# The Cellular Chamber of Doom

*Structures called proteasomes inside cells continuously destroy proteins. Several common diseases result when the process works too zealously—or not at all*

by Alfred L. Goldberg, Stephen J. Elledge and J. Wade Harper

Every minute of every day a scene straight out of an Indiana Jones movie plays out in all our cells. One second a hapless protein is tooling along just trying to do its job. The next instant it is branded for destruction and gets sucked into a dark tunnel, where it is quickly cut to pieces. Unlike Indiana Jones, for the protein there is no escape. Inside the chamber of doom, the protein is stretched out like a medieval prisoner on the rack and fed through a series of enzymatic knives that deliver the Death of a Thousand Cuts. A few seconds later the remnants emerge from the tunnel, only to be pounced on and chewed up further by simpler enzymes.

One might think that this intracellular drama is insignificant (except, perhaps, to the unfortunate protein). But scientists in many laboratories, such as our own, are now finding that these molecular abattoirs, called proteasomes (pronounced "pro-tee-ah-somes"), are crucial players in pathways that regulate an entire repertory of cellular processes. A typical cell in the body has roughly 30,000 proteasomes. When they malfunction—whether overeagerly gobbling important proteins or failing to destroy those that are damaged or improperly formed—diseases can ensue. Some viruses, such as the human immunodeficiency virus (HIV), have even developed the means to manipulate protein degradation by proteasomes for their own ends. Indeed, several of the next-generation drugs to treat cancer and other dire diseases are expected to consist of chemical compounds that act on proteasomes and the pathways that feed proteins into proteasomes. Several biopharmaceutical companies are now studying compounds that inhibit the proteasome pathway; two such potential drugs are already in clinical trials in humans.

## Turnover Is Fair Play

Proteins are the very fabric of which cells are made. Some proteins also act as enzymes, the molecular workhorses that drive the chemical reactions of life. The types of proteins a cell produces depend on which of its genes are active at any given time. Genes encode how the 20 basic protein subunits, called amino acids, are assembled into chains of various combinations. The chains fold into compact coils and loops to become different kinds of proteins, each with a specific function determined by its shape and chemistry.

What happens when proteins are no longer needed or fail to fold correctly? For years, scientists presumed that the lion's share of protein degradation occurs in lysosomes, bags of digestive enzymes present in most cells of the body. But in the early 1970s one of us (Goldberg) showed that cells lacking lysosomes, such as bacteria and immature red blood cells, can nonetheless destroy abnormal proteins rapidly. What is more, the process requires energy, whereas other degradative processes do not.

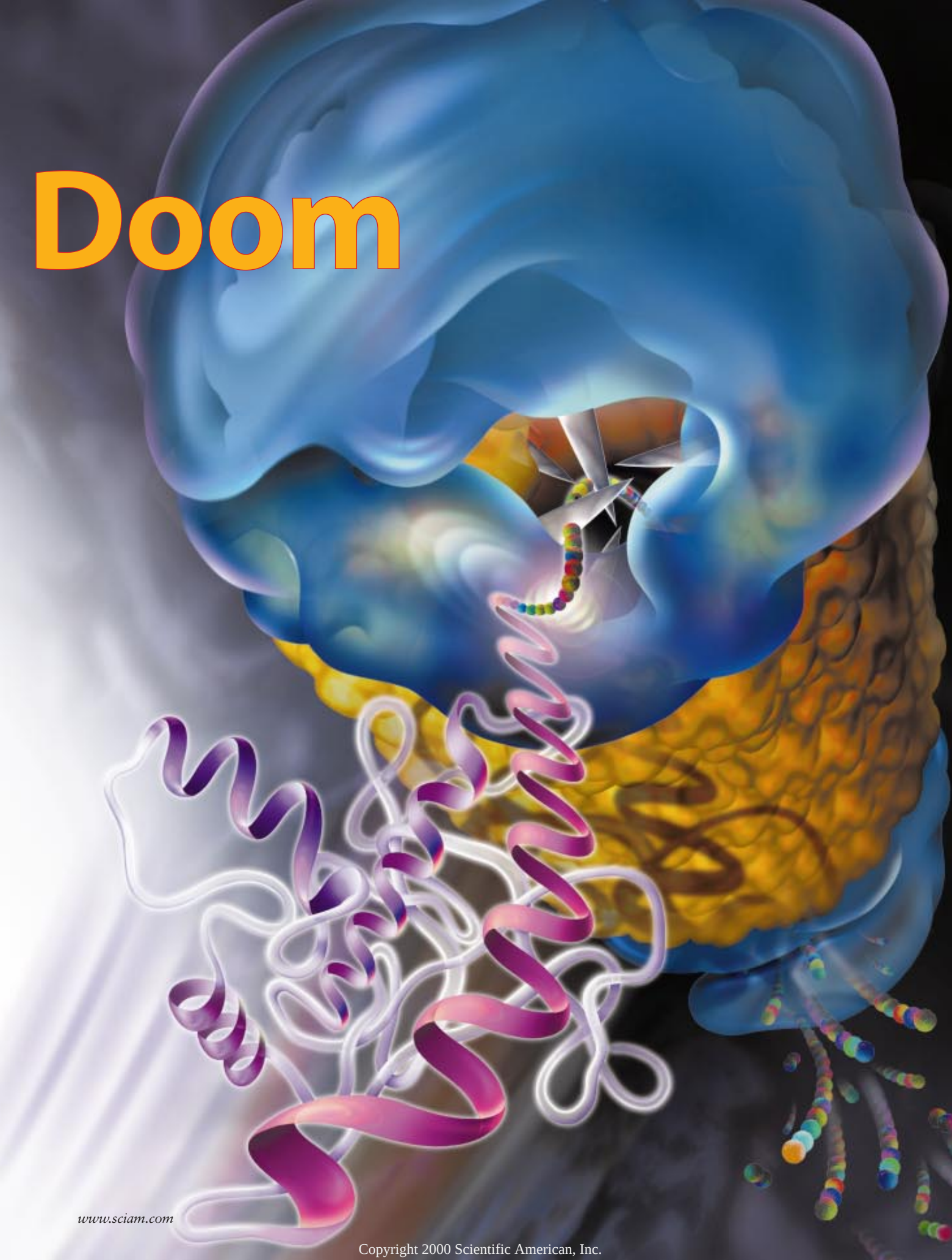
He and his colleagues were able to get the energy-requiring degradation process to work in test tubes, which enabled several research groups in the late 1970s and throughout the 1980s to discover the enzymes responsible. Eventually, in 1988, two groups—one led by Goldberg and the other by Martin C. Rechsteiner of the University of Utah—found that the proteins are broken down by large, multienzyme complexes that Goldberg's group named proteasomes.

Proteasomes were so named because they contain many proteases, enzymes that cut proteins into chunks. But proteasomes are 100 times larger and more complex than other proteases. Once a protein is laid on the doormat of a proteasome, it is taken inside the particle and ultimately disassembled like a Tinker Toy into amino acids that can be reassembled later

**PROTEASOME draws a protein (*ribbonlike structure at left in lower half*) into its maw for destruction by six specific enzymes, shown here as knives. An average body cell has thousands of proteasomes, which chop proteins the cell wishes to remove into bits of various sizes. The bits are then broken down by other enzymes into the basic building blocks of proteins—amino acids—which are eventually recycled to make new proteins.**

AUDRA GERAS

# Doom



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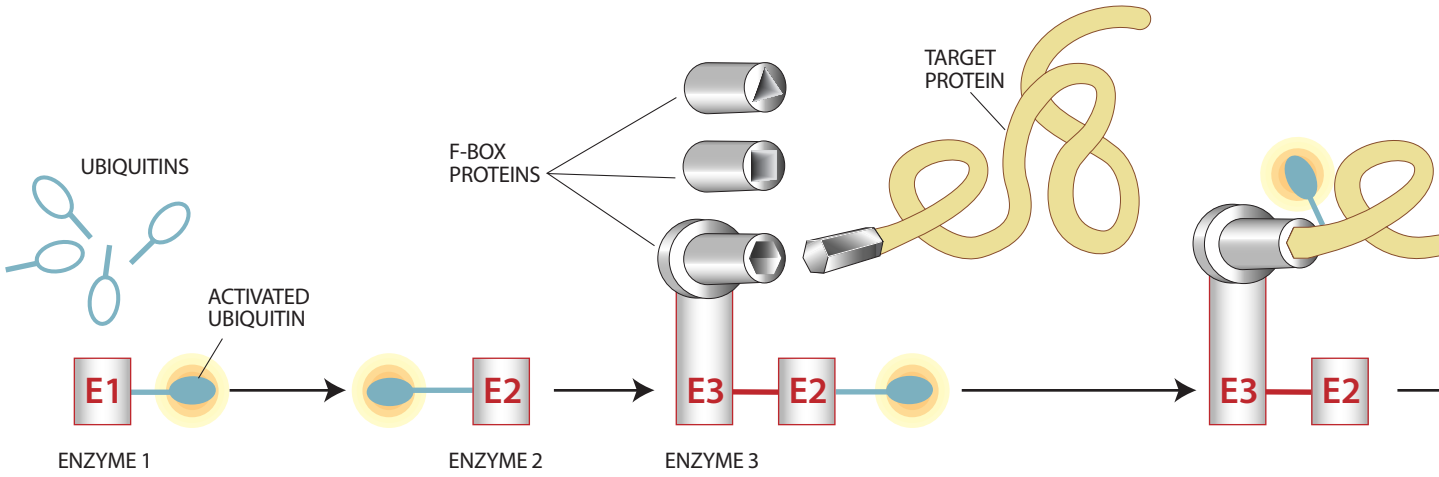



into other proteins. Most proteins are replaced every few days, even in cells that themselves divide rarely, such as those in the liver or nervous system. And different proteins are degraded at widely differing rates: some have half-lives as short as 20 minutes, whereas others in the same cell may last for days or weeks. These rates of breakdown can change drastically according to changing conditions in our bodies.

At first glance, such continuous destruction of cell constituents appears very wasteful, but it serves a number of essential functions. Degrading a crucial enzyme or regulatory protein, for example, is a common mechanism that cells use to slow or stop a biochemical reaction. On the other hand, many cellular processes are activated by the degradation of a critical inhibitory protein, just as water flows out of a bathtub when you remove the stopper. This rapid elimination of regulatory proteins is particularly important in timing the transitions between the stages of the cycle that drives cell division [*see box on page 72*].

Protein degradation also plays special roles in the overall regulation of body metabolism. In times of need, such as malnourishment or disease, the proteasome pathway becomes more active in our muscles, providing amino acids that can be converted into glucose and burned for energy. This excessive protein breakdown accounts for the muscle wasting and weakness seen in starving individuals and those with advanced cancer, AIDS and untreated diabetes.

Our immune system, in its constant search to eliminate virus-infected or cancerous cells, also depends on proteasomes to generate the flags that distinguish such dangerous cells. In this process, the immune system functions like a suspicious landlady checking whether her tenants are doing something undesirable by monitoring what they throw out in their daily trash. Although cell proteins are usually degraded all the way to amino acids, a few fragments composed of eight to 10 amino acids are released by proteasomes, captured, and ultimately displayed on the cell's surface, where the immune system can monitor whether they are normal or abnormal [*see illustration on page 73*]. Indeed, in disease states and in certain tissues such as the spleen and lymph nodes, specialized types of proteasomes termed immunoproteasomes are produced that enhance the efficiency of this surveillance mechanism.

Protein breakdown by proteasomes also serves as a kind of cellular quality-control system that prevents the accumulation of aberrant—and potentially toxic—proteins. Bacterial and mammalian cells selectively destroy proteins with highly abnormal conformations that can arise from mutation, errors in synthesis or damage.

The degradation of abnormal proteins is important in a number of human genetic diseases. In various hereditary anemias, a mutant gene leads to the production of abnormal hemoglobin molecules, which do not fold properly and are rapidly destroyed by proteasomes soon after synthesis. Similarly, cystic fibrosis is caused by a mutation in the gene encoding a porelike protein that moves chloride across a cell's outer membrane. Because these mutant chloride transporters are slightly misshapen, proteasomes degrade them before they can reach the cell membrane. The sticky mucus that builds up in the lungs and other organs of people with cystic fibrosis results from the lack of normal chloride transporters.

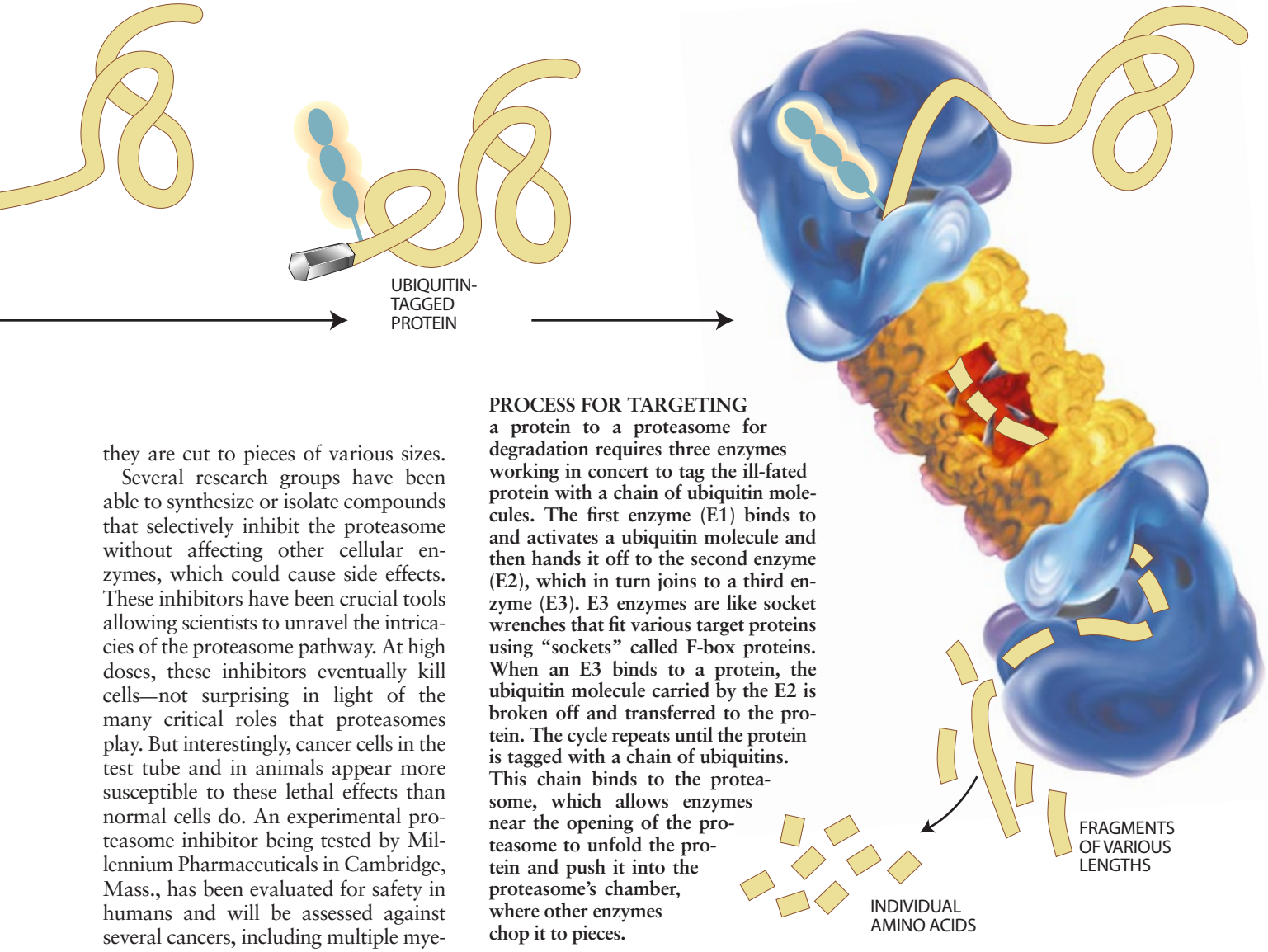
Still other diseases could result in part from the failure of abnormal proteins to be degraded by proteasomes. Scientists are finding, for example, that clumps of misfolded proteins accumulate in association with proteasomes in certain nerve cells, or neurons, in the brains of people with neurodegenerative disorders such as Parkinson's, Huntington's and Alzheimer's diseases. Why the neurons of individuals stricken with these maladies fail to degrade the abnormal proteins is a burgeoning field of research.

## In the Belly of the Beast

From a protein's humble perspective, proteasomes are enormous structures. Whereas the average protein is 40,000 to 80,000 daltons (or 40,000 to 80,000 times the molecular weight of a hydrogen atom), most proteasomes from higher organisms weigh in at a whopping two million daltons. In the mid-1990s scientists led by Wolfgang Baumeister and Robert Huber of the Max Planck Institute for Biochemistry in Martinsried, Germany, used x-ray diffraction and electron microscopy to determine the molecular architecture of proteasomes. Each one consists of a tunnellike core particle with one or two smaller, regulatory particles positioned at either or both ends like caps. The core particle is formed by four stacked rings—each composed of seven subunits—surrounding a central channel that constitutes a proteasome's digestive tract. The outer two rings appear to act as gates to keep stray proteins from accidentally bumbling into the degradation chamber.

Similarly, the regulatory "cap" particles are thought to act as highly selective gatekeepers to the core particle. These regulatory particles recognize and bind to proteins targeted for destruction, then use energy to unfold the proteins and inject them into the core particle, where

CLEO VILETT




**PROCESS FOR TARGETING** a protein to a proteasome for degradation requires three enzymes working in concert to tag the ill-fated protein with a chain of ubiquitin molecules. The first enzyme (E1) binds to and activates a ubiquitin molecule and then hands it off to the second enzyme (E2), which in turn joins to a third enzyme (E3). E3 enzymes are like socket wrenches that fit various target proteins using "sockets" called F-box proteins. When an E3 binds to a protein, the ubiquitin molecule carried by the E2 is broken off and transferred to the protein. The cycle repeats until the protein is tagged with a chain of ubiquitins. This chain binds to the proteasome, which allows enzymes near the opening of the proteasome to unfold the protein and push it into the proteasome's chamber, where other enzymes chop it to pieces.

they are cut to pieces of various sizes.

Several research groups have been able to synthesize or isolate compounds that selectively inhibit the proteasome without affecting other cellular enzymes, which could cause side effects. These inhibitors have been crucial tools allowing scientists to unravel the intricacies of the proteasome pathway. At high doses, these inhibitors eventually kill cells—not surprising in light of the many critical roles that proteasomes play. But interestingly, cancer cells in the test tube and in animals appear more susceptible to these lethal effects than normal cells do. An experimental proteasome inhibitor being tested by Millennium Pharmaceuticals in Cambridge, Mass., has been evaluated for safety in humans and will be assessed against several cancers, including multiple myeloma, in trials set to begin this winter. Another of Millennium's proteasome inhibitors is in early safety trials in humans as a possible treatment for stroke and myocardial infarction.

## The Kiss of Death

The proteasome does not just randomly pick out proteins to destroy. Instead a cell points out which proteins are doomed. Scientists have discovered that the vast majority of such proteins are first tagged with another protein called ubiquitin, for its ubiquity among many different organisms. With only 76 amino acids, ubiquitin is a relatively tiny protein that can be attached to larger proteins in long chains. These polyubiquitin tails act like postal codes that speed doomed proteins to proteasomes.

What controls the timing of a protein's demise is not its actual breakdown by the proteasome, but the process of adding the ubiquitin chains, called ubiquitination, which requires energy. The basic outline for how ubiquitin is attached to a protein has come from Avram Hershko and Aaron Ciechanover of the Technion-Israel Institute of Technology in Haifa, working with Irwin A. Rose of the Fox Chase Cancer Center in Philadelphia.

The ubiquitination process has several steps and involves three enzymes, dubbed E1, E2 and E3 [*see illustration above and on opposite page*]. The E1 enzyme activates ubiquitin and connects it to E2. The third enzyme, E3, then facilitates the transfer of the activated ubiquitin from the E2 to the protein. The process repeats until a long chain of ubiquitins dangles off the protein. That chain is then recognized by a proteasome, which draws the protein in.

The mystery of how a protein is chosen for ubiquitination revolves around the E3 proteins. Recently researchers, including two of us (Elledge and Harper), have discovered that there are hundreds of distinct E3 proteins that recognize information in the amino acid sequences of other proteins that make them targets for ubiquitination. In response to altered physiological conditions, such as infection or a lack of nutrients, cells can modify proteins by adding phosphate groups. Such phosphorylation can alter the activity of a protein or its ability to bind to E3s. Proteins that fail to fold or that become damaged are also recognized by E3s, which come along and clean up the proteins by marking them for pickup by the proteasome—a little like putting them out on the curb on garbage day. Many key cellular processes rely on protein stability, and finding out how stability is controlled therefore holds the key to many of biology's secrets.

By controlling the stability of crucial proteins, the E3 proteins regulate many cellular processes, such as limb development, the immune response, cell divi-

sion and cell-to-cell communication. Even circadian rhythms and flowering in plants are dictated by E3 enzymes. What is more, several E3s have been identified as tumor suppressors or oncogenes, tying ubiquitination to the onset of cancer.

A case in point is the Von Hippel Lindau (VHL) tumor suppressor, an E3 that is often mutated in kidney tumors. VHL's job is to retard cell growth by limiting the development of blood vessels in tissues; when it is mutated, newly formed tumors are able to generate a rich blood supply and grow rapidly. Scientists have now found that an inherited form of Parkinson's disease results from a mutation in the gene for a type of E3 enzyme that can cause proteins to build up in certain brain cells and kill them.

Viruses, which are famous for diverting cellular processes, have evolved the means to hijack the process of ubiquitination and protein degradation for their

## Why Cell Division Depends on Protein Death

One of the best examples of why a cell's ability to break down proteins is important for its life and growth comes from studying cell division in *Saccharomyces cerevisiae,* the common baker's yeast. Before a yeast cell—or even a human cell—divides, it must first copy its DNA. And to begin DNA synthesis, a cell needs to activate a particular class of proteins called S-phase Cdks, which are composed of two proteins, a cyclin and a Cdk subunit.

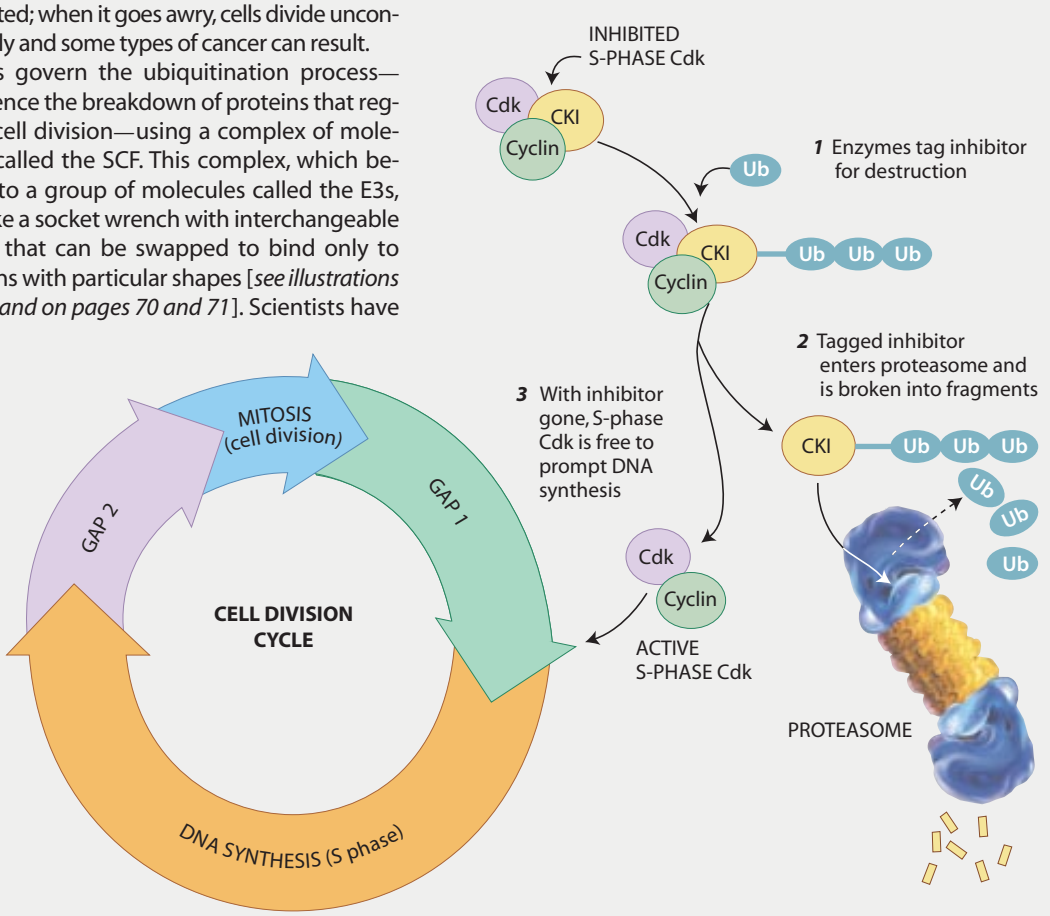
S-phase Cdks are normally inactive because they are bound to inhibitory proteins (called CKIs) that were made during the previous cell division. To activate the S-phase Cdks, a cell must get rid of the inhibitory proteins by sending them to a proteasome to be degraded (*below*).

Targeting the inhibitory proteins for destruction by a proteasome requires tagging the proteins with a death signal called ubiquitin (Ub). This tagging process is normally tightly regulated; when it goes awry, cells divide uncontrollably and some types of cancer can result.

Cells govern the ubiquitination process—and hence the breakdown of proteins that regulate cell division—using a complex of molecules called the SCF. This complex, which belongs to a group of molecules called the E3s, acts like a socket wrench with interchangeable heads that can be swapped to bind only to proteins with particular shapes [*see illustrations below and on pages 70 and 71*]. Scientists have identified more than 217 such socket heads, called F-box proteins, in the nematode worm *Caenorhabditis elegans*; several dozen have been found in human cells so far, and the count is rising.

The SCF complex uses its specific set of socket heads to recognize proteins that should be broken down by the proteasome. Indeed, cells choose which proteins to degrade by adding a phosphate group to them so that they bind to the F-box proteins of the SCF. The SCF also serves as a go-between to bring such ill-fated proteins together with the enzymes that add the ubiquitin death tag.

The variety of SCF complexes gives a cell exquisite control over which types of proteins—and how much of each one—it has on hand at any given time. Proteins regulated by SCF complexes include those that promote or inhibit the cell division cycle and those that turn on genes. —*S.J.E. and J.W.H.*

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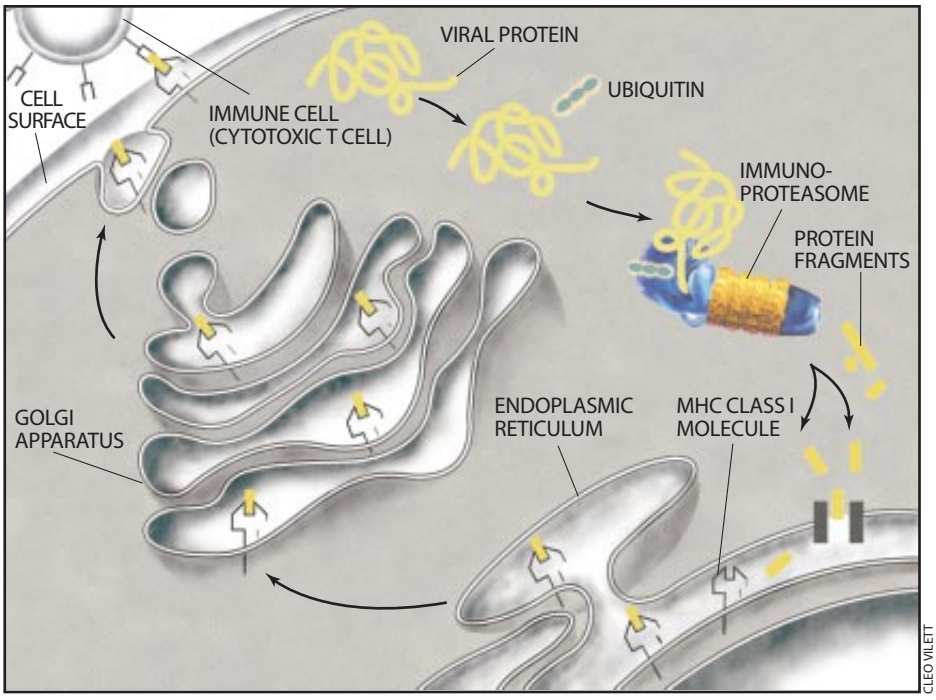


**IMMUNE SYSTEM relies on specialized proteasomes called immunoproteasomes to help it distinguish healthy cells from cancerous ones or those that have been infected by viruses. In the example shown at the right, a viral protein is tagged with ubiquitin for destruction by the immunoproteasome. Bits of the viral protein that are between eight and 10 amino acids in length then enter the endoplasmic reticulum, where they are loaded onto newly formed, forklike molecules called the major histocompatibility complex (MHC) class I. As the MHC class I molecules are transported through the Golgi complex and float to the cell surface, they take along the viral protein bits. Immune cells called cytotoxic T cells recognize the bits of virus embedded in the MHC class I molecules on the cell surface as foreign and kill the infected cell.**

own nefarious purposes. Human papillomaviruses (HPVs), which can cause genital warts or cervical or anal cancer, are examples. The transformation to cancerous growth is usually blocked by the defense protein p53, one of the body's tumor suppressor proteins. HPVs use a trick to circumvent this cellular defense system: they make a protein that binds simultaneously to both p53 and an E3 enzyme. This binding leads to the ubiquitination of p53, which destines p53 to be sliced and diced to obliteration by the enzymatic Ginsu knives of the proteasome. The defenseless cells are then more likely to become cancers.

HIV uses a similar ploy to destroy the cell-surface protein CD4, which is necessary for the virus to infect cells but which interferes with the production of more viruses later on. CD4 acts as a docking site for HIV to enter the T cells of the immune system; it binds to the gp160 protein that protrudes from the surface of the virus. But when HIV starts attempting to replicate in the newly infected cells, CD4 can present a problem: it adheres to freshly made gp160 proteins, keeping them from assembling with other viral proteins into new viruses. To circumvent this obstacle, HIV has evolved a protein called Vpu that puts CD4 on the fast track to oblivion. Vpu binds to both CD4 and a complex containing an E3 enzyme, causing CD4 to become ubiquitinated and then dropped down the chute of the proteasome to be destroyed.

New discoveries about the importance of E3s in disease are rapidly emerging, and these enzymes are likely to be targets for drug development in the future. Because each E3 is responsible for the destruction of a small number of proteins, specific inhibitors of E3s should be highly specific drugs with few side effects. The recent identification of large families of E3 enzymes have opened up whole new avenues for drug discovery. These are exciting developments that promise to enrich the understanding of diverse regulatory phenomena and human biology. The more we learn about proteasomes and the ubiquitination selection machinery, the more we appreciate how much of life is linked to protein death. SA

## *The Authors*

ALFRED L. GOLDBERG, STEPHEN J. ELLEDGE and J. WADE HARPER have built their careers on studying protein degradation and its role in disease. Goldberg is a professor of cell biology at Harvard Medical School, where he received his Ph.D. in 1968 and where he has spent most of his academic life. He has consulted widely for industry; among his honors are the 1998 Novartis-Drew Award for Biomedical Research. Elledge is the Robert A. Welch Professor of Biochemistry at Baylor College of Medicine and is an investigator for the Howard Hughes Medical Institute. He received his Ph.D. from the Massachusetts Institute of Technology in 1983 and was awarded the Michael E. DeBakey Award for Research Excellence in 1994. Harper is a professor in Baylor's department of biochemistry and molecular biology and its department of molecular physiology and biophysics. He received his Ph.D. from the Georgia Institute of Technology in 1984 and was granted the Vallee Visiting Professorship at the University of Oxford in 2000.

## *Further Information*

How the Cyclin Became a Cyclin: Regulated Proteolysis in the Cell Cycle. Deanna M. Koepp, J. Wade Harper and Stephen J. Elledge in *Cell,* Vol. 97, No. 4, pages 431–434; May 14, 1999.

Intricacies of the Proteasome. Stu Borman in *Chemical and Engineering News,* Vol. 78, No. 12, pages 43–47; March 20, 2000.

Probing the Proteasome Pathway. Alfred L. Goldberg in *Nature Biotechnology,* Vol. 18, No. 5, pages 494–496; May 18, 2000.

For more information on the history of studies of the proteasome pathway, visit the Albert and Mary Lasker Foundation Web site at www.laskerfoundation.org/library/2000/citation1.html