age that is normally lost. With the use of very short exposures, the individual "speckles" of light created by atmospheric turbulence are recorded and then the information that they contain is extracted by mathematical manipulation. Keith Hege and his colleagues at Steward Observatory in Arizona have reported that their speckle observations are "highly suggestive" of a satellite companion 175 ± 20 kilometers wide for Pallas (which is 550 ± 50 kilometers wide) and another one for Victoria. The initial reception for these observations has been generally positive. Two other groups of speckle observers have looked at a few asteroids, Victoria and Pallas included, but their preliminary analyses have not yet revealed any possible companions.

Speckle observers, not being at the mercy of celestial motions, have an edge in the effort to confirm the existence of such large asteroidal satellites; it will fall to occultation observers, catching as catch can, to demonstrate the existence of smaller companions, or to pile up negative evidence until once again asteroidal satellites appear unlikely.

—RICHARD A. KERR

Tumor Viruses and the Kinase Connection

Protein kinases, made under the direction of certain RNA tumor viruses, may produce the malignant changes evoked by those viruses

Over the past 2 to 3 years, researchers have finally begun to get a handle on how one group of tumor viruses converts normal cells to malignant ones. For each of these viruses, malignant transformation has been linked to the production in cells of a single protein, made under the direction of the viral genetic material, which is RNA.

That a single protein can bring about the numerous changes seen in transformed cells might seem surprising at first glance. As Tony Hunter of the Salk Institute puts it, "Transformation causes a major disruption of cell activities and cell shape." But the recent work suggests that the transforming proteins are enzymes of the kind called protein kinases, which transfer phosphate groups from adenosine triphosphate (ATP) to protein acceptors. Most protein kinases can phosphorylate more than one acceptor, and can thus affect more than one cell activity. Moreover, they regulate such a wide variety of cellular events that no one would be especially surprised if they also helped to regulate cell division.

In fact, epidermal growth factor (EGF), a naturally occurring stimulator of cell division, may produce its effects by first activating a protein kinase with properties similar to those of the transforming proteins. Ora Rosen of Albert Einstein College of Medicine says, "I think we are now seeing the coming together of two very different fields not previously thought to impinge on one another; they are growth factor activity and cell transformation by these RNA viruses." The kinase connection implies that the viruses might produce the abnormally rapid cell division characteristic of malignancy by subverting normal growth control machinery.

Other major—and hitherto mysterious—transformation changes may also be explained by the discovery of the transforming virus kinases. These include the disruption of the microscopic filaments that compose the cell skeleton and the Warburg effect, the increased rate of glycolysis seen in tumor cells. (Glycolysis is a relatively inefficient anaerobic pathway for obtaining energy from the sugar glucose.)

The best evidence suggesting that a viral kinase might bring about transformation comes from studies of Rous sarcoma virus (RSV), the grandfather of all the tumor viruses, which was discovered in 1911 by Francis Peyton Rous. Almost 70 years would elapse before investigators began to find out how the virus produced tumors, but by the mid-1970's work from several laboratories had made it clear that just one of the four genes in the RSV genome carried all the information needed for transformation (*Science*, 13 January 1978, p. 161). Shortly thereafter, Ray Erikson of the University of Colorado School of Medicine in Denver identified a protein with a molecular weight of 60,000 as the product of the *src* (for sarcoma) gene, as the transforming gene is called. Erikson and other investigators, including J. Michael Bishop and Harold Varmus of the University of California at San Francisco, went on to produce evidence suggesting that the protein is a kinase (*Science*, 25 August 1978, p. 702).

Since then the pace of the research has accelerated dramatically. Guided by the RSV work, investigators began looking at other RNA tumor viruses to see if they, too, direct the synthesis of protein kinases. And it seems that several of them do, although kinase production is not a universal ability of the RNA tumor viruses. Most of those that have the ability are either sarcoma viruses or leukemia viruses. The former include two sarcoma viruses of cats and Fujinami sarcoma virus, which, like RSV, infects chickens. Among the latter is the Abelson mouse leukemia virus.

The case for kinase production by the viruses is not airtight, however. Peter Duesberg of the University of California at Berkeley remarks, "The use of the word 'associated' in the papers is conspicuously high." Duesberg was referring to a problem that is the bête noire of transforming protein research. It is simply very hard to tell by the methods currently available whether an enzymatic activity is inherent in the various viral gene products as they are prepared from transformed cells or whether the activity belongs to a minor protein contaminant that is merely associated with the gene products. G. Steven Martin, also at Berkeley, says, "It is clear with RSV that the kinase activity must be very tightly associated [with the *src* gene product] or it is intrinsic. . . . But there are a lot of kinases in cells, especially in membranes."

Despite this caveat about the potential for contaminating viral proteins with cellular enzymes, the evidence for at least some of the viral kinases is beginning to approach the firmness of that for the RSV protein. One way the evidence has been strengthened is through studies of variant and mutant viral strains. For example, David Baltimore of the Massachusetts Institute of Technology and

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Owen Witte of the University of California at Los Angeles have isolated four or five variant strains of the Abelson leukemia virus. Most of them have the ability to transform cells and also produce viral proteins with kinase activity. But one of the strains, which lacks transforming ability, directs the synthesis of a viral protein that does not phosphorylate proteins. "The kinase activity is an intrinsic property of the protein, which is lost in the variant," Baltimore maintains.

Another indication that transforming and kinase activities go hand in hand comes from studies of temperature-sensitive mutants, which transform at one temperature (the permissive temperature) but not at others. Two groups of investigators, one led by Hidesaburo Hanafusa at Rockefeller University and one by Martin, have identified temperature-sensitive strains of Fujinami sarcoma virus. At nonpermissive temperatures these strains do not produce detectable kinases; but at the permissive temperature they do. Hanafusa also finds that cells produce the viral protein at all temperatures, but only the one made at the permissive temperature has kinase activity.

Even though transformation by means of kinase production may be a hallmark of this group of RNA tumor viruses, studies of the viral genomes and of the proteins themselves show that the kinases can have very dissimilar structures.

Some of the differences took investigators by surprise. Hanafusa says, "Fujinami sarcoma virus was discovered at about the same time as RSV, but no one worked on it because they assumed that it was similar to RSV." In the last year or so, however, both Duesberg and Hanafusa have reported that the Fujinami gene structure is not like that of RSV.

The structural research shows that there are two basic designs for the viral transforming genes, one exemplified by the RSV genome and the other by that of the Fujinami virus. Both designs have a common element in that the virus genomes appear to be recombinants consisting of viral sequences plus sequences derived from cellular genes. For example, evidence from Hanafusa's laboratory suggests that RSV was produced when the nontransforming parent virus picked up a cellular gene. This gene, which is present in all vertebrate cells and which codes for a kinase, became the *src* gene of RSV. When the *src* gene is expressed, the protein product is made independently of the other proteins specified by the viral genome.

In contrast, the transforming genes of Fujinami sarcoma virus and others having the second design consist of both cellular and viral sequences and the transforming proteins are themselves hybrids.

The cellular sequences found in the second group are not related to those of the *src* gene. Moreover, they need not even be related to one another. There are three distinct subgroups of viruses with hybrid transforming genes, based on the fact that three distinct cellular sequences have been found in these viruses.

These structural differences are another reason why Duesberg questions whether all the viruses transform by directing kinase synthesis. He asks, "Why should they come up with totally different designs for making the same kind of kinase?" Duesberg suggests that, while some viruses might work through kinase production, others might take a different tack.

Nevertheless, the kinases associated with all the transforming proteins studied thus far share an unusual specificity that provides part of the rationale for the current emphasis on their role in transformation. Hunter and Bartholomew Sefton, also of the Salk Institute, found that the *src* gene kinase attaches the phosphate group to residues of the amino acid tyrosine. All the previously discovered protein kinases phosphorylate either serine or threonine residues. Hunter explains, "Until we found that the *src* gene product phosphorylates tyrosine, no one had ever found a phosphorylated tyrosine residue in cells. We estimate that about 90 percent of the phosphorylated residues of normal cells are serines and about 10 percent are threonines. Phosphorylated tyrosines constitute less than 0.05 percent of the total."

When the other viral proteins were examined they turned out to have the same specificity. Moreover, Sefton and Hunter showed that when cells are transformed by RSV and some of the other viruses, the amount of phosphorylated tyrosine increases as much as tenfold.

The increase does not appear to be just the result of transformation. Other transforming agents, including infection with polyoma virus, a tumor virus that contains DNA as its genetic material, do not produce the increase.

At one time, Hunter had results hinting that a polyoma virus protein, the one called the middle T (for tumor) antigen, might be a kinase, a finding that would be truly remarkable since it would indicate that DNA and RNA tumor viruses might transform in the same way. Many kinases, including the *src* gene protein, have the ability to phosphorylate themselves. Consequently, when Hunter found that the middle T antigen isolated from transformed cells contained a phosphorylated tyrosine, the result suggested that the polyoma protein might be a kinase. However, the observation did not hold up under further scrutiny, and Hunter now thinks that the phosphorylation was performed by a cellular kinase that became associated with the middle T antigen during isolation.

Identifying the products of the transforming genes is only the first step on the road to a better understanding of malignancy. Their targets need to be identified, too. So far the major progress in this regard has been the discovery of a number of proteins that appear to be substrates for the *src* gene kinase.

One of them is a cytoplasmic protein with a molecular weight of 36,000 that occurs in relatively large quantities, constituting up to 0.5 percent of the total cell protein. The function of the 36,000-dalton protein is still unknown, however. "This is one of the problems we are having," Erikson explains, "If you find a substrate like this protein, it might be structural, not enzymatic. Then, how do you get a handle on its function?" So far there is no answer to Erikson's question, although investigators are working hard on the problem.

A recent discovery that cannot help but give impetus to their task comes from the laboratory of Stanley Cohen at Vanderbilt University School of Medicine, who has been studying EGF. The membranes of cells affected by EGF carry specific receptors for the growth stimulator, to which it must bind in order to promote cell division. According to Cohen, when EGF binds to its receptors,

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it stimulates by severalfold the activity of a cell membrane kinase. This enzyme, like the *src* gene product, phosphorylates tyrosine residues. The EGF-stimulated and the *src* gene kinases are apparently not the same, Cohen says, although they may be antigenically related. Meanwhile, Hunter's group has found that the EGF-stimulated kinase phosphorylates the same 36,000-dalton protein acted on by the *src* gene product, a finding that suggests that the two kinases might stimulate growth by a common pathway.

The 36,000-dalton protein is not the only substrate for the *src* gene kinase. Hunter's group, for example, has identified a half-dozen or so proteins that either have no phosphorylated tyrosine residues in normal cells but acquire them after transformation by RSV, or show a big increase in phosphorylated tyrosine content after transformation. Some of these may not have physiological significance, but one good candidate for a crucial target protein is vinculin.

Sefton, Hunter, and S. J. Singer of the University of California at San Diego find that the amount of phosphorylated tyrosine in vinculin increases about tenfold after transformation. This protein is interesting because it is located in membrane structures called adhesion plaques.

The plaques serve as points of attachment between cells and also anchor the actin filaments of the cytoskeleton to the inner side of the cell membrane. Benjamin Geiger and Singer have suggested that vinculin might link the actin filaments to the membrane.

Disruption of the actin filaments is one of the consequences of transformation and is thought to contribute to the altered shape of transformed cells. Moreover, transformed cells are usually much less adhesive than normal ones. "It would be really exciting," Erikson says, "if the *src* gene product changed the function of a protein involved in cell adhesion."

Because of their finding that vinculin is a substrate for the kinase, Sefton, Hunter, and Singer suggest that this might be the case. Phosphorylation of vinculin might destabilize the linkage of actin filaments to the plaques, causing the filaments to disperse and thus weakening the attachment of cells to surfaces and producing the shape changes. As further support for this hypothesis, Larry Rohrschneider of the Fred Hutchison Cancer Research Center in Seattle has shown that the *src* gene kinase is present in adhesion plaques where it would have ready access to vinculin.

Along with the characteristic changes in cell shape and adhesive properties, transformed cells undergo a number of metabolic changes. Prominent among these is the Warburg effect, discovered almost 50 years ago by the late Otto Warburg, but never adequately understood—until recently perhaps.

During the last 20 years, Efraim Racker of Cornell University has been looking at the factors that control the glycolytic pathway. In his view, one of the most important is the availability of inorganic phosphate and adenosine diphosphate (ADP), which are combined to make ATP during glycolysis. The phosphate and ADP are supplied by enzymes, called adenosinetriphosphatases (ATPases), that hydrolyze ATP, thus providing energy for cellular activities such as ion transport and biosynthesis, and at the same time regenerating the raw materials for ATP production.

One of the ATPases is located in the outer cellular membrane, where it serves to transport sodium out of, and potassium into, the cell. When Racker examined this "sodium pump" in Ehrlich ascites cells, tumor cells that can be grown in large quantities, he found that it was very inefficient, pumping little sodium in comparison to the amount of ATP hydrolyzed. This would produce an unusually large quantity of ADP and inorganic phosphate that could be used for glycolysis by the tumor cells.

"But no one believed us," Racker says, "So we decided to go after the protein [the sodium pump]. We isolated it from tumor cells, and put it into liposomes to study." (Liposomes are artificial membrane preparations of known lipid composition.) The result was that the sodium pump from the tumor cells was still inefficient in the liposomes, but a control enzyme taken from brain cells worked efficiently.

When Racker and his colleagues set out to determine what constituted the difference between the two enzymes, they found that one of the subunits of the tumor enzyme contained a phosphate group not found in the brain enzyme. When the phosphate was removed, the tumor sodium pump became just as efficient as the control. They then identified a kinase in the tumor that put the phosphate on one of the proteins of which the pump is composed.

But this was just the beginning. The first kinase turned out to be activated by phosphorylation by a second kinase, which was activated by a third kinase, which was activated by a fourth kinase. All the kinases phosphorylate tyrosine residues and the fourth turned out to be none other than the product of the cellular *src* gene.

All in all, Racker's findings not only suggest a way in which the *src* gene kinase might contribute to transformation—by inappropriately setting in motion events that ultimately lead to increased glycolysis—but also provide a possible role for the cellular enzyme.

Why the viral product, which so closely resembles a cellular kinase, leads to malignant changes still puzzles investigators, incidentally. It might be a dosage effect, in that transformed cells make about 50 times as much of the viral enzyme as normal cells make of the cellular one. Or the small changes in the structure of the viral gene might alter the specificity of the enzyme product with the result that it phosphorylates proteins that are not affected by the cellular kinase.

Kinases probably do not explain all types of transformation. Chemicals, DNA viruses, and some RNA viruses might act in different ways. But protein kinases with the unusual specificity for tyrosine now seem to lie at the regulatory heart of many of the cell's activities and have moved to a central position in transformation research.

Even if some of the viral proteins are not themselves kinases, they might act by associating with, and activating, a cellular enzyme. Martin sums it all up, "I don't feel that the question of whether the kinase activity of the transforming proteins is 'intrinsic' or 'associated' is critical; in either case the phosphorylation of cellular polypeptides is important in transformation."—JEAN L. MARX

"It would be really exciting if the *src* gene product changed the function of a protein involved in cell adhesion."

Additional Reading

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2. T. Hunter, "Proteins phosphorylated by the RSV transforming function," *Cell* **22**, 647 (December 1980).
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