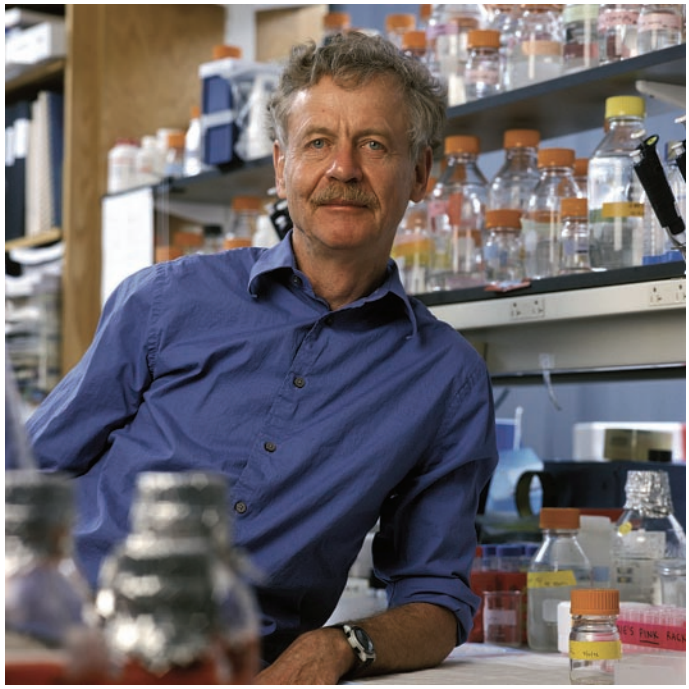
Biography of Rudolf Jaenisch



Rudolf Jaenisch

Genetics research often concentrates on identifying minute differences in the genome that give rise to assorted phenotypes. However, a large part of genetic control lies not in the makeup of particular genes or intergeneic regions but in their epigenetic conformation. The modification of DNA or of chromatin can profoundly affect gene expression without causing mutations. Rudolf Jaenisch, a member of the Whitehead Institute and Professor of Biology at Massachusetts Institute of Technology (Cambridge, MA), has made enormous contributions to the understanding of epigenetic mechanisms. Jaenisch created the first transgenic mice that enabled researchers to study epigenetic control of genomic viral DNA and advanced the epigenetics field through the studies of knockout mice and, most recently, cloned mice.

Jaenisch's work has earned him numerous awards and recognition, including the first Peter Gruber prize in Genetics (2001), the Robert Koch Prize for Excellence in Scientific Achievement (2002), and the Charles Rodolphe Bruphacher Foundation Cancer Award (2003). In 2003, Jaenisch was elected to the National Academy of Sciences. In his Inaugural Article (1), published in this issue of PNAS, Jaenisch and his colleagues show that stem cells derived from cloned embryonic carcinoma cells do not have the potential to differentiate beyond additional embryonic carcinoma cells. The article stands in stark contrast to his team's recently published research involving melanoma cells (2). These findings suggest that, although genetic factors exclusively may control the phenotype of certain cancers like embryonic carcinoma, epigenetic alterations may play a crucial role in other cancer types such as melanoma.

Independent Research

Jaenisch was born in 1942 in Germany into a family where medical careers were a tradition—both his father and grandfather were physicians. Thus, Jaenisch's enrollment in medical school at the University of Munich came as no particular surprise to his family. However, after taking basic science, anatomy, and physiology classes, Jaenisch felt his concentration waning. "By studying medicine, I really just lost interest," he said. "I didn't like how it was taught. I didn't like the whole environment."

Seeking a change, Jaenisch joined the Max Planck Institute of Biochemistry in Martinsried (Germany) to conduct experimental research while continuing his medical studies. For the first time, he considered research instead of medical practice for his future career. "I didn't take medical school very seriously after that. I didn't go to class anymore. I learned all from books for my exams, and I really worked only in the lab," he said. Although the medical programs at that time required only a relatively simple thesis, Jaenisch went beyond the requisites of undemanding library research or laboratory experiments by studying bacteriophages. In the 1960s, bacteriophages had become important experimental tools for molecular biology. One of the leading German laboratories in this fledgling field was that of P. H. Hofschneider, whose research focused on the *Escherichia coli* phages ΦX 174 and M13. Jaenisch joined Hofschneider's group as a medical student and performed his thesis work on phage replication and expression (3). Jaenisch graduated in 1967 with his M.D.

After completing 2 additional years of experimental work at the Max Planck Institute and clinical training at the University of Munich, Jaenisch chose to pursue postdoctoral training in the United States. After searching for a suitable mentor, he chose geneticist Arnold Levine, who had recently set up his first laboratory at Princeton University (Princeton, NJ). Jaenisch admired Levine's previous work on bacteriophage genetics, which was similar to his own thesis work, and also was captivated by Levine's latest line of research using animal tumor viruses to study cancer. Jaenisch corresponded with Levine about his interests; in 1970, Jaenisch became Levine's first postdoctoral fellow.

In Levine's laboratory, Jaenisch began his postdoctoral study of mammalian cells infected with simian virus 40 (SV40), a DNA tumor virus. Levine's inventive and clever guidance motivated Jaenisch. However, Jaenisch recalls that this time of chaperoned intellectual growth was short-lived. After only 2 months, Levine announced that he would be leaving Jaenisch on his own for several months: "He told me that he was going on sabbatical to Europe and that I should run the lab."

With the help of Levine's graduate students, Jaenisch continued research on the replication of SV40 (4). During this period, his interests began to take a different turn. After stumbling upon a article by developmental geneticist Beatrice Mintz at the Fox Chase Cancer Center

This is a Biography of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 13985.

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(Philadelphia, PA) on generating chimeric mice (5), Jaenisch wondered whether working with early mouse embryos might help resolve a phenomenon he had puzzled over in his own research. When Jaenisch injected adult mice with SV40 they contracted sarcoma, a type of cancer that arises only in supporting tissues like bone, muscles, cartilage, or fat. Why, Jaenisch wondered, didn't the mice get another form of cancer, such as liver cancer?

Jaenisch hypothesized two answers: either SV40 could not infect liver cells, or liver cells somehow turned off viral DNA after infection. Injecting SV40 into an early embryo theoretically would introduce the virus into all cells of the resulting mouse, showing definitively whether the virus can transform only mesenchymal cells, such as fibroblasts, or other cell types, such as liver cells.

"Hot" Discovery

Jaenisch was eager to pursue his new idea but was unable to discuss the experiment with his mentor, who was on sabbatical. Instead, Jaenisch drove from Princeton to visit Mintz in Philadelphia, and he shared his proposal with her. "She was very friendly but somewhat skeptical," he recalls. Mintz's doubts about whether the experiment would succeed temporarily dampened his enthusiasm: "I thought, maybe I don't want to do this experiment yet, maybe I don't want to do it at all." Jaenisch lacked the equipment and expertise to perform the entire experiment in Levine's laboratory, so he contacted other laboratories to gauge their interest in his idea. After securing space at another laboratory, he received a call from Mintz: "She said, 'Listen, I've decided that you can come here and do this.' I was really ecstatic." Soon after, Levine returned from sabbatical and generously gave Jaenisch permission to perform part of the work in his laboratory.

After isolating SV40 DNA at Levine's laboratory, Jaenisch took the supply with him to Mintz's laboratory. Mintz directed one of the leading laboratories in developmental biology and tutored him on isolating and culturing early mouse embryos. "Mintz is an impressive scientist," Jaenisch notes, "with deep insights into the biology of the mammalian embryo. To have been introduced by her into mouse developmental genetics has been one of the most important experiences in my career." He began injecting embryos with SV40 DNA, implanting them in surrogate mothers, and allowing them to develop. As the mice were born and grew into adults, Jaenisch was disappointed: "The mice didn't get a tumor. Nothing happened to them. They were totally normal, so I didn't know whether viral integration had occurred into the embryos and what to do with them." Because Southern blotting had not yet been developed, he was unable to view the genomic DNA to determine whether the SV40 genome had been incorporated into the mouse genome. Frustrated with his efforts, Jaenisch temporarily put the experiment on hold.

In the meantime, he received an offer to continue his research at the Salk Institute in La Jolla, CA. After setting up his laboratory there, Jaenisch found himself surrounded by knowledgeable colleagues with new ideas on how to resolve his experiment. One colleague, geneticist Paul Berg (at Stanford University in Stanford, CA), advised Jaenisch to use "hot," or radioactive, DNA as a probe for SV40. Berg had pioneered the technique of "nick translation" for use in DNA hybridization experiments. After several months of developing experiments on mice with hot DNA, Jaenisch was ecstatic to discover that the virus had indeed become incorporated into the genome of the mice (6), making them the first retrovirus-mediated transgenic mice, although the term "transgenic" had not yet been coined.

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Inevitably, his success led him to wonder whether inserted transgenes could be passed on through the germ line. By using a different system, the Moloney leukemia virus, Jaenisch began infecting early mouse embryos as he had done with SV40. As successive generations of mice contracted leukemia, he concluded that the answer to his question was a definitive yes (7).

To Germany and Back

Despite his numerous successes in creating transgenic mice, a question continued to plague Jaenisch: Why were mice infected with SV40 as embryos free of tumors but wild-type mice exposed to the virus later in life developed cancer? He and his colleagues surmised that the answer was not in the gene sequence but rather in the modification of DNA and the effects that other molecules in a cell exert on DNA without causing mutations. This control, know as "epigenetics," has formed the basis of much of Jaenisch's later research.

Jaenisch's work at Salk caught the eye of the Director of the Heinrich Pette Institute in Hamburg, Germany, who offered him a job in 1977. Taking his experiments with him, he and his Hamburg colleagues continued to study genetics over the next 7 years by infecting mice with DNA viruses and retroviruses. He discovered that some inserted viruses disrupted gene activity in the early embryo, making the viruses and insertional mutagenesis a useful tool for studying development (8).

After using insertional mutagenesis to block collagen 1, which turned out to be a key gene necessary for embryo survival, Jaenisch was curious about the mechanisms at work. Subsequent experiments showed that epigenetics again played a part, silencing the gene's promoter by methylation (9). This discovery prompted Jaenisch to further explore the mechanism behind methylation. Upon accepting an offer to continue his research in the United States at the Whitehead Institute, he and his colleagues created knockout mice with a mutation in the methyltransferase gene, the gene that establishes and maintains DNA methylation (10). The mutant embryos displayed an intriguing phenotype: they all died extremely early in development. "It was the first proof that methylation is important for survival," he said. "Now we had a genetic tool to study epigenetics." He used the methyltransferase mutant mice to establish a causal relation between DNA methylation and cancer (11).

For more than a decade, Jaenisch used a host of tools, particularly homologous recombination, for creating knockouts to study epigenetic niches in cancer, brain function, and development. In 1997, he received an unexpected inspiration: Dolly, the cloned sheep, was created (12). A year later, the first cloned mouse, dubbed Cumulina, was born (13). Jaenisch immediately saw cloning as an important new tool to study epigenetics. "This is the ultimate method to use, because cloning is nothing but an epigenetic phenomenon," he explains. "Cloning is the most unbiased test of epigenetics. The problems of nuclear cloning are not genetic; rather, they are caused by faulty reprogramming of the epigenetic state of the genome."

Excited by the possibilities for this new tool, he and his colleagues began creating mouse clones through nuclear transfer. Jaenisch's team had many subsequent successes and derived cloned mice from terminally differentiated cells such as immune cells and neurons (14,

15). But Jaenisch found that most of his clones displayed abnormal phenotypes, such as widespread faulty gene expression (16): "I think all clones are abnormal. The ones that live longer are just less abnormal than the ones that die early." However, he asserts that therapeutic cloning holds significant promise in helping human patients. In 2002, he and his colleagues "cured" mice with severe combined immune deficiency by creating embryonic stem cells from each mouse's skin cells (17). Jaenisch's team allowed the cells to differentiate into bone marrow cells and then placed the cells back into the affected mice. The bone marrow cells subsequently produced immune cells, repairing the immune systems of the mice.

Cloning Cancer

Cloning remains an important tool in Jaenisch's laboratory. In recent work published in *Genes and Development* (2), he and his colleagues used cloning to answer a fundamental question: What part of a cancer cell phenotype can be reversed?

Although cancer typically starts with a mutation in an oncogene or tumor suppressor gene, previous research suggested that epigenetic factors may determine several elements of a cancer cell's phenotype. Because these epigenetic factors are not genetic mutations, Jaenisch's team wondered whether they might be reversible. After inserting the nucleus from a mouse melanoma cell into an enucleated egg, the researchers collected stem cells from the resulting embryo. They then incorporated these stem cells into healthy mouse blastocysts. Many of the blastocysts developed into healthy adult mice, which shows that the melanoma nucleus had been "reprogrammed" to direct development of normal tissues. In contrast, injecting a melanoma cell into an adult mouse creates only more melanoma cells. "This argues that the phenotype of the cancer cell is in large part determined by epigenetic changes. [These changes] are all reversible," Jaenisch explains. However, all of the mice ultimately developed melanomas, indicating that the genetic mutations that are an important determinant of cancer are permanent and irreversible.

For his PNAS Inaugural Article (1), Jaenisch's group performed a similar experiment in mice by using embryonal carcinoma cells, a type of cancer cell derived from embryonic tumors. He and his colleagues inserted the carcinoma cells into eggs and then collected the resulting stem cells once the eggs divided. In contrast to the group's previous findings using melanoma cells, however, Jaenisch's team discovered that the stem cells were unable to differentiate into assorted cell types when they were injected into healthy mouse blastocysts; instead, they could only divide to make additional embryonic carcinoma cells. These findings suggest that the developmental restrictions of these embryonal carcinoma cells, in contrast to the tested somatic melanoma, are attributable to genetic alterations. Although the tested somatic cancers have the potential to be reversible, embryonal cancers do not. Thus, embryonal carcinoma cells are at one end of the spectrum, whereas most cancers have genetic as well as epigenetic alterations, and the combination of both determines the malignant phenotype.

In addition to his current focus on cancer, Jaenisch plans to continue studying other issues, including factors involved in brain function and the genomic reprogramming necessary for therapeutic cloning. His team especially is interested in determining what factors make cloning inefficient and in improving the technique's effectiveness. Like the majority of his career, each of these efforts concentrates on understanding epigenetics. "[Epigenetics] is a mechanism which has only recently become interesting to people," Jaenisch muses, "but I have been interested in it for 20 years. It's an important part in the big picture of gene control."

Christen Brownlee, *Science Writer*

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