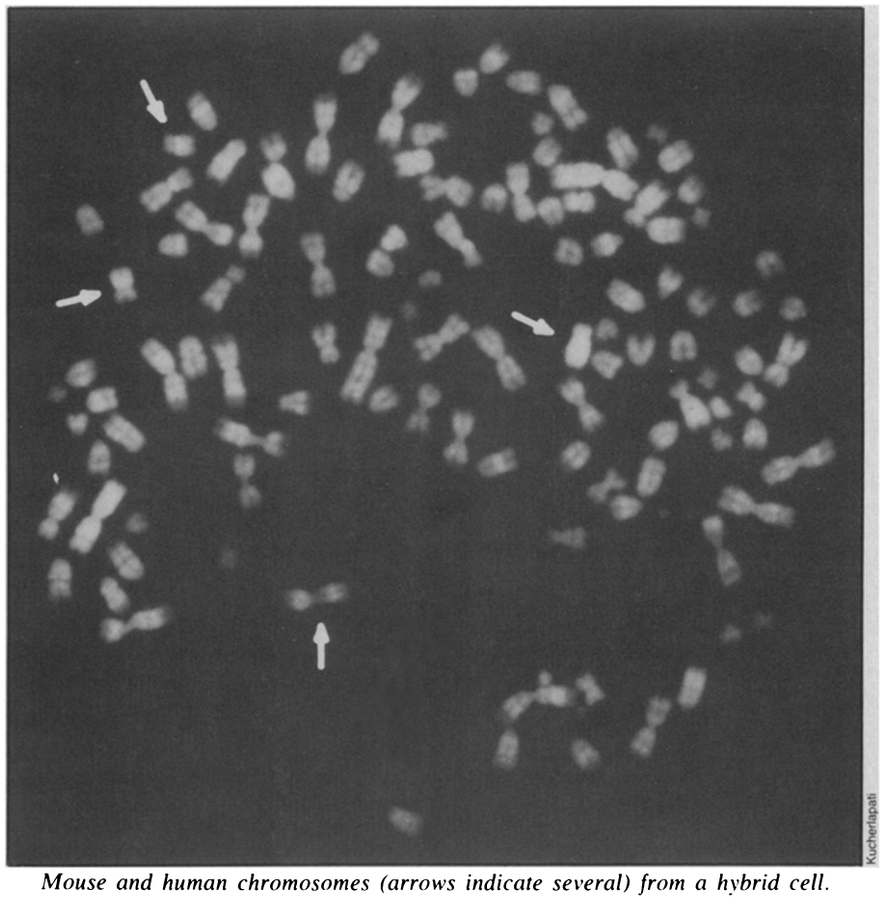
PUTTING HUMAN GENES ON THE MAP

Some 100 human genes have now been mapped on chromosomes, opening doors to understanding and prevention of human genetic diseases

BY JOAN AREHART-TREICHEL



Mouse and human chromosomes (arrows indicate several) from a hybrid cell.

In one of America's zaniest ivory towers (the Kline Biology Tower at Yale University), a team of 30 geneticists, biochemists and cell biologists are tackling one of the most ambitious projects of 20th-century molecular biology: the mapping of genes on human chromosomes.

Considering that biologists weren't even sure what genes were until the 1950's, that human chromosomes could not be clearly visualized until 1970 and that even today human genes look like tiny blobs under the microscope, their ambition is all the more impressive. But thanks to the development of new scientific techniques, some of them developed by the Yale group, the mapping of human genes is now a reality. About a hundred human genes have now been assigned to individual chromosomes or even to particular areas of those chromosomes, by the Yale investigators and others. This feat is opening the door to a better understanding and prevention of human genetic diseases.

The geneticist who heads up the Yale team is Frank Ruddle, a soft-spoken, six-foot, five-inch giant in khakis and sneakers. In his tenth floor office, back-dropped by a floor-length view of lush New England below, he describes how techniques have evolved so that human gene mapping is now a reality. . . .

Of the 1,000 genes in the one-celled bacterium, virtually all have been mapped on chromosomes, and of the 5,000 genes in each cell in the fly *Drosophila*, about 2,000 genes have been mapped. The reason is that classical Mendelian (family pedigree) experiments can be conducted on these lower organisms. The organisms can be selectively bred, offspring can be obtained quickly and in large numbers. Thus one can learn about the pattern of inheritance of particular genetic traits (gene products) and link them to certain chromosomes or areas of chromosomes. But such studies can't be conducted in people, because it's unethical and impractical. So geneticists have been forced to seek alternate methods in order to map human genes, some 100,000 of which populate each of the 100 trillion cells in the human body. The first breakthrough in this direction came in 1960.

George Barski and his colleagues at Institut Gustave Roussy in Paris discovered that different kinds of mouse cells could be fused. In 1965, Boris Ephrussi, first in France and later at Case Western Reserve University, and Henry Harris of the University of Oxford, improved on this technique so that cells from other mammals could be fused with mouse cells.

The next major advance came in 1967 when Mary Weiss and Howard Green, then of New York University School of Medicine, found that human cells could also be fused with mouse cells. If the hybrid cells were placed in a nutritive medium, they would divide into more identical copies of themselves, forming colonies of pure hybrid cells (clones). If the clones of hybrid cells were allowed to divide many times, say 30, their progeny cells contained the same number of mouse chromosomes as before, but randomly lost human chromosomes. And different clones retained different sets of human chromosomes. What's more, Weiss and Green discovered, both mouse and human genes in these cells were functional: Both sets of genes were expressed at the same time, each coding for its appropriate proteins.

Another significant development occurred in 1969, courtesy of Ruddle's team, which actually allowed the correlation of a particular gene to a particular chromosome. First one uses the technique of electrophoresis to see whether a particular gene product (enzyme or other kind of protein) is present in some 10 different clones of mouse-human hybrid cells. Each clone contains some 8 human chromosomes out of 24 possibilities. One then attempts to correlate the presence of the gene product with the presence or absence of a particular chromosome. If product X is always present in a clone when chromosome number 14 is, then one may conclude that chromosome 14 contains the gene that makes the product of interest.

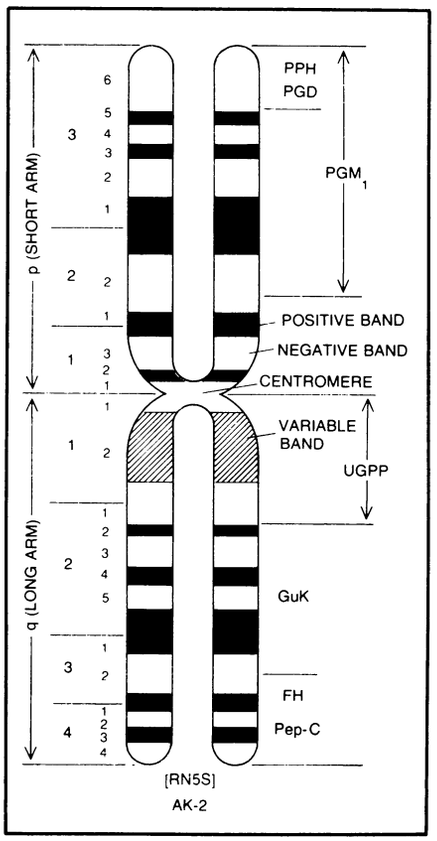
This technique, of course, doesn't allow one to locate a gene at a precise point on a chromosome or to establish the order in which several genes are arrayed along a chromosome. A method to do

these things was developed in 1973 by Ruddle's team, by Dirk Bootsman of Erasmus University in Rotterdam and by Marcello Siniscalco, then at the University of Leiden. The method depends on the use of human chromosomes that have had their parts rearranged (translocated). For example, a short arm of one chromosome gets attached to the long arm of another chromosome, and vice versa.

Human cells containing a particular translocation are fused with mouse cells, and clones are made of the hybrid cells. Again one looks for a particular gene product in different clones and attempts to correlate it with a particular chromosome, or rather this time with a particular area on the chromosome, the translocated part. Whenever the short arm of chromosome 18 is present in a clone, for example, enzyme X is too, but whenever the long arm of chromosome 18 is present, the enzyme is not. One may then conclude that the gene that makes the enzyme is somewhere on the short arm of chromosome 18.

"The key to this technique," Ruddle points out, "is in using chromosomes with different break points. That way you can build up information that tells you about chromosome intervals and which genes can be linked to them."

This is about where the state of the art is today, with the exception of one more refinement that promises even more specific gene mapping. The strategy, developed by W.O. McBride and H.L. Ozer of the National Institutes of Health, and further refined by Ruddle's group, would transfer part of a human chromosome into mouse cells that lack a particular gene product. If the human chromosome part provided the cells with the product they lack, then one could conclude that the gene that makes the product is on that area of the human chromosome. "To do this,



The human chromosome best mapped for genes to date is chromosome number one (above). Genes are designated by the initials of the protein products they code for.

however," Ruddle stresses, "one must know how much of the human chromosome material becomes functional in the recipient cell."

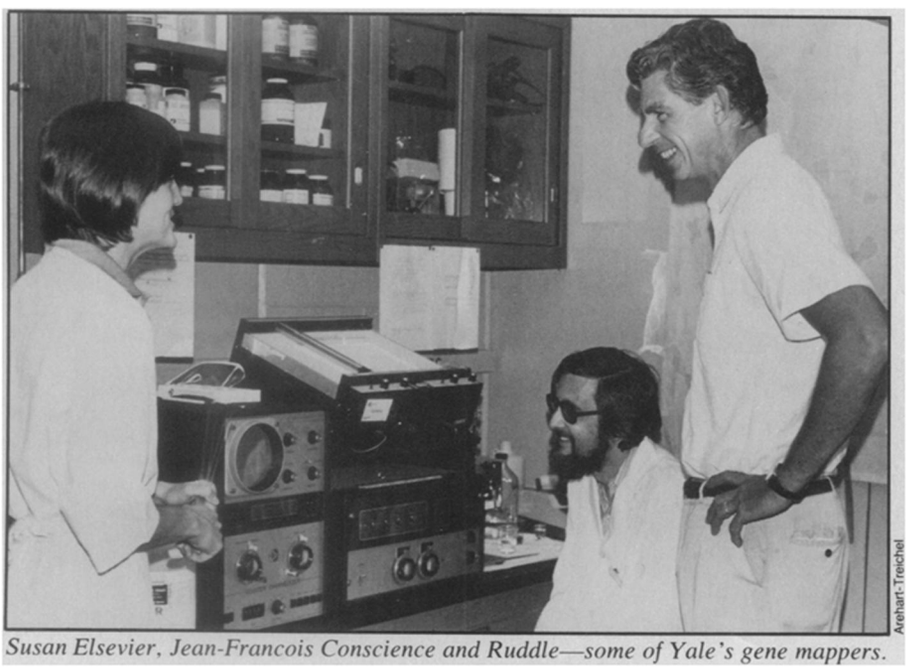
Using these various procedures, Ruddle's team, Bootsman, and the other investigators have mapped some hundred genes on the 24 chromosomes in each human cell, or even on specific regions of these chromosomes. This means, of course, conceptual mapping. While individual human chromosomes can now be distinctly visualized under the microscope (SN: 9/25/71, p. 200), human genes cannot.

What are the implications of such mapping for better understanding and preventing human genetic diseases? States Raju S. Kucherlapati, one of Ruddle's colleagues: "Although we have been able to map some of the genes that are implicated in human diseases, such as those for the Lesch-Nyhan syndrome, Tay-Sachs' disease, Sandhoff's disease and galactosemia, there are hundreds more genetic diseases that have been identified, but whose faulty genes have not yet been assigned to chromosomes. So we are not yet at the point where mapping has practical value in the prevention of human genetic diseases. Eventually, of course, as more and more genes are mapped, the diseases should become better understood, and methods of prevention will undoubtedly open up."

Meanwhile, he says, a related spinoff holds practical implications for the prenatal detection of genetic diseases affecting such specialized tissues as brain, liver and kidneys. Currently, the technique of amniocentesis is being used to withdraw skin cells from human fetuses (SN: 7/17/71, p. 44). Chromosomal translocations and other abnormalities in these cells can easily be detected, but not most genetic diseases, unless the disease concerns a gene product expressed in skin cells. The Yale team recently learned that they can fuse mouse liver cells with human fetal skin cells, and that the mouse cells activate latent genes in the human cells for liver products. "The key," Kucherlapati points out, "is that each human cell has all the genes in the human body, even if they are not expressed."

So the Yale investigators have an idea of how they might use this method to detect genetic diseases in fetuses: Suppose a fetus is suspected of lacking an important brain enzyme that could cause mental retardation. Its skin cells would be fused with mouse brain cells. If the mouse brain cells activated a latent gene in the human skin cells for the brain enzyme in question, then all would be well for the fetus because the gene would be present in the fetus's brain cells. But if the mouse cells did not activate the gene, then one could conclude that the fetus's brain cells lack the crucial gene, and that the fetus would be mentally retarded.

Ruddle swivels in his office chair and gazes through his window to the lush earth below. "I agree with Raju," he says. "Not too far off, say in five or ten years, detection of genetic diseases in fetuses should become possible. And by then we should have mapped some 1,000 human genes, opening up other, unexpected approaches to the prevention of human genetic diseases." □



Susan Elsevier, Jean-Francois Conscience and Ruddle—some of Yale's gene mappers.