X-RAY CRYSTALLOGRAPHY

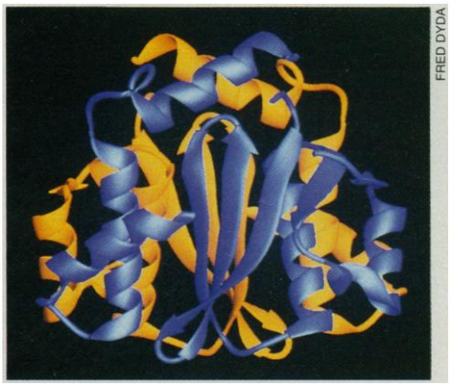
HIV Integrase Structure Catalyzes Drug Search

Like all retroviruses, HIV does its dirty work from within the genome of the cells it infects. It insinuates itself into the genetic material of its host cell, using an enzyme called HIV integrase to snip the cell's DNA and splice its own genes into the chromosome. Researchers have long realized that if they could find a way to block this enzyme, they might be able to prevent the virus from infecting new cells and thereby defeat it. But they have faced a difficult problem: The search for HIV integrase inhibitors has been a hit-or-miss operation because the precise shape of the enzyme was not known. But that may be about to change.

On page 1981 of this issue of *Science*, Robert Craigie, David Davies, and their colleagues at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) at the National Institutes of Health report the three-dimensional structure of the catalytic domain of HIV-1 integrase as determined by x-ray crystallography. Says protein crystallographer Guy Dodson of the University of York and London's National Institute for Medical Research: "This is an important development for AIDS research and structural biology as a whole—it's really going to open up the field." And in a second paper, on page 2002, a group led by Stephen Goff at Columbia University reports a finding that may help explain how HIV integrase targets specific areas of the host cell's genome—a finding that may open up yet another line of attack for drug designers.

In determining the structure of the enzyme, Craigie and Davies' group pulled off a feat that has eluded several other groups. They focused on the central core of the enzyme—amino acids 50 to 212 out of 288 in total—and essentially tricked the protein into becoming soluble. To make protein crystals, researchers start with a concentrated solution of the pure protein and slowly remove the solvent until the protein molecules crystallize. But HIV integrase has been notoriously difficult to crystallize because it is highly insoluble and tends to clump as the solvent is removed. So the NIH team took a novel approach: They made mutants of the protein in the hope that those mutants would dissolve and crystallize better. "We looked through the protein for hydrophobic amino acids that might be exposed on the surface and changed them into a lysine or an alanine," explains Craigie.

The NIH group made 30 such "point mutants," each with a different single amino acid change that might stop the molecules from clumping. "One [mutant] worked—we were lucky," says Craigie. Dodson doesn't think Craigie should be so modest. The method "deserves a medal," he says, adding that he expects it will be adopted widely and applied to other insoluble proteins.



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Last of a trio. Integrase is the third of HIV's key enzymes to have its structure worked out.

Now that they know the shape of the enzyme, researchers have a better basis for designing drugs to block its activity, for example by tailoring molecules that will bind to and obscure the enzyme's catalytic site. "This is a challenge for the synthetic chemists. ... It's going to be pursued with the utmost vigor," predicts Dodson.

In pursuing new drugs, researchers will no doubt follow up on several clues to how HIV integrase works that are generated by the NIDDK group's work. First, there is a striking resemblance between its catalytic site and that of a family of enzymes called polynucleotide transferases, which transfer chains of nucleotides in a variety of biological systems. The active site in the crystal fragment has two amino acid residues with carboxyl groups, which are part of a group of three that are highly conserved in other retroviral integrases and polynucleotide transferases. By analogy with other polynucleotide transferases, the NIH group concludes that these negatively charged residues are essential for catalysis and probably interact with positively charged metal ions such as magnesium.

Another clue to the way the enzyme does its work has come from a separate line of research pursued by Goff, a Howard Hughes Medical Institute investigator, and colleagues at Columbia and Stanford University. Goff and his colleagues report that a protein found in many types of human tissue, including white blood cells, may help direct HIV integrase to a suitable site on the host chromosome where the viral DNA is most likely to be transcribed quickly into multiple RNA copies.

The protein, which Goff's group calls Ini1, binds to HIV integrase and stimulates its DNA-joining activity in vitro. Its genetic sequence is similar to that of a yeast protein, SNF5, which promotes expression of certain genes. In yeast cells, SNF5 forms a complex with similar proteins that appear to make certain stretches of DNA more accessible by opening up the tightly bound chromatin structure in which the nucleic acid is wound up with proteins. Goff suggests that Ini1 may perform the same function for HIV integrase, and this could explain "an old observation that these viruses tend to insert [their genetic material] into active genes," thus ensuring that they will be quickly replicated. He points out that it may eventually be possible to inhibit HIV's replication by tailoring compounds that block the binding of Ini1 and HIV integrase.

These findings clearly provide drug designers with several promising lines of attack. And the recent examples of two other HIV enzymes—reverse transcriptase (RT) and HIV protease—show that, when an enzyme is crystallized, drug designers swing into action.

The crystal structure of RT complexed with an inhibitor was elucidated only recently by Thomas Steitz and his colleagues at Yale University (*Science*, 26 June 1992, p. 1783). That development has stimulated a search for compounds more precisely tailored than RT inhibitors currently in clinical use, such as AZT. "Having the structure [meant] you [could] think about completely new chemistries and interactions," says Dodson. Several companies are also developing protease inhibitors based on that enzyme's crystal structure, which was determined in 1989 by Alexander Wlodawer's group at the National Cancer Institute in Frederick, Maryland (*Science*, 11 August 1989, p. 616).

Now, with the structure of the third of the trio of key HIV enzymes in hand, researchers are hoping that they may ultimately be able to develop a cocktail of inhibitors for the three enzymes that would be used together. Such a combined attack would help reduce the chances that HIV could mutate and become resistant to drug therapy. Mutations that resist reverse transcriptase and protease inhibitors have already turned up, notes molecular biologist Ronald Plasterk of the Netherlands Cancer Institute in Amsterdam, who is one of those working on integrase inhibitors. But, because it is less likely that HIV could develop resistant mutations to all three at once, "with the third [inhibitor] we might just get it," he says.

–Claire O'Brien

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