Finding a New Target for AIDS Therapy

The crystal structure of an HIV enzyme should help in the national design of new AIDS therapies

TARGETED DRUG DESIGN—IN WHICH A therapeutic drug is aimed at a precise molecular bull's-eye—has great advantages over pulling drugs down off the shelf and trying them at random. Among the advantages: more precise action and fewer side effects. But to do targeted design, you have to know the target. Now a team of scientists at Agouron, a La Jolla, California, biotech firm, have provided a three-dimensional picture of RNase H, a critical protein used by the AIDS virus to infect cells (see article on page 88 of this issue). With that image, drug designers can narrow their search for agents that will knock out HIV.

HIV provides several likely targets for attack, but so far the most successful approaches have focused on an enzyme unique to retroviruses called reverse transcriptase. AZT, the only drug so far approved for treating AIDS, works by interfering with one reverse transcriptase function: transcribing the viral genetic instructions encoded in RNA into cellular genetic instructions, encoded in DNA. Most candidate antiviral drugs, including ddI and ddC, also work by interrupting this step.

But making DNA isn't the only thing the viral enzyme does. In addition to assembling DNA strands (a process carried out by a part of the enzyme called a DNA polymerase), reverse transcriptase plays another key role. After a new strand of DNA is made, the RNA that served as a template must be destroyed so that a second DNA strand can join with the first to form a double helix, which then gets incorporated into the host's genome. RNase H, short for ribonuclease H, is the small fragment of reverse transcriptase that chews up the RNA.

To determine the three-dimensional structure of RNase H, the Agouron researchers needed to crystallize it—which proved to be a problem. The entire reverse transcriptase molecule is a dimer with two subunits having molecular weights of about 66 and 51 kilodaltons. From what was known of its amino acid sequence, there appeared to be a natural break dividing the 66 kilodalton subunit into two domains: a large one of approximately 51 kilodaltons that provides the polymerase function, and a smaller one providing the RNase H function. But the smaller fragment just wouldn't crystallize—until two Agouron molecular biologists, Zuzana and Zdenek Hostomsky, tried lengthening the smaller fragment with an amino acid sequence copied from the larger fragment. To their delight, this slightly larger protein did form crystals, and using x-ray diffraction patterns the Agouron team was able to determine the structure to a resolution of 2.4 Å.

Agouron is making the structure available to drug designers (in the Protein Data Bank), and in the coming months researchers will begin looking for molecular arrows: compounds that can interrupt RNase H function. One point of attack might be metal-ion binding sites. It is known that metal ions are necessary for RNase H to function properly, and David A. Matthews of Agouron says he and his colleagues have determined the location of two metal binding sites and a third region that binds uranium. Matthews says drugs that could inactivate these sites would be very attractive. "We're already thinking of [candidate] molecules that might incorporate features of metals as well as more organic type structures," he says.

Before drug design based on the new structure goes too far, however, other researchers will want to confirm that Agouron does, indeed, have the correct structure. The fragment of reverse transcriptase described by the Agouron workers seems to fit the description of the desired RNase H: It crystallizes nicely, has a structure similar to another RNase H enzyme that has already been described, and it associates with the larger piece of the reverse transcriptase responsible for forming the DNA strand from the virus' RNA template. Yet by itself, the enzyme Agouron scientists have studied cannot perform its normal function of chewing up RNA. One possible explanation for this, says Matthews, is that both regions of the reverse transcriptase molecule need to be in place in order for either to function. "There [may be] a kind of a symbiosis that has evolved there," he suggests.

But there is another possible explanation, posed by work done by Stuart Legriece, a molecular biologist at Case Western Reserve University in Cleveland who has also been working with HIV's RNase H. He says the RNase H molecule he has made retains some of its biological function even in the absence of the larger subunit of the reverse transcriptase molecule. Does that mean Agouron has got the wrong molecule? Stephen H. Hughes, a virologist at the ABL basic research program at the National Cancer Institute's Frederick Cancer Research Center, says Legriece's work does not mean that Agouron is wrong, but must be treated with some caution. "The rigorous proof" that their structure is the right one "depends on [the RNase H] being active," Hughes says.

Probably the best way to resolve the issue of how the RNase H and polymerase domains interact with each other is to determine the structure of the entire reverse transcriptase. Several teams around the country are working feverishly to do that. And that should give drug designers an even better target.

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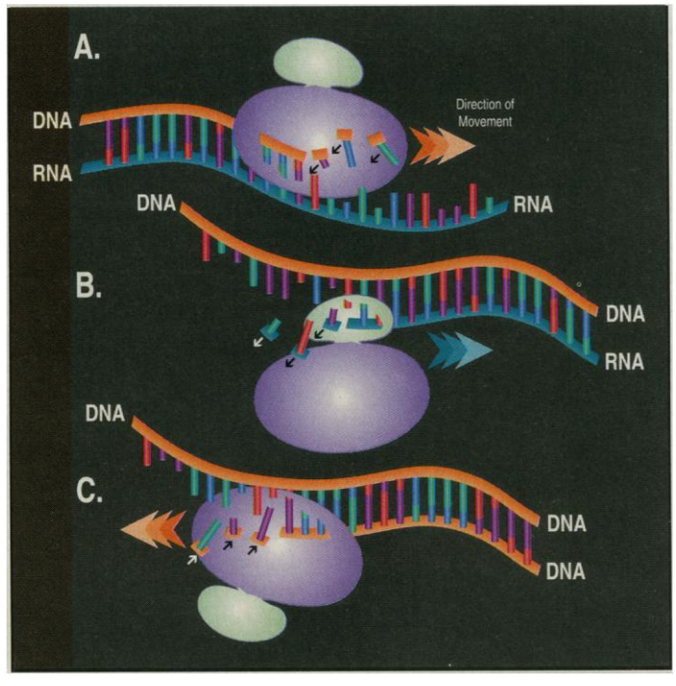

Multitalented molecule. *This cartoon depicts the three actions of the reverse transcriptase enzyme: A) the polymerase portion makes a DNA strand that complements the viral RNA, B) the RNase H region removes the RNA, and C) the polymerase creates a second DNA strand.*