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Oncogenes: Evolutionary Hitchhikers: [Dr. Hidesaburo Hanafusa]

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RESEARCH PROFILES

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Everyone who deals with the phenomena of pathology soon comes to know that nature often speaks her secrets with a still, small voice out of a dense thicket of happenings.

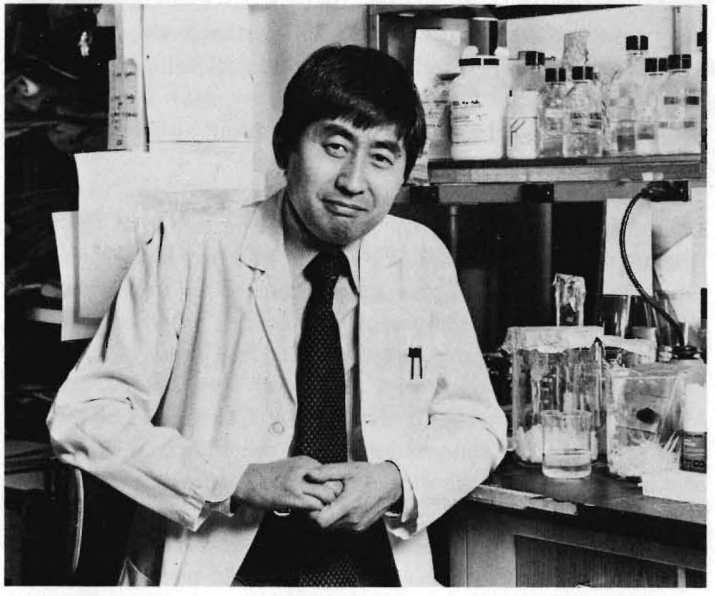
—PEYTON ROUS, *1942*

Oncogenes: Evolutionary Hitchhikers

Shortly after Peyton Rous, a young pathologist, began studying cancer at The Rockefeller Institute for Medical Research, a chicken breeder brought to his laboratory a hen with a large lump on one leg. The hen had a connective tissue tumor called spindle-cell sarcoma, which in mammals, including humans, is often highly malignant. Rous inoculated some healthy chickens with this tissue and they developed sarcomas. He then inoculated other chickens with a filtrate of cancerous material from which all cells, including the cancer cells, had been removed. Those chickens, too, developed tumors. Rous concluded that a virus, too small to be seen with the microscopes of the day, was the cause. But in 1911, the idea of virus-induced cancer was received with considerable scepticism. Rous was to experience what a colleague called a "long, dark period of acknowledgment deferred."

In 1966, near the end of a protean career marked by many honors and many other major contributions to medical science, Peyton Rous was awarded the Nobel Prize "for his discovery of tumor-inducing viruses." As Charles Huggins of the University of Chicago and a co-recipient of the prize later recollected: "A thousand Swedish medical students held a formal dinner in Stockholm in honor of Dr. Rous.... One remembers Peyton (age 87) very late in the evening standing on his chair with glass in hand singing *skoal* with the young students." Peyton Rous died on February 16, 1970. □

Whether or not viruses are an important cause of cancer in human beings remains—70 years later—an unresolved question. But since the days of Rous's discovery, it has been found that viruses cause various kinds of cancers in many animals, from chickens to apes, and scientists like Professor Hidesaburo Hanafusa of The Rockefeller University are studying



Hidesaburo Hanafusa

Teruko Hanafusa

Lu Hai Wang

these animal viruses to learn more about basic cancer mechanisms. They have learned, among other things, about specific genes, present in some viruses, that are involved in the malignant transformation of cells. This information has led recently, and surprisingly, to the identification of what a growing number of researchers believe may be potential "cancer genes" present in *all* cells, including human cells, and to a new theory of how cancer happens.

The so-called oncogene theory (*onc* meaning "mass" in Greek) has gained great momentum. The result of the converging of different lines of research in many laboratories, it represents years of dogged effort sparked by those leaps of imagination and serendipitous adventures by which science proceeds. The field of viral oncology has lately become, in Dr. Hanafusa's words, "very busy and very lively." As Dr. Michael Bishop of the University of California, San Francisco, wrote some months ago, "For the first time investigators have perceived the dim outlines of events that can induce cancerous growth."

For Dr. Hanafusa, who is one of five scientists to receive a 1982 Albert Lasker Basic Medical Research Award this November for contributions to the understanding of cancer genes, the new findings dramatically confirm his perception, 25 years ago as a student in Japan, that tumor viruses are "a very attractive biological problem." In the years since, he has used his training as a biochemist, combined with new insights and new technologies from molecular biology and genetics, to observe, isolate, control, and explain the events that occur and the elements that interact when virus meets cell.

In the viral oncology laboratory at Rockefeller, which he established in 1973, Dr. Hanafusa and his group concentrate on studies of viruses that induce tumors in birds, particularly Rous sarcoma virus (RSV), the prototype of animal tumor viruses and still a favorite laboratory model. In 1979 they reported the results of a series of experiments that have lent weight to the belief, to quote Michael Bishop (another 1982 Lasker-Award winner) again, that "a final common pathway by which all tumors arise may be part of the genetic dowry of every living cell."

In the experiments, Dr. Hanafusa and his colleagues inoculated chickens with a preparation of Rous virus, which normally brings on sarcomas within about a week. In this case, however, the viruses used in the preparation were defective mutants, lacking most of the particular DNA sequence known to constitute the tumor-inducing gene called *src* for sarcoma, within the viral gene bundle (genome). Not surprisingly, tumors did not appear in a week. Two months later, tumors did arise, however, but very far from the site of inoculation. When the viruses in these tumors were analyzed, they were found to contain *complete src* genes. To understand the significance of that finding, which Dr. Hanafusa confesses astonished him, and to understand its place in the oncogene thesis, it is necessary to know something about the course of tumor virus research in general, and of Dr. Hanafusa's work in particular.

A TALE OF TWO VIRUSES

A virus consists of its genome and some proteins sealed in a protein envelope. To survive and replicate, viruses must infil-

trate and commandeer the metabolic machinery of cells. Dr. Hanafusa's involvement with viruses began at Osaka University, where he earned his bachelor's and doctoral degrees. Wanting to understand "how such miniature parasites with such minimal genetic information could capture control of complex, healthy organisms," he did some studies of the virus of smallpox at the university's Research Institute for Microbial Diseases. His interest in viruses as cancer agents was reinforced by news from the United States that researchers there had recently developed the means for growing tumor viruses in tissue culture, making it possible to observe cell transformation in progress.

In 1961 he obtained a postdoctoral appointment at the University of California at Berkeley, to work in the laboratory of Harry Rubin, one of the pioneers in tumor virus research. (Accompanying Dr. Hanafusa across the ocean was one of his colleagues from Osaka, also a new Ph.D., an organic chemist named Teruko Inoue, who had become Teruko Hanafusa three years earlier, and who is still her husband's closest research collaborator.) "Rubin was working with Rous virus, which appealed to me," Dr. Hanafusa explains, "because its action is direct, quick, and quantitative—ideal for biochemical study. With a good viral preparation, I soon found that I could get a transformation of cells within 24 hours."

Dr. Hanafusa and lab members evaluating results of DNA sequencing. Second from right, Research Associate Ricardo Feldman. From left, Graduate Fellows Bernard Mathey-Prévôt, Fred Cross, and Teena Lerner.

The newcomer's first assignment produced his first major finding. "Rubin had noticed something that perplexed him. He had found that all the Rous virus he isolated seemed to be contaminated with another virus, which he called Rous-associated virus. A number of such associated viruses have since been identified. He wanted me to see if I could isolate pure Rous virus. In trying to do that, what we found was that the Rous virus was defective. It could transform cells—make them cancerous—but it could not replicate itself. It needed the other virus to help it do that, which is why it was not found alone. The defect, we subsequently learned, was that it could not make the envelope glycoproteins necessary for entry into the cell.

"Defective viruses had been seen in bacteriophages—viruses that infect bacteria—but not in animal viruses. They hadn't even been suspected. So our finding was rather shocking. What was even more surprising was that the Rous virus being studied in Europe wasn't defective, even though all of the viruses, here and abroad, had been cultured from ones originally isolated by Rous himself. Whether they represent different strains or evolved differently from the same strain is still a question. The defective virus made an excellent tool for study. By changing the helper virus we could begin to alter properties and activities of the Rous virus." (The find also made the young Dr. Hanafusa "just a little famous" in the relatively small world of viral oncology.)

In 1964 he went to Paris, as a visiting scientist at the College de France, and continued the line of investigation he had

begun with Rubin. Two years later, he returned to the United States to accept an appointment as chief of viral oncology at the Public Health Research Institute in New York, where he remained until coming to Rockefeller.

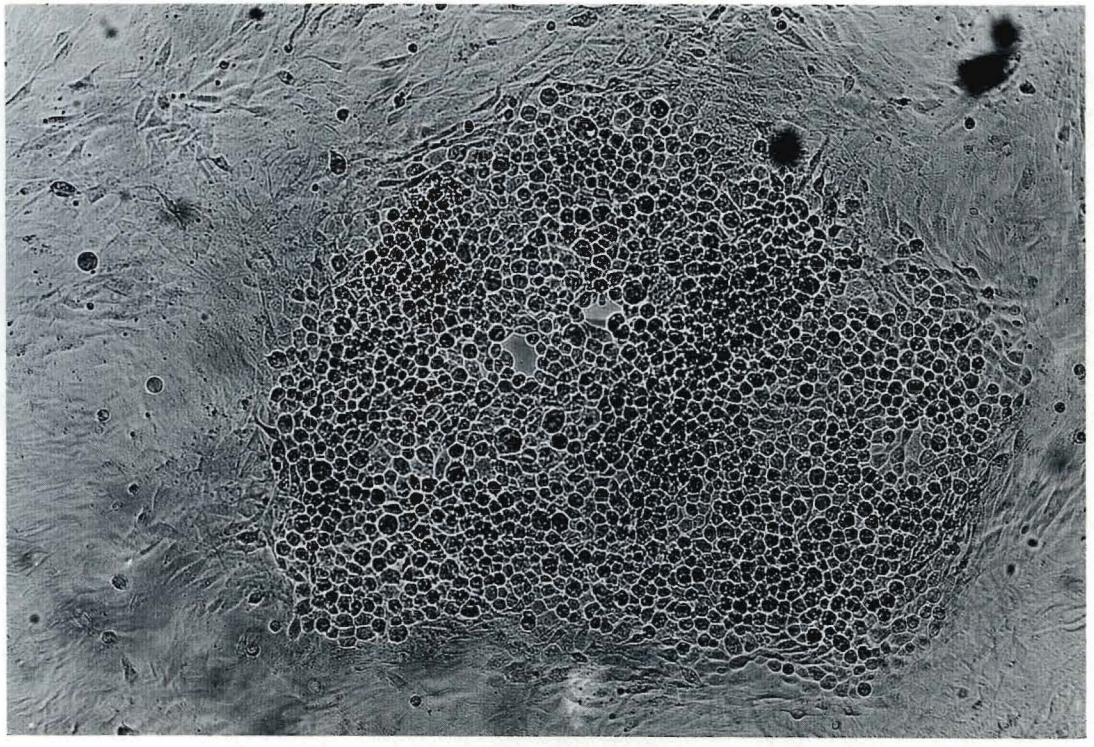
"BACKWARD" VIRUSES

In cells, genetic instructions are transcribed from strips of DNA—deoxyribonucleic acid—into strips of RNA, another nucleic acid. This messenger RNA, as it is called, then transmits the DNA's orders which specify primarily what protein to assemble. Unlike cellular genes, which are always DNA, viruses can be made of DNA or RNA. For a long time, the mode of action of RNA viruses, such as Rous sarcoma virus, mystified scientists because the accepted dogma was that genetic instructions could flow only from DNA to RNA. But it turned out that RNA viruses, now also called retroviruses, work backward: their RNA is transcribed into virus DNA, which then hooks into the cellular DNA.

The clue to solving the backward-virus mystery was found independently in the laboratories of David Baltimore of MIT (who earned his Ph.D. at Rockefeller) and Howard Temin of the University of Wisconsin. They isolated an enzyme, appropriately named reverse transcriptase, that they demonstrated to be the mechanism for transcribing viral RNA into provirus DNA. Experimenting with a mutant strain of Rous sarcoma virus deficient in reverse transcriptase, Dr. Hanafusa confirmed the enzyme's essential role in viral replication.

The discovery of reverse transcriptase was a giant step forward both conceptually and technically. "Until then," Dr. Hanafusa points out, "the analysis of viral-specific RNA in cells infected with retrovirus was extremely difficult since infected cells contain abundant amounts of cellular RNA. Using reverse transcriptase, it became possible for us to synthesize DNA complementary to RNA of the viral genome and to use the complementary DNA as a probe to detect the viral RNA in the cell, with which it will link."

The finding, around the same time, of an RSV mutant which is temperature-sensitive in its transforming ability but not in its ability to replicate gave the first direct proof that a viral gene was responsible for inducing transformation. Because these mutants can be turned on and off by varying the temperature to which they are exposed, they have become invaluable aids in tumor research. The most widely used of these mutants was isolated in Dr. Hanafusa's laboratory.



A cluster of cells transformed by Rous sarcoma virus. The shape and refractility of these transformed cells are very different from the surrounding normal chicken cells.

"In the 1970s," says Dr. Hanafusa, "genetic analysis of RNA tumor viruses came into full blossom." The genome of Rous sarcoma virus was the first to be mapped. It turned out to be very small, even for a virus, with only four genes: one coding for structural proteins, one for reverse transcriptase, one for the envelope proteins, and one—the *src* gene—for

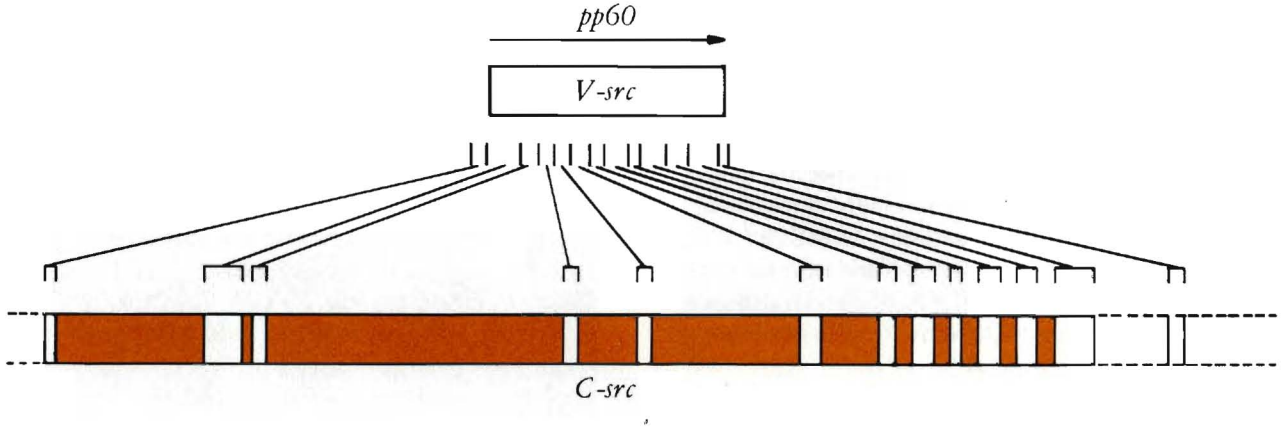
transformation. Dr. Hanafusa's experiments with mutant viruses lacking *src* helped to establish its function. Other genes were later pinpointed in a variety of tumor viruses that infect different animal species.

THE GENETIC DOWRY

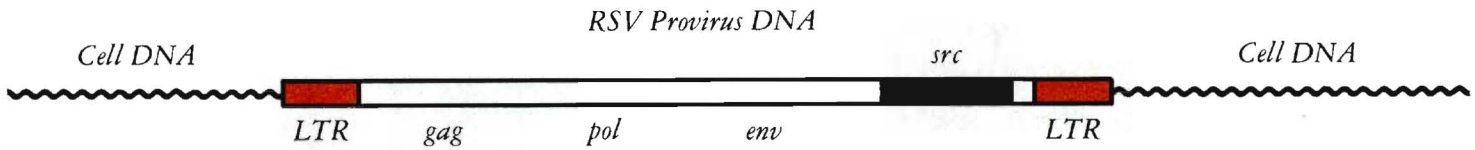
As stated earlier, viruses with transforming genes induce tumors quickly and directly, in living animals as in tissue culture. But there are other viruses, *without* cancer genes, that also cause tumors, although less directly and more slowly. And there are the cancers, including human cancers, that cannot be ascribed to viruses, that stem from chemical or other carcinogens. How do their mechanisms relate? For a long time it was thought that they did not. Then, some years ago, researchers studying viral transforming genes began to notice DNA sequences in uninfected cells that looked very much like viral oncogenes.

"DNA sequences homologous to the RNA sequences of the *src* gene in Rous virus were found in the chromosomes of normal chickens and many other vertebrates," says Dr. Hanafusa. "This unexpected finding raised the possibility, which later studies proved to be the case, that viral transforming genes originated as cellular genes and were picked up long ago by viruses, by means of natural genetic recombination." The cellular genes that became viral genes began, in effect, as hitchhikers on the evolutionary road. Presumably, they recombined with viral genes much as, in the experiment Dr. Hanafusa reported in 1979, *src*-defective viruses recombined with homologous sequences in the cells of the chickens he infected. The significant point about Dr. Hanafusa's recombined *src* gene is not that it looked like a viral *src* but that it behaved like one: it caused tumors.

Most researchers assume that oncogenes have persisted in cells because they have a vital function in normal cell life: to produce a protein the cell needs. They further speculate that it may be only when that protein is overproduced that it adversely affects other proteins, which in turn bring about such changes as cellular distortion and unrestrained growth characteristic of cancer. The protein for which the *src* gene codes in chicken cells has been identified as kinase, an enzyme that catalyses the addition of phosphate on other proteins. Dr. Hanafusa's recovered viruses, in which the *src* gene had been put back together, produced this kinase. He has found that the protein is also present in much smaller amounts in normal chickens. How are these potential onco-



Comparison of the viral src gene (v-src) with its homolog in chicken cells (c-src). The cellular gene contains many intervening sequences (shown by orange bars) that are lost in the viral gene. Both cellular and viral genes encode a similar protein, shown as pp60.



A schematic drawing of Rous virus DNA which becomes a part of the cellular chromosomal DNA after infection. The LTR triggers active formation of the src product.

genes in cells turned on? Provirus DNA contains sequences at both ends that are thought to contain a triggering device. These sequences are called LTR, for "long terminal repeats." When a normally dormant cellular oncogene is captured by a virus, the expression of the oncogene comes under the control of this trigger.

Avian leukosis virus is a virus *without* a specific cancer gene but *with* LTR sequences. Adjunct Professor William Hayward, now at Memorial Sloan-Kettering Cancer Center, who has been a member of Dr. Hanafusa's laboratory for a number of years, discovered that these leukosis virus LTR sequences turned on a cellular gene, called *myc,* which is homologous to a viral transforming gene. His studies showed that, contrary to the cellular oncogene being captured by a virus, in this case the leukosis virus' LTR is incorporated within the chromosome in front of the cellular *myc* sequences. In more recent studies in other laboratories, oncogenes, homologous to viral transforming genes, have been isolated from human bladder and colon cancers. "It seems," says Dr. Hanafusa, "that LTR-like sequences, or promoters, are present in normal chromosomes but are not 'important,' from the point of view of cancer, unless near an oncogene. Thus, we can now speculate that either relocation of these sequences or mutation of the cellular promoter can activate the cellular oncogene and result in cancer."

TO UNDERSTAND CANCER

On the door of Dr. Hanafusa's office, there is a large lapel pin, the kind given out by an airline company as souvenirs, a gift from a student. Written on it, in Hawaiian, are the words, "I'm the boss." In the laboratory, the "boss" looks barely older than his graduate students. It is hard to imagine these young people being daunted by a man who enjoys relating that when, in his own student days in Osaka, he was allowed to use a brand new piece of expensive equipment (imported from America and, coincidentally, designed at Rockefeller), "I broke it!"

Dr. Hanafusa and his colleagues, principal among them Dr. Teruko Hanafusa, who has been largely responsible for the isolation and characterization of the mutant viruses through which so much has been learned, and Dr. Lu Hai Wang, who has made an enormous contribution by his analysis of viral RNA and who is now actively working to elucidate the mechanisms of viral RNA processing, are governed by one objective: to understand cancer.

As Dr. Hanafusa stresses: "The new ideas are very exciting. They look good, but the phenomenon of cell transformation is incredibly complicated. We know that in Rous virus infection the *src* gene action is a primary event, but whether we can correlate that with the activity of cellular oncogenes remains to be seen. Then there's the question of how the gene product—the kinase protein—converts normal cells. We don't have any real fix on how many changes occur in the course of transformation. Those are the crucial questions, the tough ones." Then he adds: "I have always believed that virus research would prove applicable to all cancerous transformation. It seems to be the case. I hope." □

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