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## A Simplex Plan

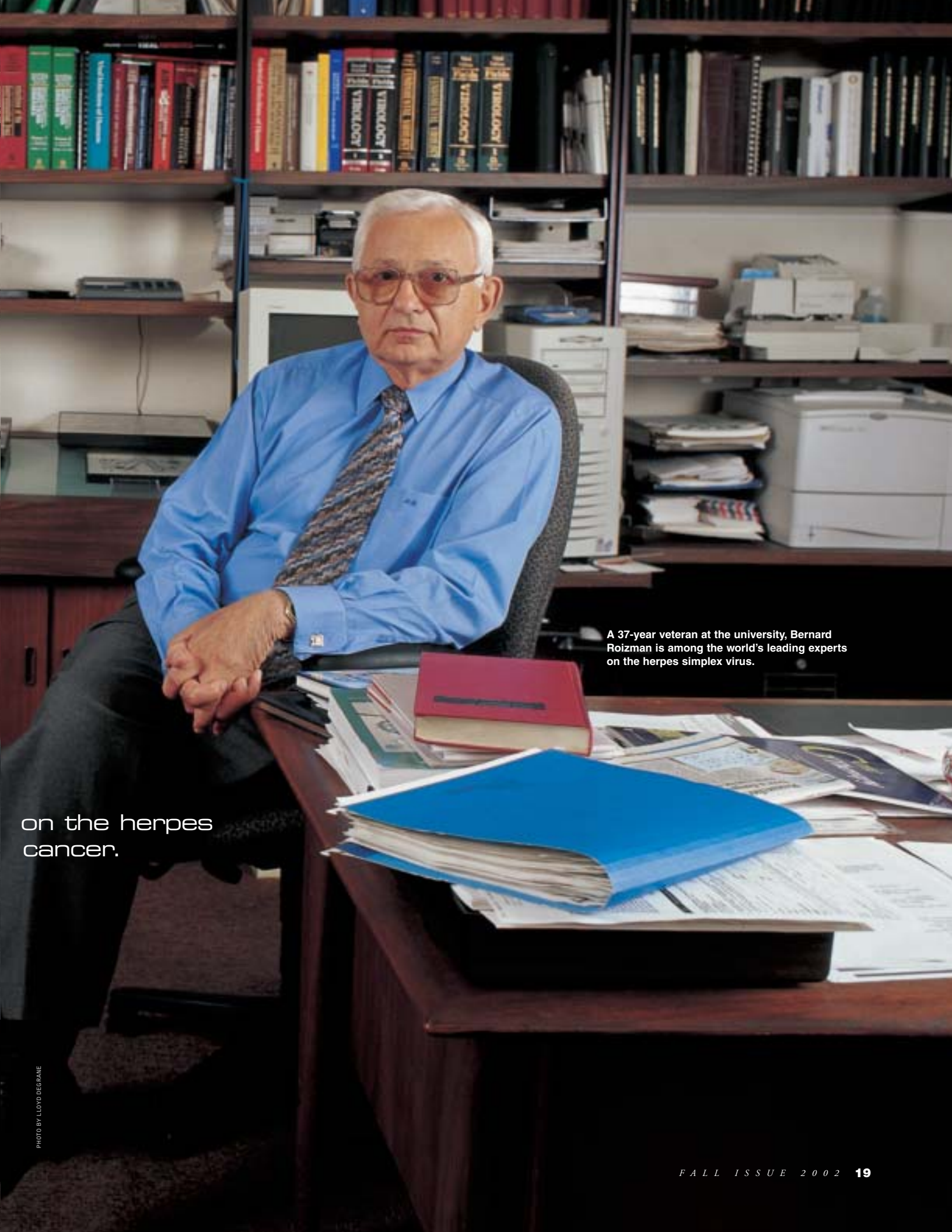
by Paul Karr

Bernard Roizman is one of the world’s leading experts on the virus. Now he is trying to use that knowledge to fight

**I**N LABORATORIES A SHORT STROLL FROM WASHINGTON PARK, BERNARD ROIZMAN performs groundbreaking research on the virus nobody wants to talk about. Soon, though, everyone may be talking about it. Roizman is widely considered the world’s foremost expert on the herpes

simplex virus (HSV). His persistent, half-century effort to unlock the secrets of that pathogen now are bearing fruit — and not only in ways that might take the teeth out of a clever, dangerous virus. His work also is providing unexpected bonuses, including one that seems useful in treating previously incurable cancers.





A 37-year veteran at the university, Bernard Roizman is among the world's leading experts on the herpes simplex virus.

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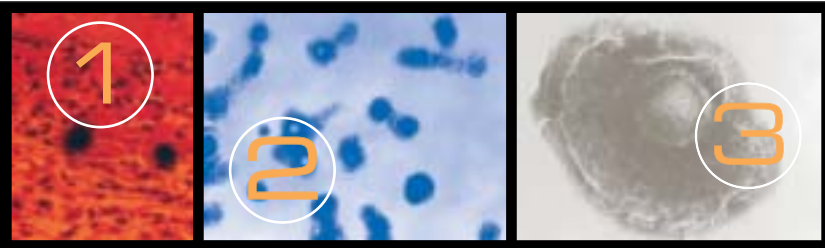
The University of Chicago's Joseph Regenstein Distinguished Service Professor of Virology and chairman of the Viral Oncology Laboratory, Roizman was born in the city of Kishinev, in what was then called Bessarabia — an area variously under Turkish, Russian, Romanian and Soviet control, now part of the independent country known as Moldova. Displaced during World War II, the young Roizman moved with his parents through several countries, settling eventually in Italy. Roizman began reading law in Turin, but found that it didn't pique his interest; he soon moved to Philadelphia, where he studied biology and sharpened his facility for languages (he speaks a half dozen) before "the science bug somehow bit me" at Temple University.

the life of the host. Unlike many other viruses, it survives in the body for life, lodging in the sensory neurons packed along the spinal cord. In the process, the virus transforms human cellular machinery to block the body's natural resistance to it — something like "converting an entire automobile factory to make one kind of hairpin," in Roizman's words. An infection with HSV-1 is usually harmless, but some people are at special risk: newborns, for example, who have underdeveloped immune systems for the first seven to 10 days after birth. Herpes contracted at delivery or during that first week of life — often from an infected relative's innocent kiss — can cause a viral infection that, if not treated promptly, can result in the death of the infant.

Roizman's decades-long effort in the laboratory has been to try to understand just how HSV takes over the body's cells and how each of the virus's 84 known genes acts during that process. Some of the genes may have as many as six functions each, making the job extremely complicated. Yet it is essential to understand how the viral genes tick before designing a vaccine — or before attempting to turn HSV's insidious methods toward a positive goal.

"Some people want to destroy cells, some people want to cure viruses," Roizman said. "I would like to do both. But no matter what our ambition is, we need to know what all of these viral genes do."

In the late 1960s, his lab began the long work of purifying HSV particles to determine the structure of the virus's DNA. What he found was intriguing: Herpes, it turns out, is enormously complex, both in the composition of its particles and the structure of its DNA. Each virus particle, for example, contains more than 30 different proteins, a large number; the DNA consists of two linked components that, unusually, are flip-flopped relative to each other. In the 1970s, Roizman's team began identifying the genes that make up HSV, their gene products and the order in which they are made.



Nerve cell with latent virus (1); virus-infected cells (2, 3).

PHOTOS COURTESY OF BERNARD ROIZMAN

Following doctoral studies and nine years of teaching at Johns Hopkins University in Baltimore, Roizman moved to Chicago in 1965 and took up virology research full time. He since has become the world's leading expert on HSV, reaping numerous awards and honors for his research and training dozens of top researchers in the award-winning Kovler Viral Oncology Laboratories.

"I have long been fascinated by viruses," Roizman said, "because they are extremely small — some are so small that they contain as few as five genes — yet they take over and completely subvert a body cell containing more than 50,000 genes. It's curious that so small an organism can take over a larger one so effectively and dramatically."

Herpes viruses are especially good at doing so. The word herpes, in fact, comes from the Greek "herpein," meaning "to creep," which is precisely what most forms of the virus do as they ascend the nerve trunk to take residence in the friendlier territory of the sensory neurons. The large and diverse family of herpes viruses includes more than 100 members, some of which are associated with chicken pox, shingles, mononucleosis and Kaposi's sarcoma, among other diseases. Humans are infected by eight types of herpes, including zoster, responsible for shingles and chicken pox; herpes simplex 1 and herpes simplex 2, associated with oral and genital lesions respectively; the virus that causes mononucleosis in adults; and Kaposi's sarcoma.

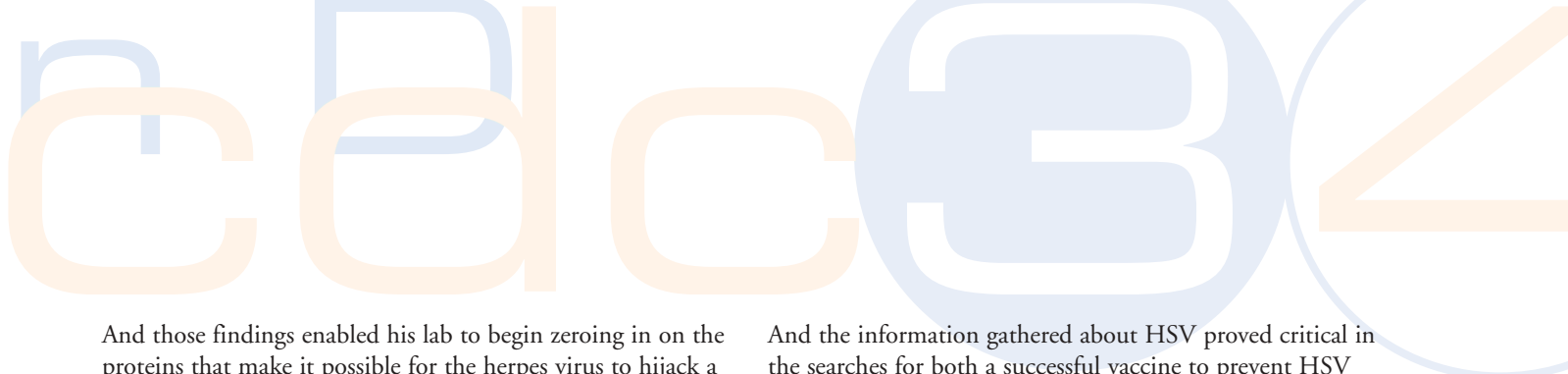
HSV-1 and HSV-2 are particularly interesting because, in spite of their names, the viruses are extremely complex and cunning in their methods.

At transmission, HSV infects the mouth and genitals but then quickly establishes itself in cells where it can remain for



Bernard Roizman in his lab in May 1972.





And those findings enabled his lab to begin zeroing in on the proteins that make it possible for the herpes virus to hijack a body cell.

Most recently, the team has detailed the process that helps keep the virus growing. Cyclin D is a protein present in healthy cells but normally gets destroyed by a complex of proteins — including one known as *cdc34* — before cellular DNA synthesis occurs. HSV needs cyclin D for its own purposes, however, and to keep it from being destroyed by normal cellular processes the virus produces a ubiquitin ligase — a special protein that targets *cdc34* for destruction instead of the beneficial cyclin D.

“The cyclin D creates an environment that’s more advantageous for the virus to grow in,” said Ryan Hagglund, a graduate researcher in Roizman’s virology laboratory. *(See related story on next page.)*

And the information gathered about HSV proved critical in the searches for both a successful vaccine to prevent HSV infections and a genetically altered virus that might attack hard-to-cure cancers.

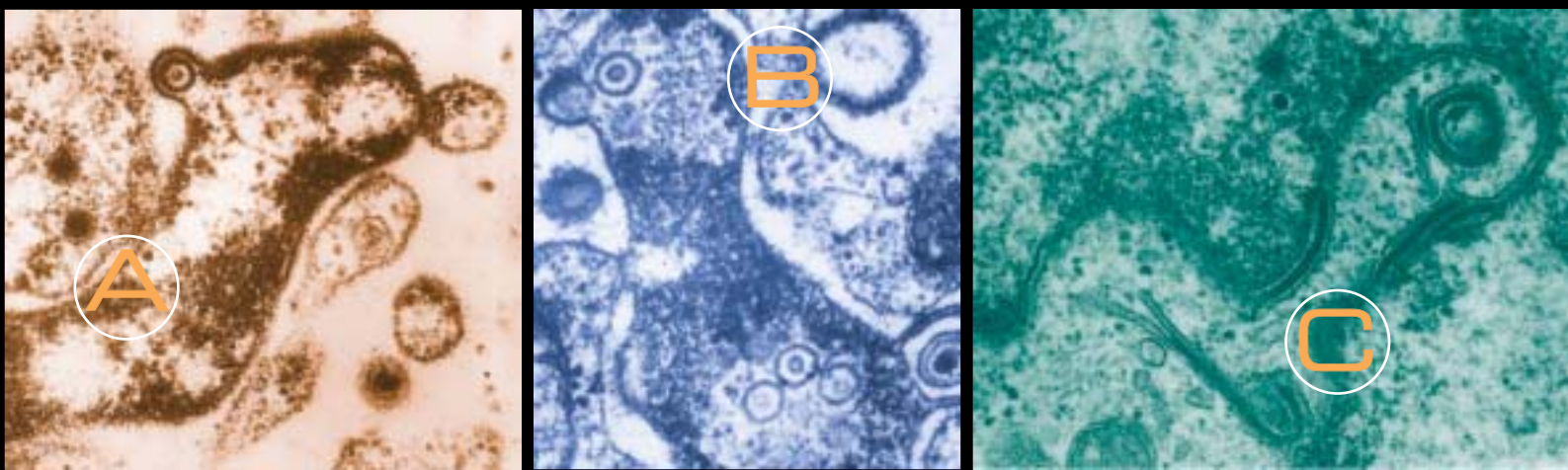
### A potential weapon against brain cancer

It was Roizman’s University of Chicago lab that first developed the trick of modifying viral DNA — genetically engineering it — into something slightly different in order to destroy it. The quantity of DNA in HSV is enormous, far too large to clone in its entirety, so Roizman did something clever. To manipulate the viral DNA, he and his associates took advantage of the observation that, in infected cells, a snippet of altered DNA will recombine with a similar piece

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— Virologist Bernard Roizman

PHOTOS COURTESY OF BERNARD ROIZMAN



Herpes simplex virus-induced protrusions in an infected cell nucleus can take various shapes, including: a “camel” (A) and a “turtle” (C). In the “duck” photo (B), Roizman’s favorite, the “beak” shows a capsid in the process of being enveloped by the inner nucleus membrane; the “eye” is an unenveloped capsid; and the “tail” contains a complete virus particle.

This research has proved tremendously useful in the fight against herpes. Roizman’s work on the mapping of HSV proved that hospital nurses were unwittingly transmitting the virus from infant to infant by failing to wash their hands — research that led to new, stricter rules for hospital hygiene. His gene-mapping techniques have been used to assist prosecutions involving disease transmission, such as that of a Florida dentist convicted of passing the AIDS virus to a patient.

of intact viral DNA. Such recombinants are extremely valuable when they are created, but they occur only rarely and are difficult to find and isolate — a process Roizman has compared with searching for a specific car in an entire airport parking lot.

To find them more easily, Roizman tried something else novel. He made them stand out.

Roizman and a postdoctoral fellow inserted a gene encoding a “selectable marker” — an enzyme known as thymidine kinase — into the middle of each snippet. By then growing the cells in special environments, it became possible to easily identify the altered recombinants. Much like a bright, familiar band around an anonymous suitcase on a baggage conveyor belt, the simple addition of the thymidine kinase turned those special, hard-to-find pieces of virus into the equivalent of red flags for the researchers.

That replacement technique has since been applied both to viruses and to cells, leading to the generation of hundreds of recombinant viruses. The technique — and many of the recombinants — have been licensed by the university to private firms working on anti-viral vaccines and therapeutic viruses.

With a private pharmaceutical laboratory taking up the bulk of the vaccine research and testing, Roizman and his lab team are now free to focus on viral gene functions. In so doing, a new application of HSV has emerged: developing a virus that specifically targets malignant brain cancer cells known as gliomas.

Some 15,000 new cases of that type of brain cancer are diagnosed in the United States each year. “Once diagnosed, the median life expectancy of a patient with malignant gliomas is

less than a year” Roizman said. “They are presently incurable.”

Among the viruses constructed in Roizman’s laboratory, several specifically turned out to target malignant glioma cells and had no effect on normal cells. That finding was exciting, because HSV is normally extremely hazardous when it invades the brain. The viruses engineered in Roizman’s laboratory, however, proved safe in an animal that would normally have been killed by it; they also have been found to be safe in phase I studies on human patients with malignant gliomas. In collaboration with Chicago’s Ludwig Professor of Radiation and Cellular Oncology Ralph Weichselbaum and a group of scientists from the University of Alabama, Roizman and his associates now are working on second-generation recombinant viruses that specifically recognize and infect cancer cells.

For such advancements in a lifetime of work on herpes, Roizman has won considerable acclaim.

“He is the undisputed dean of herpes virology,” said Peter Palese, chairman of the microbiology department at New York’s Mount Sinai School of Medicine and an expert in the

## Tagged for destruction

As he struggles to learn what makes the herpes simplex virus tick, Bernard Roizman follows a simple, structured plan of action.

“We’re taking it apart piece by piece,” Roizman said.

His research team pays special attention to how the virus takes over a cell’s normal “degradation tagging” mechanism. This process allows a cell to target specific proteins to be destroyed, a normal housekeeping chore.

But when it takes control of a cell, the herpes virus hijacks that mechanism and targets entirely different proteins for destruction.

“The goal of the viral genes is to create an environment conducive for the virus to replicate,” said virologist Ryan Haggglund, a third-year graduate student and one of nearly two dozen graduate students, post docs and technicians in Roizman’s award-winning lab. “The elimination of certain cellular

proteins is part of that environment.”

Scientists discovered that the virus hijacks the degradation-tagging system by studying the levels of cyclin D, a protein that helps regulate the normal cell cycle. Cyclin D is present in a healthy cell but gets destroyed by other proteins, including *cdc34*, prior to the cell’s DNA synthesis. Scientists hypothesize that in a herpes-infected cell, the viral protein ICP0 tags *cdc34* for degradation, which in turn, enables cyclin D to remain throughout the cell cycle.

So researchers took a closer look at the tagging mechanism of ICP0. They found that this protein has the only known dual-degradation mechanism. All other similar proteins have only one domain, or chamber, with a mechanism to tag proteins for degradation. ICP0 has two.

A normal tagging machine works this way: Through a complex metabolic pathway, three different enzymes help transfer ubiquitin, a small protein that serves as

the tag, to various cellular proteins, which targets them to be destroyed. In the ICP0 tagging machine, the same thing happens except different proteins are tagged.

“The cell has its own interest in regulating the presence of certain proteins at certain times,” Haggglund said. “When the virus invades a cell and takes over this tagging machine, it causes the degradation of proteins that aren’t normally degraded. By causing the destruction of these proteins, the virus creates a more favorable cellular environment to replicate.”

Normally, the *cdc34* enzyme helps transfer the ubiquitin tags to the various cell proteins. But the ICP0 tagging machine makes *cdc34* tag itself to be destroyed. ICP0 has a second chamber with its own tagging machine that doesn’t need *cdc34* to transfer the tags. So even though the *cdc34* is destroyed, the viral protein keeps tagging other

related field of RNA viruses. “He has shepherded the field from humble beginnings in the middle of the last century into the modern era of molecular virology. He

has extraordinary insight and the energy of a person half his age.” Roizman was elected to the National Academy of Sciences in 1979. He has been the recipient of honorary degrees from United States, Italy, France and Spain, and an honorary professorship from Peking Union Medical College — the most prestigious medical school in China. He also is a foreign member of academies in Hungary and China. He has received the Pasteur Award, the first ICN International Prize in Virology, the J. Allyn Taylor International Prize in Medicine and the Bristol-Myers Squibb Award for Distinguished Achievement in Infectious Disease Research.

He is proudest, however, of his laboratory’s reputation for turning out top young scientists even as it publishes groundbreaking research findings. The list of full professors

trained in his laboratory includes faculty members at Harvard, Stanford, Columbia, Johns Hopkins, the University of California-Berkeley, the University of California-San Francisco, Northwestern and many other top medical institutions.

“The object of this lab is to do good science and to train future contributors to science,” Roizman said. “I am very pleased that so many of our trainees are distinguished scientists in some of the major universities and research institutions in the United States and abroad.”

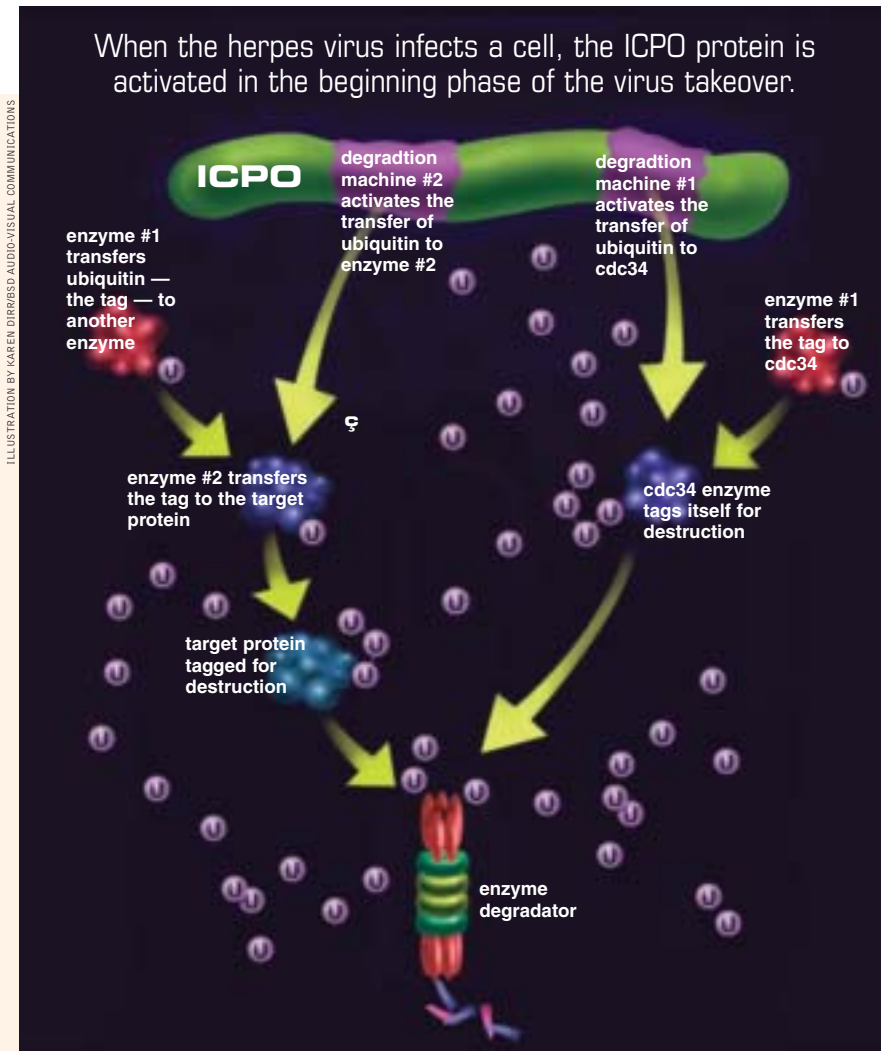
Mount Sinai’s Palese agreed.

“He is one of the guiding lights in microbiology,” Palese said, “someone the younger ones look up to.”

As for his continuing herpes research, Roizman remains optimistic that genetically engineered strains of HSV will prove useful in the fight against other hard-to-treat ailments.

“Really good projects have no end,” he said. “Discovery and rediscovery lead to new questions that require solutions. We will continue to try to solve them.”

When the herpes virus infects a cell, the ICPO protein is activated in the beginning phase of the virus takeover.



proteins for degradation, and eventually the virus takes complete control of the cell.

“It makes sense for the virus to have this arrangement in order to carry out the destruction of the cell,” Hagglund said. “And it’s the only such arrangement that’s ever been described in this type of viral or cellular protein.”

Recently, the researchers identified five amino acids that are required for ICPO to tag cdc34 for degradation. By introducing various mutations of those specific amino acids into a virus-infected cell, they now are studying the effects of the growth of the virus.

“We’re going to continue working with this piece because we want to know exactly why the virus wants to tag specific proteins,” Roizman said. “The more we understand how it works, the more we can control it and manipulate it to work *for* us instead of against us.”