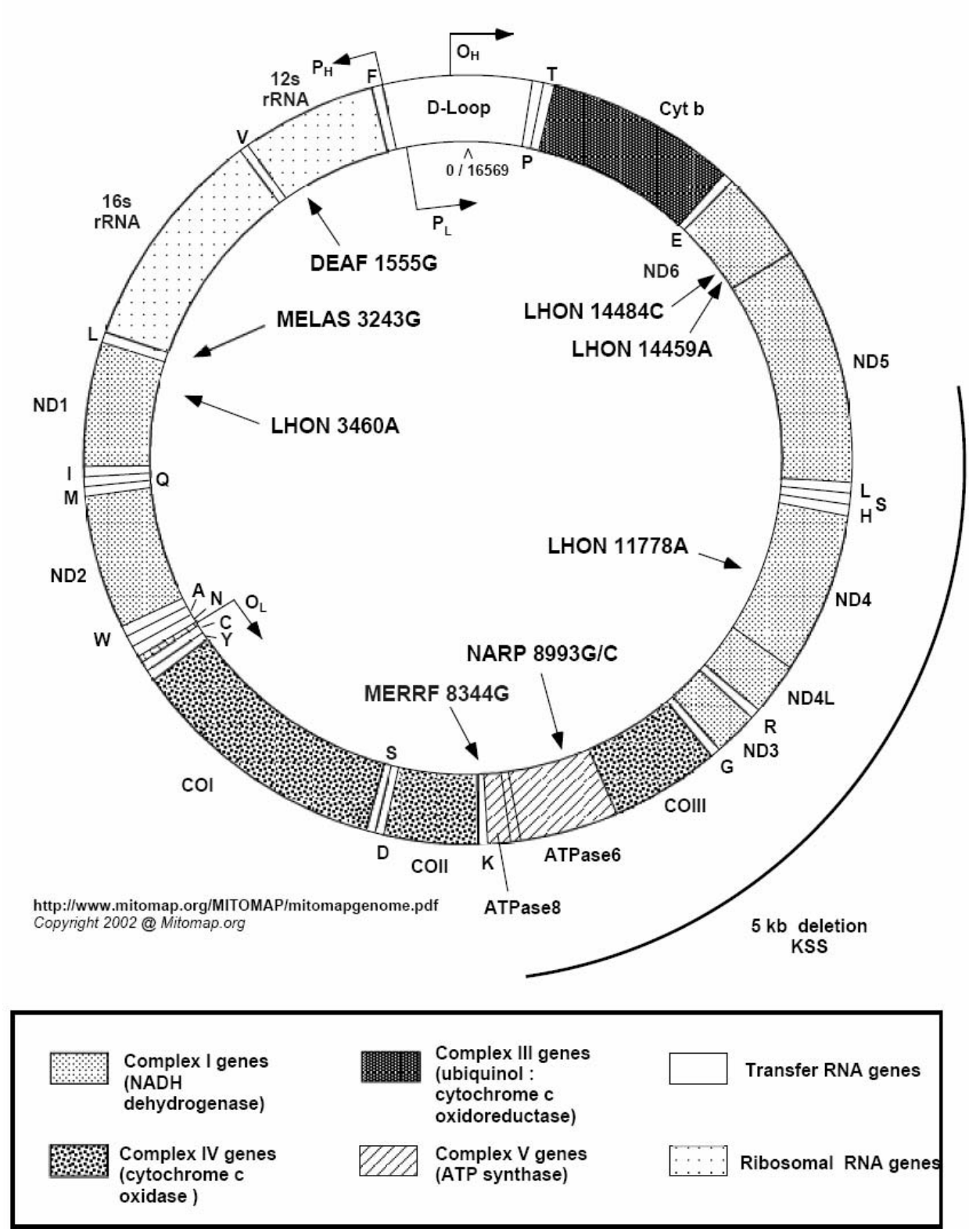
APPENDIX A A Map of Mitochondrial DNA



Appendix B
The Fire Within: The Unfolding Story of Human Mitochondrial DNA by Kenneth R. Miller

Nearly every cell of the human body contains scores of mitochondria, tiny organelles that play a key role in releasing cellular energy. Every student of biology learns (some more willingly than others) that mitochondria are home to a complex series of biochemical pathways, including the Krebs cycle and the electron transport chain (see pp. 123-131 in Biology by Miller & Levine). Mitochondria have always been interesting, ever since they were first recognized as important subcellular organelles by Altmann in 1890. He called them "bioblasts," and suggested that they might be tiny independent organisms within eukaryotic cells. He was wrong about that, but not quite as wrong as biologists once believed.

When sugars are broken down to release energy, most first enter a pathway in the cytoplasm known as glycolysis which produces a modest amount of adenosine triphosphate ATP. The end product of that pathway, pyruvate, enters the mitochondrion and then proceeds into the Krebs cycle. The reactions of the cycle systematically strip high-energy electrons away from the intermediates of the cycle, and these electrons enter the electron transport pathway, which is bound to the inner mitochondrial membrane. The oxygen we breathe serves as the final electron acceptor of the chain. As Peter Mitchell showed, this electron flow produces a proton gradient across the membrane which, like the pressure of water against a dam, can be used to generate energy. The proton "pressure" across the inner mitochondrial membrane produces not electricity but chemical energy in the form of ATP.

If any part of a human cell truly contains what the ancients called "the fire of life," it's the mitochondrion. Interrupt, even for a moment, the flow of electrons to oxygen, and that fire will go out. Indeed, some of the most lethal poisons, including the cyanides, act by blocking mitochondrial electron transport, and that is precisely why they are so deadly. There are a host of connections between the pathways in these tiny organelles and the larger organism, many of which I've shared with my students over the years. However, until very recently, one of those stories was nothing but a minor footnote. It's a footnote no more, and that's why it's the subject of this article: Mitochondria have DNA.

In the early 1960s a number of experiments showed that mitochondria could not be produced by cells de novo, but instead always arose from the division of preexisting mitochondria. In other words, they were self-replicating. By the end of the decade it was clear that mitochondria had their own DNA, and some researchers began to speculate that they might indeed be the semi-independent creatures that Altmann had wondered about. Led by Lynn Margulis (now of the University of Massachusetts), a number of researchers suggested that today's mitochondria are the descendants of ancient prokaryotes that took up residence within eukaryotic cells, and provided them with important biochemical benefits, notably the ability to oxidize food compounds and produce ATP (Biology p. 349). This is an intriguing idea that continues to influence thinking about the evolution of the eukaryotic cell.

How big is mitochondrial DNA? Well, it's not big at all. In fact, human mitochondria DNA (one of the smallest known) is only 16,569 base pairs in length, less than 1/300,000th of the total length of DNA molecules in the nucleus of a human cell. We now know the complete DNA sequence of human mitochondrial DNA. It codes for ribosomal RNAs and transfer RNAs used in the mitochondrion, and contains only 13 recognizable genes that code for polypeptides. It's not a very impressive piece of DNA, although it is efficient Ñ nearly every part of the molecule is transcribed, and there is precious little unused space between the genes. What are those 13 genes? Each codes for a different polypeptide that makes up part of the electron transport chain in the inner mitochondrial membrane. It looks as though the location of these genes inside the mitochondrion makes it easier for their gene products, including some nearly insoluble polypeptides, to be inserted into the inner mitochondrial membrane.

This is interesting enough, but it's hardly the stuff of medical mystery or international intrigue. At least that's the way things were. They may never be the same.

A few years ago, Doug Wallace, a researcher at Emory University in Atlanta, was puzzled by an unusual inherited

disorder known as Leber's Hereditary Optic Neuropathy ("Leber's" for short). Leber's results in a rapid loss of vision that usually begins in adolescence and can result in total blindness due to degeneration of the optic nerve. Leber's runs in families, so it had long been suspected as a genetic disorder, but there were two puzzling aspects to the disorder. First, the disorder was highly variable, causing complete blindness in some people and only minor loss of vision in others. Second, and most puzzling, was the fact that only women seemed to be able to pass the disorder along to their children. The children of men with Leber's never inherited the disorder, but the children of women with Leber's very often did. This did not mean that Leber's was sex-linked. Remember that sex-linked genes, although they are expressed more frequently in men, may be inherited from either parent, since they are carried on the X-chromosome. These two characteristics -- variable effect and maternal inheritance -- seemed to violate Mendel's principles of genetics. Wallace, and everyone else who had worked on Leber's, was puzzled.

What could account for this strange pattern of inheritance? Wallace was familiar with work on human mitochondrial DNA, and he wondered whether there was a chance that Leber's might be due to a mitochondrial gene. Using restriction enzymes to analyze fragments of mitochondrial DNA, he discovered that Leber's patients had a point mutation -- a single DNA base change – in their mtDNA that normal patients did not. Of what possible consequence could this single altered base be? Well, as it turns out, the base change changed a single codon in the gene for a protein in the electron transport pathway. When the slightly altered mRNA from this gene is translated, a single amino acid in the protein is changed. The protein still works, still transfers electrons, but it's just a bit less efficient.

This reduced efficiency of electron transport, which slightly lowers the rate at which ATP can be made, is at the heart of Leber's. In a connection still being worked out, Wallace and his coworkers theorize that the tiny defect becomes critical only in cells with very high demands for ATP. Such cells include the neurons of the optic nerve. Late in adolescence, a few of the cells in the nerve cannot keep up with ATP demand, weaken, and die. This increases the load on the remaining nerve cells until an increasing number of them malfunction, resulting in total blindness, first in one and often in the other. As interesting as this was, the most interesting part of the story may be how the mitochondrial connection fits in with Leber's non-Mendelian inheritance.

When sperm and egg fuse to form a diploid zygote, the new individual gets half of its nuclear genetic information, 23 chromosomes, from each parent. That 50/50 split is the basis of Mendelian inheritance. However, due to the sheer size of the egg cell, all (or nearly all) of the mitochondria in the embryo come from the mother. In other words, mitochondrial inheritance is maternal, and that's why Leber's is passed only from mother to child. What about the variability of Leber's? Well, if a particular allele is located, say, on chromosome #7, since we carry two copies of chromosome #7, one carries 0, 1, or 2 copies of the allele. Those are the only possibilities, barring a major chromosomal rearrangement. This is not the case for mitochondria. An egg cell contains more than 1,000 mitochondria, each with its own DNA "mini-chromosome." This means that several mitochondrial genotypes may exist side-by-side in the egg. Furthermore, because mitochondria are not carefully separated by the mitotic spindle (as chromosomes are), a mixture mitochondria are randomly split between two daughter cells during mitosis.

Wallace suspected that this meant that Leber's variability might simply be due to the percentage of defective mitochondria carried by an individual. Sure enough, his clinical studies revealed that individuals suffering from Leber's-induced blindness carried more than 70% defective mitochondria, while those with milder forms had no more than 30%. Apparently, the presence of a large number of "healthy" mitochondria can compensate for the loss of respiratory efficiency in those carrying the Leber's base change as long as there are enough of them around.

The Leber's story is not unique. In the last few years at least 6 genetic disorders have been traced to mitochondrial DNA, and there is no doubt that more will be discovered. To date, the discovery of the causes of these disorders has not resulted in treatments, but there are many possibilities to investigate. It's not at all improbable to speculate that in the near future individuals who carry the Leber's defect may wish to have their egg cells undergo an injection of "healthy" mitochondria to ensure that their children will not suffer premature blindness. Other therapies are possible, too, all made possible by the recognition that within there is a second, tiny genome in these remarkable organelles. The mitochondrial story provides a superb example of the links between molecular biology, genetics, neurobiology, and medicine. It also opens a new way to study inheritance, which has already had application in some surprising areas. And that's the next part of the story.

It is unfortunately true that the 20th century has been home not only to a series of flourishing democracies, but also to a number of brutal and repressive dictatorships around the world. We can be thankful that many of these regimes have fallen in the past few years, but in many cases they have left behind some appalling human wreckage after many years in power. One case in point is found in Argentina, where a new democracy is struggling with the legacy of a long-standing military dictatorship. The military's techniques for staying in power included the routine kidnapping, torture, and murder of political opponents. An estimated 12,000 people were killed during this reign of terror. In most cases, the victims were simply snatched from the streets and vanished from view. More than 200 children were either kidnapped along with their parents, or born to them in captivity. Now that democracy has been restored to Argentina, the families of the disappeared have begun a frenzied search to find out what happened to their loved ones.

In many cases there are no records to help them, and the only evidence of the disappeared are mass graves containing hundreds of bodies. The families of the disappeared are eager to identify the remains of their relatives, of course, but they are particularly eager to find their disappeared grandchildren. Many of these children were literally stolen from their parents, and sold on an active black market for babies.

As you may know, it is possible to identify individuals by means of unique sequences in their DNA. Because such sequences are inherited, they can also be used to establish family relationships. At first, molecular biologists tried to use such techniques to identify the children of the disappeared and to match them with their grandparents. However, after a few successes, they ran into a problem. In most cases the closest living relatives of the disappeared are their grandmothers. Unfortunately, a given DNA sequence has only a 50% chance of being passed from parent to child, so that all 4 grandparents would have to be checked for a match to the DNA sequence of a particular child. Where one or two of the four grandparents were missing or deceased, definitive identification was often impossible. Could there be a better way? Perhaps a DNA sequence that was always passed from mother to child?

No doubt, you already have the answer. So did Mary-Claire King, a molecular geneticist from Berkeley. Realizing that DNA sequences in mitochondria were passed directly from mother to child, she searched mitochondrial DNA until she found a 600-base pair region in which the variability from one individual to another was so great that they could be used to link mother and child unambiguously. Because mtDNA is passed directly from grandmother to mother to child it serves as a perfect recognition marker to establish identity. Supported by private foundations and the new Argentine government, Dr. King organized a systematic study of mtDNA from the grandmothers, and has now begun to use this data to identify the children of the disappeared. Mary-Claire King's work has won her international recognition and acclaim, as well as the thanks of scores of families who now have a chance to identify their missing children.

The fact that the tiny mitochondrial genome, unlike the much larger nuclear genome, is directly transmitted through the maternal line, makes it an ideal piece DNA with which to trace family lineage's. Small families, and large ones, too. The last few years have seen an extraordinary number of studies on the relationships of human population groups throughout the world. These studies have approached important questions, not the least of which is how long ago the great native populations of Africa, Europe, and Asia diverged from each other. The genetic markers used for these studies, naturally enough, are mitochondrial DNA.

It's already clear that mtDNA has become a powerful too for analyzing relationships in humans and other animals, and that the influence of mitochondrial genetics on human health can be substantial. These tiny organelles within our cells may indeed be the remnants of ancient strangers, as Lynn Margulis has suggested, and the fire of life which they represent has only just begun to fire human curiosity about their miniature world.