

Cancer

CURRENT RESEARCH AT THE SALK INSTITUTE

FALL 2009

AS DISEASES GO, CANCER IS THE ULTIMATE shape shifter. Thought to start with just a single mutation in the DNA of a single cell, it spawns generation after generation of quirky, out-of-control progeny whose genetic instability results in many additional mutations and wild proliferation leading to solid tumors and blood cancers.

Moreover, even when scientists discover drugs capable of reining in its destructive behavior, it tends to outsmart them by morphing, freeing itself to head once more down its renegade path.

Cancer is on course to overtake heart disease as the leading killer of Americans, but despite the urgent need to find new therapies and prevention strategies, scientists conducting cancer research are still working their way through a maze of unparalleled complexity. For one thing, cancer actually refers to more than 100 different diseases. All are fundamentally disorders of tissue growth regulation, and nearly all are caused by abnormalities in the affected cells' genetic material. Yet that inherent heterogeneity means that researchers continually are racing to keep up with a collection of especially dynamic and perplexing adversaries.

In 1971, President Richard M. Nixon signed the National Cancer Act, dramatically increasing funding for cancer research and launching "our great crusade against cancer [which] should be a cause for new hope among people everywhere." Throughout the intervening decades, Salk Institute researchers have been at the front lines of that fight. Their studies of the diverse genetic mutations that drive the development of individual cancers have resulted in important contributions that have increased understanding and helped change the landscape for cancer treatment.

The following pages summarize some of their most recent discoveries.

The Salk NCI Cancer Center

The Salk Institute's Cancer Center, a National Cancer Institute–designated basic research center, was established in 1970 by **Jonas Salk**. The first NCI Cancer Center Support Grant was awarded in 1973, with Nobel Prize winner **Robert Holley** as director. Today, under the leadership of **Tony Hunter**, researchers in the Cancer Center probe the fundamental aspects of cancer biology, with the ultimate goal of reducing incidence, morbidity, and mortality.

Stemming from a philosophy that basic research has the power to illuminate underlying causes of cancer, often in unexpected ways, the center's research is divided into three programs: Metabolism and Cancer, Mouse Models and Stem Cells, and Growth Control and Genomic Stability. The Salk Cancer Center, which currently includes 30 faculty members, 161 postdoctoral researchers, 70 graduate students and 105 research assistants, comprises more than half the research at the Salk Institute.

Among eminent Salk faculty who have made seminal contributions to the field is Nobel Laureate **Renato Dulbecco**, who discovered that tumor viruses cause cancer by inserting their own genes into the chromosomes of infected cells. This first clue to the genetic nature of cancer revolutionized how scientists think about the disease.



Going viral

WHEN IT COMES TO CANCER prevention, the tumor suppressor p53 is something of a jack-of-all-trades. It can activate repair proteins when a cell's DNA has sustained damage. It can stop the cell's cycle of growth and division to give the DNA repair proteins time to fix the damage. And it can order the cell to commit suicide, or apoptosis, if the DNA damage proves to be irreparable.

Clodagh O'Shea and her team are capitalizing on the fact that cancer cells, like many viruses, need to get p53 out of the way to proliferate successfully. They are developing viruses that unerringly home in on p53-deficient cancer cells throughout the body and implode them from the inside. Such oncolytic viruses promise a novel and potentially self-perpetuating cancer therapy: Each time a virus infects a cancer cell and multiplies, the virus ultimately will burst the cancer cell open to release thousands of viral offspring. The next generation

then will seek out remaining tumor cells and distant micro-metastases but leave normal cells unharmed.

In clinical trials, however, the first generation of oncolytic viruses did not produce the expected patient responses. When O'Shea followed up, she discovered a novel viral factor that neutralizes p53's action and that had been overlooked. This same viral protein subverts cellular targets that are also disrupted by tumor mutations in acute promyelocytic leukemia and thyroid cancer. She and her team are now exploiting this new viral protein as a powerful tool to both pinpoint and connect critical new targets in the cellular p53 tumor suppressor network and to develop the next generation of oncolytic viruses.

Glossary

Apoptosis—The mechanism by which old or damaged cells normally self-destruct. Apoptosis, which is the body's normal way of getting rid of unneeded or abnormal cells, may be blocked in cancer cells.

Cell cycle—The process a cell goes through each time it divides.

DNA repair gene—A gene that codes for proteins whose normal function is to correct errors arising when cells duplicate their DNA prior to cell division.

Histone—A type of protein found in chromosomes. Histones bind to DNA, help give chromosomes their shape, and help control the activity of genes.

Invasion—The direct migration and penetration by cancer cells into neighboring tissues.

Metastasis—The ability of cancer cells to penetrate into lymphatic and blood vessels, circulate through the bloodstream, and then invade normal tissues elsewhere in the body.

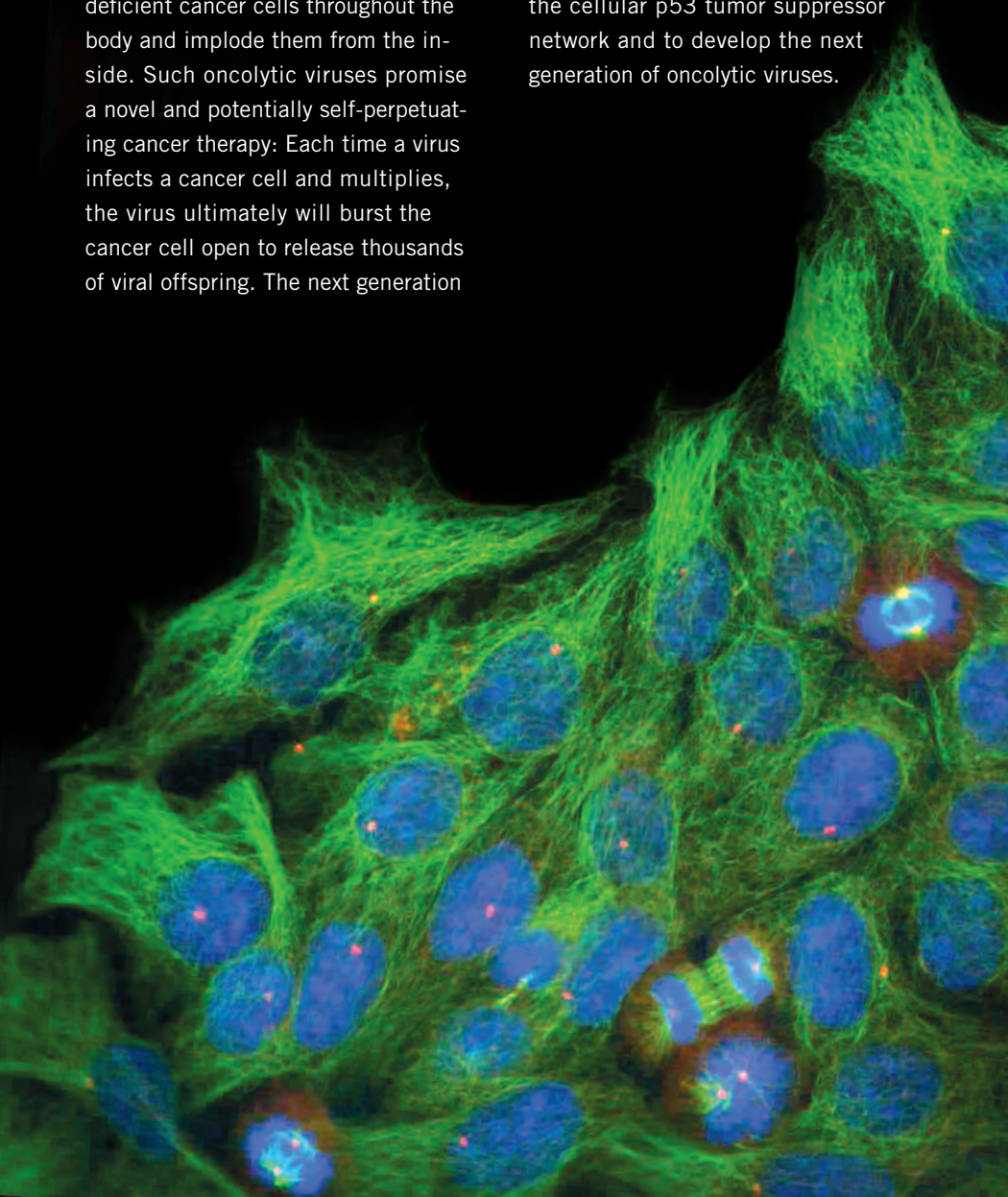
Mutation—Any change in a cell's DNA. Mutations may be caused by errors during cell division or by exposure to DNA-damaging agents in the environment.

Oncogene—A mutated form of a gene involved in normal cell growth. Oncogenes can contribute to the development of cancer by instructing cells to make proteins that stimulate excessive cell growth and division.

Oncolytic virus—A type of virus that infects and breaks down cancer cells but not normal cells. Oncolytic viruses can occur naturally or can be made in the laboratory by changing other viruses.

Signaling pathway—A group of molecules in a cell that work together to control one or more cell functions, such as cell division or cell death. Abnormal activation of signaling pathways can lead to cancer.

Tumor suppressor gene—A family of normal genes that instruct cells to produce proteins that restrain cell growth and division, much like the brakes in a car. Their loss allows a cell to grow and divide uncontrollably.



Enlisting the fly on the wall

THE MASTERMINDS BEHIND THE SCI-FI classic *The Fly* may have been on to more than they realized. Fruit flies and humans actually share most of their genes, including 70% of all known human disease

genes. Taking advantage of this remarkable evolutionary conservation, researchers in the laboratory of **John Thomas** have transformed the fruit fly into a laboratory model for an innovative study of gliomas, the most common and malignant brain tumors. About 77% of malignant brain tumors are gliomas, and their prognosis is usually bleak.

Gliomas seem to have one thing in common: Most, if not all, carry mutations that activate two particular signaling pathways. These mutations are also thought to play a key role in developing drug resistance. Because fruit flies possess genes that correspond to many human genes, including the

two genes in question, a team of scientists led by postdoctoral researcher **Renee Read** developed the *Drosophila* model to figure out how these genes regulate brain tumor pathogenesis and to discover new ways to attack the tumors. When the researchers activated both signaling pathways in genetically engineered fruit flies, they found that the pathways gave rise to rapidly dividing, invasive cells that created tumor-like growths in the fly brain, mimicking the human disease.

The Salk investigators are now using their fly model to search for genes and drugs that might block brain tumors associated with these pathways.

Double duty

ORDINARILY, THE PROTEINS KNOWN AS NUP98, NUP214, and NUP88 are among the 30 gene products serving as the bricks and mortar of nuclear pore complexes, the communication channels that regulate the passage of molecules to and from a cell's nucleus. But recent research in the laboratory of **Martin Hetzer** suggests that the trio also moonlights at a second job. And like honor students who secretly commit acts of vandalism, they sometimes stray from the straight and narrow to play a role in cancer as well.

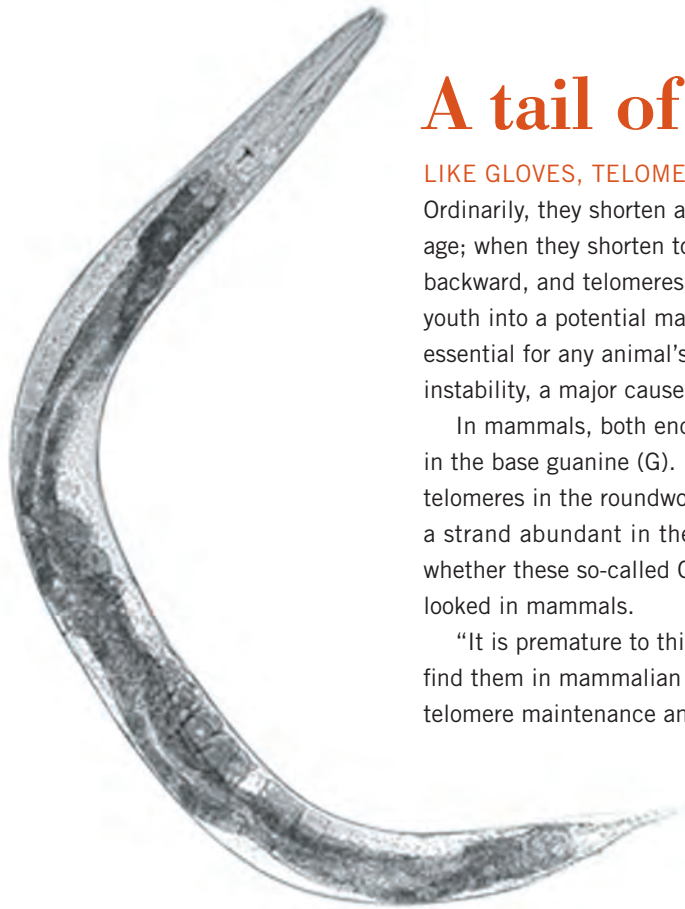
For more than a decade, scientists have known that when NUP98 abnormally fuses with certain proteins that regulate gene

expression, the marriage causes leukemia. Moreover, in many cancers, NUP214 and NUP88 are misregulated and in particular are associated with very aggressive forms of lung cancer.

Investigators have long questioned why these components of the cell's transport channel are implicated in cancer and have theorized that the connection relates to a problem in the conveyance of molecules in and out of the nucleus. But Hetzer offers a different explanation. He and his team believe these proteins also function as a new class of gene transcription regulators, which turn specific genes on and off during cell differentiation or tissue development.

“We think they are not only part of the transport channels but play a role in the organization of the genome and a very direct role in gene expression.” ~Martin Hetzer





A tail of two nucleotides

LIKE GLOVES, TELOMERES PROTECT THE ENDS OF CHROMOSOMES FROM DAMAGE.

Ordinarily, they shorten as cells divide, acting as a kind of cellular clock ticking down a cell's age; when they shorten to a critical point, the cell dies. In cancer, however, the clock runs backward, and telomeres aberrantly elongate, turning what could be a cellular fountain of youth into a potential malignancy. Therefore, safeguarding the ends of linear chromosomes is essential for any animal's survival. "Telomere loss can lead to a condition known as genome instability, a major cause of cancer," explains **Jan Karlseder**.

In mammals, both ends of every chromosome normally terminate with a string of DNA rich in the base guanine (G). Researchers in Karlseder's laboratory, however, have discovered that telomeres in the roundworm *C. elegans*, unlike those in mammals, sometimes also terminate in a strand abundant in the base cytosine (C). An obvious question emerging from the study is whether these so-called C-tails are unique to worms or whether they have simply been overlooked in mammals.

"It is premature to think that C-tails do not exist in human cells," says Karlseder. "We may find them in mammalian cells under certain circumstances, and if so, they could play a role in telomere maintenance and in cancer."

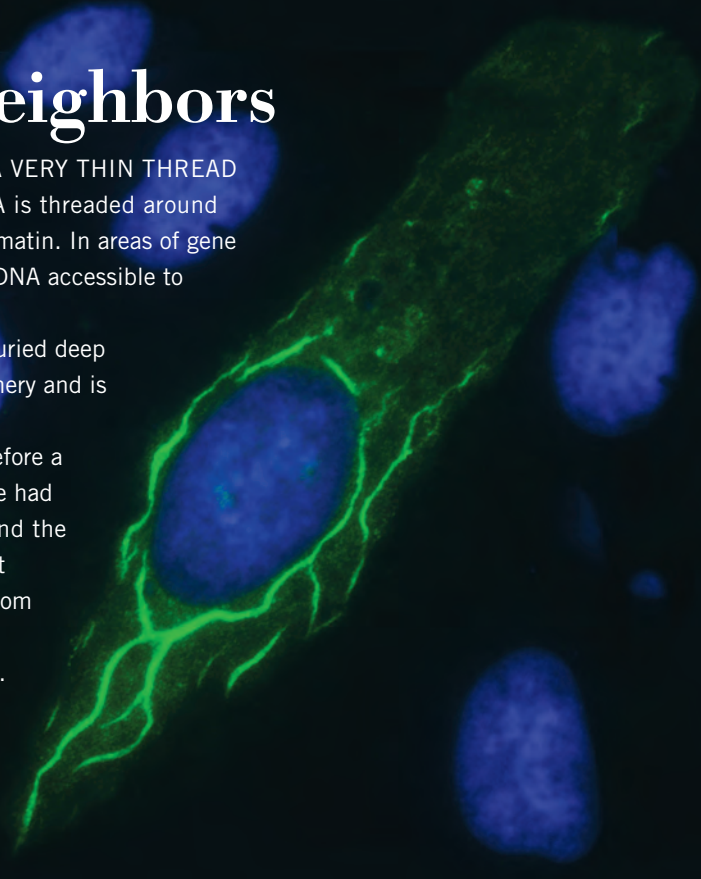
Good fences make good neighbors

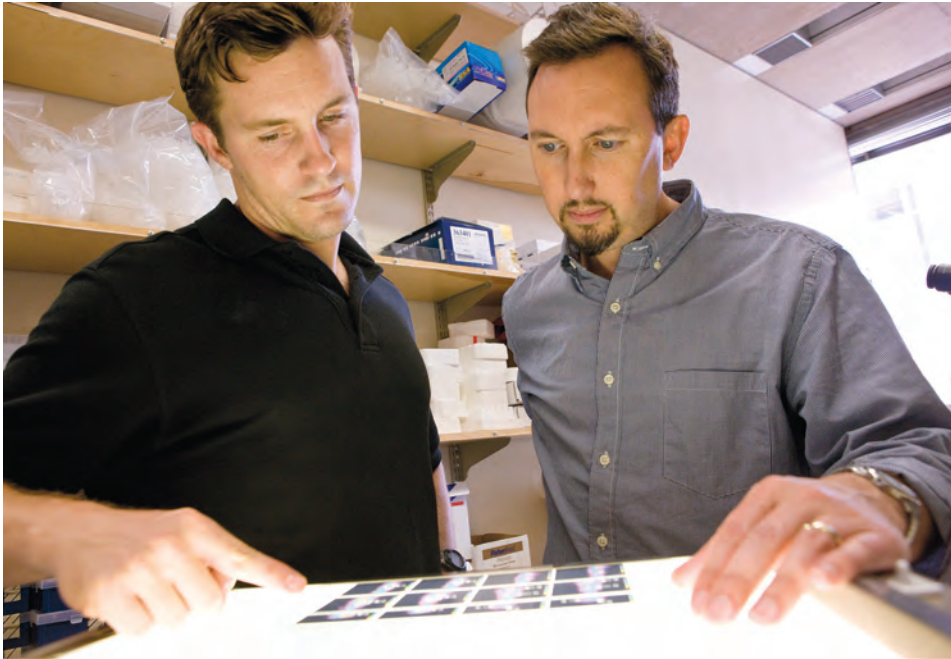
IF STRETCHED OUT, THE DNA OF A SINGLE HUMAN CELL WOULD FORM A VERY THIN THREAD about 6 feet long. To fit such a long molecule inside a cell's nucleus, the DNA is threaded around histone proteins and coiled up in a highly condensed structure called heterochromatin. In areas of gene activity, the tightly packed heterochromatin is unfurled just enough to make the DNA accessible to regulatory proteins.

In many different types of cancers, however, the tumor suppressor p16 gets buried deep inside heterochromatin. As a result, it cannot be read by the transcription machinery and is unable keep watch over cell growth.

Researchers had known for a long time that p16 is frequently silenced long before a cell turns cancerous, yet why that particular stretch of DNA becomes inaccessible had remained a mystery. A team in the laboratory of **Beverly Emerson** has now found the answer. Postdoctoral researcher **Michael Witcher** discovered a DNA sequence that forms the centerpiece of the molecular fence posts separating heterochromatin from the rest of the genome. "We found that several fence posts are lost in numerous types of cancer cells, leading to the collapse of the molecular boundary," he says. "Once the boundary was gone, the adjacent heterochromatin encroached and silenced the nearest gene."

"For a really long time people have been trying to understand how tumor suppressor genes get silenced in cancer," he adds. "Now that we have figured out one of the key events that leads to their inactivation, we might be able to exploit this mechanism to develop novel therapies."





Building a better mouse model

THE LOWLY MOUSE HAS LONG PROVIDED researchers with valuable insights about cancer. But the most commonly used technique for producing a cancer mouse model—transplanting human tumor tissue or cancer cell lines in immunocompromised mice—ignores the role of the immune system in cancer. Other animal models either express oncogenes in a tissue-specific manner or shut down the expression of tumor suppressor genes in the whole tissue. But tumors generally develop from a single cell or a small number of cells of a specific cell type.

To create a better mouse model, researchers in the laboratory of **Inder Verma** turned to gene therapy techniques, using modified viruses to infect cells and ferry activated oncogenes into a small number of cells in adult, fully immunocompetent mice. After initial experiments confirmed that the approach was working, postdoctoral researcher **Tomotoshi Marumoto** injected viruses carrying two well-known oncogenes into three brain regions of mice lacking one copy of the gene encoding the tumor suppressor p53. He specifically targeted astrocytes, star-shaped support cells that are suspected of being the source of glioblastoma, the most common and deadly human brain cancer. Within a few months, massive tumors that displayed all the histological characteristics of glioblastoma developed in two of the regions.

To test whether the induced glioblastomas contained cancer stem cells, Marumoto isolated and cultured individual tumor cells in the lab, which looked and behaved just like neural stem cells. Less than 100 and as few as 10 cells were enough to initiate a tumor when injected into immunodeficient mice. “These findings show that our cancer model will not only allow us to start understanding the biology of glioblastoma but will also allow us to answer many questions surrounding cancer stem cells,” says Verma. He and his team are currently using this methodology to investigate lung, pancreatic, and pituitary cancers.

Sugar rush

PEOPLE WHO SUFFER FROM PEUTZ-JEGHERS SYNDROME, A rare inherited cancer syndrome caused by a mutation in the tumor suppressor LKB1, develop gastrointestinal polyps and are predisposed to colon cancer and other tumor types. Currently there is no treatment for Peutz-Jeghers; patients must undergo continual surgeries to remove the polyps and tumors as they arise. The LKB1 gene is also mutated in 20 percent of cervical carcinomas and 30 percent of non-small cell lung carcinoma, one of the world’s most widespread and lethal cancers.

During earlier work, **Reuben Shaw** had discovered that LKB1 operates via the mTOR pathway, short for “mammalian target of rapamycin.” Since a loss of LKB1 results in a hyperactive mTOR signal, **David Shackelford**, a postdoctoral researcher in Shaw’s lab, wondered whether rapamycin, a powerful immunosuppressant that binds and inactivates mTOR, could be used as a drug to treat the tumors that arise as a result of Peutz-Jeghers. When he used rapamycin to treat mice that had intestinal polyps because of an LKB1 mutation, their polyps shrank and in most cases disappeared altogether.

He then asked whether he could visualize the drug’s effectiveness using a technique called FDG-PET, which reveals the uptake of radioactively labeled glucose into cells. Normally, heart cells are the most ravenous consumers of glucose, but in patients with cancer, tumors light up. Most people assumed that polyps weren’t far enough along on the road to malignancy to be visible on an FDG-PET scan, but Shackelford’s experiment revealed that the LKB1 mutation resulted in altered glucose metabolism in cells and tumors, allowing even benign LKB1 polyps to be clearly visible.

“This result really could change the life of people with Peutz-Jeghers syndrome.” ~ Reuben Shaw

Balancing act

AS A POWERFUL TUMOR SUPPRESSOR, p53 turns on genes that either halt cell division to allow time for repair of damaged DNA or, when all rescue attempts prove futile, prevent cells with genetic defects from dividing, as this would fuel the development of cancer. Consequently, before any tumor cell can start proliferating willfully, it needs to escape from p53's iron grip.

Under normal circumstances the two related proteins Mdm2 and Mdmx are part of a system of checks and balances that keep p53 from wreaking havoc in healthy cells even as it maintains a tight lid on unchecked cell growth. In an earlier study, **Geoffrey Wahl** and his team discovered that the two proteins cooperate to

prevent p53 from being activated. But how p53 shakes off its negative regulators when cells experience one of the myriad stresses that activate it has been the topic of much discussion.

Recently, researchers in Wahl's lab sought to ascertain the mechanisms behind p53 activation. What they discovered was that just slightly increasing the amount of available Mdmx made mice remarkably resistant to the harmful effects of radiation but very susceptible to the development of oncogene-induced lymphomas.

"Our experiments emphasize how subtle and precarious the balance is," says postdoctoral researcher **Yunyan V. Wang**. "A slight shift of balance and

the mice survive the equivalent of Chernobyl but are in big trouble when an oncogene is activated."

Their findings could explain why some tumors don't respond to radiation or chemotherapy and provide novel routes for the development of new anti-cancer therapies.

Marshaling first responders

MATTHEW WEITZMAN STUDIES THE cell machinery that responds to the DNA damage cells sustain as a result of viral infection. Because viruses deliver DNA into the cell nucleus and have developed many different, and ingenious, ways of tricking the cellular alert system, they are excellent models for understanding the same process of DNA damage recognition and repair that goes awry in cancer, as well as determining how to prevent cells from turning malignant in the first place.

In a recent study, Weitzman and his group discovered that when adenovirus invades a cell, it brings along extra muscle: a protein charged with immobilizing the cellular proteins that sound the alarm when DNA needs repairing. Herding the proteins into abnormal clusters, it ensures that the signaling cascades ordinarily activated when the cell's DNA is damaged are blocked, and the cell cannot respond when it needs to repair its DNA. The virus thus gives

itself carte blanche to continue replicating and running amok.

"It is essential that our cells can recognize and respond to DNA damage," Weitzman says.

"If damage is not repaired correctly, then mistakes are incorporated into our DNA, and these are the initial triggers for the process of transformation in cancer."

This issue of *From the Bench* is part of a series of updates on key areas of scientific research conducted at the Salk Institute for Biological Studies. Our goal is to keep you informed of Salk researchers' most recent findings in areas such as stem cells and regeneration, vision, plant biology, neuroscience, behavior, and more. We are very interested in your feedback regarding this update.

For more information, or to share your comments, please contact Judy Hodges at the Institute's Office of Development at 858.453.4100 x1882 or email hodges@salk.edu.

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