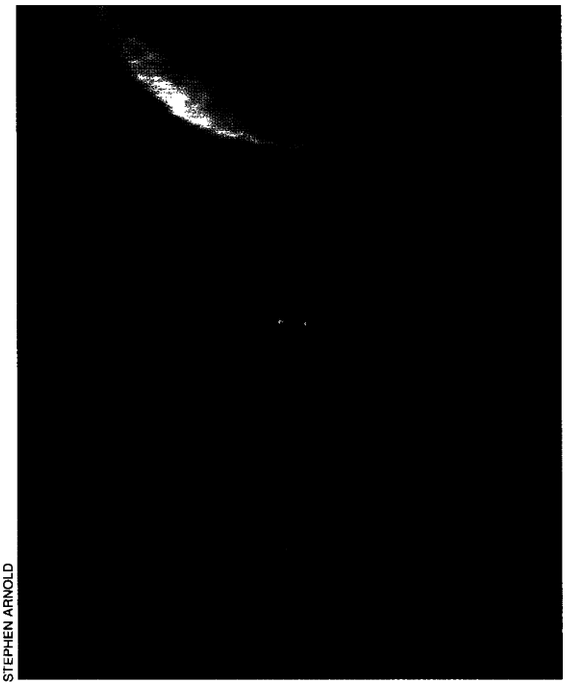


STEPHEN ARNOLD

Point of light. *Micron-sized spheres—in this case a glycerin droplet suspended between electrodes—can ensnare light waves.*

shined on a transparent solid riddled with light-absorbing impurity molecules. When the laser is tuned to a frequency absorbed by some of the impurities and its intensity is cranked up, the light-absorbing impurities can be excited until some of their molecular bonds are broken or they give up electrons. The disrupted molecules can no longer absorb light at that frequency, creating a "spectral hole" where there is almost no absorption—a hole that can later be "read" with a low-intensity laser.

Best of all, because each patch contains impurities that absorb at many different frequencies, several holes, each at its own frequency, can be written onto the same patch, each hole potentially representing a "0" or "1" in a computer memory. As a result, such a memory could offer densities tens of thousands of times greater than conventional optical disks. The catch is that the frequencies of the holes would broaden and overlap one another at temperatures above that of liquid nitrogen—77 degrees Kelvin.

But Arnold's Almaden visit got him thinking. His photonic atoms responded to the same frequencies no matter what the temperature, and a collection of randomly sized spheres would certainly provide the broad array of different frequencies needed for a stacked memory. Though there was no good way to assemble dye-laden glycerin droplets into a memory, the polystyrene beads would do the trick, if they could somehow be combined with molecules that could be altered by laser light.

Working with William Whitten and Michael Ramsey at Oak Ridge National Laboratory, Arnold decided to imitate the original glycerin droplets by embedding fluorescent dye molecules in the plastic spheres. The dye should fluoresce at the spheres' size-dependent resonant frequencies, and a high-intensity laser could destroy the fluorescence to burn a hole at any one of those frequencies. To test the scheme, Whitten and Ramsey soaked an assortment of different-sized beads in dye, placed them in water on a glass slide, and beamed a low-intensity laser at them at a range of different frequencies. As hoped, the beads fluoresced at such a wide variety of frequencies that the spectrum was essentially flat. Then Whitten and Ramsey turned up the intensity at one frequency to try and write a spot on the sample. When they came back with the low-intensity "read" laser, they found the sample no longer fluoresced at that frequency; they had indeed burned in a hole. Arnold later repeated the experiment, using various frequencies to create multiple holes.

All that has made Arnold optimistic that the beads could form the basis of a practical hole-burning memory, in which multiple bits of data could be written on the same small cluster of beads. Moerner, too, is intrigued by the possibilities, though he is quick to point out a problem. The beads, he notes, are thousands of times bigger than molecules, which might eliminate the density advantage of a hole-burning memory. "Arnold's work is new, and we have to see if the [density] problem can be solved," he says. Besides, he adds, the competition just got tougher: a Swiss team recently claimed some success at getting a specially doped crystal to retain spectral holes at room temperature.

Arnold concedes his beads are a long shot for commercial memories. But he notes that even though the beads are big by molecular standards, they would still have a hefty density advantage over conventional memories if they were packed in a three-dimensional array. In any case, Moerner and other researchers agree that, memory or no, Arnold's spheres make for some interesting physics—a physics that might center on a new sort of artificial matter. "Photonic atoms may turn out to be able, in effect, to exchange photons," Arnold says, much as atoms in a compound exchange electrons. "We're only just starting to look at this question, but if they do we'd consider them to be photonic molecules." Could the next hot field be photonic chemistry? ■ DAVID H. FREEDMAN

David H. Freedman is a science writer based in Boston.

Antigen Processing:

Immunologists have long known that the immune system must recognize an invading organism from portions of its proteins before it can destroy the invader, but one key question in immunology has been: How do cells chop up foreign proteins from intracellular invaders like viruses and present them to the immune system? The mechanism used by cells for degrading and presenting proteins has become a field of its own, known as antigen processing, that is one of the fastest-moving in all of science today. Indeed, antigen processing studies have moved so fast that if you had asked insiders for a status report only a few weeks ago, they would have told you that researchers were putting the finishing touches on their picture of the biochemical pathway by which antigens are processed. Ah, but that was then, and now another group has come up with an alternate—and unexpected—pathway.

Behind this revisionism are Donald Hunt, Victor Engelhard, and colleagues at the University of Virginia School of Medicine in Charlottesville, along with collaborators Kazuyasu Sakaguchi and Ettore Appella at the National Cancer Institute in Bethesda, Maryland. Referring to their paper on page 1264 of this issue of *Science*, Jonathan Yewdell, a specialist in antigen processing at the National Institutes of Health, says: "Now it appears that there are two ways of feeding [protein fragments] into the system."

And while, for the moment, this new information remains primarily a matter of basic research, knowledgeable immunologists believe that it could ultimately have important clinical implications. "The hope," says David Margulies of NIH, "has always been that by understanding what [protein fragments] are presented to the immune system, we might have a better understanding of the etiology of diseases and be in a better position to design drugs to modulate [the] immune response." So, the more knowledge the merrier—even if it upsets prior conceptions.

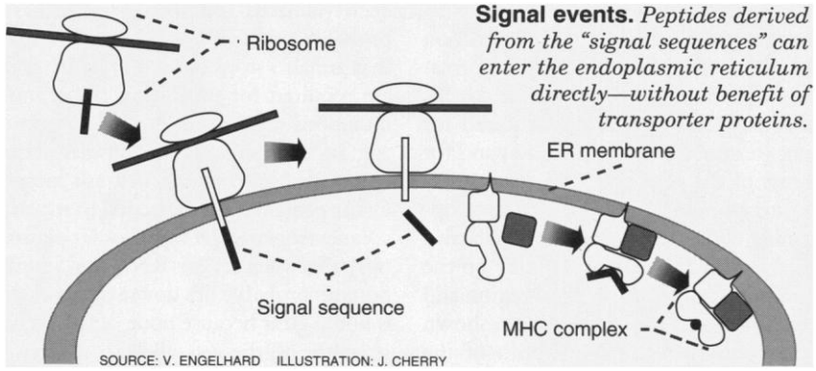
The "old" path to antigen processing begins after infection, when viral proteins from the cell's cytoplasm are chopped up into fragments called peptides by a ball of degradative enzymes known as the low-molecular mass polypeptide (LMP) complex. The peptides are then transferred into the endoplasmic reticulum (ER), a membranous compartment in the cell that serves as a conduit for newly synthesized proteins to reach the cell surface. But the peptides can't get into the ER on their own; to cross the lipid membrane that bounds the compart-

A New Pathway Discovered

ment, they must be ushered in by specialized transport proteins. Once inside the ER, the peptides meet and bind to proteins known as class I major histocompatibility complex (MHC) proteins, which are destined for the cell surface. When they've been bound to the MHC molecules, the viral peptides get carried through the ER to the cell's outer membrane. The MHC-peptide complex is inserted into the membrane with the peptide pointed outward—where it can be detected and recognized by receptors on immune cells.

A key feature of this pathway is that the peptides are required to stabilize the class I MHC protein structure: Without the peptide, the MHC molecule probably will not make it to the cell surface, and even if it does, it will fall apart rapidly. But when Engelhard and his colleagues noticed MHC molecules where they weren't expected, they were alerted that something unusual was going on.

Engelhard and his co-workers were working in mutant cells called T2 cells that lack the machinery ordinarily needed for antigen presentation by class I MHC molecules. Specifically, they don't make part of the degradative LMP complex or the peptide transporters, and so peptides produced in the cytoplasm are not transported into the ER. And since those peptides are required for stable expression of MHC class I molecules on cell surfaces, the lack of peptides in the ER should have implied a lack of MHC class I molecules on the cell surface. But, strikingly, that wasn't the case in T2.



Signal events. *Peptides derived from the "signal sequences" can enter the endoplasmic reticulum directly—without benefit of transporter proteins.*

In fact, one kind of MHC class I molecule—known to immunologists as HLA.A21—was expressed on the surfaces of the mutant cells at nearly normal levels. Engelhard and his colleagues were puzzled by this finding—and they immediately began exploring two hypotheses. Either, they speculated, the HLA.A21 molecules in T2 were unusually stable and could somehow make it to the surface without the peptides, or peptides were being made and getting into the ER—in spite of the lack of the usual machinery.

Their first guess was that the peptides somehow weren't needed in T2. "Our earliest interpretation was that HLA.A21 was out there without any peptide," says Engelhard. But that didn't turn out to be true: Surprisingly, the HLA.A21 molecules do contain peptides. The next question, recalls Engelhard, was to find out "where these peptides came from, since the antigen processing machinery, as defined, was missing in T2 cells." To get an answer, they relied on a new application of a technique originated by Engelhard's collaborators Donald Hunt and Jeffrey Shabanowitz and described in a separate article on page 1261 of this issue of *Science*. The method, which could be of great help to workers who want to know the amino-acid sequences of minuscule amounts of protein fragments, joins high-pressure liquid chromatography with mass spectometry. It allows a mixture of peptide molecules to be sequenced simultaneously and requires far less cellular material than is needed for current chemical techniques. "The technology is absolutely spectacular," says immunologist Michael Bevan of the University of Washington at Seattle.

Applying this spectacular new technowizardry to the peptides bound to the HLA.A21 molecules on the T2 mutant cells, Hunt, Engelhard, and their colleagues found that all the peptides shared one important property: they derived from the so-called "signal sequences" that are included in certain membrane-bound and secretory proteins. Those sequences are highly soluble in lipids (of which cellular membranes are made), and therefore they pull the proteins they're attached to through membranes. As Engelhard points out, "The signal peptide can get across the ER membrane independent from the transporter proteins."

But these signal peptides weren't found in their ordinary location. Most signal sequences are thought to remain embedded in the ER membrane, and "one of the great unknowns," of the new pathway, says Engelhard, is how some signal peptides become dislodged from the membrane in order to enter the ER and continue down the path to the cell surface.

There are other questions that remain to be answered, but for the moment, Engelhard's team has devised a possible model for their newly discovered antigen processing pathway. In that model, a newly synthesized protein is inserted into the ER directly from the synthetic machinery of the ribosome. As the protein is being inserted into the ER, the 9 to 12 amino acids of the signal sequence are cut off (probably by an enzyme called a signal peptidase). The signal sequence is then free to join with MHC molecules that take the entire complex to the cellular surface.

Since this alternate pathway was originally found in mutant cells, Engelhard and his colleagues were curious to know whether it was also operating in normal cells. So they looked, and, sure enough, they found that the signal sequence peptides were presented on the cell surfaces of normal cells. "We can say that this pathway is operating in normal cells as well as in mutants," says Engelhard. Even if it is operating in normal cells, the current indications are that the new pathway will turn out to be the road less traveled. Data from Engelhard's group suggest that the vast majority of peptides are processed by the established path. Nonetheless, for some specific invaders, processing by the new pathway could be the primary —if not the only—way the immune system knows of the pathogen's existence. Hence for some diseases, the new pathway could turn out to be crucial.

Furthermore, the new pathway may sometimes play a much larger role in maintaining health, speculates John Monaco of the Virginia Commonwealth University in Richmond, Virginia. Monaco is one of the immunologists who, 2 years ago, first found evidence for the transporters that shunt peptides into the ER. Since then, Monaco says, he has wondered, "Why haven't we found any humans with defects in transporter proteins?" The answer, might be, says Monaco, that such people aren't very sick. "My suspicion is that they are not very immunocompromised. It may be that this second pathway is kicking in and compensating for a loss in the first," he says. If Monaco is right the increased flexibility provided by a second antigen processing pathway could turn out to be an important element of how our immune system protects us from the horde of pathogens that are always in and around us. ■ MICHELLE HOFFMAN