Two Disease-Causing Genes Found

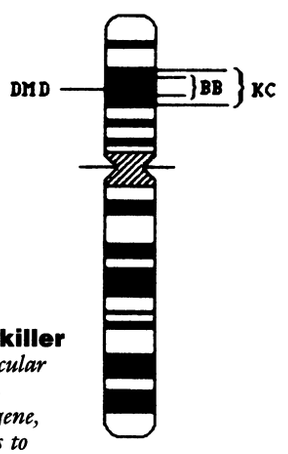
Researchers hope that their findings will lead them to understand what goes wrong in muscular dystrophy and how a recessive gene causes two kinds of cancer

Two disease-causing genes that have long been sought are now pinned down. One group of investigators, headed by Louis Kunkel of Children's Hospital in Boston, isolated a DNA segment within the Duchenne muscular dystrophy gene. The other group, headed by Thaddeus Dryja of the Massachusetts Eye and Ear Infirmary, and by Stephen Friend and Robert Weinberg of the Whitehead Institute, report isolation of the entire retinoblastoma gene. Both groups published their work in the 16 October issue of *Nature*. The results constitute a major step toward understanding how the two genes cause inherited diseases and why.

The work on the muscular dystrophy gene, says Donald Wood, director of research for the Muscular Dystrophy Association, is "science at its best." Investigators have known for several years approximately where the gene is located on the X-chromosome and three groups—headed by Kunkel, Ronald Worton of the Hospital for Sick Children in Toronto, and Kay Davies of Oxford University—have been looking for the gene in earnest.

"They were going neck-and-neck trying to find it," says Wood. "Any one of them could have come up with the piece of DNA that is a candidate for the gene first. But they all shared their data and they all cooperated with each other."

In the end, it was Kunkel's group that did it. Anthony Monaco, working with Kunkel, identified two segments within the gene region that are conserved among mammals and therefore are presumed to be coding regions. They sequenced those regions and then used them as probes to look for RNA transcripts from muscle cells. The probes picked out a 16 kilobase RNA and the investigators made complementary DNA (cDNA) clones from it. When mapped back to the chromosome, the cDNA hybridized to exons spread across 130 kilobases of DNA. This means the muscular dystrophy gene could be as long as 1 to 2 million base pairs, according to Kunkel.

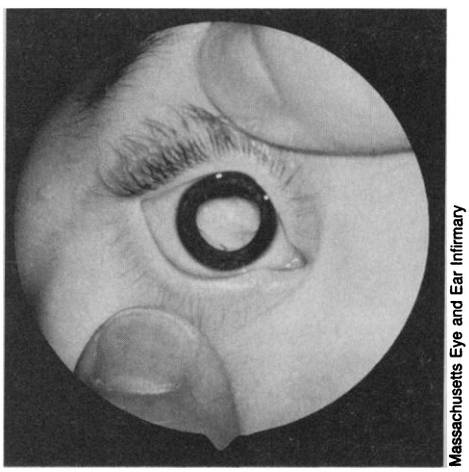


Portrait of a killer gene. *The muscular dystrophy gene, a mutant normal gene, causes muscle cells to degenerate and die. It is located on the short arm of the X-chromosome and apparently codes for a protein that is unique to muscle cells. (From* Trends in Genetics, *July 1985, p. 206)*

The finding, says Wood, "is a great scientific achievement, but it is not a medical one." Because the entire muscular dystrophy gene is not yet isolated, the cDNA probe cannot be used to determine who is a carrier for the disease. A woman could have a mutation at the other end of the gene from the end detected by the probe, for example, and the probe would never establish that anything was amiss. But carriers can be detected with other probes that can establish a family genetic pattern (*Science*, 18 October 1985, p. 307).

But, says Kunkel, "now we have a handle to get at the question: Where is this gene?" The first surprise is that it is expressed only in muscle. Although only muscle cells show the tremendous damage that is characteristic of Duchenne muscular dystrophy—starting with a generalized weakness and ending with total destruction—no one has demonstrated scientifically that the gene is expressed only in muscle. "In retrospect, people wasted a lot of time looking at red blood cells and in other places" for signs of change associated with muscular dystrophy, according to Wood. "There have been more than 20 different disorders of red blood cells described in muscular dystrophy patients, but I don't know of any that were ever confirmed in two different labs," he says.

Now investigators have a valuable hint in the discovery that the mutation should be seen only in a protein in muscle. Of course, they long ago looked at all the obvious muscle proteins, and found them all to be normal in muscular dystrophy patients. Therefore, it must be a protein that is not so obvious. Kunkel and others are going to try to determine just what that protein is by completely isolating and sequencing the gene. "Once the protein is identified, we can put a name to the answer of what causes muscular dystrophy," says Wood. "At that point, we will know if we have a long way to go or a short way to go to get a treatment."



Massachusetts Eye and Ear Infirmary

An inherited cancer. *Retinoblastoma, a cancer of the eye, is caused by a recessive gene on chromosome 13. The same gene apparently can lead to osteosarcoma, a bone cancer.*

The retinoblastoma gene, which causes the most common eye tumor in children, is of interest not because it will lead to a treatment, but because it is the first recessive cancer-causing gene ever isolated. All the other oncogenes are dominant—one copy of the gene leads to cancer. This means, researchers say, that the dominant genes code for products that apparently direct cells to grow uncontrollably. Because a recessive gene only causes cancer when there is no good copy of the homologous gene present, the interpretation is that these genes may be coding for substances that tell a cell to stop proliferating.

The retinoblastoma gene is thought to be a rare mutation of a normal gene. Children develop retinoblastoma when they inherit one copy of the gene and then, by chance, the homologous segment of their other chromosome in one or more of their retinal cells is deleted or mutated. As a result, they have no normal gene to counter the effects of the retinoblastoma gene and they develop cancer.

The search for the retinoblastoma gene began in the 1960's when Jorge Yunis, a

geneticist at the University of Minnesota, noticed that a patient who had this eye tumor also had a deletion on chromosome 13. Yunis and other geneticists were intrigued by this cancer because it is hereditary, it strikes young children between birth and age four, and those children who get retinoblastoma are hundreds of times more likely than normal to develop osteosarcoma, a bone cancer, when they are teenagers. (The exact frequency of osteosarcoma in retinoblastoma patients is not known.) The patient with the deletion in chromosome 13 provided the first hint of where the retinoblastoma gene might be located.

By 1974, geneticists had identified eight additional patients with a chromosome 13 deletion. But it was a discouraging search. Their techniques were far from sensitive and they could only spot the largest deletions. Between 1976 and 1980, they examined the chromosomes of 1200 retinoblastoma patients. Only 24 had noticeable deletions in chromosome 13.

Then Brenda Gallie and her colleagues at the University of Toronto noticed that the enzyme esterase D is coded for by a gene in the region of the putative retinoblastoma gene. This meant that researchers could look for retinoblastoma mutations by looking for normal variations in the nearby esterase gene. This technique enabled geneticists to identify chromosome 13 mutations in as many as one-third of all retinoblastoma patients who were studied.

Finally, Dryja and Webster Cavenee of the University of Cincinnati School of Medicine showed that nearly all retinoblastoma patients have mutations in the q14 band of chromosome 13. Then Dryja used chromosome walking techniques to isolate and map a 30-kilobase region of DNA in the q14 region. He found a fragment that is conserved in mouse and humans, suggesting that it constitutes a coding region. Finally, Friend and Weinberg looked for RNA transcripts in retinoblastoma cells from four patients and in normal retinal cells that hybridize to this conserved region. They report that a 4.7-kilobase RNA transcript from the normal retinal cells hybridizes to the DNA segment and that this transcript is missing in the retinoblastoma cells, indicating that the transcript may be the normal counterpart of the gene that is deleted or missing in retinoblastoma. They extended their analysis by making a cDNA copy of this RNA transcript and using it to screen four retinal cell lines, four retinoblastoma cell lines, and one osteosarcoma cell line. The cDNA probe hybridizes to a transcript in the retinal cells but not the retinoblastoma cells nor the osteosarcoma cells.

Now that they apparently have located the retinoblastoma gene, the researchers plan to add good copies of it to cells that have only mutant genes to see if the good copies restore normal growth. And they hope to learn what sort of protein the gene normally codes for. Now, says Friend, "we can do the experiments we always wanted to do." ■ GINA KOLATA

Plate Tectonics Is the Key to the Distant Past

Field geologists taking a closer look at 3-billion-year-old rocks are deciding that drifting plates formed them after all

GEOLOGIST Gregory Harper was intrigued. Here he was in the middle of Wyoming, sitting on rocks more than two and a half billion years old, more than half as old as the earth itself. Yet there was something familiar about these rocks of the Archean eon that reminded him of the rocks he had studied in northern California, a jumble of crust assembled only a few hundred million years ago.

According to conventional thinking, geologic processes quite unknown during the past billion years formed these exotic Archean crustal rocks in Wyoming's Wind River Mountains. The drifting and colliding of the continents evident in today's plate tectonics supposedly had nothing to do with this sort of rock, which constitutes the most ancient cores of the continents. But Harper eventually convinced himself otherwise. The familiar Archean rocks led him to propose that a nearly complete slice of ocean crust sits in the middle of Wyoming, ocean crust of the sort shoved up on the continents by drifting plates throughout the past billion years.

A growing number of geologists, apparently now a solid majority, have through one avenue or another arrived at the same conclusion as Harper—the present is the key to the past, no matter how distant that past. With only cosmetic differences in the end result, the same basic mechanics of crustal generation and destruction have shaped the surface of the earth from its early days until now. The division of geologic time, as well as geologic specialties, into epochs of unique behavior of the earth would appear to be unfounded.

Harper's claim of a beached slice of Archean ocean crust, called an ophiolite, is one of three being presented as evidence of modern-style plate tectonics in the Archean. Like the other two Archean ophiolite proponents, Harper was not trained as a specialist in the study of Archean rocks. He knew ophiolites from younger terranes, but he was not looking for them in Wyoming. He was only there to teach undergraduates at the University of Utah's annual summer field camp. What first caught his eye was a spot on a geologic map indicating a patch of rock a few hundred meters in diameter. It was labeled ultramafic, the type of dark rock found at the base of the oceanic crust and the uppermost mantle. "That's how you smell an ophiolite," says Harper, now at the State University of New York at Albany. "That's what got me going."

On close inspection, Harper found what he believes to be all but one of the components of a typical ophiolite of the past billion years—the mounded lava that flowed out onto the sea floor, the conduits or dykes of unique structure that carried magma to the sea floor where new crust was forming, and the ultramafic mineral crystals that fell to the floor of the underlying magma chamber. Missing was a piece of the uppermost mantle. That would be understandable, says Harper, if the higher temperatures of the Archean earth, temperatures that had been presumed to radically transform the behavior of the crust, merely raised the point at which the ophiolite was sliced off from the upper mantle to the lower crust.

The other two reported Archean ophiolites are related in a nongeologic way. For several years Maarten de Wit of the University of the Witwatersrand, Johannesburg, has been building a case for a 3.6-billion-year-old Archean ophiolite on the far northeastern border of South Africa. After showing it off to Herwart Helmstaedt of Queen's University, Kingston, Canada, during a week-long field trip, de Wit visited Helmstaedt's field site on the northern shores of Great Slave Lake in north-central Canada.