New Rule Proposed for Protein Degradation

The terminal amino acid of a protein may be the factor that determine how stable the protein is

A group of researchers at Massachusetts Institute of Technology has discovered what may be a simple code that determines the length of time proteins exist in cells before they are degraded. Reporting on page 179 of this issue of *Science*, Andreas Bachmair, Daniel Finley, and Alexander Varshavsky argue that the amino-terminal amino acid of a protein determines the protein's half-life.

Varshavsky notes that he and his colleagues did not go out looking for a protein degradation code; when they came upon it, they were startled. Their discovery of what they call the N (for amino)-end rule came about, he says, because they did the right experiment for the wrong reasons.

Different proteins have vastly different lifetimes in cells. Some survive as long as or even longer than the cell does, whereas others survive for just seconds. The question of what determines these protein lifetimes is of practical as well as theoretical interest. Molecular biologists who design and clone genes to make new pharmaceutical products, for example, must be sure that the products are not immediately degraded. But, although many have worked on the problem, no one has been able to determine why some proteins are orders of magnitude more stable than others. Varshavsky's hypothesis is an attempt at an answer.

The story begins with Varshavsky's interest in ubiquitin—a mysterious protein that is, as its name implies, ubiquitous. About 3 years ago, Masa-Atsu Yamada of the University of Tokyo isolated a mutant line of cells that fail to go through the normal cycle of growth and division when they are grown at high temperatures. The difficulty seemed to be a temperature-sensitive mutation in a gene for an enzyme that acts on ubiquitin. This enzyme normally helps join ubiquitin to other cell proteins. At high temperatures, the enzyme fails to work, ubiquitin is not added to other proteins in the cell, the cell fails to grow and divide, and, as a result, the cell dies.

Varshavsky immediately thought of using this mutant to answer a fundamental question about ubiquitin and protein degradation. A number of researchers believed that at least one determinant of a protein's lifetime is ubiquitin. According to this hypothesis, ubiquitin binds to proteins that are to be destroyed, marking them for degradation. Varshavsky and his colleagues reasoned that if the hypothesis were true, the mutant cells would stop degrading proteins at high temperatures. "It was an acid test," Varshavsky remarks.

So he and his colleagues did a standard experiment. They grew cells at a high temperature and observed that the short-lived proteins were immediately stabilized. It was, says Varshavsky, "the first direct evidence that if you don't add ubiquitin, proteins are not degraded."

The discovery of the N-end rule came about because they did the right experiment for the wrong reasons.

But there were a lot of loose ends. "It was clear that the system would be horribly complex," Varshavsky recalls. He and his colleagues decided to use genetic techniques to determine how ubiquitin marks proteins for degradation. Ubiquitin is added to proteins at amino groups, which occur at one end of proteins and also at the end of the side chain of the amino acid lysine. So they could create, genetically, a protein-ubiquitin combination by joining a ubiquitin gene to a gene for a protein and cloning the protein-ubiquitin gene. Then they could determine how much less stable the protein-ubiquitin is than the protein alone.

Varshavsky decided to hook a ubiquitin gene to the bacterial gene for β-galactosidase and to clone the product in yeast. But as soon as the gene product was made, the cells cleaved the ubiquitin from the β-galactosidase. "We ended up with nothing but β-galactosidase," Varshavsky remarks. The researchers were surprised and disappointed. "It denied us what we wanted. Since ubiquitin was cleaved in no time, we could not ask our question," says Varshavsky.

"Then we decided to deceive the enzyme. We can change an amino acid by changing a gene, so we decided to change an amino acid at the β-galactosidase–ubiquitin junction, hoping to fool the enzyme so that it would not cleave the product." Bachmair made 16 plasmids containing altered β-galactosidase–ubiquitin genes, and each plasmid differed from the others only in the DNA coding for the single amino acid at the junction between β-galactosidase and ubiquitin. Then they repeated the experiment.

"We discovered that we were wrong again. The protease doesn't care about that amino acid. No matter what you do, it cleaves," Varshavsky reports. But the researchers discovered something else that was completely unexpected. They were able to synthesize proteins that should have whatever amino acid they wanted at the amino terminus. (They are still in the process of determining whether the proteins in fact have the predicted terminal amino acids.)

Because AUG, the genetic code for methionine, is also the start signal for protein synthesis, all proteins have methionine at the amino terminus. After synthesis, enzymes may clip off a portion of the amino terminus, causing the protein to end with something other than methionine, but this methionine dependence has meant that molecular biologists have not been able to synthesize proteins with whatever amino terminus they please. However, when ubiquitin is cleaved from the hybrid protein, and when the methionine that is normally on the end of the protein has been changed to another amino acid, what is left should be a protein that has something other than methionine at its amino terminus.

The next discovery was that the amino acid at the amino terminus of β-galactosidase apparently determines how stable the protein is. This, then, is the N-end rule. Methionine, serine, alanine, threonine, valine, and glycine give half-lives of more than 20 hours. Isoleucine and glutamic acid give half-lives of about 30 minutes. Tyrosine and glutamic acid give half-lives of about 10 minutes. Phenylalanine, leucine, aspartic acid, and lysine give half-lives of about 3 minutes. And arginine gives a half-life of about 2 minutes. The only exception is proline, which defeats the enzyme—it does not cleave the β-galactosidase–ubiquitin bond when proline is at the junction. The half-lives of the altered β-galactosidase molecules "span the entire range of protein half-lives known to man," Varshavsky says.

To test the N-end rule, Varshavsky and his colleagues looked at all 208 intracellular proteins whose amino termini and half-lives are known. All except one of these proteins is long-lived—unstable proteins are much more difficult to synthesize and sequence.

Still, the rule works for all of these and also for the one unstable protein, which is from the bacterial virus lambda. They found no exceptions to the rule. The investigators are also doing the β-galactosidase–ubiquitin experiment with another protein, dihydrofolate reductase, that is much smaller and different in shape from β-galactosidase.

Even if the N-end rule is correct, there are still some unanswered questions. For example, How does the rule operate? What recognizes the amino-terminal amino acid of proteins, and how does it do it? What determines how much of the amino terminus is clipped after a protein is made? The clipping of the amino terminus exposes amino acids other than methionine to whatever it is that causes the N-end rule to operate. But Varshavsky is unperturbed. He makes an analogy with the genetic code. "The genetic code is very simple," he says, "but the underlying machinery to decode it is extremely complex."

Finally, whatever happened to ubiquitin, which was where the story started? Does it or does it not have anything to do with protein degradation? Varshavsky now thinks that ubiquitin was a red herring. It does not mark proteins for degradation, he says, and is not even added to the amino termini of proteins since, if it were, it would immediately be chopped off. It must be added only at lysine side chains. Its role may be to help destabilize proteins that are already marked for destruction. The N-end rule also helps explain why bacteria, which have no ubiquitin, manage to degrade proteins perfectly well.

It remains to be seen, of course, how general the N-end rule really is. But, more on this subject, undoubtedly, is yet to come. ■ GINA KOLATA

In Search of Dark Matter

Through the telescope, some 90% of the mass in the universe seems to be invisible; in the laboratory, however, a new generation of detectors may bring the dark matter to light

FOR more than a decade now, astronomers and cosmologists have been forced to explain the things they do see by appealing to something they do *not* see—a necessity that gives their field an undeniable air of mysticism. Yet the evidence is compelling. The motions of galaxies, the distribution of galaxies, the very existence of galaxies—even the overall expansion of the universe, all seem to be dominated by a cosmic ectoplasm known as the dark matter. Whatever this stuff is, it comprises as much as 90% of the mass of the universe. It is also utterly invisible. Indeed, it only makes itself felt by its gravitational effects; the ordinary "baryonic" matter that comprises the visible stars and galaxies is only a kind of flotsam, drifting along wherever the dark matter carries it.

From an empirical point of view this situation is unsatisfactory, to say the least. Fortunately, however, it may also be changing. A number of techniques have recently been proposed for detecting the dark matter in the laboratory. These experiments will stretch detector technology to the limit, and a little beyond. But several teams of researchers have begun development work nonetheless. The first clear detection of dark matter—an event as significant as the discovery of the 2.7 K cosmic background radiation in 1965—could conceivably be announced within the next few years.

All the proposed detection techniques are based on the assumption that the dark matter consists of a haze of elementary particles left over from the Big Bang. Presumably, each particle interacts very weakly with ordinary matter, thus rendering the haze invisible. And presumably, each particle also possesses a tiny mass, thus allowing the haze to produce a large gravitational effect. A wide variety of such particles have, in fact, been predicted in the physicists' current theories of grand unification and supersymmetry; they fall broadly into two major classes—light and heavy—and require two corresponding classes of detectors.

Among the light particles, for example, perhaps the likeliest candidate is the so-called axion, which arises in certain versions of quantum chromodynamics. (In technical terms, the axion is needed to explain the absence of CP violations in the strong interactions.) Taken at face value, the axion is elusive in the extreme. Its predicted mass lies somewhere in the range of 10^{-5} to 10^{-2} electron volts, while its coupling to ordinary matter is thought to be a trillion times weaker than the already feeble coupling of neutrinos.

However, as Pierre Sikivie of the University of Florida and his co-workers first pointed out in 1983, the structure of axion theory is such that the particles would have an effective coupling to photons; in fact, in the proper circumstances an axion might be induced to turn into a photon, which could then be detected by conventional means.

Sikivie thus proposes a microwave cavity located in a strong, uniform magnetic field. According to axion equations, he points out, the magnetic field would serve to trigger the conversion, producing a photon with an energy equal to the axion's mass plus its kinetic energy. If one assumes that the axions do comprise the dark matter, then a short cosmological calculation yields an estimated photon frequency of about 2.4 gigahertz, which lies in the microwave region of the spectrum. Thus the microwave cavity: if it is tuned to resonate at precisely the axion frequency, Sikivie says, it will greatly enhance the probability of conversion. With the use of a state-of-the-art cryogenic amplifier, in fact, the axion-generated photon might actually be detectable.

Sikivie and his colleagues at the University of Florida have begun development work on an axion detector, as have several other groups. In fact, one such group, including Adrian Melissinos of the University of Rochester, together with colleagues from Brookhaven National Laboratory and Fermilab, began taking data on 1 August with a small detector located at Brookhaven.

The real challenge in axion detection is not detection per se, Melissinos explains. It is the signal's needle-in-a-haystack aspect. On the one hand the signal itself is thought to be less than 1000 hertz wide. (It would be smeared out slightly because axions orbiting through the galaxy would have a spread of velocities.) On the other hand its location at 2.4 gigahertz is only an estimate, which is why the Brookhaven detector is searching all the way from 1 gigahertz to 6 gigahertz—a region that gives the axion signal 5 million places to hide. "That's an enormous amount of data," says Melissinos. "If we knew exactly where it was, we could get the sensitivity way up just by sitting on that frequency and integrating longer." As it is, however, any practical experiment has to scan its allotted range within a reasonable amount of time; in the case of the Brookhaven experiment, that means scanning from 1 to 6 gigahertz within about a year. Unfortunately, says