completely understood, and an action in one might be transmitted to the other.

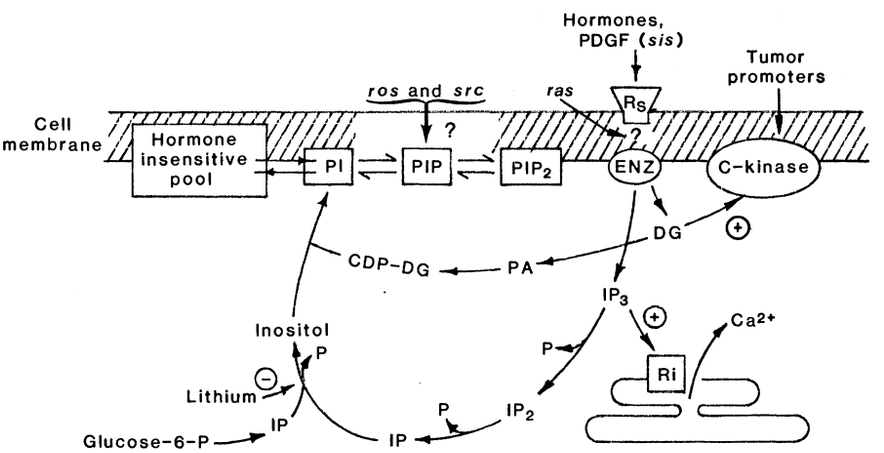
Ultimately a signal to divide, which is received at the cellular membrane, must be transmitted to the nucleus to be carried out. There are strong indications that cellular oncogene counterparts may be on the receiving end of these signals as well as helping to initiate them. Investigators have shown that one of the consequences of exposing cells to growth factors such as PDGF and to tumor promoters is an activation of the *myc* and *fos* oncogenes, the products of which are localized in the nucleus where they may be involved in controlling the expression of other genes.

Exactly how the signals are transmitted to the nucleus is unclear, although there are a number of possibilities. For example, activation of the polyphosphoinositide system affects cellular ion concentrations, increasing those of calcium and sodium ions and decreasing those of hydrogen ions. Changes such as these might influence the activities of enzymes throughout the cell.

Work from Michael Berridge's laboratory at Cambridge University, which he reported at the receptor meeting, has introduced a new player in the polyphosphoinositide system—another candidate for transmitting signals to the interior. The inositol 1,4,5-trisphosphate, which is released when PIP_2 is split, has a very short half-life. It appears within seconds of receptor activation and then disappears rapidly. According to Berridge, there is a second inositol trisphosphate, this one the 1,3,4-isomer, that appears much more slowly and builds up to a maximum about 30 minutes after an appropriate hormone binds to its receptor.

The source of inositol 1,3,4-trisphosphate has not yet been identified. It does not appear to be produced from the 1,4,5-isomer but the Cambridge workers have so far been unable to detect any other precursor. The function of inositol 1,3,4-trisphosphate is also currently unknown. Berridge speculates that it may help produce the long-term effects of receptor activation, perhaps including transmission of signals to the nucleus, with inositol 1,4,5-trisphosphate effecting the short-term actions.

Work by Howard Rasmussen, Itaru Kojima, Kumiko Kojima, and Paula Barrett of Yale University School of Medicine adds another level of complexity to the polyphosphoinositide system. They propose that hormone responses occur in two phases, but find a role for inositol trisphosphate only during the first. Treatment of adrenal glomerulosa cells with the peptide angiotensin II induces a sustained increase in the secretion of aldosterone, a hormone that helps to regulate sodium and potassium ion balance in the body. The first stage in this response, Rasmussen says, is a transient increase in the calcium ion concentration inside the cell, which is mediated by inositol 1,4,5-trisphosphate. The increase lasts only seconds, even though aldosterone secretion continues as long as angiotensin II is present. "The calcium concentration goes down," Rasmussen explains, "because there is a regulatory mechanism that increases calcium ion efflux from the cell in response to its influx. The cell says in effect, 'I'm not going to tolerate a sustained increase in cytosolar calcium.' " Calcium ions continue to flow into the cell but the pump removes them as fast as they enter.



Some possible ways in which oncogene products might interact with the polyphosphoinositide system.

The question then is: How does the cell get a sustained response out of a transient calcium ion increase? According to Rasmussen, it is largely through the activity of protein kinase C. The enzyme is first turned on by diacylglycerol working in concert with the briefly elevated concentration of calcium ions in the cytosol. The continued influx of calcium ions then controls the turnover rate of the calcium-sensitive form of the kinase.

Moreover, there are detectable differences in the patterns of proteins that become phosphorylated during the initial and later stages of the hormone response. "We can clearly distinguish a temporal pattern of phosphorylation—just as we see an altered pattern of calcium movements and aldosterone secretion," Rasmussen says. He postulates that this two-stage model may be generally applicable to understanding how hormones and growth factors work.

—JEAN L. MARX