PUZZLING OUT THE CELL'S POWER PLANT

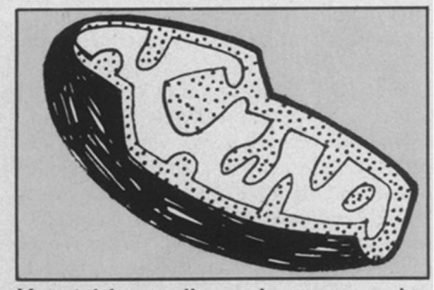
The construction and heredity of mitochondria have surprising differences from other cell processes

BY JULIE ANN MILLER

Mitochondria, the powerhouses of plant and animal cells, are in the cells but not entirely of them. These essential organelles are the product of two genetic systems — that of the cell and that of a separate hereditary mechanism within each mitochondrion. Assembly of all the mitochondrial components, produced within and without, has been a puzzle to biologists, but work by Gottfried Schatz and colleagues at the University of Basel and by Günter Blobel at Rockefeller University now explains a mechanism by which mitochondrial proteins are transported across membranes. Research in several laboratories has also uncovered surprising information about the mitochondrial genes.

Construction of a mitochondrion requires that protein components made outside the organelle, as well as those made within, reach their correct locations. Some externally produced components go into the mitochondrion's outer membrane, some go into the convoluted inner membrane and others end up in the space in between. Schatz and collaborators examined subunits of an enzyme called F1 ATPase. This enzyme is part of a complex that serves both in producing and breaking down ATP, the cell's major energy-storing molecule. Some of its subunits are made outside the yeast mitochondrion and then transported across both the outer and inner membranes. For several of the subunits, Schatz has discovered that transport into mitochondria is coupled with a final trimming of the subunit.

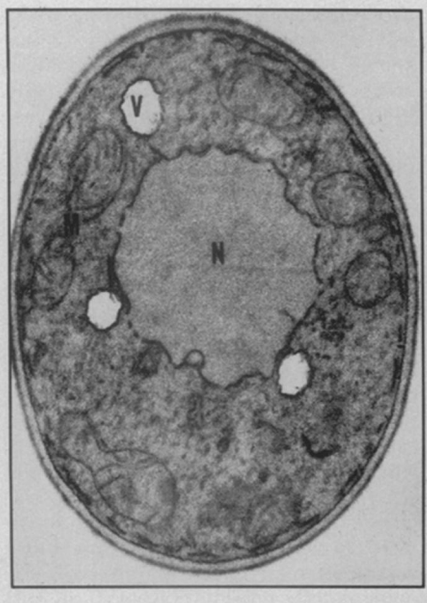
The F1 ATPase subunits are made as larger, precursor molecules that can accumulate outside the mitochondria, Schatz told the recent XIth International Congress of Biochemistry in Toronto. Only when protein-cutting enzymes, called proteases, clip off the extra material do the subunits cross into the mitochondria.



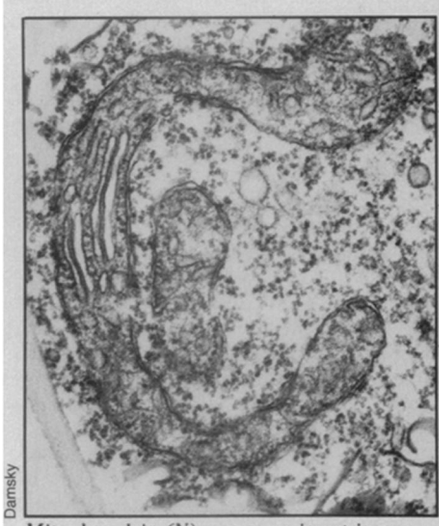
Material from cell must be transported to the various mitochondrial compartments.

Subunits already of the mature size cannot cross the membranes. The trimming and transport require ATP, and Schatz proposes that the import is driven by the trimming process. This novel mechanism is not required for all proteins that end up in mitochondria. Some mitochondrial components do not seem to have larger precursors, Schatz says. The novel mechanism contrasts other situations, such as hormone packaging in secretory cells, in which protein synthesis is coupled directly with transport across membranes (SN: 7/30/77, p. 73).

While most of the protein in a mitochondrion is imported from the surrounding cell, about 10 percent is coded by genes within. Research in the past 10 years has revealed that those mitochondrial genes direct an independent system of protein synthesis. Within a mitochondrion

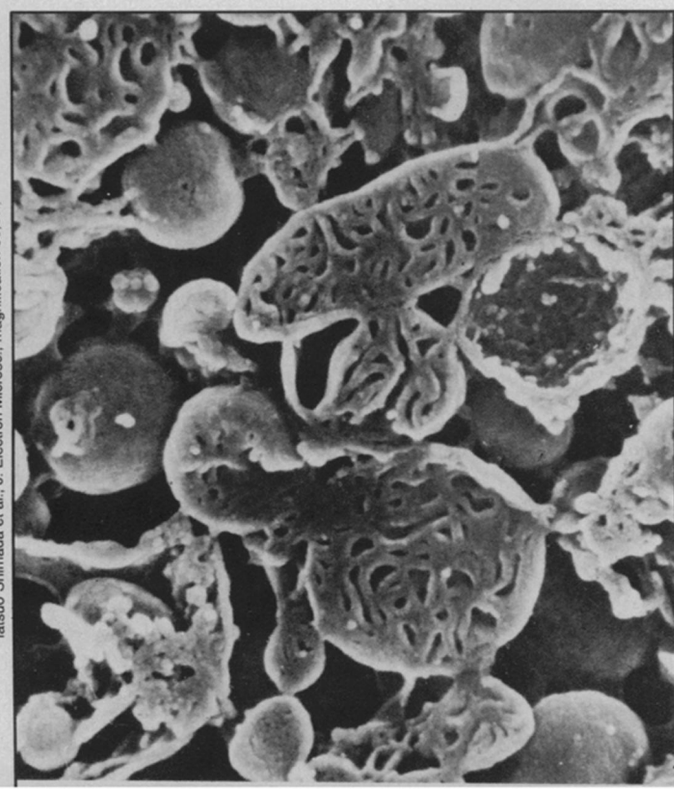


Caroline Damsky, Wistar Institute, magnification 21,000



Damsky

Mitochondria (N) are prominent in yeast cells grown with oxygen (top). Inner membrane is site of energy reactions.



Tatsuo Shimada et al., J. Electron Microsc., magnification 36,000

Convoluted inner membranes, which divide the structures into compartments, are visible in this scanning electron micrograph of fractured mitochondria isolated from monkey heart muscle.

special ribosomes, containing mitochondrial coded RNA, carry out production of polypeptide chains using at least some mitochondrial transfer RNA's. Those chains turn out to be very insoluble in water; therefore some scientists suggest that their special synthesis avoids transport problems. The mitochondrion-coded polypeptide chains eventually combine with protein subunits imported from the cell cytoplasm to make proteins of the mitochondrial inner membrane. The inner membrane is responsible for the mitochondrion's crucial functions: respiration and production of most of a cell's ATP molecules.

Every step of protein production within the mitochondrion is somewhat different from that step outside in the cytoplasm, Schatz says. Researchers have now found that even the genetic code is a bit different in mitochondrial genes (see box). Another surprise, relating to the origin of mitochondria, arises from the mitochondrial genes for two proteins. Some scientists believe the organelles are descended from ancient bacteria that became trapped in a symbiotic relationship. Mitochondrial genes, however, appear to be segmented by intervening sequences of DNA. Such an arrangement has been considered the hallmark of a plant or animal gene, Schatz says. The findings indicate that, at least genetically, the mitochondria do not follow modern bacterial style.

Both Schatz and a group at the University of Amsterdam, including Gert-Jan B. van Ommen and Piet Borst, found that in some strains of yeast the mitochondrial gene for the protein cytochrome b is divided into three coding regions by two intervening sequences. The total length of the non-coding regions in the gene is six times that of the coding stretches. In other strains of yeast, the Amsterdam biologists find four intervening sequences instead of two, van Ommen reported at a seminar at the National Institutes of Health.

Van Ommen and Borst also find evidence of intervening sequences in the yeast mitochondrial gene for a subunit of cytochrome oxidase. One strain of yeast has four intervening sequences in the gene and another has just two. In a third strain the gene is not segmented at all. "The variability between strains doesn't affect expression," van Ommen says.

The function of the intervening regions in plant and animal genes is still uncertain (SN: 7/7/79, p. 12) but the mitochondrial work may provide important clues. Both Schatz and van Ommen have found that mutations in the "silent" region can alter protein production. This observation is among the first evidence that an intervening sequence plays a role in gene operation. (At the International Congress of Biochemistry, Benjamin Hall of the University of Washington also reported that a mutation in the intervening sequence of a nuclear yeast gene interferes with normal transfer RNA production [SN: 8/4/79, p. 88]). The genetic change may interfere with the process by which the non-coding regions are trimmed from messenger RNA before it directs protein synthesis. Van Ommen reports that a mutation in the second intervening sequence of cytochrome b results in a larger protein that can bind antibodies to cytochrome b and therefore appears to contain cytochrome b within it.

Both groups report that, surprisingly, a mutation in the intervening sequence of the cytochrome b mitochondrial gene also interferes with production of cytochrome oxidase — a protein whose gene is quite a distance away. Van Ommen suggests that the cytochrome oxidase subunit is still made, but absence of normal cytochrome b somehow leads to degradation.

Since the mitochondrial DNA is orders of magnitude smaller than even the smallest nuclear DNA, it will probably be the first set of genes to be completely sequenced. The study of mitochondria should provide further insight into the organelle's unique genetics and construction, but may also provide key information for understanding the organization and regulation of plant and animal nuclear genes. □

Genetic Code Not Universal

Genetic material directs protein production according to a set code. The "universality" of that code — in bacteria, people and all the plants and animals in between — has been an important rule of modern genetics. Now investigations of the genes of mitochondria, the powerhouse structures within cells, reveal the first variations of the genetic code. A few of the code words have been found to have a different meaning in mitochondria than they have in the surrounding cell cytoplasm.

In the genetic code, a stretch of three nucleotide "letters" spells a codon "word" that specifies an amino acid of a protein or a "terminate protein" signal. Use of the same code from organism to organism has allowed recombinant DNA techniques for example, to produce a bacterium that correctly translates genetic information obtained from other bacteria, plants or animals.

The nucleotide sequences that make up several mitochondrial genes have been determined recently in a number of laboratories. To their surprise investigators found that the mitochondrial protein-making machinery speeds right through a "stop" sign, previously thought to be universal. The codon UGA (uridine, guanine, adenine) in mitochondria specifies the amino acid called tryptophan, rather than acting as a termination signal. That result was independently found in human placental mitochondria by Bart Barrell and Fred Sanger at the Medical Research Council in Cambridge, England, and in yeast mitochondria by Giuseppe Macino and Alexander Tzagoloff at Columbia University. In addition, Tzagoloff finds another genetic code variation. The codon CUA (cytosine, uridine, adenine) that in the cell cytoplasm specifies amino acid leucine is used in mitochondria for amino acid threonine.

Tzagoloff and collaborators suggest that the mitochondrial code has resulted from an evolutionary simplification. Because there are 64 possible 3-nucleotide codons and only 20 amino acids, some codons specify the same amino acid. For reasons still unknown, mitochondria show a strong bias against using the codons with G in the third position. For most codons, however, substitution of an A for a G in the third position does not change the amino acid specificity. (The 1965 "wobble hypothesis" explains that observation by proposing that the third nucleotide of a codon can form more than one type of base pair with "anticodons" of transfer RNA's, the carriers of amino acids.)

In the standard genetic code of cell cytoplasm, the codon UGA is one of two exceptions to the equivalence of G and A in the third codon position. UGG specifies tryptophan, while UGA specifies protein termination. However, in mitochondria the equivalence seems to hold — UGA does specify tryptophan.

Tzagoloff points out that instead of being a simplication of the standard genetic code, the mitochondrial code may be the original form. "It is equally plausible ... that mitochondrial codons have undergone little change and are therefore representative of a highly primitive code," Tzagoloff and colleagues say in the August PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES.

With the near-universality of the genetic code, a yeast cell can translate a gene from a foreign bacterium. But that yeast would not be able to use genes from its own mitochondria. Perhaps that distinction points to an advantage of mitochondria's slightly different genetic code. The differences could ensure that the quite separate mitochondrial and nuclear genes don't accidentally swap their cellular roles.

GAA glutamate *glutamate*	UAA stop *stop*	UGA stop *tryptophan*	CUA leucine *threonine*
GAG glutamate	UAG stop	UGG tryptophan	CUG leucine

Three-nucleotide codons specify different amino acids in a few cases for the genetic codes of mitochondria (italics) and organisms. In most cases changing a G to an A in the third position does not alter the amino acid specificity.