Low-Resolution Physical Mapping

Chromosomal map. In a chromosomal map, genes or other identifiable DNA fragments are assigned to their respective chromosomes, with distances measured in base pairs. These markers can be physically associated with particular bands (identified by cytogenetic staining) primarily by in situ hybridization, a technique that involves tagging the DNA marker with an observable label (e.g., one that fluoresces or is radioactive). The location of the labeled probe can be detected after it binds to its complementary DNA strand in an intact chromosome.

As with genetic linkage mapping, chromosomal mapping can be used to locate genetic markers defined by traits observable only in whole organisms. Because chromosomal maps are based on estimates of physical distance, they are considered to be physical maps. The number of base pairs within a band can only be estimated.

Until recently, even the best chromosomal maps could be used to locate a DNA fragment only to a region of about 10 Mb, the size of a typical band seen on a chromosome. Improvements in fluorescence in situ hybridization (FISH) methods allow orientation of DNA sequences that lie as close as 2 to 5 Mb. Modifications to in situ hybridization methods, using chromosomes at a stage in cell division (interphase) when they are less compact, increase map resolution to around 100,000 bp. Further banding refinement might allow chromosomal bands to be associated with specific amplified DNA fragments, an improvement that could be useful in analyzing observable physical traits associated with chromosomal abnormalities.

cDNA map. A cDNA map shows the positions of expressed DNA regions (exons) relative to particular chromosomal regions or bands. (Expressed DNA regions are those transcribed into mRNA.) cDNA is synthesized in the laboratory using the mRNA molecule as a template; base-pairing rules are followed (i.e., an A on the mRNA molecule will pair with a T on the new DNA strand). This cDNA can then be mapped to genomic regions.

Because they represent expressed genomic regions, cDNAs are thought to identify the parts of the genome with the most biological and medical significance. A cDNA map can provide the chromosomal location for genes whose functions are currently unknown. For disease-gene hunters, the map can also suggest a set of candidate genes to test when the approximate location of a disease gene has been mapped by genetic linkage techniques.

High-Resolution Physical Mapping

The two approaches to high-resolution physical mapping are termed "top-down" (producing a macrorestriction map) and "bottom-up" (resulting in a contig map). With either strategy (described on pp. 14–15) the maps represent ordered sets of DNA fragments that are generated by cutting genomic DNA with restriction enzymes (see Restriction Enzymes box at right, p. 13). The fragments are then amplified by cloning or by polymerase chain reaction (PCR) methods (see DNA Amplification, pp. 18–20). Electrophoretic techniques are used to separate the fragments according to size into different bands, which can be visualized by direct DNA staining or by hybridization with DNA probes of interest. The use of purified chromosomes separated either by flow sorting from human cell lines or in hybrid cell lines allows a single chromosome to be mapped (see Separating Chromosomes box at right).