RNA Tumor Viruses: Getting a Handle on Transformation

Today, cancer viruses rarely win the headlines they rated when the "War on Cancer" was declared some 6 years ago. Overly optimistic promises and the failure to deliver a quick fix—a vaccine, for example—for human cancer no doubt contributed to the decline in interest. But loss of glamour does not mean lack of progress. While virologists may not have found an answer for the most dreaded of human diseases, investigators have been quietly concentrating their efforts on viruses that cause cancers in animals and now they appear to be on the verge of discovering how some of these agents produce their malignant effects.

In particular, they have made great strides toward understanding the genetics and biochemistry of the type C RNA tumor viruses. The type C viruses, which have RNA as their genetic material, produce several kinds of animal cancers. Some of them also have the ability to transform cultured cells. Transformation is thought to be equivalent to the process by which normal cells become malignant or cancerous in vivo.

Transformed cells differ from normal cells and resemble cancerous ones in several ways. For example, both transformed and cancerous cells are often capable of multiplying in conditions in which normal ones are not, and they are usually locked in a more primitive state of development than their normal counterparts. How these changes come about is completely unknown; for that matter, even the normal mechanisms for controlling cell division and development are mysteries.

Lack of this information has greatly handicapped cancer researchers, but the new virus work appears to have opened the door to explaining the biochemical basis of transformation by at least some of the type C viruses. Investigators have identified in one—and possibly more—of the viruses a specific gene that is necessary for transformation to occur. They have also identified the proteins coded for by the gene. The functions of these proteins will be vigorously examined, since they may provide the keys to both transformation and the normal control mechanisms.

A second striking aspect of the work is that several of the type C viruses, which are otherwise unrelated, seem to have developed by similar mechanisms. They apparently gained the ability to transform, or at least increased their malignant potential, as a result of acquiring new genetic information from the cells they infect.

Moreover, results with the murine leukemia viruses indicate that the genetic transfer from the host cells might create a slightly different virus each time it occurs. This situation would represent a marked change from the way investigators viewed the situation a few years ago. As Richard Lerner of the Scripps Clinic and Research Foundation puts it, "Instead of having one or a few leukemia viruses as we once thought, there may be an infinite number of them."

Recently, avian sarcoma virus (ASV, which is also called Rous sarcoma virus) has been attracting a great deal of attention. Genetic studies of ASV and ASV mutants have been carried out in a number of laboratories, including those of Peter Duesberg of the University of California at Berkeley, Peter Vogt of the University of California at Los Angeles, and Hidesaburo Hanafusa at Rockefeller University. These investigators have shown that the virus contains a gene (designated *src* for sarcoma) that codes for a protein product that must be produced in order for transformation to occur.

Now, the proteins whose synthesis is directed by the *src* gene have been identified. When the work was first reported, however, there appeared to be significant—and hard to reconcile—differences in the results. Two groups of researchers suggested that proteins with masses of 17,000 and 25,000 daltons were the *src* gene products, whereas a third found a protein with a mass of 60,000 daltons.

The investigators who found the smaller proteins, John Buchanan and James Kamine of the Massachusetts Institute of Technology and Karen Beemon and Tony Hunter of the Salk Institute, used similar approaches. They prepared messenger RNA's (mRNA's) from a transforming strain of ASV and from a mutant that does not transform. These mRNA's were then used to direct the synthesis of proteins in the test tube. Only the mRNA from the transforming virus produced the two proteins.

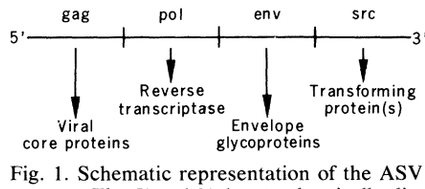

Fig. 1. Schematic representation of the ASV genome. The 5′ and 3′ denote chemically distinguishable ends of the RNA molecule. Transcription of the RNA to form DNA, which is carried out by the enzyme reverse transcriptase, and translation of the RNA to form protein both begin at the 5′ end of the molecule.

Meanwhile, Ray Erikson and his colleagues at the University of Colorado developed an immunological probe for detecting the *src* gene products and came up with the larger protein. They found the protein only in cells infected with a transforming ASV strain and not in those infected with a nontransforming mutant. There are also temperature-sensitive ASV mutants that transform cells at one temperature (the permissive temperature) but not at higher temperatures even though the virus replicates at both. The Colorado workers detected the 60,000-dalton protein only in cells infected with the temperature-sensitive mutant and kept at the permissive temperature. They subsequently showed that a messenger from a transforming ASV strain directed the synthesis of the same protein.

After Erikson and his colleagues reported finding the larger protein, the MIT and Salk groups both decided to look again for the *src* gene products. Further experimentation in both laboratories uncovered proteins with masses of 55,000 to 60,000 daltons. Preliminary evidence indicates that these proteins are identical to that identified by the Colorado group, but more work is needed to confirm this.

Beemon says that they might have missed the larger protein in their first experiments because they used a different ASV strain from Erikson. Buchanan attributes their failure to find it to the possibility that they used the wrong messenger. They originally translated a mRNA encompassing the entire ASV genome (Fig. 1). Translation begins at the left-hand end of the messenger and proceeds toward the right-hand end. Since the *src* gene is located near the right-hand terminal and translation decreases as the messenger is read across, the 60,000-dalton protein might not have been synthesized in quantities sufficient for detection. Normally, the synthesis of the products of genes on the right-hand portion of the ASV genome is directed by shorter messengers that begin nearer the *src* gene re-

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gion. For the newer work, Buchanan and his colleagues used such a messenger.

All three groups now have evidence from peptide mapping that a 25,000-dalton protein is contained within the 60,000-dalton protein. Whether the same is true for the 17,000-dalton molecule is still unclear. And, at the present time, no one knows which of these molecules is active or what they do. The large protein may be an inactive precursor of the smaller molecules. Or the latter might be simply breakdown products of the former. Or all might have some kind of activity. An intense effort to resolve these issues and determine how the *src* gene product transforms cells is now under way.

The function of the *src* gene and its products may not be limited just to transformed cells, however. Normal cells contain DNA sequences related to the RNA sequence of this gene, according to Harold Varmus, J. Michael Bishop, and Deborah Spector of the University of California at San Francisco, and Dominique Stehelin, now at the Pasteur Institute in Lille, France. They find that cells from all the vertebrate species they have examined—ranging from fish to primates—have one or at most a very few DNA sequences related to the *src* gene.

Their data indicate that the *src*-related sequence of the chicken is only 3 to 4 percent different from that of the ASV gene.

Speaking of Science

Chemicals: How Many Are There?

Like it or not, the world around us is filled with chemicals. The clothes we wear, the foods we eat, the magazines we read, and virtually all of the other things that nurture our civilization are made possible by the use of chemicals. Recognition of these facts, and of some of the potential dangers of chemicals, often prompts the question How many chemicals are there? A definitive answer to that question has proved elusive, but one measure of the answer is provided by the American Chemical Society's Chemical Abstracts Service (CAS). As of November 1977, CAS's unique computer registry of chemicals contained 4,039,907 distinct entities. The number of chemicals in the register, moreover, has been growing at an average rate of about 6000 per week.

CAS began the computer registry in 1965 to assist in the indexing of chemical substances reported in the scientific literature. The system identifies chemicals on the basis of an unambiguous computer-language description of their composition and molecular structure and automatically assigns a permanent identifying number to each unique substance. The registry contains all compounds that have been mentioned in the literature since 1965.

The system is not set up to provide a detailed breakdown of different classes of chemicals. Some broad generalizations can be made, though. About 96 percent of the chemicals, for example, contain carbon. The average compound in the registry, if it is possible to define such a thing as an average compound, contains 43 atoms, 22 of which are hydrogen. The fictitious average compound also contains one and a half ring systems with eight atoms per ring.

About 3.4 million of the chemicals are organic or inorganic chemicals whose structures are fully defined. Some 3 million of these contain at least one ring system. Another 258,000 entities are coordination compounds, which require a somewhat different registration procedure. About 59,000 are organic compounds whose structures are not completely defined; these generally are compounds in which the exact location of a substituent or a double bond or the site of an esterification is uncertain. About 120,000 entries are listed only by name or molecular formula; these are specifically identified substances for which not enough is known or has been disclosed about structure to permit machine structure registration. The registry also lists 72,000 alloys, 120,000 polymers, and 10,000 mixtures with specific names.

The list contains some apparent duplication in that stereoisomers are listed individually. There are, for example, four listings for aspartic acid: D-aspartic acid, L-aspartic acid, DL-aspartic acid, and aspartic acid of unspecified stereochemistry. Since each of these has its own biological characteristics, however, they can justifiably be considered distinct entities.

The vast majority of chemicals in the registry are esoteric materials that have been isolated from natural products or synthesized for research purposes. A more interesting problem, then, might be to define the number of chemicals that are in everyday use. That problem is much more difficult, but at least a partial answer may soon be available. As part of the Toxic Substances Control Act, the Environmental Protection Agency (EPA) has been charged with maintaining an inventory of chemical substances manufactured, imported, or processed in the United States for commercial purposes. Because there are so many different names for many of the chemicals used in industry, EPA has contracted with CAS to process reports submitted by manufacturers, determine the precise identity of the reported chemicals, and create and maintain a computer file on the chemicals and their manufacturers.

To initiate the project, CAS has submitted to EPA a preliminary list of some 33,000 chemicals that are thought to be in common use. The complexity of the registration problem is illustrated by the fact that CAS has already found in its files more than 183,000 different names for those chemicals. Current estimates from EPA, moreover, indicate that there may be as many as 50,000 chemicals in everyday use, not including pesticides, pharmaceuticals, and food additives. EPA estimates that there may be as many as 1500 different active ingredients in pesticides. The Food and Drug Administration estimates that there are about 4000 active ingredients in drugs and about 2000 other compounds used as excipients to promote stability, cut down on growth of bacteria, and so forth. FDA also estimates that there are about 2500 additives used for nutritional value and flavoring and 3000 chemicals used to promote product life. The best estimate thus is that there are about 63,000 chemicals in common use. Small wonder then that determination of the safety of all commonly used chemicals is a massive project that may never be completely finished.

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(The virus was originally isolated from chicken cells.) The degree of similarity between the cellular sequences and the viral gene decreases as the evolutionary distance between the species and the chicken increases. But even the least related cellular sequences share most of their nucleotides with the *src* gene. Thus, the gene has changed relatively little during the long course of evolution from fish to primates. Varmus points out that this implies that the *src*-related sequence performs some essential function in normal cells but provides no clue as to what that function might be.

However, the San Francisco workers have found small quantities of mRNA complementary to the cellular *src*-related sequence in all the cell lines they have studied, including one derived from a chemically induced tumor. This suggests that the sequence is expressed and that products derived from it may function in some way in the cells.

Investigators have postulated that the sequence might act in normal cells to regulate cell division, and that growth control becomes deranged when the sequence is removed from its normal environment and incorporated into a virus. If that is the case it is not reflected in the amounts of mRNA produced in the cells studied by Varmus, Bishop, and their colleagues; they detected no differences in the quantities produced by the tumor and other cells.

The functions of the cellular and viral genes may not be the same, however. Even the chicken sequence is about 4 percent different from the one in ASV. Since a change in a single base may theoretically produce a gene product with an altered function, the *src* gene might have acquired its transforming function only as a result of undergoing changes in its base sequence after incorporation into the virus.

Another implication of the findings of Varmus, Bishop, Spector, and Stehelin is that the sarcoma virus originated as a result of a precursor virus picking up the *src* gene from the chicken cells and thus gaining the ability to transform. Because ASV formed spontaneously in nature, this hypothesis cannot be proved directly but there is indirect evidence for it.

Hanafusa and his colleagues have shown that they can reproduce in the laboratory a phenomenon similar to the one postulated. To do this, they used nontransforming mutants of the virus from which most, but not quite all, of the *src* gene had been deleted. Two months after the injection of the mutants into chickens, the animals developed sarcomas at sites distant from the point of injection. (Usually, tumors develop rapidly near the site where ASV is injected.) The investigators isolated viruses from the tumors that again bear a *src* gene and are capable of transforming cultured cells. Because the nontransforming mutants contained short segments of the *src* gene, Hanufusa thinks that base-pairing with the cellular sequence would be facilitated; this would increase the chances that the mutants would regain the genetic information required for transformation. In addition, the ability of the newly formed viruses to transform cells supports the hypothesis that the products of the viral and cellular genes perform very similar, perhaps identical, functions.

The possibility that viruses may spontaneously acquire the ability to transform cells by incorporating one or more genes from an infected cell is supported by studies of the development of several mouse viruses unrelated to ASV. For example, the Kirsten and Moloney strains of murine leukemia virus do not themselves transform cultured cells, but as a result of serial infection of rats or mice with the leukemia viruses and their reproduction in these animals, altered viruses are produced that have the capacity to transform in vitro and cause sarcomas in vivo.

Both Rat and Mouse RNA

According to Edward Scolnick, Wade Parks, Thomas Shih, and Robert Goldberg of the National Cancer Institute (NCI), about 15 percent of the RNA of the sarcoma virus derived from the Kirsten leukemia virus is leukemia virus RNA and the remainder consists of sequences originating in the rat. They identified these sequences as belonging to an endogenous type C rat virus. (The genetic information of endogenous viruses is carried in the genome of the host cells and passed from parents to progeny through the germ cells. Endogenous viruses have been found in a wide variety of species.) From their structural analysis of the sarcoma virus RNA, the NCI investigators concluded that the malignant potential of the virus resides either in rat sequences or in a region near the 3′ end of the RNA that contains a combination of rat and mouse virus information.

The origin of the genetic information that transforms a cell or makes it cancerous has been a topic of discussion for several years now. Two of the major hypotheses, which are not necessarily mutually exclusive, are the oncogene theory proposed by Robert Huebner and George Todaro of NCI and the protovirus theory suggested by Howard Temin of the University of Wisconsin Medical School [*Science* **183**, 1181 (1974)].

The oncogene is described as a gene present in all cells that may become activated in some manner and make them cancerous. Aspects of the new work with ASV and the murine sarcoma viruses suggests that an oncogene may actually be present in cells. However, Varmus and Bishop say that not all of their findings with ASV are consistent with the predictions of the theory. Neither are they totally consistent with the predictions of the protovirus theory, although certain aspects of the work are reminiscent of this hypothesis, too.

Exchange of genetic material among the endogenous viruses of mice also appears to play a role in the development of the murine leukemia viruses, especially in the development of the agents causing leukemia in AKR mice. This strain of mouse is especially susceptible to the cancer; 95 percent of the animals develop the condition between the ages of 6 and 18 months. Janet Hartley, Wallace Rowe, and their colleagues at the National Institute of Allergy and Infectious Diseases determined that viral information necessary for leukemia development is carried at two different sites on the AKR genome. Each site contains the information necessary for the formation of a virus. The two viruses, which are designated Akv-1 and Akv-2, are indistinguishable by a number of biological and biochemical criteria.

Although the expression of one or the other of these viruses is necessary for the development of leukemia in the mice, the evidence indicates that the Akv viruses are not sufficient. For example, young mice produce large quantities of virus but the animals do not develop the disease until they are about 6 months old. Moreover, extracts from the thymus glands of young and old mice often contain comparable concentrations of Akv viruses, but only extracts from old animals produce leukemia when they are injected into other mice.

Hartley and Rowe have evidence that the agent causing leukemia in AKR mice is formed when the Akv viruses exchange one or more genes with another endogenous virus by a process called recombination. The first pathological changes signaling the onset of the leukemia appear in the thymus glands. Lloyd Old, Elisabeth Stockert, and their colleagues at Memorial Sloan-Kettering Cancer Center detected a marked increase in the production of leukemia virus antigen by thymus cells undergoing the pathological changes. Hartley and Rowe observed no increase in the production of Akv viruses in the changing

cells but they did find in the thymus a new virus with properties unlike those of any of the known endogenous viruses of mice.

The previously known endogenous mouse viruses are of two types. These are the ecotropic viruses that reproduce in mouse cells but not in those of other species and the xenotropic viruses that reproduce in cells of other species, such as mink, rabbit, and so forth, but not in mouse cells. The Akv viruses are ecotropic but the new agents isolated by Hartley and Rowe have some of the biological characteristics of an ecotropic virus and some of a xenotropic virus. They reproduce in both mink and mouse cells, for example. The new viruses alter mink cells in ways that ordinary mouse leukemia viruses do not; in some cases, the alterations include transformation. Because of their pathological effects in mink cells, Hartley and Rowe called the new agents mink cell focus-inducing (MCF) viruses.

They hypothesize that the MCF viruses were formed by recombination between the ecotropic Akv virus and a xenotropic virus. One distinction between ecotropic and xenotropic viruses is that each has a different form of a glycoprotein (designated gp70 because it has a mass of 70,000 daltons) located on its membrane; this protein determines what kind of cells the virus can infect. Consequently, Hartley and Rowe suggest that the recombination event takes place between the genes coding for the glycoproteins of the two viruses. The result would be a virus bearing a hybrid glycoprotein with some of the characteristics of each parent molecule. Both hypotheses were subsequently confirmed by structural analyses of RNA and glycoprotein from the MCF viruses.

Lerner and his colleagues compared the peptide "fingerprints" of gp70's from four of Hartley and Rowe's MCF viruses with those of the membrane glycoproteins from the Akv viruses and a mouse xenotropic virus. They found that the MCF glycoproteins contain some but not all of the peptides found in the Akv glycoproteins and one peptide found only in the glycoprotein from the xenotropic virus. This indicates that the suggested recombination did occur.

Meanwhile Nancy Hopkins, Jean Rommelaere, and Douglas Faller of MIT were analyzing genomic RNA from the same four MCF viruses and finding that the envelope gene of each has RNA sequences derived from an Akv virus and from another source, presumably a xenotropic virus. Both groups say that their results show that the left-hand (or 5′) end of the MCF virus is Akv material and the right-hand (3′) end is from the xenotropic virus.

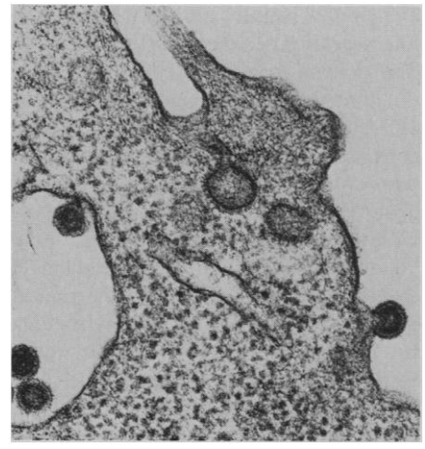

Fig. 2. Electron micrograph of an Akv virus budding from an AKR mouse cell. The dark outer membrane of the budding virus contains viral glycoprotein, which was incorporated into the mouse cell membrane, and possibly cell membrane components, also. [Source: Nelson Wivel, National Cancer Institute]

In addition, Lerner and his colleagues showed that the gp70's of the four MCF strains are similar but not identical to one another. This may mean that the recombination occurred at different locations within the envelope gene of each, a prediction confirmed by the RNA studies of Hopkins and her colleagues. It could also mean that every individual case of mouse leukemia is caused by a slightly different virus.

The fact that the viral glycoproteins differ somewhat in structure is consistent with the observations of Hartley and Rowe that each of the four induces somewhat different changes in mink cells. In addition, some MCF viruses, when injected into young AKR mice, accelerate the development of leukemia in the animals by at least 2 to 4 months; other MCF preparations have not thus far had this effect. This finding may be especially useful since structural analyses of both the envelope genes and their products should permit investigators to determine just which characteristics are needed for leukemia production and which are not.

The evidence that the MCF viruses are the immediate cause of leukemia in AKR mice is highly suggestive but still circumstantial. For example, they are in the right place at the right time and some of them do accelerate development of the cancer. Moreover, Hartley and Rowe have shown that MCF viruses are present in all strains of mice having a high incidence of cancers affecting the lymphatic cells of the thymus or spleen. Nevertheless, additional work will be needed to confirm that the MCF viruses are the actual cause of the cancers.

Just how a change in the envelope glycoproteins of the viruses might transform normal cells to malignant ones is unclear. During the reproduction of the type C viruses, however, viral envelope components, including gp70, become incorporated into the cellular membrane (Fig. 2). A large body of evidence suggests that components of the cell membrane are involved in regulating such functions as differentiation and growth. Thus incorporation of an altered glycoprotein into the membrane might be the cause of the derangement.

Changes in the transforming capacities of viruses other than the Akv viruses may also be linked to recombination involving envelope genes. Scolnick and David Troxler, also of NCI, have shown that a leukemia virus called the spleen focus-forming virus (SFFV) is a type C virus that originated from the recombination of two separate parental type C viruses. The two parents are the Friend strain of murine leukemia virus and a mouse xenotropic virus. The recombinant SFFV is a more malignant virus than the murine leukemia parent. And again the recombination involved the envelope gene of the xenotropic parent.

This does not mean, however, that all alterations in malignant potential involve the same gene. The *src* and envelope genes of ASV are distinct entities. In addition, Duesberg and Vogt and also Bishop and Diana Sheiness of the University of California at San Francisco, have evidence that an avian leukemia virus designated MC29 is a different kind of transforming virus than ASV. They have shown that the RNA genome of the MC29 virus, which transforms cultured cells, does not contain any sequences related to the *src* gene of ASV. Thus, the MC29 virus apparently has its own transforming sequence and may act by a mechanism different from that of ASV.

Although these results indicate that there is probably more than one route to malignancy, the type C viruses are providing investigators with valuable tools for probing both transformation and such fundamental cell processes as growth control and differentiation. Whether the results with these animal systems will also prove applicable to human cancers is uncertain. All the investigators have noted the striking similarities in the results being obtained with the mouse and avian viruses. And Varmus points out that mice and men are more closely related than mice and chickens; thus he thinks that the information may eventually prove pertinent to humans.

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