Restriction Enzymes: Prenatal Diagnosis of Genetic Disease

In the few years since they were discovered, restriction enzymes have revolutionized the analysis of gene structure (see accompanying story on the Nobel Prize for Physiology or Medicine). The enzymes and the gene mapping techniques they have made possible are now resulting in the development of safer and more sensitive methods for the prenatal diagnosis of genetic diseases. The current gene mapping procedures can only be used to diagnose diseases caused by defects in the genes coding for the polypeptide chains of which hemoglobin, the oxygen-carrying protein of blood, is composed. In theory, however, the procedures are applicable to the diagnosis of any condition resulting from harmful changes in gene structure.

The hemoglobin disorders that can be detected by gene mapping with restriction enzymes include two forms of thalassemia and also sickle cell anemia. Although the thalassemias are rare in this country, sickle cell anemia is relatively common. About 60,000 black Americans have sickle cell anemia and another 2 million are carriers of the sickle cell gene.

In the past, prenatal diagnosis of these conditions could only be done by obtaining samples of fetal blood, a hazardous procedure that results in the deaths of 5 to 10 percent of the fetuses undergoing diagnosis. Thus, one of the big advantages of the new gene mapping procedures is that they do not require fetal blood but can be carried out on the fetal cells present in the amniotic fluid. These cells are fibroblasts; they do not make hemoglobin but they carry a complete set of genes, including those for the chains composing the hemoglobin molecule.

The cells are obtained by inserting a needle through the abdominal wall of the mother and withdrawing from the womb a sample of amniotic fluid. This procedure, which is called amniocentesis, appears to be safe. A large clinical study carried out under the aegis of the National Institutes of Health indicates that amniocentesis causes no significant risks to either mother or child.

Cells obtained in this way are used as a source of the DNA for the gene mapping procedure recently developed by Yuet Wai Kan and Andrée Dozy of the University of California Medical School (San Francisco) for the prenatal diagnosis of sickle cell anemia. This disease is caused by the inheritance of two defective genes for the β chain of hemoglobin. (A hemoglobin molecule consists of two identical α chains and two identical β chains.) The condition, which is characterized by severe anemia and the blockage of blood vessels by the deformed or "sickled" red blood cells, is often debilitating and even fatal.

Persons who inherit one defective and one normal globin gene do not develop sickle cell anemia, but they are carriers of the condition and they may pass it on to their children. For example, if two carriers conceive a child there is one chance in four that it will inherit two sickle cell genes and thus suffer from the disease. Such couples may seek prenatal diagnosis to determine whether their unborn child does have the condition.

To accomplish the diagnosis, Kan and Dozy first use the restriction enzyme designated Hpa I to digest the fetal DNA. A given restriction enzyme recognizes a specific nucleotide sequence in the DNA and cuts the DNA wherever that sequence appears. Each enzyme produces a characteristic pattern of fragments; the fragments bearing a specific gene or gene portion can then be identified if a probe for the gene is available. (The probe is usually a radioactive copy of the gene.) There is a probe for the β globin gene, and the fragment carrying it can thus be spotted in the mixture of fragments produced by the action of Hpa I on fetal DNA.

The ability to distinguish fetuses that have sickle cell disease from fetuses that are carriers and fetuses that have not inherited the defective gene at all depends on observations made in Kan's laboratory concerning the nature of the DNA fragments from the three types of individuals. Kan and his colleagues found that the normal β globin gene is located on a DNA fragment either 7000 or 7600 nucleotides (7 or 7.6 kilobases) long some 97 percent of the time; the rest of the time it is found on a 13-kilobase fragment. In contrast, the sickle cell gene is located on the 13-kilobase fragment about 87 percent of the time (Fig. 1). Apparently, the defective gene is associated with the loss of a recognition site for Hpa I about 5000 kilobases from the end of the gene, thus resulting in the production of the large fragment when the enzyme is used to digest the DNA.

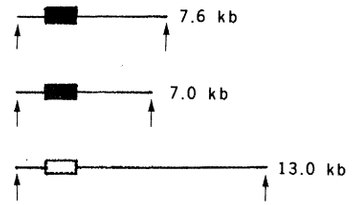

Fig. 1. The three types fragments bearing the β globin gene that are produced by digesting human DNA with the restriction enzyme Hpa I. The solid bars represent the normal gene and the open bar represents the defective sickle cell gene. The arrows indicate the sites cut by the enzyme. The site that is about 5000 nucleotides away from the end of the normal genes is missing in the fragment carrying the sickle cell gene. The lengths of the fragments are given in kilobases (*kb*).

In order to diagnose the disease in the fetus, the fragment patterns produced by the parental DNA must also be determined. Both members of the first couple studied by Kan and Dozy were known carriers who had previously produced a child with severe sickle cell disease. The woman was again pregnant and wanted to know if this child would also have the disease. Gene mapping showed that DNA from each of the parents produced both the 7.6- and 13-kilobase fragments, which is the expected result for carriers who have one normal and one defective β globin gene. The DNA from their sick child produced two 13-kilobase fragments, again as expected. But restriction mapping of DNA from their unborn child produced the same pattern as that of the parents. Thus, Kan and Dozy could tell the couple that their baby would be a carrier like themselves but would not have sickle cell disease.

Because the defective gene is on the 13-kilobase fragment only 87 percent of the time, the technique developed by Kan and Dozy sometimes gives ambiguous results. They estimate, however, that they can make an unequivocal diagnosis in about 60 to 70 percent of the cases where sickle cell disease is suspected. Thus restriction mapping for the diagnosis of this condition should greatly reduce the need for the more hazardous method requiring fetal blood sampling.

In addition, Kan says that their new technique is so sensitive that the cells from just 15 milliliters of amniotic fluid are sufficient for making the diagnosis.

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They do not have to grow the cells in culture for several weeks to get enough to analyze, and they can usually have the results of the test as early as the 16th or 17th week of the pregnancy.

The genetic defect causing sickle cell anemia is both simple and well understood; there is a substitution of a single wrong nucleotide in the β globin gene. In contrast, the thalassemias are neither so simple nor so well understood. Rather, they constitute a complex of several anemias, of varying degrees of severity, that may be caused by any of a number of different defects in the genes coding for the hemoglobin chains. Not all of these defects have been characterized but some have been described—again largely as a result of the availability of restriction mapping techniques. Among the laboratories contributing to the progress in understanding the gene defects underlying the thalassemias are those of Kan, Arthur Bank of the College of Physicians and Surgeons of Columbia University, Bernard Forget of Yale University Medical School, and Stuart Orkin of Harvard Medical School.

For example, the α thalassemias are caused by the deletion of some or all of the four copies of the α globin gene that are carried by most persons. The α thalassemias are relatively common in Southeast Asia, where as many as 15 percent of the population of some countries are carriers. In the severest form of the disease all four genes are missing. Fetuses with this condition are stillborn because the abnormal hemoglobin they produce cannot release its oxygen to the tissues.

About 2 years ago, Kan and his colleagues devised a way to detect the most severe forms of α thalassemia. They prepare fetal DNA from fibroblasts acquired by amniocentesis, shear it into fragments, and then measure the extent to which a probe for the α globin gene binds (hybridizes) to the fetal DNA fragments. Although this hybridization procedure can determine whether the gene is missing, it is technically difficult and requires large quantities of fetal cells.

In a more recent development, reported in July of this year, Orkin, in collaboration with investigators from Harvard, Yale, and Haceteppe University in Ankara, Turkey, showed that restriction mapping could be used for the prenatal diagnosis of a rare form of thalassemia.

Orkin says the restriction method is superior to the hybridization technique for prenatal diagnosis because mapping is more sensitive. It requires only 10 to 15 percent of the DNA required by the hybridization method and is generally easier to do.

The fetus they diagnosed was at risk from a thalassemia caused by a lack of both copies of the genes for β and δ globin. (δ globin, which is structurally very similar to β globin, is produced in small quantities by normal adults.) The mapping procedure showed that the genes were present, although in reduced quantities, and the Harvard investigators concluded that the fetus was a carrier but would not be severely afflicted by the disease. This diagnosis was confirmed both by examination of fetal blood and of blood taken from the umbilical cord at the infant's birth.

Thus far, only the rare forms of thalassemia caused by gene deletions can be diagnosed by restriction mapping. Some of the more common thalassemias, including Cooley's anemia, do not involve deletion of genes. (Exact figures on the prevalence of Cooley's anemia are hard to come by. According to some estimates, a few to several percent of the populations in such Mediterranean countries as Italy and Greece may be carriers of the condition.) But the common thalassemias may also prove amenable to prenatal diagnosis by restriction mapping once the gene defects underlying them have been revealed.

Any change in DNA that results in the gain or loss of a site recognized by a restriction enzyme will alter the pattern of restriction fragments produced by digestion of the DNA by the enzyme. Thus, at least in theory, any genetic defect is detectable by restriction mapping provided the appropriate tools—restriction enzymes and gene probes with the right specificities—are available. And they may become available. Already about 80 restriction enzymes that recognize different DNA sequences have been identified and more are still being found. Moreover, as Kan's work has shown, the altered restriction site need not be within the gene of interest, although it must be closely linked to that gene.

The principal reason why the current work has centered around defects in globin genes has been the ready availability of the appropriate probes. But probes for other genes may also be produced soon. For example, Thomas Maniatis of the California Institute of Technology is currently developing a library of cloned human genes that should prove useful for preparing probes. Thus, in the future, restriction mapping may prove feasible for prenatal diagnosis of a wide range of genetic defects.—JEAN L. MARX

The 1978 Nobel Prize in Physiology or Medicine

The 1978 Nobel Prize in Physiology or Medicine was awarded to Werner Arber, 49, of the Biozentrum in Basel, Switzerland; Hamilton O. Smith, 47, of Johns Hopkins University; and to Daniel Nathans, 50, also of Johns Hopkins University. The awards recognize the development of restriction endonucleases, enzymes that can be used to study genetic organization and to manipulate DNA for "genetic engineering." Arber is credited with having first predicted the existence of the enzymes, Smith with having isolated the first such enzyme and describing its specific reaction, and Nathans with having first applied these enzymes to the study of gene organization and regulation.

The development and application of the restriction endonucleases is a prime example of how scientific progress can be achieved through interlaboratory communication, with a concomitant "explosion" of results and developments simultaneously in many institutions throughout the world.

The story began in the early 1950's with the description of host-controlled variation among bacterial viruses (bacteriophages), probably most systematically presented by Salvador E. Luria of MIT and by Giuseppe Bertani and Jean J. Weigle. For example, when the bacteriophage lambda (Table 1), is isolated after growth on a particular strain of bacteria and replated on the same strain, it forms plaques (infects) with a relatively high efficiency. However, when these phage particles infect a different strain, only a few of them survive to form plaques. But the progeny of these survivors are adapted to the new strain in that they now plate with high efficiency on this strain. Thus, for example, when grown on *Escherichia coli* B, lambda is

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