introns. Then, just a few weeks ago, the distinction between nuclear and mitochondrial introns was suddenly breached.

Tom Cech and his colleagues at the University of Colorado published their latest information on a remarkable intron in the nuclear ribosomal RNA gene of *Tetrahymena thermophila*. The intron, just 413 nucleotides long, is excised and the remaining exons ligated in the absence of enzymes. The splicing reaction appeared to be mediated by catalytic activity inherent to the RNA. Perlman, while sitting in on a graduate seminar on the self-splicing intron, noticed that the intron contained the box 9L sequence. Intrigued with this discovery, he looked for box 2, which he quickly found. Further scrutiny revealed the 5′ and box 9R sequences too. And, over the next few days, careful study showed up the A and B sequences. Burke independently noticed the coincidence of box 9 between mitochondrial introns and the ribosomal intron of *T. thermophila*. That made six out of six signatures of mitochondrial introns in this rather short nuclear intron, a surprising finding indeed.

The coincidence in the conserved sequences between the long mitochondrial introns, many of which have open reading frames for maturases, and the short nuclear self-splicing intron, which has no substantial reading frame, has many possible ramifications. Could it be that, like the nuclear intron from *Tetrahymena*, the mitochondrial introns are self-splicing? This seems unlikely in view of the data on maturases, but splicing-enzyme involvement has yet to be conclusively demonstrated. Perlman wonders whether mitochondrial introns might once have been self-splicing but that, as they acquired more and more sequences through insertions, they came under selection pressure to evolve maturase activity. At the very least there is the implication that normal self-splicing of the *Tetrahymena* intron might require the binding of proteins to parts of the higher order structure induced by the interaction between the conserved sequences. This might explain why self-splicing is slower in the total absence of protein than when it occurs in the cell.

The *Physarum* nuclear ribosomal RNA introns have four of the six functionally important elements: they lack sequences A and B. Examination of nuclear introns from other lower eukaryotes promises to turn up other examples like *Physarum* and *Tetrahymena*. And the discovery of new conserved sequences involved in splicing can be expected too. In any case, as Perlman points out, there could have been relatively recent exchange between nuclear and mitochondrial introns. Whether this involved the wholesale movement of genes between the compartments, or just the intervening sequences acting as transposable elements, remains to be established.

The probability that higher order structure is important in the splicing of mitochondrial, and some nuclear, introns now seems to have moved close to certainty. As yeast mitochondria offer an extremely suitable experimental system in which to isolate splicing-defective mutants, this, plus the study of revertants, promises a fruitful avenue of inquiry in which to pin down further the structures involved.—**ROGER LEWIN**

Additional Reading

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Fetal Hemoglobin Genes Turned On in Adults

Scientists used a cancer drug to turn on fetal hemoglobin genes and thereby corrected anemia in patients with thalassemia and sickle cell anemia

In an experiment that has been described as bringing molecular biology to the bedside, researchers have used a drug to unmask fetal hemoglobin genes that normally are suppressed before birth. As a consequence, they have been able to partially correct severe anemia in patients with β thalassemia and sickle cell anemia. Although these results are a natural culmination of a large body of recent research in molecular biology and genetics, the scientists themselves express amazement that the experiments worked so dramatically well in humans.

Over the past several years, it was learned that patients with the genetic disease β thalassemia, like those with sickle cell anemia, have mutations in β globin genes. Adult hemoglobin consists of two β globin subunits and two α globin subunits. But during fetal life a different but equally effective hemoglobin is made in which two subunits from a gene called γ replace the β globin subunits. Fetal hemoglobin, then, consists of two α globin subunits and two γ globin subunits. It was known that fetal hemoglobin is perfectly effective in adults since patients with a rare genetic disorder that causes them to produce only fetal hemoglobin throughout their lives appear quite normal and healthy. So, the investigators reasoned, if they could just turn on γ globin genes in patients with β thalassemia or sickle cell anemia they could substitute for the defective β globin genes.

The next thing that was learned is that the γ globin DNA seems to be chemically modified when these genes are turned off in adult life. Gary Felsenfeld of the National Institutes of Health and other molecular biologists discovered that active genes frequently have few methyl groups attached to them. In contrast, genes that are not being expressed often are covered with methyl groups. About 2 years ago Richard Flavell and L. H. T. van der Ploeg, then at the University of Amsterdam, reported that DNA in the region of the γ globin genes is undermethylated during fetal life and is methylated in adult life. The clue to turning on γ globin genes, then, might be to remove methyl groups from them.

At about the same time as these methylation discoveries were being made, several groups of researchers, including Mark Groudin and Harold Weintraub of the Fred Hutchinson Cancer Research Center in Seattle, found that 5-azacytidine, a drug used to treat leukemia patients, might do the trick. This drug is an analog of cytidine, one of the DNA bases, and is incorporated into newly synthesized DNA. When 5-azacytidine is supplied to cells in tissue culture, newly synthesized DNA is undermethylated and repressed genes sometimes become active.

A year ago Joseph DeSimone, Paul Heller, and their colleagues at the Uni-

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versity of Illinois College of Medicine decided to use 5-azacytidine on baboons to see if it leads to fetal hemoglobin production in these animals. Baboons have globin genes that resemble those of humans, but when the animals lose a lot of blood or are given too little oxygen, they start making fetal, in addition to adult, hemoglobin. The amount of fetal hemoglobin they make under these circumstances is genetically determined. DeSimone and Heller learned that baboons will produce much more fetal hemoglobin if they are given 5-azacytidine.

Encouraged by these results with baboons, Timothy Ley, Arthur Nienhuis, Nicholas Anagnou, R. Keith Humphries, Patricia Turner, and Neal Young at the National Heart, Lung, and Blood Institute working with DeSimone and Heller and, independently, George Dover, Samuel Charache, and Kirby Smith at Johns Hopkins University School of Medicine decided to try the drug on patients. The NIH researchers, who reported their results in the 9 December issue of the *New England Journal of Medicine*, chose first a 42-year-old black man with β thalassemia. Starting in his early 30's, this man had been increasingly debilitated by his disease. He had severe bone pain and, because he needed many transfusions, he had begun to build up iron deposits which were causing testicular failure, cirrhosis of the liver, and heart failure.

The patient was given 5-azacytidine intravenously for 7 days with dramatic results. His γ globin synthesis increased sevenfold by day 7 and remained elevated for an additional 14 days. His bone pain went away and his red blood cell count increased more than 25 percent and remained high for nearly 5 weeks. When the NIH group looked at this man's bone marrow DNA, it found that the DNA in the region of the γ gene was undermethylated following the 5-azacytidine treatment.

These results, of course, do not prove that the 5-azacytidine stimulated fetal hemoglobin synthesis by causing undermethylation of γ globin genes. "Another interpretation is that methylation is not related at all," says Nienhuis. It could be that the fetal genes are turned on by another mechanism entirely and that, as a consequence of being turned on, they are undermethylated. The NIH researchers favor the hypothesis that 5-azacytidine acts by demethylating but they point out that demethylation is, apparently, necessary but not sufficient for gene expression. "We believe the γ globin gene is primed somehow to be expressed," Ley says.

The NIH researchers next treated two more patients with severe β thalassemia and, working with Constance Noguchi and Alan Schecter who are also at the NIH, they treated two patients with sickle cell disease. Each patient began making large amounts of fetal hemoglobin and, in each patient, the γ globin genes were undermethylated following the 5-azacytidine treatment.

The Johns Hopkins group gave 5-azacytidine to a 32-year-old black man with sickle cell anemia. Like the NIH patients, this man was severely affected by his disease. He had been hospitalized for half of the past year for sickle cell crisis. This occurs when red blood cells, which are distorted by the abnormal β globin subunit, get stuck in small blood vessels. The resulting logjam causes decreased blood flow to tissues which can cause severe pain. The only treatment until now, says Dover, has been supportive, meaning bed rest and narcotics to control the pain.

"We gave the drug for 3 days. To our surprise, the patient's fetal hemoglobin increased very rapidly. It began increasing within 3 days after the drug was given, it peaked at 10 days, and it continued to be synthesized for 25 days," Dover says. The patient's sickle cell crisis was not relieved but that was not unexpected because, once a patient is in the midst of a crisis, new blood will not bring him out of it. The way the drug might help in sickle cell anemia is by preventing the crises in the first place.

The scientists themselves express amazement that the experiments worked so dramatically well.

Dover and his associates next tried giving 5-azacytidine a second time just after the fetal hemoglobin synthesis began to fall after reaching its peak. They learned that when they did this, the patient maintained his fetal hemoglobin synthesis. "We used the drug repeatedly. Each time there was a significant and dramatic increase in fetal hemoglobin," Dover says. In addition, there was a reciprocal relationship between fetal hemoglobin and sickle cell hemoglobin synthesis. The more fetal hemoglobin produced, the less sickle cell hemoglobin was made.

The NIH group also noticed such a reciprocal relationship when they treated sickle cell patients. "There was a striking decrease in abnormal cells," says Nienhuis. "The effect was greater than we would predict from just the increase in fetal hemoglobin." The reason for this unexpectedly good result remains a mystery.

But Ley, Nienhuis, and Dover caution that the 5-azacytidine treatment is not yet ready for clinical application. Dover, who treated only sickle cell anemia, says, "What we saw were *very promising* laboratory signs but no therapeutic benefits. It is too early to see benefits or side effects." Nienhuis adds, "The most important thing is that this treatment perhaps offers some hope but we must find the optimum drug dose and frequency. With thalassemia, the important question is to find whether we can eliminate the transfusion requirement. With sickle cell anemia, the important question is whether it has any effect on the clinical course of the disease. That may eventually require a double-blind trial." And Ley stresses that, "We don't know whether repeated doses of the drug are toxic. We have been very careful with the patients we select to be sure they understand these uncertainties."

The question of long-term toxicity is a serious one. In leukemia patients, 5-azacytidine suppresses the production of white blood cells and platelets, leaving patients vulnerable to infections and bleeding disorders. It also causes nausea and vomiting. The drug is a carcinogen in animals and it is not known whether it also causes cancer in humans. The NIH and Johns Hopkins groups gave much lower doses of the drug to their patients than are normally given to cancer patients and they have, as yet, seen no adverse side effects.

Dover emphasizes also that he is as interested in the mechanism of action of 5-azacytidine as he is in its effects on patients with sickle cell anemia or β thalassemia. An understanding of how this drug works, he notes, "may lead to other drugs or methods for turning on other genes. If we can learn to turn one gene on and another off, that would be *enormously* important."

But despite their caveats, the investigators clearly are elated by their dramatic results. Edward Benz, Jr., of Yale University School of Medicine, commenting on the work in an editorial in the *New England Journal of Medicine*, remarks, "Their clinical applications of the concepts and techniques of molecular biology demonstrate beyond doubt that this discipline has come to the bedside."—**GINA KOLATA**