AUTHOR COMMENTARIES - 2009

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Rudolf Jaenisch

Featured *Science Watch*® Newsletter Interview

Genes have always received far more than the lion's share of credit for determining our physical characteristics—genotype determines phenotype, as the old biological cliché has it. But genes aren't everything. Brain, liver, heart, skin, and nerve cells, just to name a few, all have the same set of genes—the same DNA in the chromosomes—and yet entirely different structures and functions. So why does the DNA of one cell direct it to become a neuron, while the DNA of another might preordain a skin cell, or a liver cell, or any other cell type in the human body? This is the world of "epigenetic" gene regulation—the molecular signals outside the genome that determine which genes will be activated and which silenced during development and cell proliferation.

The science of epigenetics blossomed in the mid-1990s and has remained in full flower ever since. Epigenetic regulation is now known to play fundamental roles in everything from cancer to cloning to stem cells. Leading this epigenetic revolution is Rudolf Jaenisch of the Massachusetts Institute of Technology. In the last two years, Jaenisch has contributed to a baker's dozen Hot Papers on reprogramming fibroblasts into a pluripotent state comparable to embryonic stem cells—a distinction that earned him a spot in this issue's annual roundup of "hot" authors. And his prior citation record is no less impressive: a 1992 *Cell* paper on mutation of the methyltransferase gene, for example, has amassed over 1,600 citations, while his *Nature Genetics* review on the epigenetic regulation of gene expression has been cited more than 700 times in just six years (see the second below, paper #1). More recently, Jaenisch's 2007 *Nature* article on "*In vitro* reprogramming of fibroblasts into a pluripotent ES-cell-like state" has repeatedly appeared in the Biology Top Ten and now ranks at #9.

Jaenisch, 66, received his M.D. from the University of Munich in 1967. Over the next five years, he did postdoctoral research at the Max Planck Institute for Biochemistry in Munich, at Princeton University, and at the Fox Chase Cancer Research Center in Philadelphia. In 1972, he became an assistant research professor at the Salk Institute in La Jolla, and then in 1977 moved back to Germany to become head of the department of tumor virology at the Heinrich Pette Institute for Experimental Virology and Immunology in Hamburg. Since 1984, Jaenisch has been a professor of biology at MIT and a member of the Whitehead Institute for Biomedical Research.

Professor Jaenisch spoke to Science Watch from his office in Cambridge.

SW: You've been a driving force in modern epigenetic research since its earliest days. Can you tell us what prompted your move into the science of epigenetics?

It came from an odd observation in the 1970s, when I was interested in making what would turn out to be the first transgenic mice, although that term wasn't even invented yet. The idea was to use retroviruses to put some foreign genetic information into the germ line of mice. A group from the Wistar Institute had reported that when you do this, these viruses get activated. But when I infected embryos with retroviruses, I couldn't reproduce what they saw. In my lab, viruses could infect the embryos and integrate into the genome but they were silenced. Retroviruses—in this case, leukemia viruses—have very strong promoters. They are strongly expressed in somatic cells, in blood cells, in fibroblasts, but when they infect embryos they are not expressed at all—they seemed to get silenced. I was very puzzled by this. I also observed that if I put these viruses into early embryonic cells such as ES cells, they were not expressed. They did efficiently integrate into the genome, but became silenced. If I infected cells from later stages in development, the viruses expressed very highly. So what was the reason for this?

I then realized that as soon as these viruses integrate into the genome they become methylated in cytosine residues. This is what's called CpG methylation, a phenomenon that had just been discovered and was very new at the time. Methylation seemed to correlate with gene inactivity. If a gene was methylated, it was inactive. If it wasn't methylated, it was active. What we found was that as soon as a virus integrates into the genome it becomes methylated, but this happens only in the embryonic cell. In a somatic cell or a later cell, the viral DNA stays unmethylated and so it's expressed. We were the first to demonstrate this, and many people confirmed it.

Highly Cited Papers by Rudolf Jaenisch and Colleagues, Published Since 2003
(Ranked by total citations)

Rank	Papers	Cites
1	R. Jaenisch, A. Bird, **"Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals,"** *Nature Genetics*, 33: 245-54, 2003.	759
2	L.A. Boyer, *et al.*, **"Core transcriptional regulatory circuitry in human embryonic stem cells,"** *Cell*, 122(6): 947-56, 2005.	500
3	F. Gaudet, *et al.*, **"Induction of tumors in mice by genomic hypomethylation,"** *Science*, 300(5618): 489-92, 2003.	375
4	B.E. Bernstein, *et al.*, **"A bivalent chromatic structure marks key developmental genes in embryonic stem cells,"** *Cell*, 125(2): 315-26, 21 April 2006.	354
5	T.I. Lee, *et al.*, **"Control of developmental regulators by polycomb in human embryonic stem cells,"** *Cell*, 125(2): 301-13, 21 April 2006.	349

SOURCE: Thomson Reuters *Web of Science*®

SW: Were you able to show that methylation was the causal factor in silencing these genes? And what promoted the methylation?

For this we needed more genetic tools, so we made one of the first knockouts, knocking out the gene for methyltransferase, which is the main enzyme that accomplishes this methylation. Knocking out that gene is lethal; the embryos die in mid-gestation. So that made it clear that DNA methylation is absolutely essential for the development and health of the organism, and that led me into this concept of epigenetic control of gene expression. When we infected embryonic stem cells with these viruses, and the methyltransferase gene had been knocked out, the virus would be expressed; it would not be silenced. This told us that there was a causal relationship between methylation and silencing. It was the methylation that silenced the gene in the embryonic stem cells. And this methyltransferase knockout led us to many, many more interesting insights into epigenetics. It told us not only that methylation is essential for life—but also that it has a causal role in cancer development and in genomic imprinting. Many issues came into focus when we used this particular mutant to understand epigenetic phenomena.

SW: What determines whether the viral DNA is methylated or non-methylated at different stages of development?

You have to realize that the genome is full of viruses, and they are generally silent, which means they're methylated. And organisms have a major interest in silencing viral genes that get into the genome. Otherwise they'd screw up the genome. So this is an evolutionarily conserved mechanism to silence these transposable elements, these viruses. And you can argue that this is most important in the early embryonic lineage, which gives rise to germ cells. We later found out that these early embryonic cells express several methyltransferases. The one we knocked out is the main one, but it only serves to maintain methylation once methylation has already been established by a different enzyme. It

propagates the methylation signal from one cell division to the next. It doesn't establish new methylation on its own.

The job of establishing methylation is done by enzymes called *de novo* methyltransferases. So if you put a virus into an early cell and it's not methylated, then the maintenance methyltransferase that we used would not do anything for this virus, but the *de novo* methyltransferase will—or it will if the viral DNA is a target for it. That's how these two enzymes collaborate. As it turns out, only embryonic cells express these *de novo* methyltransferases. Later they're not expressed. And this explains why if a virus gets into a later cell, it's not methylated. If it gets into an earlier cell, it is. This resolved the issue that you rightly raised.

SW: Did you ever knock out *de novo* methyltransferases?

That was done by a former student of mine, En Li. He actually did the first methyltransferase knockout in my lab as a student. He started his own lab and knocked out the *de novo* methyltransferases, and that really showed us that they are very important in cancer development.

SW: So what is the role of DNA methylation in cancer development, and how do you use that knowledge, if you can, to treat or prevent cancer?

We know now, for example, that cancers need to silence tumor suppressor genes. This can be done either by a mutation, which disrupts the gene and is irreversible, or by silencing the gene with methylation. In colon cancers, for instance, certain tumor suppressor genes are always silenced by methylation. We wondered what the mechanism for that is, and we learned that in these colon cancers, the *de novo* methyltransferases become inappropriately re-expressed. Then they target certain genes—specific sequences—and they become causally involved in cancer by silencing key tumor suppressor genes. And that, of course, immediately provokes the question of whether or not these insights are useful for therapy. I always thought it would be. In looking at this maintenance methyltransferase in our original experiments, we saw that when it was inhibited, either genetically by a mutation, or by a drug, it really prevented cancer development in these mice. The drug is actually a weird drug. It is very similar to cytosine. So DNA incorporates this drug, and when it replicates it does so with this altered cytosine, and now it cannot be methylated. It binds the enzyme covalently and leads to demethylation of DNA. And when we did this in mice, it protected the mice against cancer. This approach is now being tested in some big clinical trials for certain leukemias and head and neck cancers—at Johns Hopkins and at M.D. Anderson.

SW: What about side effects, since it seems you're dealing with some very fundamental mechanisms that could have effects system-wide?

If you use it for a cancer patient, it seems to be okay. But if you want to use it for children, for instance, who have these mutations that predispose them to colon cancer, you can't use any drug that has these kinds of potential side effects. But I think this is where the *de novo* methyltransferase will come in very handy. If you inhibit this with a drug there are virtually no side effects, based on what we understand from all this work. I think that will be very interesting to do, and we're hoping that some drug company finds a drug that inhibits the *de novo* methyltransferase.

SW: Why virtually no side effects?

Well, as I told you, the enzyme, *de novo* methyltransferase, is never expressed at high level in somatic cells—it has no role in somatic cells. It gets re-expressed in cancer, but that's an error. The maintenance methyltransferase is expressed in all cells that replicate DNA. So when that's inhibited, it's lethal to the cell, which dies. This enzyme is needed in somatic cells. *De novo* methyltransferases are only needed in embryonic cells. When you knock them out in later cells, there's really no phenotype. So if you inactivate them by a drug, we would predict that they wouldn't have any phenotype and so would have no effect—except in the cancer cells.

SW: A couple years ago you got into the stem cell field in a big way, reprogramming fibroblasts into pluripotent stem cells. How did that come about?

When Dolly the sheep was born back in 1996, I immediately got interested in the technique that was used, called nuclear transplantation. That's nothing more than an epigenetic phenomenon. So I immediately went to Hawaii, where they cloned the first mice, and began a collaboration learning how to do this. We tried to understand the mechanism of the reprogramming that goes on in this procedure. But nuclear transplantation is a very complicated procedure and not many can do it well.

SW: Why is this nothing other than an epigenetic phenomenon?

Well, in Dolly they took the nucleus from a mammary cell and put it into an egg cell that had its nucleus removed. Normally, development goes in one direction. It goes from pluripotent stem cells to more restricted stem cells to differentiated cells. In nuclear transplantation, the egg reverses that. It makes out of a differentiated cell—a mammary cell, in Dolly's case—an embryonic cell. So somehow the clock is turned back, and that means you have to turn the clock of epigenetic changes back to the embryonic ground state. It's all about epigenetics. Nothing else.

SW: So how does the egg accomplish that? What's the precise mechanism?

I was thinking about that a lot for many years, while I made all the tools to address this question. It's a very interesting question. Then Shinya Yamanaka's paper came out in 2006 (K. Takahashi, S. Yamanaka, *Nature*, 126(4): 663-76, 1006).That was a really important paper, showing that you could reprogram adult fibroblast cells into pluripotent stem cells by using pretty much exactly what we were thinking about. He used the same genes, but he also added two cancer genes, two oncogenes, to do this. That was really brilliant and a very important finding. It made the process efficient enough that he could detect it. You don't need the two cancer genes but they make it much more efficient. That was a very important insight. When Yamanaka's paper came out nobody really believed it—it looked just too fantastic. We had all the tools to do this, and so we used the same genes he used, including the two oncogenes, and it worked very robustly. We showed—along with Yamanaka and Konrad Hochedlinger, a former student of mine now at Massachusetts General Hospital, all working independently—that we could make mice out of these reprogrammed stem cells, and so these cells were really indistinguishable from embryonic stem cells. This really electrified the field; it clearly showed that these cells were pluripotent, and now three independent groups had shown it. Nobody could doubt anymore that this was correct.

"We and two other groups independently showed that we could make mice out of these reprogrammed stem cells, and so these cells were really indistinguishable from embryonic stem cells," says Rudolf Jaenisch. "This really electrified the field."

SW: In the couple of years since these papers were published, how has this reprogramming technique been extended?

We've all done a lot. We can do this now in human cells. We've also done it with different cell types.

SW: And this avoids the ethical and moral issues about the use of human embryos for stem cells?

Yes.

SW: Is it reasonable to assume, then, that the ethical debates about the use of embryos are now over?

I think the sharp edges are over. I think nuclear transplantation using human embryos won't be done often. I don't think it has any place in therapy. But some people may still do it for research. So embryonic stem cells are still needed. When you make iPS cells, as these are called—"induced pluripotent stem cells"—you have to compare them to embryonic stem cells. So you still need additional embryonic stem cells for the comparison, to standardize the iPS cells.

SW: How do you see this research progressing over the next five years?

I think we'll see rapid and enormous progress. It's a very, very active field. What we really have to do, though, and it's very expensive proposition, is get into human cells more and make patient-specific cells, really analyze them, and try to develop potential therapies for some of these major diseases. This research, however, is very expensive, but if we could do it, progress could be rapid. *In vitro* reprogramming allows us to take skin cells from a patient with Parkinson's disease, for instance, and make *in vitro* pluripotent stem cells; these could then be differentiated into the neurons which are the problems in these patients. Then we can study the disease, a major human complex disease, in a Petri dish. The potential is enormous.

Rudolf Jaenisch's current most-cited paper in *Essential Science Indicators*, with 706 cites:

Jaenisch, R, *et al.*, "Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals," *Nat. Genet.* 33: 245-254 Suppl. S, March 2003. Source: *Essential Science Indicators* from Thomson Reuters.

Additional Information:

Rudolf Jaenisch is listed in the Special Topic of Epigenetics. He is ranked in the top author- and top paper lists.

Rudolf Jaenisch is featured in ISIHighlyCited.com.

KEYWORDS: RUDOLF JAENISCH, MIT, WHITEHEAD INSTITUTE, EPIGENETICS, METHYLATION, METHYLTRANSFERASE, NUCLEAR TRANSPLANTATION, REPROGRAMMING CELLS, INDUCED PLURIPOTENT STEM CELLS, IPS.

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