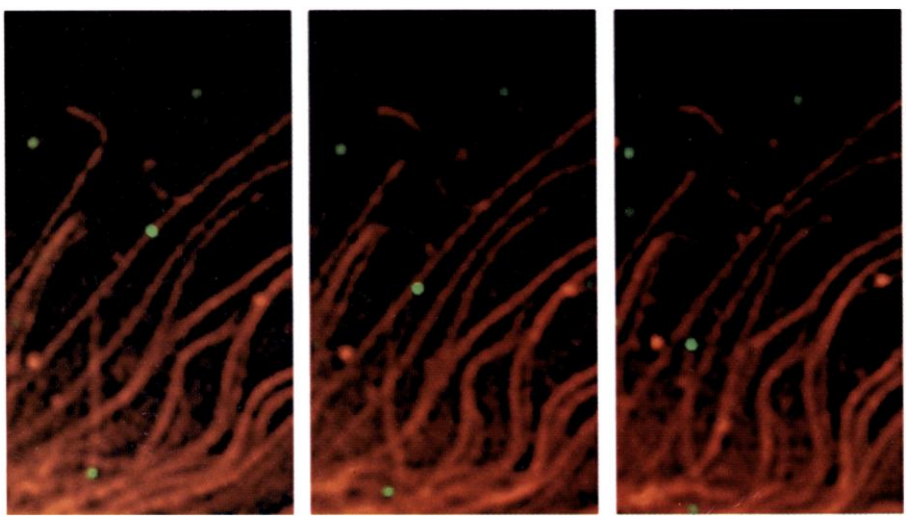
AIDS researchers are optimistic that preliminary findings on how HIV enters cell nuclei will lead to new therapeutics in the fight against the epidemic

Probing HIV's Elusive Activities Within the Host Cell

Like a lock-picking burglar, HIV slips into cells by tinkering with their outer membranes. Once inside, the virus makes its way to the nucleus, where it commandeers the cell's machinery and begins churning out copies of itself. Several promising efforts to outfox the virus with either vaccines or antiviral drugs are focusing on this first interaction—when HIV binds to and fuses with the cell—the idea being to stop the infection before it starts. But a handful of researchers have been eyeing HIV's trek from its entry site to the nucleus of its host cell. Their findings, although still preliminary, promise to reveal new—and badly needed—drug targets to fight the ever-mounting AIDS epidemic.



Guided transport. These time-lapse photos, taken at 5-minute intervals, show HIV particles apparently traveling along the host cell's microtubules.

It's one of the most exciting frontiers in AIDS research, says Robert Gallo, director of the Institute of Human Virology at the University of Maryland in Baltimore. And like any frontier, this emerging field is a wild place, replete with conflicting views and scientific tussles. Even so, says Gallo, "this research can lead to things we can't even yet anticipate. It'll open more and more ideas on understanding HIV infection, its replication cycle, and its capacity to cause disease. It's obvious that it also will give us new targets for blocking HIV infection, just as the fundamental work on how HIV enters cells gave us new insights." The work may even provide new tools for gene therapy (see sidebar).

For all the billions of dollars that have been poured into HIV research over the past 2 decades, this is largely uncharted territory. "All the interactions that the virus does inside the cell with the cellular components are not known in most cases," says Ari Helenius of the Swiss Institute of Technology in Zürich. As researchers begin to explore this landscape, a common theme is emerging: Soon after viruses enter cells, they enlist components of their hosts to assist them in their journey. Stephen Goff of Columbia University in New York City, for example, has identified cell mutants that block the progression of HIV infection at various stages, suggesting that the virus depends on several intact cellular factors to cause infection. A growing number of studies indicate that this may be the case for almost every stage of HIV's long trek, which includes the production of a DNA copy of its RNA genome, transit through the cytoplasm to the nucleus, transport across the nuclear envelope, integration into a chromosome, and the final challenge, ensuring that its genes are efficiently expressed.

Hijacking

Recent studies point to the host's scaffold of protein filaments—the cytoskeleton—as one of the key cellular components HIV enlists in its cause. Soon after HIV enters a cell, it forms a reverse transcription complex, a particle rigged to produce a DNA copy of the virus's RNA genome. In studies published in the *Journal of Experimental Medicine* in 1998, Mario Stevenson and his team at the University of Massachusetts Medical Center in Worcester suggest that the complex interacts with actin filaments of the cytoskeleton. What's more, the complex seems to require an intact filament network to perform its job efficiently. Stevenson thinks the complex might use the filaments to gain access to other factors in the cell that help it make the DNA copy, although other interpretations are possible.

Beyond making a copy of its genome that is compatible with its host's, HIV must transport that copy to the host cell nucleus, where it directs the production of new viral particles. That is no easy task. HIV's site of entry can be as much as 20 micrometers away from the nucleus—a formidable distance for HIV reverse transcription complexes that are only between 50 and 120 nanometers in diameter. Yet HIV arrives at the nucleus within minutes after infection. "Now that probably doesn't happen by chance," says Stevenson. "The virus must have a roadmap."

Thomas Hope of the University of Illinois at Chicago Medical School thinks he might have found the highway, at least. In unpublished work, Hope labeled HIV particles by fusing a viral protein called Vpr to green fluorescent protein, a marker used to track proteins in living cells. "What we saw was striking. The particles were moving in fairly linear paths within the cell," he says. Then Hope lit up the cells' microtubules—filaments that, like actin, form part of the cytoskeleton—by injecting microtubule building blocks labeled with a different fluorescent tag. Most HIV particles appeared to be associated with the microtubules; in some cases, Hope could see particles moving in bursts along the lengths of microtubules, like stop-and-go traffic on a busy street.

CREDITS: DAVE MCDONALD AND TOM HOPE/UNIVERSITY OF ILLINOIS AT CHICAGO COLLEGE OF MEDICINE

"I think the movies are very cool," says John Coffin, a leading HIV expert at Tufts University School of Medicine in Boston, of the images Hope has captured. "But one has to be very cautious when one's results are cool." Coffin notes, for example, that in a typical infection, less than 1% of viral particles successfully convert their host cells into HIV factories. That means that most of the particles that Hope labels are dysfunctional. But Hope has managed to partially solve this problem by using fluorescent deoxynucleotides to tag only those particles capable of synthesizing DNA; he is also timing his experiments to avoid monitoring nonfunctional particles that are quickly destroyed by the host cell.

Breaking and entering

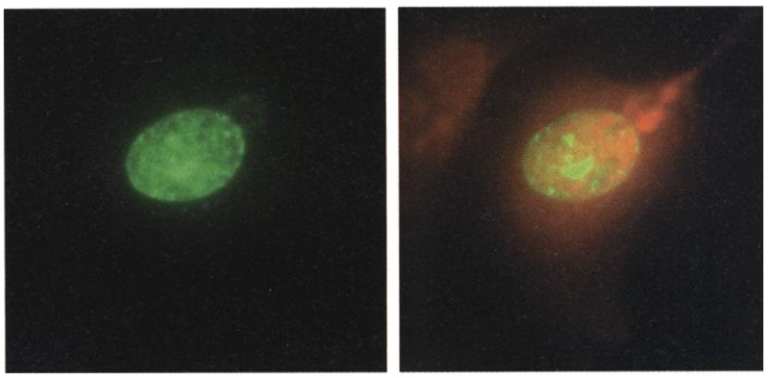
After traversing the cytoplasm, HIV encounters its next formidable task: getting through the nuclear envelope. In nondividing cells, the only way in is through the nuclear pores, which normally allow entry to cargo no more than 28 nanometers wide. Although estimates vary, the HIV particles, called preintegration complexes, that arrive at the nuclear envelope may be twice this size.

Most retroviruses solve the problem by bypassing the pores and infecting only dividing cells, taking advantage of the stage when the nuclear envelope breaks down during cell division. But not HIV. The virus's targets include both dividing cells, such as activated T cells, and nondividing cells, such as macrophages. "It's clear that the virus has gone to great lengths to do this," Stevenson says. "So macrophages must be important for some aspect of the biology of these viruses."

Outside a host cell, HIV is exposed and vulnerable. So Stevenson thinks that by infecting macrophages, one of the first cells it encounters, HIV protects itself. In addition, Stevenson reported last year in *Nature Medicine* that HIV-infected macrophages secrete signals that attract T cells, HIV's preferred host, and prime them for infection. "Our suspicion is that macrophages actually disseminate the virus on to other cells," he says.

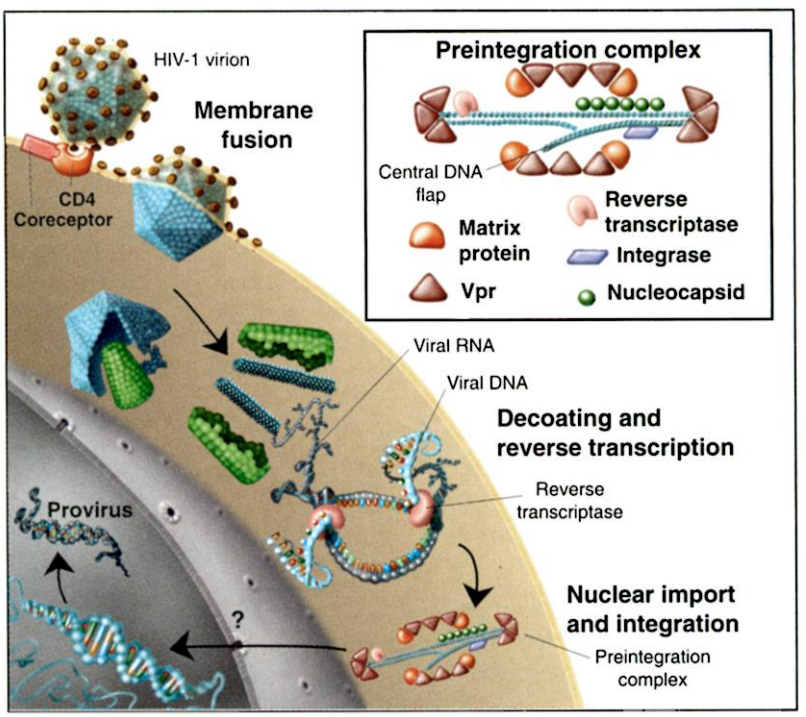
So how do HIV particles get through the nuclear envelope of macrophages and other nondividing cells? It's the $64,000 question, says Goff. Four viral components have emerged as candidates for helping move the particles into the nucleus, but their relative contributions remain elusive and controversial. "It's a ridiculously intense controversy," says Didier Trono of the University of Geneva in Switzerland. "I say 'ridiculously' because I think we are much too arrogant in our assertions and we underestimate the complexity of biological phenomena."

One of the first hints of HIV's capacity to penetrate nuclei surfaced in 1991 when Michael Malim, now at the University of Pennsylvania in Philadelphia, reported in the *Journal of Experimental Medicine* that HIV was capable of integrating its DNA into the chromosomes of nondividing cells. A year later, Stevenson helped explain this finding: He reported in the *Proceedings of the National Academy of Sciences (PNAS)* that preintegration complexes could traverse their host's nuclear envelope. Stevenson's team then set out to search for ways in which the virus might be tapping into the cell's transport system. Like packages moving through a postal system, cellular proteins often bear address labels that help the cell route them to their proper destinations. So the researchers hypothesized that HIV could be sneaking into the nucleus by sporting a nuclear address label, a short sequence called a nuclear localization signal that is recognized by the cellular proteins that ferry cargo into the nucleus. Indeed, in 1993, the researchers found that a viral protein called the matrix protein contains such a sequence.



Nuclear disruption. Viral protein Vpr disrupts the nuclear membrane by causing a defect in the underlying lamin. Left, a hernialike disruption is evident in the lamin. Right, the nuclear membrane protrudes, or blebs (shown in red), from the site of the defect.

A flurry of studies followed this initial observation, some supporting the matrix protein's role in transport, others offering evidence against it. Today the protein's role remains uncertain, but most agree it cannot be the sole mediator of HIV nuclear import. A study published in the *EMBO Journal* by Heinrich Gottlinger's lab at the Dana-Farber Cancer Institute and Harvard Medical School in Boston in 1998, for example, claimed that mutant viruses lacking most of the matrix protein are still capable of infecting nondividing cells.



The long trek. Before it can wreak its havoc, HIV must enter the cell and make a DNA copy of its RNA genome. This copy is transported through the cytoplasm and moves across the nuclear envelope as part of a particle called a preintegration complex. Within the nucleus, the virus integrates into the host chromosome and begins producing more viruses.

Buds and blebs

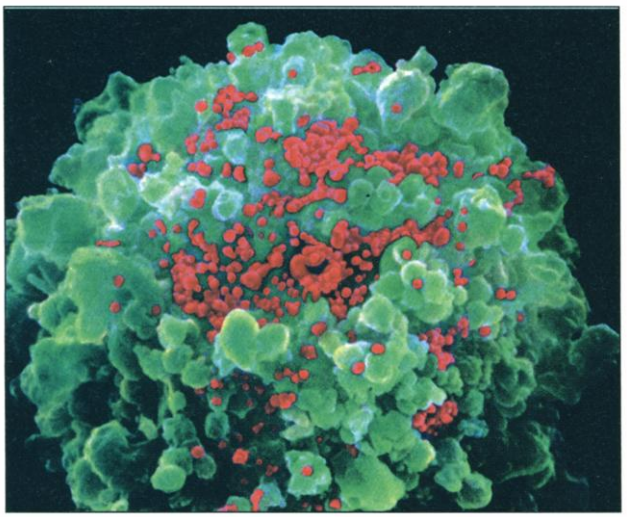
A year after the matrix protein first emerged as a candidate for transporting HIV particles into the nucleus, another viral protein, Vpr, stepped into the spotlight. A team led by Stevenson and Michael Emerman of the Fred Hutchinson Cancer Research Center in Seattle, Washington, reported in *PNAS* that mutations in the matrix protein's nuclear localization signal didn't interfere much with the transport of

CREDITS: (TOP TO BOTTOM) CARLOS DE NORONHA AND WARNER C. GREENE/GLADSTONE INSTITUTE OF VIROLOGY AND IMMUNOLOGY, UCSF; ILLUSTRATION: CAMERON SLAYDEN, ADAPTED FROM E. O. FREED, *VIROLOGY* 251, 1 (1998)

HIV DNA into the nuclei of nondividing cells unless Vpr was mutated as well. Subsequent studies showed that Vpr sports sequences that may enable it to bind directly to the nuclear pores or to associate with cellular proteins that dock nuclear-bound cargo at the pores.

Recent studies suggest that Vpr might also help disrupt the nuclear membrane itself. Working in Warner Greene's lab at the Gladstone Institute of Virology and Immunology of the University of California, San Francisco, Carlos de Noronha genetically engineered cells to produce Vpr and monitored their behavior using labeled proteins. In one set of experiments, described at the 2000 International Meeting of the Institute of Virology in Baltimore last September, they fluorescently labeled nuclear lamin C, a protein of the nuclear envelope that forms an array of filaments that enclose the nucleus like a burlap bag. "It was unbelievably striking," says Greene. "When Vpr is present, the nuclear membrane begins to bleb, forming transient protrusions from the nucleus that look like solar flares. And then, intermittently, these blebs rupture, and soluble components of the nucleus flood out into the cytoplasm, and cytoplasmic soluble components flood into the nucleus." Greene speculates that, among other things, this disruption may allow HIV preintegration complexes to sneak into the nucleus.

In 1997, Trono's group came up with yet another candidate for carrying these HIV particles across the nuclear envelope: integrase, the enzyme that inserts HIV DNA into its host's chromosomes. As Trono described in *PNAS,* integrase also sports a nuclear localization signal, which when fused to marker proteins can transport them into the nucleus. Unpublished work from Malim's lab suggests that integrase is indeed essential for transporting HIV preintegration complexes into its host's nucleus. But Fabrizio Mammano at INSERM in Paris and his colleagues claim their work, published in August in the *Journal of Virology*, indicates otherwise. Mammano thinks that integrase's nuclear localization sequences are required for hauling integrase itself, as a single protein, across the nuclear envelope but are dispensable for importing bona fide preintegration complexes, which contain several components in addition to integrase, including DNA, Vpr, and the matrix protein.



Exit strategy. After HIV has tricked the cell into making many copies of itself, the virus (shown in red) leaves the cell, a T lymphocyte blood cell (green).

Adding yet another twist to the story, Pierre Charneau of the Pasteur Institute in Paris reported in the April issue of *Cell* that a short segment of viral DNA plays an important role in the import of HIV particles. Although DNA usually exists as a double-stranded molecule, a small region of HIV DNA called the "central DNA flap" is triple stranded. Charneau found that mutant viruses lacking this unusual structure pile up at the outer edge of the nucleus, seemingly unable to get in. One possibility is that the flap helps shape the bulky particles so they can crawl through the pores.

Several researchers have developed working hypotheses to explain the complexity. "No matter what cell the virus gets into, it will face the need for traveling from the plasma membrane to the nucleus and getting through the nuclear envelope," says Trono. "And those circumstances will most likely vary from one cell to another. I think this may well explain the diversity of mediators of nuclear import."

Others note that the problem of hauling a large particle through a small and selective pore is so onerous that HIV may need multiple mechanisms to do it efficiently. In support of this idea, cell biology studies have shown that the more nuclear localization signals a particle carries, the faster and more likely it is to be transferred across the nucleus. In addition, different signals may cooperate with each other or act sequentially. And because getting into the nucleus is of

Capitalizing on HIV's Talents For Gene Therapy

The emerging understanding of HIV's journey from the cell membrane to the nucleus may help researchers design better vectors for gene therapy for a variety of diseases. In the early 1990s, genetic engineers were using mouse retroviruses, which are incapable of infecting nondividing cells, as gene-delivery vectors. Despite their efforts to shape the retroviruses' genomes and endow them with the ability to infect nondividing cells, prime targets for gene therapy, the vectors failed repeatedly.

Didier Trono of the University of Geneva, who was working on HIV and was also interested in developing vectors for gene therapy, reasoned that if he could understand how HIV managed to infect nondividing cells, he might be able to engineer the mouse retroviruses to do likewise. But as the complexity of HIV's nuclear transport emerged, Trono began to doubt that strategy. "At that time we were working on nuclear import and were saying, 'Oh, gosh, there's matrix, there's Vpr, there seems to be another guy. ... This complex may be very sophisticated; how can we think of just putting a nuclear localization signal somewhere and expect it to work?' " he recalls. So instead, working with Luigi Naldini and Inder Verma of the Salk Institute for Biological Studies in La Jolla, California, Trono decided to use HIV itself as a vector. They proposed modifying HIV to eliminate its ability to cause disease, while capitalizing on its talent for delivering genes into the nucleus.

The radical proposal worked, leading to the development of a new class of vectors for gene therapy of nondividing cells (*Science,* 12 April 1996, p. 263). Further modifications to this original vector have now led to safer and more effective varieties that are beginning to prove their clinical potential. Last month, for example, a vector developed in Trono's lab, known as a lentiviral vector, was used to successfully treat monkeys suffering from a condition similar to Parkinson's disease (*Science,* 27 October, p. 767). And even better vectors may soon become available as researchers understand the mechanisms behind HIV's talent for delivering genes to the nucleus. Pierre Charneau of the Pasteur Institute in Paris describes in an April *Cell* article, for example, that adding a DNA flap to Trono's original HIV vector dramatically improved its efficiency. As Ari Helenius of the Swiss Institute of Technology in Zürich explains, "Viruses have had millions of years to evolve gene therapy."

–M.C.

CREDIT: NIBSC/PHOTO RESEARCHERS

such vital importance to HIV, the multiple transport mechanisms may serve as backups for each other. "It's not uncommon for HIV to incorporate redundancy—to have multiple ways of getting a job done," says Greene. Still others, like Emerman, have already placed their bets on a single candidate—in his case, on integrase.

"I don't think everybody is right here, but I don't yet have a betting line on who might be and who's not," says Coffin. "I'm standing aside waiting for the dust to clear."

Inside the black box

While controversy reigns over nuclear import, mystery shrouds HIV's movements within the nucleus itself. "If the trip from the plasma membrane to the nuclear envelope is a gray box, the rest is a black box," says Trono. "Biologists don't know much about intranuclear trafficking, in general."

Despite this ignorance, researchers are beginning to realize that, just as HIV co-opts cytoplasmic factors, it also takes control of nuclear factors. Trono's unpublished observations, for example, suggest that shortly after HIV enters a cell, it triggers factors involved in regulating gene expression to rush out of the nucleus. Trono stresses that the meaning of the observation remains unclear. But he hypothesizes that HIV might be recruiting these factors to ensure that its genes are expressed efficiently once they are integrated in the host's chromosomes. Most locations on a chromosome are not suitable for gene expression. Yet HIV's genes seem to be expressed very effectively, almost regardless of where they integrate. "That is quite a success that you need to explain," says Trono. "These factors might help to create a sort of transcriptional honeymoon for the provirus right after it integrates."

Turning these emerging insights into therapeutics will clearly take some time. A few researchers are already trying to block viral nuclear localization signals, for instance. But because these sequences are similar to those of many cellular proteins that reside in the nucleus, some fear this strategy could lead to serious side effects. Also, the complexity of the challenge causes many drug companies to shy away from developing drugs that block multisite interactions between large proteins, says Coffin, such as those that occur as HIV travels through the cell.

But Gallo is optimistic, comparing the study of HIV's trek through the cell to the early days of research on viral entry. "Look at what happened in a short time in that area," he says, alluding to the new drug candidates spawned by viral entry research. "Who knows what can happen here. You can't predict what'll come, but that something will come is very likely." **–MARINA CHICUREL**

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PLANETARY SCIENCE

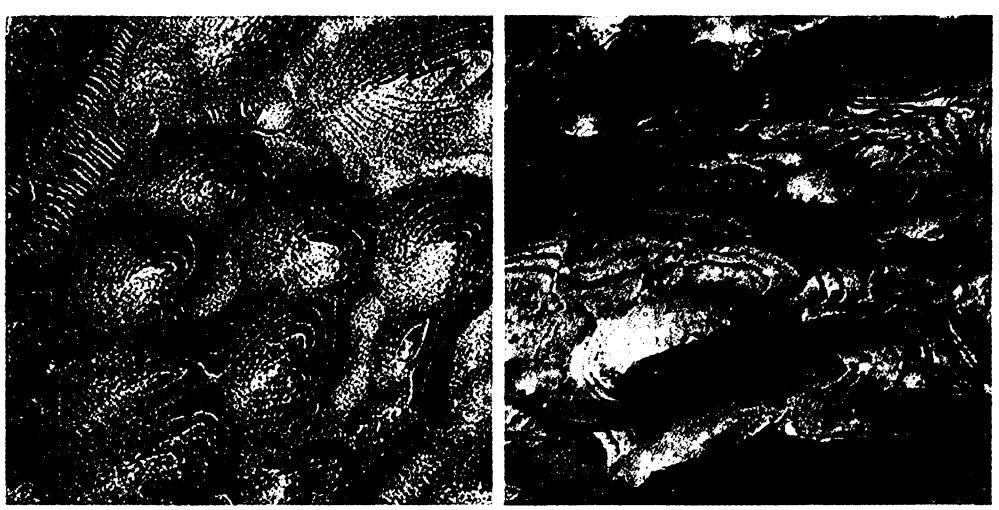
A Dripping Wet Early Mars Emerging From New Pictures

The latest images from the Red Planet are suggesting that water ponded across its equatorial region eons ago, just when life might have been emerging

Mars, water, life. That explosive mixture comes together again on page 1927 of this issue, where a sampling of hundreds of spacecraft images shows crisply detailed sediment layers on Mars. Although the authors offer more than one interpretation, the one they prefer has the sediments laid down beneath broad lakes and shallow seas at a relatively clement time in the planet's history. The images don't have the visceral impact of the springlike seeps reported earlier this year, but the geologic implications of the pictures plus supportive signs from earlier missions mean that these possible lake sediments will be prime candidates for NASA missions seeking signs of past life on Mars.

The new hints of extensive standing water on Mars as much as 4 billion years ago—about when life got started on Earth—comes after 3 decades of studies of layered martian terrains. "A lot of the pieces are not new" in the *Science* paper, says Mars geologist Michael Carr of the U.S. Geological Survey, but the new study "brings all these bits and pieces together with much better support for layered deposits and makes a good story out of it"—not that the story will lack controversy. "They make a good case that [the terrain] is layered," says planetary geologist James Head of Brown University, "and the most likely interpretation is they are sediments. That's pretty impressive. The part that will be debated will be the origin of the sediments. Sediments don't necessarily mean water."

The controversy over how much water Mars ever had and when, if ever, liquid water flowed on the surface began with the first successful Mars orbiter, the U.S. Mariner 9 spacecraft. Arriving in 1971, it confirmed the dry, desolate, moonlike appearance of large areas of Mars imaged by earlier flyby spacecraft. But Mariner 9 also discovered so-called valley networks, reminiscent of river-carved valleys on Earth. Many researchers argued that groundwater oozing from the headwalls of valleys, rather than rain and running rivers, could sculpt such features, obviating the need for a "warm and wet" early Mars. But more signs of water—water that once stood in pools, lakes, and even oceans—appeared in many of the 50,000 images returned by the pair of Viking orbiters that arrived in 1976. Hints of a shoreline around the northern lowlands suggested to Mars geologist Timothy Parker of the Jet Propulsion Laboratory in Pasadena, California, that there had been an early



CREDIT: MALIN AND EDGETT, MSSS/JPL

An edgy face of Mars. Some erosive force, perhaps the wind, has sculpted layered sediments into stair-stepped hills inside an impact crater *(left)* and a chasm. Images are about 1.2 kilometers square.