How the HTLV's Might Cause Cancer

The human leukemia viruses HTLV-I and -II may transform cells by a novel mechanism for RNA-containing tumor viruses

Two of the human T cell leukemia viruses, HTLV-I and -II, cause human blood cell malignancies, producing leukemias or lymphomas of T cells. Since the first identification of an HTLV about 5 years ago, the manner in which the viruses might cause the malignant transformation of cells has remained a mystery. New results from William Haseltine of Harvard Medical School and the Harvard School of Public Health and his colleagues, which are reported in this issue of *Science*,* provide the first clues to the mechanism by which the viruses may transform cells. "It is the first thing that has come up concretely that might explain how the viruses cause leukemias," says Myron Essex of the Harvard School of Public Health.

The results are important not only because they open up potential approaches to the prevention and cure of the T-cell malignancies caused by the viruses, but also because they may provide new insights into the regulation of growth and other activities in cells that have a central role in controlling immune responses. In addition, the two viruses have a close relative, HTLV-III, which has been implicated as a likely cause of acquired immune deficiency syndrome (AIDS), and the work may help in understanding this devastating disease.

The model being proposed to explain transformation by HTLV-I and -II represents a new kind of transforming mechanism for a tumor virus of the retrovirus group. (Retroviruses have RNA genomes.) It postulates the production by the viruses of a protein that stimulates both their replication and that of their host cells.

Retroviruses, including the HTLV's, contain long terminal repeating sequences (LTR's) at the ends of their genomes. The LTR's carry regulatory segments, known as promoters and enhancers, which activate transcription of the viral genes into messenger RNA, the first step of protein synthesis. What Haseltine, with Joseph Sodroski and Craig Rosen, who are also at Harvard, have shown is that T cells that have been infected with HTLV-I or -II produce a factor, presumably a protein encoded by the viral genomes themselves, that increases transcription from the viral promoter in the LTR.

The results indicate that the factor can facilitate transcription from the viral promoter wherever this is located in the genome. This type of regulation is called *trans* regulation in molecular biology jargon. "It's nice because you don't need specific integration sites," Sodroski explains. "No matter where the virus goes it can make the factor."

The new findings may help to explain some of the peculiarities of transformation by the two HTLV's. Up until now retroviruses have been thought to transform cells by one of two general mechanisms. The transforming potential of the acute or rapidly acting retroviruses is encoded in specific viral genes, called oncogenes, which are derived from cellular DNA sequences that became incorporated in the viral genomes during the course of infection. These oncogenes can cause transformation when reintroduced by the viruses into cells, both in living animals and in culture.

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The chronic or slow-acting retroviruses, which generally cause leukemias, do not carry oncogenes but may transform by inserting an LTR near one of the cellular oncogene counterparts, thus eliciting its abnormal activation. These viruses do not transform cultured cells.

The HTLV's fit neither of the two models. They do not carry any of the two dozen or so known oncogenes. Moreover, none of their DNA sequences appear to be related to cellular sequences, making it unlikely that they carry any oncogene as these are commonly understood. In spite of the absence of an oncogene, however, HTLV-I and -II do transform cultured cells. It is also unlikely that they act by LTR insertion because the HTLV genome may integrate anywhere in the genome of transformed cells.

A regulatory factor that stimulates transcription from a viral promoter should increase replication of the virus. Haseltine, Sodroski, and Rosen propose that, in the case of HTLV-I and -II infected cells, the factor may also increase transcription of cellular genes, such as those that turn on cell division, and that this is what leads to the uncontrolled growth and other abnormalities of transformed cells. Work from Robert Gallo's laboratory at the National Cancer Institute, where the first HTLV was discovered, has shown that expression of some cellular genes, including that for the receptor for T-cell growth factor, is increased in cells transformed by the viruses. "It's opened up a new way of thinking about how a virus like this might transform a cell," says Dani Bolognesi of Duke University School of Medicine of the Harvard group's suggestion.

In contrast to the malignancies, AIDS is caused by T-cell death, not proliferation. If HTLV-III also produces a *trans*-acting regulatory factor, Haseltine, Sodroski, and Rosen suggest, it may act either to turn off genes that stimulate growth or to turn on those that cause cells to stop dividing.

There are precedents, incidentally, although not from the retroviruses, for transforming-virus production of a *trans*-acting regulatory factor that activates expression of both viral and cellular genes. Researchers from several laboratories have evidence that DNA-containing viruses, including adenovirus and SV40, act in this way.

The likely location in the HTLV genome of the gene coding for the *trans*-acting regulatory factor is in a region that was identified by Mitsuaki Yoshida and his colleagues at the Cancer Institute in Tokyo, Japan, as having four open reading frames and thus the potential of coding for four proteins. This region lies between the genes coding for the viral structural proteins and the 3′ (right-hand) LTR, a region where retroviral oncogenes may be located, although, as already mentioned, the HTLV's do not carry any known oncogenes. The slowly transforming retroviruses do not contain a comparable stretch of RNA.

*J. G. Sodroski, C. A. Rosen, and W. A. Haseltine, p. 381; W. A. Haseltine, J. G. Sodroski, R. Patarca, D. Briggs, D. Perkins, and F. Wong-Staal, p. 419.

Haseltine and his colleagues have now compared the sequences of this region, which is roughly 1600 base pairs long, from HTLV-I and -II. They find the first 600 base pairs to be very different in the two viruses, whereas the last 1000 are very highly conserved. More than 80 percent of the amino acids in the proteins encoded by the conserved regions should be identical. "No other open reading frame has such a high degree of conservation," Haseltine says. "We predict that it will encode a protein that will affect transcription."

Essex and Tun-Hou Lee, also at the Harvard School of Public Health, have identified in HTLV-I transformed cell lines a viral protein with a molecular weight of 42,000, about the right size for a protein encoded by the long open reading frame. In one transformed line, which originated in Gallo's laboratory, it is the only viral protein made. This line is just as effective in stimulating transcription from the HTLV-I LTR as lines that make infectious viral particles. The Haseltine and Essex groups have shown that the 42-kilodalton protein is not related to the viral polymerase or structural proteins and that it is at least partly derived from the conserved reading frame. "So it proves that the long open reading frame is used," Haseltine says.

The HTLV's may not be the only RNA-transforming viruses that operate by *trans* regulation of gene transcription. Bovine leukemia virus induces a disease very similar to the leukemia associated with HTLV-I. The sequence of the bovine virus has recently been determined by Arsene Burny of the University of Brussels. It, too, contains a long open reading frame just inside the 3′ LTR. The amino acid sequence of the protein encoded by this region is different from that of the comparable HTLV protein, but they both have similar arrangements of hydrophobic and hydrophilic regions.

If the proposed mechanism for transformation by the HTLV's is correct, then the way might be open to finding means of preventing or treating the cancers caused by the agents. For example, a vaccine might be derived from a virus modified so that it no longer produces the transcription-stimulating protein. For therapy, it might be possible to attach a lethal gene to an LTR that has been altered so that it works only in tumor cells that were transformed by HTLV-I or -II. That gene, if introduced into cells, would be transcribed in—and kill—only the tumor cells. At the very least, the new results on the HTLV's should open new lines of inquiry.

—JEAN L. MARX

A Look at the Surface of Platinum

Despite the importance of platinum in industrial catalysis and in various types of electrodes, particularly those in fuel cells, remarkably little is known about what happens to it when it is degraded during use. In particular, there has been a great controversy over the oxidation state of its surface under such conditions; different investigators have assigned as many as three different oxidation states to materials produced under a given set of conditions.

Some of the controversy may be reduced by work reported recently* by Marcell Peuckert of the Institut für Grenzflächenforschung und Vakuumphysik der Kernforschungsanlage Jülich GmbH in West Germany. Peuckert has used x-ray photoelectron spectroscopy (XPS), Auger spectroscopy, electron energy loss spectroscopy (EELS), and thermal decomposition techniques—perhaps the broadest array of investigative methods that has been applied to the problem—to define the surface composition of platinum oxides produced by four different techniques. He has found that the composition is different in each case.

Peuckert used one surface of a single platinum crystal because problems have been created in the past by grain boundaries in polycrystalline materials. He then oxidized the surface by one of four different treatments: (i) heating at 900 K in flowing oxygen, (ii) electrochemical oxidation at 3 volts in a sulfuric acid electrolyte, (iii) anodic polarization in sodium hydroxide electrolyte, and (iv) etching in hot concentrated nitric acid.

Perhaps his least controversial result is for acid oxidation. Peuckert finds that the species produced is the tetravalent hydroxide, $Pt(OH)_4$. This is in agreement with results produced by others, especially Phillip Ross of the Lawrence Berkeley Laboratory. In fact, Peuckert performed the experiment at only one potential, whereas Ross will report soon in *Surface Science* that he has observed the same composition over a broad range of potentials.

Somewhat more controversy surrounds the results for gas phase oxidation. Some investigators, such as Gabor Somorjai of the University of California, Berkeley, have found that only a small amount of oxygen is chemisorbed to the platinum surface before atomic oxygen begins to penetrate into the metal. Others, including Ross and Peter Norton of Atomic Energy Canada, Ltd., have observed very high concentrations of chemisorbed oxygen. Peuckert's results support the latter observation, and he finds that the surface composition is PtO_2. A question remains about the generality of his observation, however. Rustum Roy of the Pennsylvania State University has found that Pt_3O_4 can be formed in certain regions of temperature and pressure; Roy was working with bulk materials, however, and it is not clear whether his results can be extrapolated to surfaces.

Peuckert's other results are probably less controversial if only because there has been very little study of those conditions. In hot acid, he finds that the surface species is hydrated platinum oxide, $PtO_2 \cdot nH_2O$. James Hoare of the General Motors Research Center has found that the surface species under similar conditions is PtO_2, but his studies were performed with techniques that would not necessarily have revealed the water of hydration. Peuckert's results in the basic electrolyte are perhaps the least clear cut: he finds that the composition is "approximately" $PtO(OH)_2$, and he is working to refine his measurement.

Peuckert's results may help to clear up some of the controversy about the composition of passivation layers of platinum, but it leaves one problem area unresolved. "What we would really like to know," says Ross, "is the nature of the surface states formed at the potentials present in a fuel cell. Unfortunately, those species all disappear when the electrode is removed from the cell and placed in the vacuum chamber for study. Our best hope now is that if we can understand what is happening at higher potentials, such as those measured by Marcell and us, perhaps we can extrapolate back to what is happening in the real world."—THOMAS H. MAUGH II

*Presented 6 June at a symposium, "New Eyes/New Insights," at the Center for Catalytic Science and Technology at the University of Delaware.