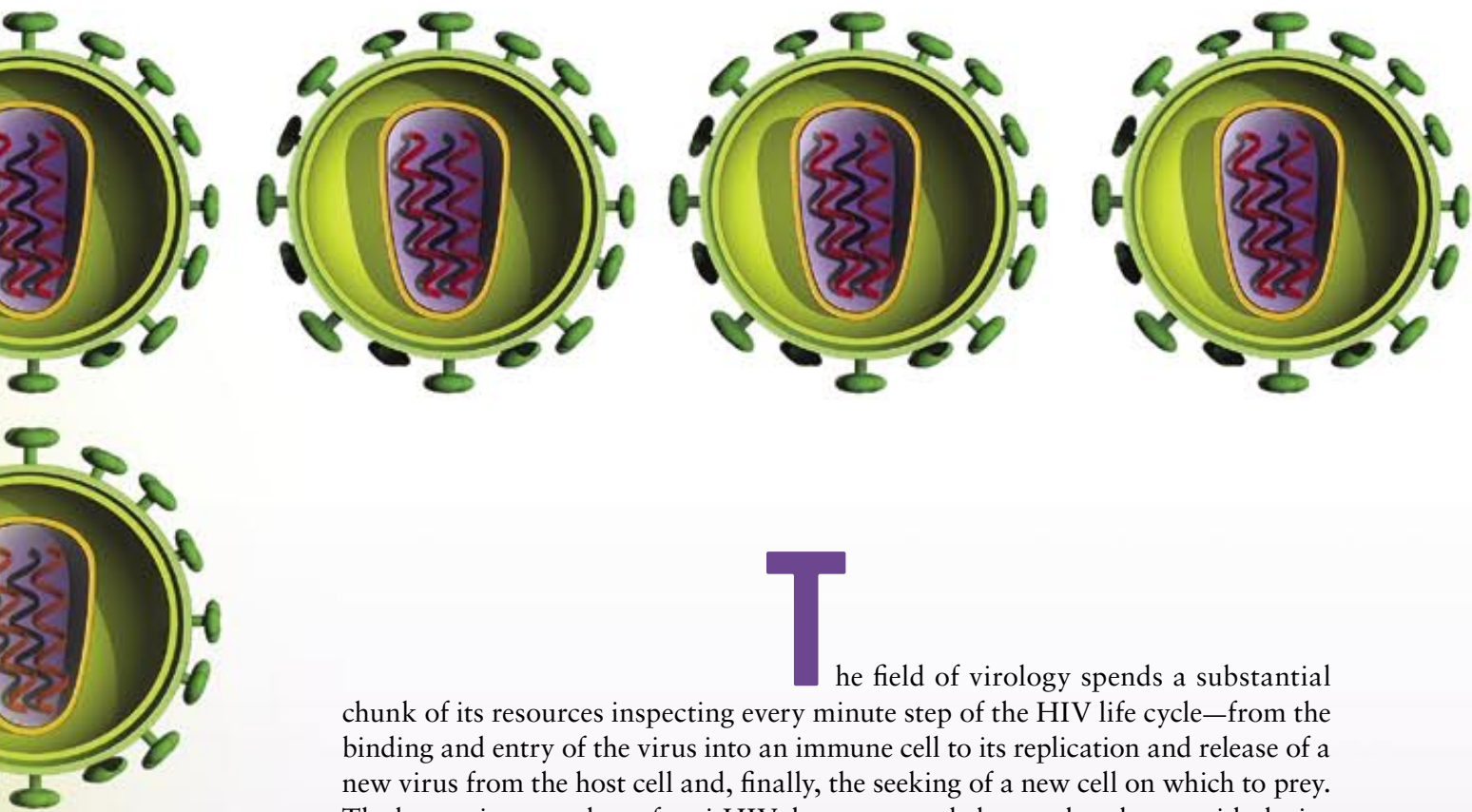
The background of the entire page is a repeating pattern of HIV virus particles. The top half features larger, more detailed green and purple virus particles with a red and blue internal structure. The bottom half features smaller, lighter-colored virus particles. The text is overlaid on this background.

A New ASSAULT on **HIV**

The constant search for weak points in the virus yields ideas for

By Gary Stix



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he field of virology spends a substantial chunk of its resources inspecting every minute step of the HIV life cycle—from the binding and entry of the virus into an immune cell to its replication and release of a new virus from the host cell and, finally, the seeking of a new cell on which to prey. The last major new class of anti-HIV drugs emerged about a decade ago with the introduction of the protease inhibitors, which curb the action of an enzyme that is critical to a late stage of viral replication.

a wholly new class of drug

At the time, a few members of the HIV research community wondered whether protease inhibitors could provide the basis for a cure. The ingenuity of the virus has proved the hollowness of that hope. As many as half of HIV-positive patients under treatment in the U.S. were found in one study to be infected with viruses that have developed resistance to at least one of the drugs in their regimen. Clinicians can choose from more than 20 pharmaceuticals among protease inhibitors and two classes of drug that prevent the invading virus from copying its RNA into DNA, thereby sabotaging viral replication. Combinations of these agents are administered to counteract the virus's inherent mutability, but that strategy does not always ward off resistance to the medicines, including the protease inhibitors. "Given increasing resistance to protease inhibitors, it's of paramount importance to identify new ways to interfere with the virus replication cycle," asserts Eric Freed, a researcher in the HIV drug resistance program at the National Institutes of Health.

Drugs that interrupt the beginning, middle and end of viral processing within the host are now in various stages of development. Academic researchers and Panacos, a small biotechnology outfit based in Watertown, Mass., are taking inspiration from the success of protease inhibitors by developing drug candidates known as maturation inhibitors that block protease activity in a novel way. Protease inhibitors mount a direct attack on the HIV protease, preventing the enzyme from processing a viral protein called GAG. When GAG proteins are cut properly, pieces spliced out of it form the conical protective core, or capsid, that encloses RNA. In contrast, the Panacos maturation inhibitor blocks a site on the GAG protein where the protease normally binds, keeping the protease from clipping GAG correctly. As a result, the capsid does

not form appropriately and the virus cannot infect another cell.

Looking for Leads

THE PATH TO THE PANACOS drug candidate began in the mid-1990s, when the company Boston Biomedica undertook a collaboration with a professor from the University of North Carolina at Chapel Hill to screen compounds from a collection of traditional Chinese herbs for biochemical activity against HIV. Kuo-Hsiung Lee's laboratory turned up a potential drug lead in a Taiwanese herb.

The substance, betulinic acid, had weak activity against HIV. After the lab separated the compound into its chemical constituents, the investigators found that one of these components, when chemically modified, exhibited a much stronger effect. "Betulinic acid had activity against HIV at the micromolar level," says Graham Allaway, Panacos's chief operating officer. "This derivative had activity at the nanomolar level."

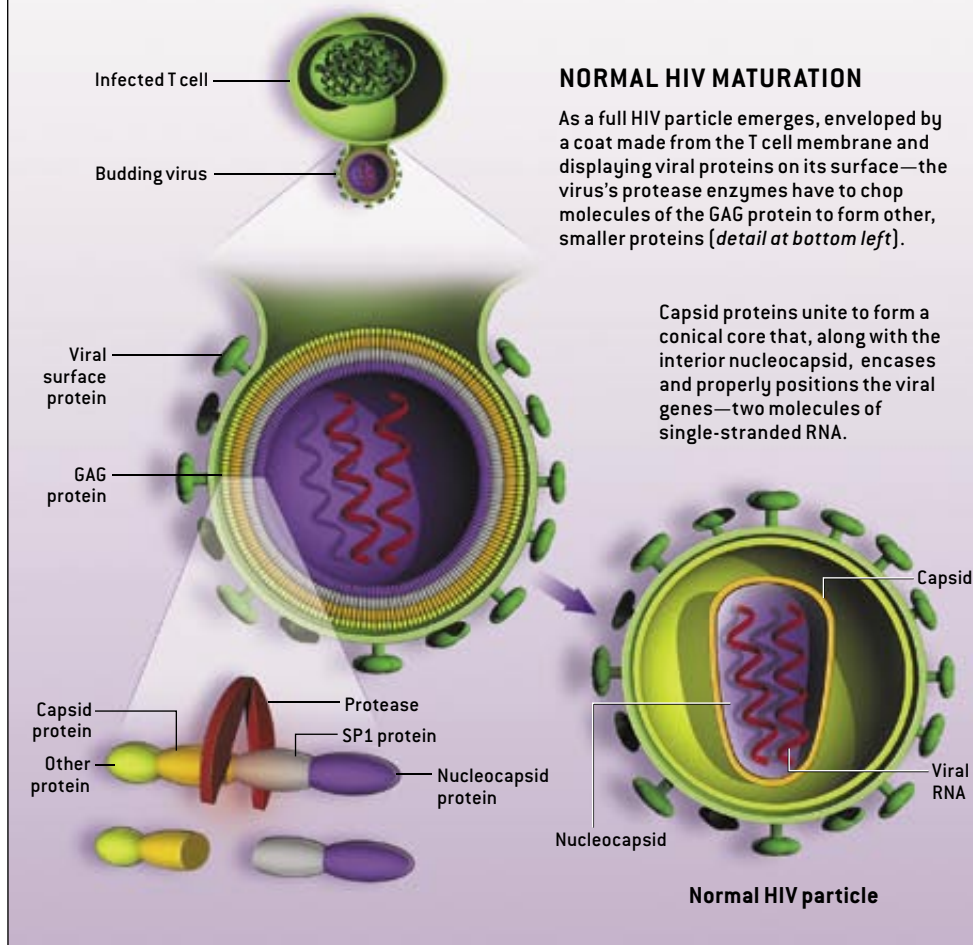
Six years ago Boston Biomedica spun off its HIV research unit into Panacos, which began to investigate the compound, by then named PA-457. PA-457 was not just another Taxol, the anticancer drug that required the controversial felling of rare yew trees until a semisynthetic substitute was found. Panacos did not need a steady source of Taiwanese herbs. Betulinic acid could be extracted from ubiquitous plane and birch trees, and a subsequent processing step yielded the desired molecule.

Even though researchers understood that PA-457 seemed to have activity against all strains of HIV, they needed to find out how the betulinic acid derivative worked against the virus on the molecular level. The company wanted a new class of drug, not just another protease inhibitor. It contacted Freed's laboratory at the NIH, which studies the virus life cycle.

Freed's group and Panacos determined that the drug worked late in the viral replication process, apparently at the stage of capsid formation. The researchers already knew that the HIV capsid forms when newly made GAG at-

A NOVEL TREATMENT STRATEGY FOR HIV

Maturation inhibitors constitute a new class of HIV drugs under study. They attack the virus at a late stage in its life cycle—when freshly made components of the virus are coming together



taches from inside the host T cell to the cell's membrane and is then chopped by the HIV protease into smaller pieces. They knew as well, from the development of protease inhibitors, that any disruption to the processing of GAG would cause the virus to become noninfectious. So they began to study PA-457's interaction with GAG to see exactly how it bollixed up the cutting of GAG into its requisite parts.

Cultivation of Resistance

TO UNDERSTAND HOW a compound works, scientists often begin by creating resistance, which lets them pin down the exact spot where the drug interacts with its target. To nurture resistance, Freed and his colleagues administered low doses of PA-457 to HIV-infected T cells

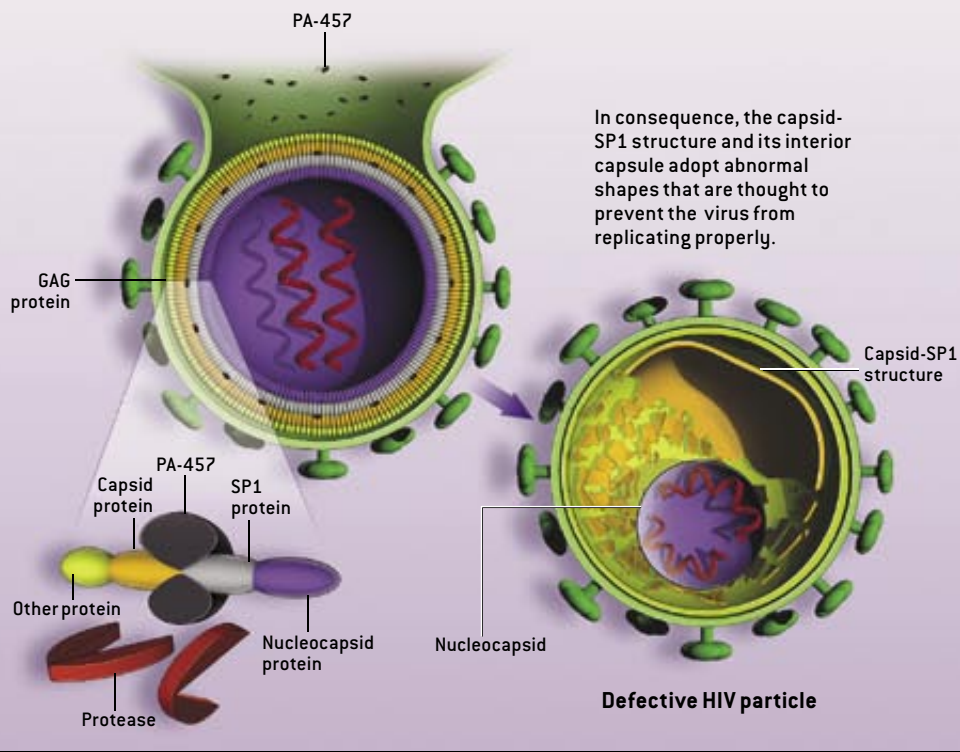
in culture. The genome of the resistant viruses was sequenced and compared with that of viruses that succumbed to the drug. That analysis located the site on the newly produced viruses that changed in the resistant versions. It turned out to be a site on GAG where the protease binds, and this alteration prevented PA-457 from blocking the enzyme's activity.

Analyzing the resistant strains allowed the researchers to ascertain that PA-457 was not simply another protease inhibitor. Most drugs, not just HIV inhibitors, work by tinkering with enzymes. "Targeting the substrate [instead of the enzyme] was unknown and surprising," Allaway comments. "As a result, we believe we will have a fairly strong patent position."

into new infectious particles that are beginning to “bud” from one infected T cell so that they can move on to infect another cell.

TREATED VIRUS

The drug candidate PA-457 works by attaching to the GAG protein and preventing the protease from separating the capsid protein from its neighbor in GAG—the SP1 protein (*detail*).



Cultivating resistant strains does not necessarily mean that the drug will have a limited therapeutic life span. In fact, resistance to PA-457 may not develop quickly, because the site where it binds on the GAG protein does not readily change from one HIV strain to another through mutations.

PA-457 has already passed through a midphase clinical trial that checked for drug activity in patients who took it for 10 days while another group received a placebo. HIV replicates so rapidly that a short trial can be used to determine whether a drug is attacking the pathogen in the body. Viral levels averaged a drop of 92 percent at the highest dose of 200 milligrams. The study looked for a decrease in so-called viral load of at least 70 percent as a preliminary sign of

the drug’s effectiveness. Some patients, however, did not respond—and the company will determine in the next phase of testing whether it can give higher doses. “The main message is that this is an active drug and research should go forward,” says Jeffrey M. Jacobson, chief of infectious diseases at Drexel University College of Medicine, who is the lead researcher in the clinical trials.

During the next round, investigators will be looking for interactions with other drugs, an essential test of any HIV drug prospect, because no treatment consists of a single drug therapy, given the threat of resistance. The Food and Drug Administration is encouraging tests earlier during clinical trials these days. In developing new HIV drugs, re-

searchers have at times detected these interactions only much later in the clinical trial process. If all goes according to plan, Panacos could file its final FDA approval application by 2008.

Other Immaturity Preservers

PA-457 IS NOT THE ONLY example of a maturation inhibitor, although it has progressed the furthest toward commercialization. At the University of Alabama and the University of Maryland, researchers working independently have identified small organic molecules that prevent the multitude of capsid subunits from joining up to form the finished casing. “We’re trying to jam the parts so they don’t fit together,” says Peter Prevelige, a professor in the department of microbiology at the University of Alabama.

This strategy goes along with other approaches under development to sabotage the viral life cycle. Entry inhibitors, including one Panacos is working on, prevent the virus from entering the cell. (One injectable entry inhibitor has already received FDA approval, but the Panacos drug would be taken orally.) Among the other classes of drugs that have reached late-stage trials are integrase inhibitors, which undermine an enzyme that allows the viral-made DNA to integrate into the host DNA to produce new viral RNA. All these biological agents are needed—and more. Absent a vaccine—not a near-term prospect—the lowly virus, a nanometer-scale capsule of single-stranded RNA, will continue to outwit the best ideas that molecular biologists conjure. SA

MORE TO EXPLORE

PA-457: A Potent HIV Inhibitor That Disrupts Core Condensation by Targeting a Late Step in Gag Processing. F. Li et al. in *Proceedings of the National Academy of Sciences USA*, Vol. 100, No. 23, pages 13555–13560; November 11, 2003.

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The Discovery of a Class of Novel HIV-1 Maturation Inhibitors and Their Potential in the Therapy of HIV. Donglei Yu et al. in *Expert Opinion on Investigational Drugs*, Vol. 14, No. 6, pages 681–693; June 2005.