Research in Progress

A New Infectious Agent

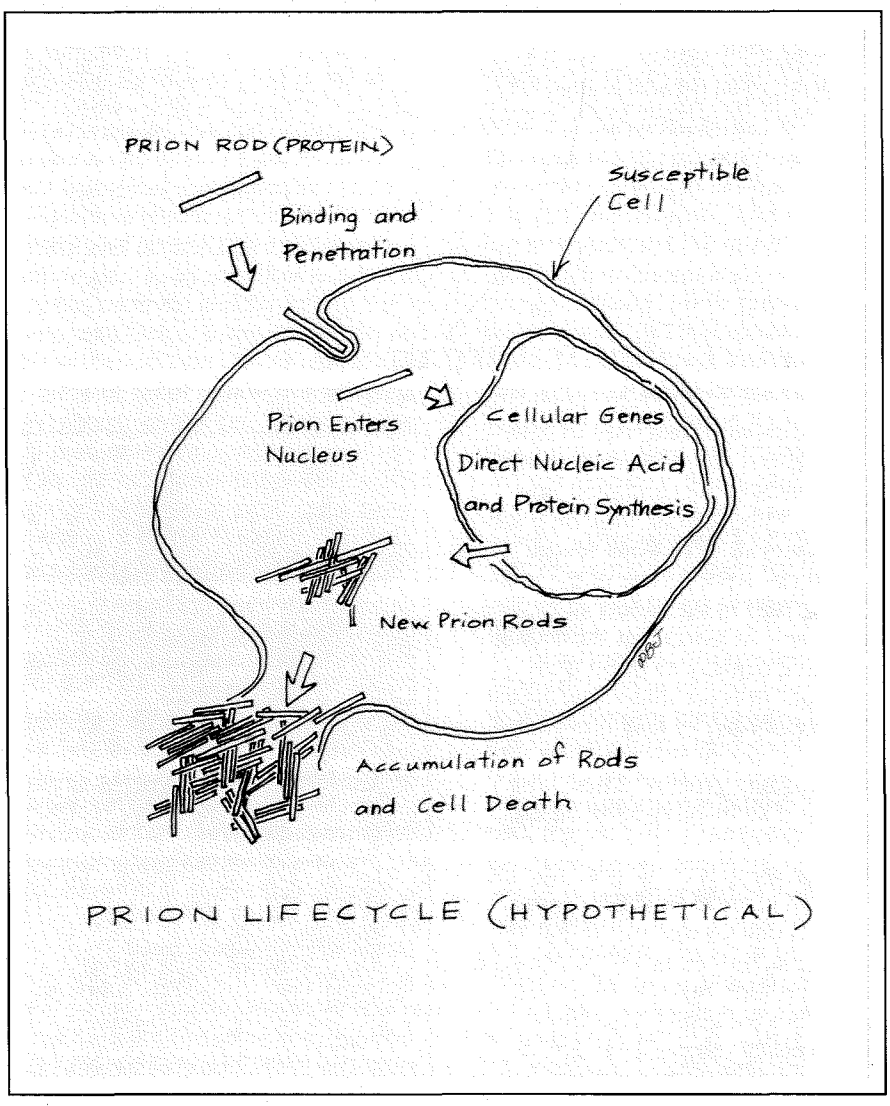
CALTECH SCIENTISTS are studying something new under the sun — an infectious agent that appears to violate the central dogma of biology. This agent, called the prion (pronounced PREE-on), seems to be a self-replicating protein, and as such it either bypasses totally or adds a new wrinkle to the normal sequence of information flow in the cell: DNA→RNA→protein. Named by Stanley Prusiner of UCSF, the prion is known to cause scrapie, a brain disease of sheep and goats.

Scrapie is one of a group of so-called slow virus diseases, a group that includes Creutzfeldt-Jakob Disease and kuru, two devastating neurological diseases of humans. The hallmark of a slow virus disease is its extremely long incubation period, which can extend for months or years. And these diseases share another common feature — the brains of those afflicted contain numerous amyloid plaques: large, regular arrays containing a rod-shaped protein. Amyloid plaques are also seen in Alzheimer's disease.

Despite the name, though, slow virus diseases seem not to be caused by conventional viruses. Viruses consist of molecules of nucleic acid — DNA or RNA — surrounded by a protein coat. When a virus invades a cell, it uses its nucleic acid to subvert the cell's genetic machinery, reproducing many copies of itself and often killing the cell in the process. But as far as anyone can tell, prions consist entirely of protein and contain no nucleic acid. In a lengthy series of experiments, Prusiner has isolated scrapie prions and has shown that they maintain their infectivity when subjected to treatments that should destroy nucleic acid, while they lose their infectivity when treated with protein-destroying agents.

Caltech researchers have obtained additional detailed information on the scrapie prion by using the powerful resources of the Division of Biology's Program in Advanced Biological Instrumentation, which has developed state-of-the-art instruments that make detailed analysis and high-yield synthesis of proteins and nucleic acids possible. The researchers determined that the scrapie prion is a glycoprotein with a molecular weight of between 27,000 and 30,000 and they've named it PrP 27-30. They analyzed its amino acid composition and determined the sequence of a 15-amino-acid segment of the protein. With this sequence in hand, they synthesized a corresponding oligonucleotide probe that enabled a group of Swiss researchers, led by Charles Weissmann, to isolate a gene coding for PrP 27-30 from scrapie-infected hamster brains. (The Caltech group includes Leroy Hood, the Ethel Wilson Bowles and Robert Bowles Professor of Biology; Stephen Kent, senior research associate; David Teplow, research biologist; Ruedi Aebersold and Paul Tempst, both research fellows; and Eric Heer, now a Stanford undergraduate.)

The Swiss researchers determined that even normal, uninfected mammalian cells contain a very similar gene. This gene produces PrP 33-35, a protein somewhat larger than, but closely related to, PrP 27-30. PrP 33-35 is not infectious and is easily bro-



PRION LIFECYCLE (HYPOTHETICAL)

ken down by enzyme treatment. The infectious PrP 27-30, on the other hand, is strongly resistant to degradation, which may explain why it accumulates in cells, kills them, and forms large amyloid plaques. In another series of experiments, the researchers are using synthetic fragments of the prion to raise antibodies. They hope these antibodies will cross-react with both proteins and will be useful in immunocytochemical studies. These studies may help in determining the function of the normal prion protein and in following the course of the infection.

The lifecycle of the infectious prion is still the subject of conjecture. According to one hypothesis, a point mutation occurs in the gene coding for the normal PrP 33-35. This mutation would render the protein infectious and immune to degradation. According to another hypothesis, illustrated here, alterations in the cell's genetic material would be unnecessary. The infectious prion protein could exert a regulatory effect leading to increased synthesis of the normal prion protein. In yet another hypothesis, the infectious protein increases the half-life of messenger RNA, thereby causing overproduction of the protein. There are other hypotheses as well, all of which are currently under evaluation.

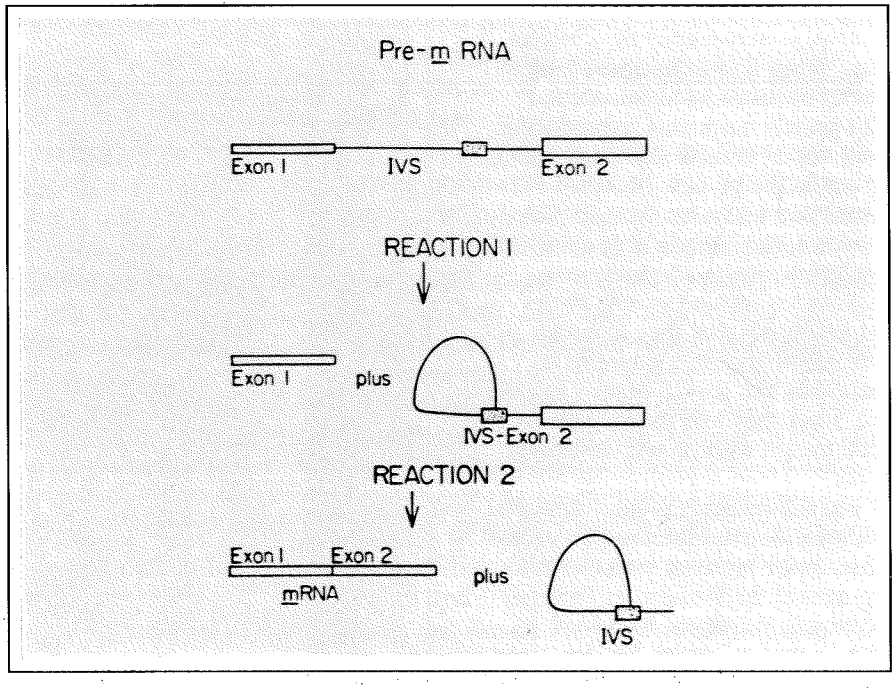
But according to many researchers, the most important unanswered question is whether the prion is truly an infectious protein containing no nucleic acid. Although all results to date point to this conclusion, it's so counter-intuitive that many researchers insist that there must be some nucleic acid somewhere, possibly buried so deeply in the protein molecule that the methods used so far can neither detect nor destroy it. Says Stephen Kent, "We just plain don't know. Speculation is always fruitful and one should indulge in it, but in this case we just don't have enough reference points to guide useful speculation. That's what makes the thing so interesting. If I were a guessing man, which I am, I would guess that it's a DNA virus. But it really doesn't matter whether it turns out to be a virus or an infectious protein. Whatever it is, it's really, really different from known infectious agents."

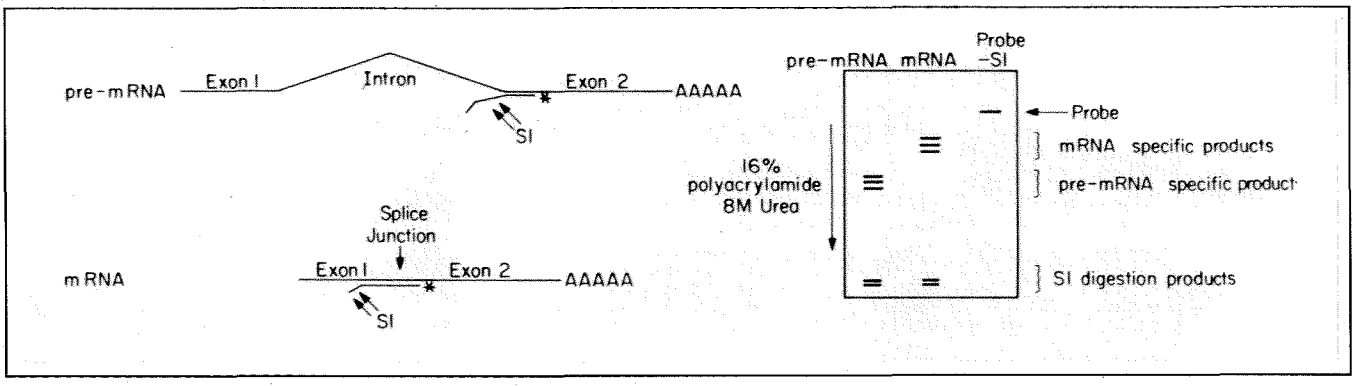
One ambitious series of experiments, currently under way, could provide an answer. The Caltech researchers are trying to confirm the amino acid sequence of PrP 27-30 deduced by the Swiss group from the gene they found. Once this is done, copies of this protein can be synthesized chemically. If the synthetic protein proved infectious, then need for nucleic acids could definitely be ruled out. But if the synthetic protein were not infectious, this would not prove that nucleic acids were necessary. It could be that the difficult-to-analyze carbohydrate portion of the glycoprotein is needed for infectivity.

Meanwhile, the Caltech researchers are working on detailing the differences between PrP 27-30 and PrP 33-35. And they're excited about the possibilities. Says David Teplow, "This thing is as controversial as the germ theory of disease was in the 19th century. It's very unusual and could really upset the whole foundation of scientific thinking." □ — *RF*

A New Cellular Organelle

BIOLOGISTS HAVE LONG tried to determine how cells process messenger RNA (mRNA) for translation into protein. This question took on a special urgency in 1977 when several laboratories reported one of the most startling facts in the history of molecular biology. It seems that the coding regions of virtually all genes from eukaryotic cells are interrupted, sometimes repeatedly, by long non-coding regions — regions that are never translated into protein. These long sections of DNA came to be called intervening sequences, or "introns," while the sections of the gene that are translated came to be called "exons." Scientists quickly discovered that the entire gene, introns as well as exons, is transcribed into mRNA. But these pre-mRNA molecules have their introns neatly snipped away before they're translated into proteins.





In the splicing assay, first an oligonucleotide probe is synthesized. The probe is designed so that part of it will bind or "hybridize" to one exon of the mRNA, part will hybridize to the other exon, and part will not hybridize at all. After hybridization, everything is digested with an enzyme called S1. The products of this digestion are separated with gel electrophoresis and will depend on whether or not the intron has been excised and splicing has taken place.

The mechanism the cell uses to cut away the introns and splice the exons together must be extraordinarily precise. If the splicing mechanism makes its cut a single nucleotide too soon or a single nucleotide too late, the entire reading frame of the mRNA will be thrown off and the cell will construct a nonsense protein. The search for the splicing mechanism has presented enormous technical and methodological difficulties, but these difficulties have recently been overcome by John Abelson, professor of biology, and his co-workers: Edward Brody, visiting associate in biology (now back at the Institut de Biologie Physico-chimique in Paris); Andrew Newman, former senior research fellow in biology; postdocs Soo-Chen Cheng, Ren-Jang Lin, and Gloria McFarland; and graduate student Usha Vijayraghavan.

Working with yeast, they have discovered that mRNA splicing takes place on a previously unknown organelle that they have dubbed the "spliceosome." Splicing proceeds in two stages. In the first stage, the exon separates from the 5′ end of the intron, which loops around to form a "lariat." [In nucleic acids, the 5′ hydroxyl group of one nucleotide normally connects to the 3′ hydroxyl group of the next one in the chain, so nucleic acids or sections of nucleic acids are said to have a 3′ end and a 5′ end.] In the second stage, the 3′ end of the looped intron separates from the second exon and the two exons join together.

These details on the splicing mechanism emerged only after seven years of labor. One major advance came with the development by Abelson's group of an ingenious assay capable of detecting mRNA splicing *in vitro*. To do this they first synthesize a radioactively tagged oligonucleotide probe. Part of this probe matches the nucleotide sequence of one exon and another part matches the sequence of the exon on the other side of the intron. When mixed with unspliced pre-mRNA, only one end of the probe will bond. Digesting the probe with S1, an enzyme that specifically degrades single-stranded nucleic acids, will then yield a characteristic set of radioactive products that can be separated and detected on a polyacrylamide gel. If, on the other hand, splicing has occurred and the intron has been removed, a larger proportion of the probe will bond to the mRNA and a different set of radioactive products will appear on the gel. The inclusion on the probe of a small section that does not match any part of the mRNA allows a distinction to be made between probe molecules that had been protected by bonding and residual probe molecules that had simply remained undigested after S1 treatment.

With the splicing assay in hand, Abelson's group then began looking for the site at which splicing takes place. They incubated radioactively tagged pre-mRNA with a yeast-cell extract and immediately centrifuged the mixture in a glycerol gradient. This procedure demonstrated that much of the radioactive pre-mRNA was associated with a 40S particle. (The Svedberg unit — S — is a measure of the speed at which a particle sediments in a centrifuge. The ribosome, for comparison, sediments at 80S.) But when a mutation was introduced into the pre-mRNA, a mutation that prevented the formation of the lariat and thus prevented splicing, there was no radioactivity detected at 40S. Abelson inferred from this that the 40S particle was the site of splicing — the spliceosome.

Work on spliceosomes is proceeding on several fronts in Abelson's lab. Says Abelson, "We'd like to know what's in the spliceosome. We're fractionating the extract and trying to reassemble spliceosomes from the fractionated components. And we're trying to understand the role of ATP [adenosine triphosphate — the cell's energy currency] in the formation of spliceosomes. ATP is required in the reaction and we don't know what its role is." ATP could be the energy source for the cutting and splicing reactions, but these are phosphotransfer reactions, which generally do not require an outside source of energy. Alternatively, ATP could provide the energy necessary for the correct folding of the pre-mRNA molecule.

In another set of experiments, Abelson is trying to find spliceosomes with the electron microscope. "We haven't seen anything distinctive yet. It's a very tricky experiment because we're putting a crude extract together with the pre-mRNA and sedimenting it. Anything will sediment. You're bound to see some particles and it's easy to ascribe any particle you see to the spliceosome. So we're being very cautious, but we're looking. My guess is that once we know what it looks like someone will say, 'Oh yeah, I saw that 15 years ago.' There must be a lot of them in the cell." □ — *RF*