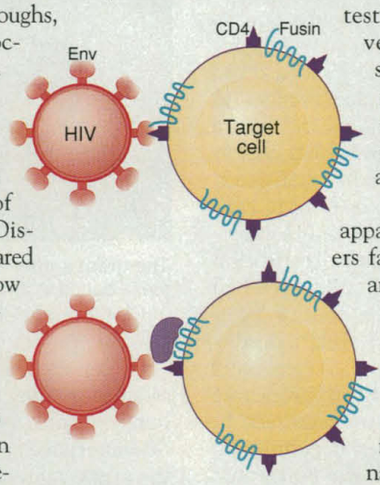
Likely HIV Cofactor Found

The long-sought "cofactor" that helps the AIDS virus enter cells may have been identified—opening the way to a better understanding of HIV action and possibly new AIDS therapies

In the language of science, not all dirty words have four letters. Take "breakthrough," disdained by many researchers for good reason: Few scientific advances by themselves push a field more than a few millimeters forward. And many so-called breakthroughs—think cold fusion—are ultimately seen as distractions or worse. Still, on rare occasions, scientists do make true breakthroughs, and one may have just occurred in AIDS research. After a decade-long search by investigators around the world, a team led by biochemist Edward Berger of the National Institute of Allergy and Infectious Diseases (NIAID) may have cleared up a major mystery about how HIV, the AIDS virus, enters and infects cells.



SOURCE: E. BERGER

Dynamic duo. If HIV needs both CD4 and fusin to enter cells, blocking fusin with, say, a chemokine *(violet)* might prevent infection.

Researchers have known since 1984 that HIV enters cells by docking onto a receptor protein known as CD4, located on immune-cell surfaces. But 2 years later, they found that CD4 by itself was not sufficient for HIV infectivity; some unknown "cofactor," only found in human cells, was also required. Since then several putative cofactors have been dragged into the interrogation room and put under the harsh light, only to reveal that they could not be guilty. Now, however, Berger's team, including his NIAID colleagues Yu Feng, Christopher Broder, and Paul Kennedy, have collared by far the best suspect yet.

On page 872, they report the discovery of a membrane protein they call "fusin," which has the expected characteristics of the elusive HIV cofactor. The researchers found, for example, that together with CD4, it permits cells to fuse (hence the name fusin) with HIV's surface—a key step in the infection process.

In contrast to previous cofactor sightings (*Science*, 24 December 1993, pp. 1971 and 2045), this time around AIDS experts are roundly hailing the result. "It's a great triumph of perseverance, and technically, he's done it very well," says John Moore, a biochemist at the Aaron Diamond AIDS Research Center in New York City. University of Pennsylvania hematologist James Hoxie, who has been searching for the cofactor for years, agrees: "This is a tremendous advance. It's very exciting."

The discovery is winning such plaudits because it could help clear up several puzzles about HIV, including how it destroys cells and why it appears not to harm some infected people. The work could also lead to a small-animal model in which to study AIDS and test vaccines, something investigators have long been seeking. And it raises the prospect of new therapies that, by blocking binding to fusin, would limit HIV's ability to infect cells.

Why did the Berger lab apparently succeed where others failed? "It's the sensitivity and versatility of our assay," Berger says. This assay basically uses vaccinia virus to convert fibroblast cells into what amount to mock HIVs that can give an easily detectable chemical signal whenever they fuse with mouse cells modified to express human CD4. The NIAID team could then add a variety of DNAs encoding human proteins to the mouse CD4 cells, which normally shrug off the virus, until they found just one—the missing cofactor—that enabled the cells to fuse with the HIV surrogates.

Berger and co-workers homed in on fusin through a process of elimination. They began by taking a complete "library" of cDNAs from human cells known to be susceptible to HIV and transfecting them into the CD4-bearing mouse cells. They then mixed in the mock HIV—which contains the virus's surface protein—and found, as they had hoped, a low level of fusion, indicating that one of the cDNAs encoded the cofactor. By repeatedly identifying smaller and smaller portions of the cDNA library that still allowed fusion to occur, the Berger team isolated a piece of cDNA that seemed to encode the necessary cofactor, the protein they named fusin.

To confirm that they had the right protein, the team went on to show that adding fusin cDNA to mink and monkey cells expressing CD4—and to human cells, such as certain types from the nervous system, that typically cannot be infected with HIV—leads them all to fuse with the sham AIDS virus. The researchers also found that antibodies against fusin blocked both fusion and infection of susceptible cells by HIV itself.

Winning plaudits. NIAID'S Ed Berger.

Without naming names, Moore and others say at least two labs have already confirmed the Berger group's finding. And several labs, including Moore's, are racing to build on the work. "We're picking over the carcass," says Moore. "Ed has killed it for us. Ed is *T. Rex*."

Adding to the excitement of fusin's discovery is the possibility that it may tie into results published late last year by a team led by Paolo Lusso and Robert Gallo of the University of Maryland (*Science*, 15 December 1995, p. 1811). They found that certain chemokines, proteins that help bring about inflammatory responses, are potent suppressors of HIV's ability to infect cells. And when the Berger team sequenced the fusin gene, they found that the protein it encodes resembles one of the receptors through which chemokines exert their effects.

That resemblance raises an intriguing possibility, says the University of Pennsylvania's Hoxie. The chemokines identified by Lusso and Gallo could be suppressing HIV infection by blocking the fusin receptor directly or by sending signals that make cells produce fewer receptors. If so, then together the two sets of findings might help explain why a small percentage of people have been able to resist HIV's ability to destroy the immune system even after being infected with HIV for a decade: They might have higher levels of these chemokines, fewer fusinlike receptors, or both.

Indeed, there is already some evidence in favor of that idea. In the April issue of *Nature Medicine*, Richard Koup and his colleagues at Aaron Diamond reported that CD4 cells from people who are repeatedly exposed to HIV, but remain uninfected, are resistant to infection with HIV. They also found that these resistant individuals secrete higher

levels of the chemokines identified by Lusso and Gallo.

The fusin discovery may point the way not only to understanding natural resistance but also to new therapies—drugs that render cells invulnerable to HIV infection by blocking its binding to the protein. And AIDS researchers hope that by stitching the fusin gene into a small animal, such as a rabbit, they can develop a relatively inexpensive lab model for testing AIDS vaccines and studying exactly how HIV destroys the immune system.

One cautious note comes from Berger, who stresses that there are likely to be several different fusinlike cofactors. As his paper explains, the mock HIV he and his colleagues used contained the surface protein from a version of the virus that was grown in a line of T cells maintained in lab culture. Isolates from such cell lines resemble HIVs typically found in people late in the course of their disease. The more commonly found HIV isolates from infected people differ markedly. In keeping with this, experiments described in the paper suggest that fusin acts as a cofactor only for HIV that can grow in the cultured cell lines. Other fusinlike proteins might serve the same role for different HIV strains. "This is not going to be the only cofactor," predicts Hoxie.

Researchers are optimistic that they will soon begin identifying the cofactors used by a wide range of AIDS viruses. Hoxie, for example, is already hard at work hunting for fusin relatives that might play a role in infection with SIV, the simian cousin of HIV, and HIV-2. "This paper is really a beginning," he says.

–Jon Cohen

MOLECULAR EVOLUTION

Just How Old Is That DNA, Anyway?

Ancient DNA: a treasure chest for molecular evolution, or fool's gold? A handful of research groups have reported recovering DNA from insects trapped in amber, plant leaves buried in clay, and even dinosaurs entombed in coal—samples as old as 135 million years (*Nature*, 10 June 1993). The finders hope to get at evolution's nitty-gritty by tracking genetic changes over millions of years. But DNA is not the most stable of molecules, and skeptics have shot back that intact DNA from old sources is more likely to be from some modern interloper in the sample, such as bacteria. About the only thing everyone agrees on is that they need—and haven't had—an independent test of ancient DNA authenticity.

Now they have one, based on changes in the organic material from which the DNA came: insect bodies, for instance, or dinosaur bone. On page 864, an international team of researchers reports that a chemical change that converts amino acids in proteins from one mirror-image form to another—a process known as racemization—takes place at virtually the same rate as the degradation of DNA. If the amino acids show this conversion to even a modest degree, then the original DNA in the sample is likely long gone, suggesting that any remaining genetic material is a contaminant. And when the researchers then used this test on a variety of ancient DNA samples, they found that only those from insects trapped in amber appear to stand the test of time.

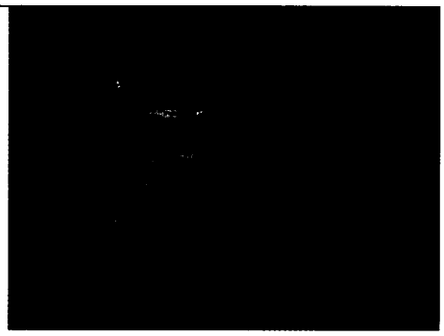
"It's really nice work," says Rob DeSalle, an ancient DNA expert at the American Museum of Natural History in New York City. "It's kind of seeing how nucleic acids [in DNA] break down by proxy." In addition, the test should also be "extremely useful" in preserving rare fossils: Researchers have to damage fossils to get at the DNA, and a quick scan, using the test, will let them know when such destruction isn't warranted, adds Tomas Lindahl, a biochemist at the Imperial Cancer Research Fund in South Mimms, England.

The test relies on the type of amino acids that organisms use to make proteins. Amino acids naturally occur in mirror-image pairs, like a left and right hand, but biological systems only use the left-handed versions to build proteins. Once an amino acid is in a protein, however, water and other factors drive a chemical transformation that slowly racemizes this left-handed, or L form, converting it into a right-handed, or D form.

Jeffrey Bada of the Scripps Institution of Oceanography and his colleagues had worked out the racemization rates of different amino acids. And in 1993, Bada noticed—and later published—that the conversion of amino acids occurs at virtually the same rate as the major DNA degradation process. In that process, which is called depurination, free radicals in water break the bonds that hold certain nucleic acids to the sugar-phosphate backbone of DNA, ultimately severing the DNA strand.

Bada realized these similar rates could allow him to test ancient DNA provenance. If amino acids from a 25-million-year-old insect were highly racemized, for example, then any DNA accompanying them should be damaged. Intact DNA would be recent.

Together with ancient DNA experts Hendrik Poinar, Mattias Höss, and Svante Pääbo at the University of Munich, Bada tested this notion by examining the amount of amino acid racemization in 26 different samples of organic remains ranging from 50 to 40,000 years old. Some of the samples had yielded intact DNA whose authenticity had been verified, in part, by comparing it with DNA from related living species: A DNA sequence from an ancient horse bone, for instance, looked very much like modern horse DNA. Others didn't contain any intact DNA. In the samples yielding intact DNA, almost all the amino acids were indeed of the L form. But in those where no DNA could be isolated, a higher concentration of the amino acids turned up D. For aspartic acid, which racemizes faster than other amino acids, the D/L ratio was always 0.08 or higher when no DNA was present.



G. POINAR

The great amber hope. A test indicates specimens bound in amber, like this ancient bee, are the only ones likely to yield ancient DNA.

When they looked at a variety of older samples, ranging from 17 million to 65 million years old, the scientists found that the racemization of aspartic acid remained below the magic value of 0.08 only in material embedded in amber. The D/L ratio in dinosaur bones said to have yielded ancient DNA, in contrast, was a minimum of 0.17. "That makes it unlikely they should be able to find ancient DNA in these samples," says Pääbo. Moreover, in all of the dino samples, the amount of racemization of other amino acids, such as alanine, was equal to if not higher than that of the aspartic acid. Because aspartic acid normally racemizes faster than alanine, this indicates something is seriously wrong with the entire sample, not just the DNA. Poinar calls this pattern the "red flag of contamination." The group reached similar conclusions for 17-million-year-old plant samples found in Idaho.

Amber, which is a form of fossilized tree resin, likely owes its unique ability to preserve biological molecules to its ability to seal out water, Pääbo says. As for dino DNA, "it looks bleak," acknowledges Scott Woodward, a molecular biologist at Brigham Young University in Provo, Utah, who in 1994 claimed to have found some from a bone (*Science*, 18 November 1994, pp. 1159 and 1229). He adds, however, that the new test is not a direct measure of DNA integrity but a correlation, so there is still room for hope. And hope, if not DNA, springs eternal.

–Robert F. Service