Primer on Molecular Genetics

Markers must be polymorphic to be useful in mapping; that is, alternative forms must exist among individuals so that they are detectable among different members in family studies. Polymorphisms are variations in DNA sequence that occur on average once every 300 to 500 bp. Variations within exon sequences can lead to observable changes, such as differences in eye color, blood type, and disease susceptibility. Most variations occur within introns and have little or no effect on an organism's appearance or function, yet they are detectable at the DNA level and can be used as markers. Examples of these types of markers include (1) restriction fragment length polymorphisms (RFLPs), which reflect sequence variations in DNA sites that can be cleaved by DNA restriction enzymes (see box, p. 13), and (2) variable number of tandem repeat sequences, which are short repeated sequences that vary in the number of repeated units and, therefore, in length (a characteristic easily measured). The human genetic linkage map is constructed by observing how frequently two markers are inherited together.

Two markers located near each other on the same chromosome will tend to be passed together from parent to child. During the normal production of sperm and egg cells, DNA strands occasionally break and rejoin in different places on the same chromosome or on the other copy of the same chromosome (i.e., the homologous chromosome). This process (called meiotic recombination) can result in the separation of two markers originally on the same chromosome (Fig. 8). The closer the markers are to each other—the more "tightly linked"—the less likely a recombination event will fall between and separate them. Recombination frequency thus provides an estimate of the distance between two markers.

On the genetic map, distances between markers are measured in terms of centimorgans (cM), named after the American geneticist Thomas Hunt Morgan. Two markers are said to be 1 cM apart if they are separated by recombination 1% of the time. A genetic distance of 1 cM is roughly equal to a physical distance of 1 million bp (1 Mb). The current resolution of most human genetic map regions is about 10 Mb.

The value of the genetic map is that an inherited disease can be located on the map by following the inheritance of a DNA marker present in affected individuals (but absent in unaffected individuals), even though the molecular basis of the disease may not yet be understood nor the responsible gene identified. Genetic maps have been used to find the exact chromosomal location of several important disease genes, including cystic fibrosis, sickle cell disease, Tay-Sachs disease, fragile-X syndrome, and myotonic dystrophy.

One short-term goal of the genome project is to develop a high-resolution genetic map (2 to 5 cM); recent consensus maps of some chromosomes have averaged 7 to 10 cM between genetic markers. Genetic mapping resolution has been increased through the application of recombinant DNA technology, including in vitro radiation-induced chromosome fragmentation and cell fusions (joining human cells with those of other species to form hybrid cells) to create panels of cells with specific and varied human

HUMAN GENOME PROJECT GOALS

	Resolution
• Complete a detailed human genetic map	2 Mb
• Complete a physical map	0.1 Mb
• Acquire the genome as clones	5 kb
• Determine the complete sequence	1 bp
• Find all the genes	

With the data generated by the project, investigators will determine the functions of the genes and develop tools for biological and medical applications.