ton, Ralph Brinster of the University of Pennsylvania School of Veterinary Medicine, and their colleagues have found another type of insertional mutation that apparently disrupts sperm formation by male mice.

In addition, Rudolf Jaenisch of the University of Hamburg and his colleagues have produced a lethal developmental mutation by introducing a virus into early embryos. The mutation, which results in the deaths of the embryos around the 12th day of gestation, was caused by integration of the viral genome in a gene for collagen, one of the body's major structural proteins.

Neither the Mintz nor the Palmiter-Brinster groups have as yet determined the functions of the genes interrupted by the inserts, although they have been able to pull out the mouse sequences flanking the DNA inserts. According to Mintz, integration of the foreign gene, one coding for human growth hormone, produced some unanticipated complications. "In both mutants, there have definitely been rearrangements in the recipient mouse DNA," she explains. "I believe that this is more prevalent than had been expected." The rearrangements may include deletions as well as insertions and inversions of the DNA.

The relative ease with which the DNA alterations occur during these experiments suggest, Mintz points out, that similar rearrangements might have given rise to some of the many mutations known to occur in the mouse. Examination of the bithorax complex, a set of genes that help to control *Drosophila* development, has shown that such major DNA rearrangements are common causes of mutations. Whatever role the rearrangements play naturally, their occurrence during insertional mutagenesis will make unraveling the gene functions much more difficult.

In addition to the general problem of determining gene function, especially when the gene has rearranged, there is also the possibility that an insertional mutation may cause an apparent developmental defect without actually interrupting a developmentally specific gene. For example, it could disrupt a gene needed for one of the cell's common synthetic or energy-producing pathways.

Nevertheless, Mintz remains convinced that insertional mutagenesis is a good way of homing in on the genes that control mouse development. She estimates that incorporation of transferred DNA into one of the recipient animal's genes occurs in up to 20 percent of the animals that develop from injected eggs. That is a sufficiently high frequency to make it worthwhile to do the breeding experiments needed to detect a developmental mutation, especially if the investigator is already doing egg injections. "What has come out of the work so far," Mintz explains, "is an indication that insertional mutagenesis is not a rare event, and with just some luck you may be able to get something interesting." And that is what the Leder group appears to have done.—**JEAN L. MARX**

Additional Reading

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How Do Proteins Find Mitochondria?

They have signaling sequences, investigators find, that tell them where to go

Liver cells are packed wall to wall with hundreds of mitochondria—the oval shaped organelles that are responsible for the cells' energy metabolism. Other cells may have as few as a dozen or so mitochondria. But every animal cell contains these organelles and every cell presents researchers with a problem: Nearly all of the several hundred proteins contained in the mitochondria are synthesized not in the mitochondria but in the cell's cytoplasm. How, then, do these proteins find their way to the mitochondria and, once there, how do they end up in the correct portion of this organelle, which has inner and outer membranes and a convoluted interior?

The answer seems to be that most proteins destined for mitochondria are synthesized as precursor proteins carrying with them specific amino acid sequences that are recognized by the organelles and that allow the proteins to be taken up by the mitochondria. In most cases, these sequences, called leader sequences, are cleaved after the proteins enter the mitochondria.

So crucial are the leader sequences to mitochondrial import that when researchers purposely put these sequences on non-mitochondrial proteins, they, too, are taken up by mitochondria. But this finding raises further questions, such as, What is it about the leader sequence that the mitochondria recognize and how does this system compare to systems used to direct proteins to other parts of the cell? Several groups of cellular and molecular biologists are actively working on these problems, motivated not just by the fact that technology allows them to address these issues but also by their belief that an understanding of how proteins are imported to mitochondria may have real clinical significance.

Leon Rosenberg of Yale University School of Medicine explains: "One thing I'm sure about is that we'll find that certain diseases of mitochondrial proteins will be diseases of traffic rather than diseases of enzyme activity. The proteins will not function in the mitochondria because they don't get there." Geneticists have described 30 to 40 inherited metabolic disorders characterized by specific deficiencies of imported mitochondrial enzymes. "Until recently, we thought of mutations leading to such enzyme deficiencies in classic terms, that is, as a point mutation, an elongation, or a deletion," Rosenberg says. "Now here's another possible metabolic basis of these diseases—proteins that don't get where they're supposed to go." Defects in the import of mitochondrial proteins might also explain acquired disorders. For example, Reye's syndrome is an acquired disease of mitochondria.

The study of how proteins are imported to mitochondria began in earnest around 1979 when Gottfried Schatz and his colleagues in Basel, Switzerland, and Rosenberg and his associates began to establish that these proteins are synthesized as precursors, that their leader sequences are cleaved in the mitochondria, and that these cleavages occur after the proteins are translated. A second phase, which focuses on understanding the molecular biology of the import of mitochondrial proteins, began in 1982 when Gunter Blobel and his colleagues at Rockefeller cloned the gene for the yeast mitochondrial enzyme cytochrome

c peroxidase and found that it has an extra segment on one end that does not appear on the protein in the mitochondria. Walter Sebald and his colleagues in Germany did the same for a subunit of ATP synthase, a mitochondrial enzyme of *Neurospora*, and now there are more than a dozen of these leader sequences for mitochondrial proteins published. They range in length from 0 to about 80 amino acids. The sequences with 0 amino acids are those on mitochondrial proteins whose leader sequences are incorporated into the sequence of protein itself and are not cleaved.

As the mitochondria story unfolds, it takes its place in a broader picture of how proteins find their way in cells. The best studied system is proteins that are to be secreted from cells or that are to go to lysosomes, where they help degrade other proteins. These proteins, too, are made with leader sequences that enable them to be taken up into the endoplasmic reticulum and, ultimately, packaged in secretory granules or lysosomes. But the proteins do not have to travel through the cytoplasm before being taken up. Instead, they are made on ribosomes that are bound to the endoplasmic reticulum that takes them up.

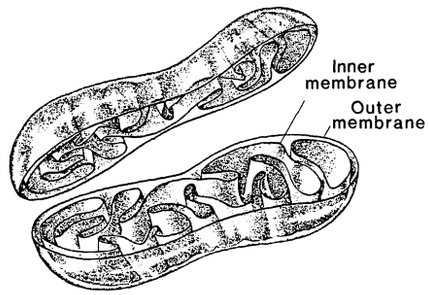
On the other hand, proteins that stay in the nucleus seem to have sequences built into them that serve as signals. The theory is that nuclear proteins cannot have sequences that are made and then cleaved, like those of mitochondrial proteins and proteins that are secreted, because, when a cell divides, its nuclear membrane dissolves. If a nuclear protein's signal sequence has been cleaved, it would have no way of getting back to the nucleus.

At Yale, Rosenberg, Arthur Horwich, and their associates are focusing on one particular protein—ornithine transcarbamylase (OTC)—a liver mitochondrial enzyme involved in the synthesis of urea and the detoxification of ammonia. Patients without properly functioning OTC often die in early infancy from a buildup of ammonia. Some of these patients lack OTC entirely, others make nonfunctional OTC, and others—the Yale researchers are convinced—make OTC that does not get into their mitochondria.

When OTC is synthesized, the first portion of the precursor to be made is a 32 amino acid sequence that serves as a signal for its uptake into mitochondria. This precursor protein subunit somehow drifts or is carried to mitochondria where it is taken up, transported to the innermost mitochondrial compartment—the matrix—and cleaved of its leader sequence. There, groups of three OTC subunits assemble themselves into the finished protein.

The Yale group first asked whether the 32 amino acid leader sequence contains sufficient information for the import of proteins into mitochondria. The answer is yes. They decided to join the OTC leader sequence to a protein, dihydrofolate reductase, that normally does not enter mitochondria. When they did so, this chimeric protein was taken up by mitochondria.

Independently, Schatz and his associates fused a 25 amino acid leader sequence from a yeast mitochondrial protein to dihydrofolate reductase and found that the protein then entered yeast mitochondria and the leader sequence



A mitochondrion

*Several hundred proteins find their way here and, once at the mitochondrion, go to appropriate positions in this organelle. [Source: BSCS/*Biological Science: Molecules to Man, ed. 3, *Copyright © 1973, p. 193]*

was cleaved. Both groups did their experiments in vitro and in vivo, thereby demonstrating, Schatz notes, that "the information for targeting is wholly contained in the pre-sequence."

Next, the two groups of researchers asked what it is about the leader sequence that makes it work. The dozen or so leader sequences that are known come from mammals, yeast, and the mold *Neurospora*. Horwich, Rosenberg, and their colleagues first compared these sequences, looking for similarities. They found none, but they did notice that there is a virtual absence of acidic amino acids in these sequences. The sequences, says Horwich, "all are strikingly basic."

As a result of these comparisons, Horwich remarks, "We were left thinking that basic residues play an important role. So we decided to change them to neutral residues." They took the distal three out of the four arginines of the OTC leader sequence and changed them to the neutral amino acid glycine. They then joined this changed leader sequence to OTC and mixed the synthesized OTC with mitochondria in vitro. The result, Horwich says, is that "nothing happened. The OTC was not imported and the leader sequence was not cleaved."

In a separate experiment, the Yale researchers incubated their altered OTC protein with a preparation of the mitochondrial matrix that contains the enzyme that normally cleaves the leader. They saw, says Horwich, "absolutely no evidence of cleavage." Rosenberg, reflecting on these results, remarks, "Here we've changed three out of 32 amino acids in the leader and three out of 350 amino acids in the total molecule and we've rendered the protein functionless."

The three possible explanations for this result are that the positive charge of arginine itself plays a major role in recognition; that it is not so much the charge as the particular sequence itself that is crucial; or that the conformation of the leader sequence was altered when the three amino acids were changed, making it no longer functional.

On the basis of his own results, Schatz favors the third hypothesis. He and his colleague Eduard Hurt began by chopping amino acids off the 25 amino acid leader sequence of the yeast mitochondrial protein, starting from the end attached to the protein, and found, he reports, that only the first 12 of them at the amino terminus are necessary for mitochondrial import. But even though proteins carrying this reduced leader sequence get to the mitochondria, the leader sequence is no longer cleaved there.

Schatz proposes that proteins destined to stay in the inner membrane of mitochondria have a leader sequence that first specifies that they are destined for the mitochondria. Immediately following this "N-terminal address" there is what Schatz calls a "trans-membrane sequence" that specifies that the proteins are to go to the inner-membrane space. The trans-membrane sequence, Schatz and his associate Dolf Van Loon find, consists of uncharged amino acids flanked by charged amino acids. He proposes that what is recognized in these different sub-sequences of the leader sequence are three-dimensional structures. But, he says, "until we have x-ray data on the molecules and can look at structure, we really don't know what's going on."

Although many of the key questions about mitochondrial proteins are still unanswered, no one doubts that the answers will soon be found. Schatz states firmly that, "the study of protein import will only be interesting for another 5 years or so." But, Rosenberg points out, clinical applications of the work will undoubtedly take much longer than that.

—GINA KOLATA