

had been contaminated by plutonium, the 64,000 villagers drank its water, washed their clothes in it, and bathed in it for decades. Among other nuclear accidents at the plant, 217 villages of 272,000 inhabitants were also exposed to 2 million curies of radiation released when a liquid-waste storage tank blew up in 1957. Unlike any other region in the world, at least 400,000 people have been continuously exposed to both external radiation, the gamma rays deposited throughout the area, and internal radiation, the strontium-90 and cesium-137 absorbed from drinking water and contaminated vegetables, according to a February article in *Science*.

Soviet scientists carefully studied the villagers for three decades. Soviet secrecy, however, prevented any results from becoming public; even the villagers were never told why they were being examined. But in early January, a team of radiation biologists from the United States, Europe, and Japan traveled to the city of Chelyabinsk, home of the long-secret nuclear facility Chelyabinsk-65 and its Mayak plutonium production plant, to meet their Russian counterparts and take a look at the research for the first time. Such data represent the only known studies in the world on long-term, low-dose radiation exposure; studies in Hiroshima and Nagasaki, in contrast, were based on short-term, high-dose exposure.

"The Russian scientists have carried out some unique studies, including the only reliable research on the long-term effects of plutonium exposure," writes Michael Balter in his article in *Science*. One epidemiological study of 28,000 Techa River villagers "found a statistically significant increase in leukemia incidence, as well as an overall increase in cancer mortality, compared to control populations that did

not live in the contaminated zone. Still, the leukemia risk per unit of radiation dose was at least two times smaller than that of the atomic bomb survivors," he says.

Over the years, several local physicians had tried to gain access to the data being collected on their patients by the Institute of Biophysics Branch Number Four. According to Diahanna Lynch, coordinator of the Russian Environment and Energy Project at the Natural Resources Defense Council, Russian doctor Gulfarida Galimova threatened to prevent the institute's researchers from continuing to examine her patients if they did not provide more information on their condition. In 1993, the researchers gave her a list of 285 patients diagnosed with chronic radiation sickness in her village of Muslyumova, 50 miles downstream on the Techa River from Chelyabinsk-65.

"In 1993, Dr. Galimova determined that of the more than 4,000 residents in the village, about 3,000 were examined by the institute," says Lynch. "Of these, she says, 92% had some kind of chronic illness, ranging from circulatory problems to birth defects such as missing kidneys. Dr. Galimova has also been a local activist in the Chelyabinsk Movement for Nuclear Safety, encouraging people to lobby the government to resettle the village in a cleaner area, and to demand compensation for the damage to the villagers," said Lynch.

Traces of plutonium have been found in the organs and tissues of the villagers and local animals, according to a recent article in *Surviving Together*, published by the environmental organization ISAR (formerly the Institute for Soviet-American Relations), in Washington, DC. In addition, an article distributed by the Japanese Kyodo News Service after the January

1995 meeting in Chelyabinsk reported that villagers along the Techa River have more lymphatic genetic mutations than people who suffered radiation from the atomic bombing of Hiroshima. Scientists also discovered a buildup of strontium-90 and other radioactive isotopes in the livers and in other organs of the local residents, as well as an increasing incidence of mutations of the gene responsible for T-cell antigen receptors in lymphocytes in peripheral blood, according to the article.

In January, President Boris Yeltsin's former environment adviser, Alexei Yablokov, now in charge of environmental matters for the country's top policy-making body, the Security Council, warned that radiation from the Chelyabinsk site could ultimately spread to the North Pole. He said that radioactive groundwater was now contaminating the Tobol River, which feeds into the Ob River system. The Ob system empties into the Barents Sea, which flows toward the North Pole. He also said that total radiation around Chelyabinsk-65 is 22 times the radiation released in the 1986 explosion at the Chernobyl nuclear reactor in Ukraine. Although the Mayak facility's five industrial uranium-graphite reactors have been shut down, the plant is still used for reprocessing spent fuel.

As the Cell Cycles

Scientists have known for decades that exposure to certain environmental agents can lead to cancer, and many have suspected that this occurs through the alteration of cell cycle controls. Until recently, however, not enough was known about the molecular basis of cell growth and division to understand the specific pathways by which such agents could alter cell growth in a way that leads to cancer. In the last few years, a large number of specific control points in the cell cycle have been identified, as have the individual genes and proteins that regulate these checkpoints. Researchers have observed that alteration of such controls can disrupt normal cell cycle regulation, but the mechanisms by which chemical treatment or exposure affects these critical functions are largely unknown. Recent research in this area, however, has shed some light on how environmental agents and external cell signals affect cell cycle regulation.

All eukaryotes, from yeast to humans, share many features in the process of cell division. Cells that are actively growing and dividing pass through four stages: G_1 (gap), followed by the S-phase in which the chromosomal DNA replicates, G_2 , and finally M (mitosis), in which the chromosomes move to opposite ends of the cell



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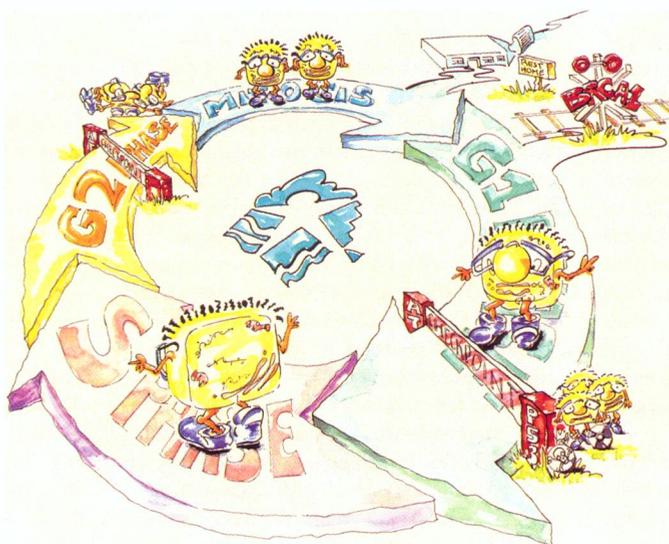
Downriver risk. A family in Muslyumovo grows vegetables on the banks of the contaminated Techa river, 50 miles from Chelyabinsk-65.

and the cell then divides. Research has recently indicated that the transitions between cell cycle states are regulated at checkpoints by a family of protein kinases, the cyclin-dependent kinases (CDKs), and their activating partners, the cyclins.

One of the most important checkpoints is START in late G₁, at which the cell commits itself to another round of DNA replication and at which both positive and negative signals are integrated into the cell cycle. Many checkpoints are deregulated in oncogenesis, and this is often due to changes in cyclin-CDK complexes. In particular, the deregulation of START may allow cell growth and division to become insensitive to external cues. Research has shown that this insensitivity can be a consequence of either the aberrant expression of positive regulators, such as the cyclins, or the loss of negative regulators, such as the cyclin-dependent inhibitor proteins (CDIs). Another consequence of abnormal START checkpoint control is that cells can bypass the normal restriction on entry into the S-phase that is normally imposed by damaged DNA, and this may allow the cells to replicate unrepaired mutations and thus accumulate genetic changes that contribute to carcinogenesis.

Much current research is focused on identifying factors internal to the cell nucleus that regulate cell growth, and how the over- or underexpression of those factors perturbs the cell cycle. At the NIEHS, Richard Paules heads a growth control and cancer group that is conducting *in vitro* studies on mouse and human cells. By overexpressing an oncoprotein, called *mos*, that can affect the *ras/raf*/MAP (mitogen activated protein) kinase pathway in mouse fibroblasts, Paules's group has observed that cells cannot exit G₁ and go into resting mode. Rather, the cells are pushed by abnormally high levels of cyclin A and the cell division cycle gene, *CDC2*, and become unstable and thus vulnerable to further genomic alteration that can lead to uncontrolled growth. Similar studies are underway with the MAP kinase (*MEK1*) and *v-Ha-ras* transformed mouse fibroblasts.

Paules's team, in collaboration with William K. Kaufmann of the University of North Carolina-Chapel Hill School of Medicine, is also investigating checkpoint responses to the kind of damage that may result from exposure to environmental toxicants. Previous research has shown that a lack of the p53 tumor-suppressor gene can



The Cell Cycle

lead to genomic instability. Paules's team has shown that one consequence of this may be the loss of the G₂ checkpoint function. G₂ provides a protective delay, preventing entry into mitosis when there is DNA damage. Without this checkpoint, cells are vulnerable to the chromosomal aberrations frequently seen in cancers.

"We are very excited about the possibility of understanding the molecular consequences of exposure to a variety of environmental agents that impact normal cell cycle control," Paules said. "The hope for the future would be to develop intelligent approaches for early detection and better chemotherapeutic strategies exploiting these pathways."

Other researchers are examining chemical interactions with the cell cycle. Thomas Goldsworthy and his colleagues at the Chemical Industry Institute of Toxicology have teamed up with NIEHS researchers George Lucier and Robert Maronpot to examine pathways by which certain environmental agents affect the cell cycle. Goldsworthy's team is particularly interested in how nongenotoxic, carcinogenic agents affect the cell cycle.

"We know that genotoxic agents can cause direct mutation of some of the key cell cycle regulators, but we also believe that nongenotoxic agents can indirectly lead to these changes," said Goldsworthy. "Our hypothesis is that exposure to certain chemicals can cause aberrant expression of certain genes, such as the p53 tumor suppressor, which in turn prompts certain cell cycle events. Once you have an altered response to the growth signals, that can lead to cell cycle dysregulation. This can allow the cell to proceed to DNA synthesis and replication without repairing any DNA damage, and that in turn leads to altered growth, genomic instability, and

the accumulation of DNA mutations—the hallmarks of cancer."

Goldsworthy has investigated unleaded gasoline and its mechanism of carcinogenesis in mouse liver and observed that precancerous cells exposed to gasoline lose their response to inhibitory growth factors and exhibit aberrant growth. The challenge now is to understand the dose and species susceptibility to these processes. "Although specific cell cycle genes may not be identical between mice and humans, we can say that the processes for controlling cell growth are similar, and certain chemicals, such as gasoline, do appear to affect these processes," Goldsworthy says. "Are these the

critical changes that result in cancer? We don't know."

Goldsworthy's work with Maronpot is focusing on identifying the growth factors and oncogenes that are involved in chemically induced mouse liver neoplasms and relating those changes to cell proliferation and cell death. Chemicals being studied are mainly agents shown not to directly interact with DNA, including chlorinated hydrocarbons, furan, and phenobarbital. The team has identified a number of novel genes that have the potential to affect the regulation of the cell cycle and appear to be involved in mouse hepatocarcinogenesis.

Goldsworthy and Lucier have teamed up to study receptor-mediated carcinogenesis, particularly in response to dioxin exposure. The team has been examining dose-response effects, hormonal effects on receptor binding, gene expression, cell growth, and the induction of liver cancers.

"I believe this is the future of toxicology," says Goldsworthy of research examining the interaction of environmental agents and cell cycle controls. "We've been characterizing the cancer process with respect to altered cell growth, but we don't really understand the exact interaction between the chemical and the growth process and its role in inducing the cancer. The tools are now available to really understand the interactions of chemicals with the critical cellular and molecular components of the cell cycle, which will lead to better species extrapolations and, ultimately, improved risk assessment."

Richard Paules/Elizabeth West