Genetic Probes Become Ever Sharper

Rapid detection of multiple-pathogen infections, including major drug-resistance genes, may be possible using a newly developed technique

A group of researchers, based at Yale University, is developing a technique for identifying and locating RNA or DNA sequences in genetic material that rivals, and may soon surpass in sensitivity, standard isotopic methods. The basic technology, which has been patented by Yale University and is under exclusive license to Enzo Biochemicals, is already superior to traditional methods in its rapidity: results are obtained in hours rather than days. The stability and safety of the reagents used makes the method especially valuable in routine clinical diagnosis as well as in basic research.

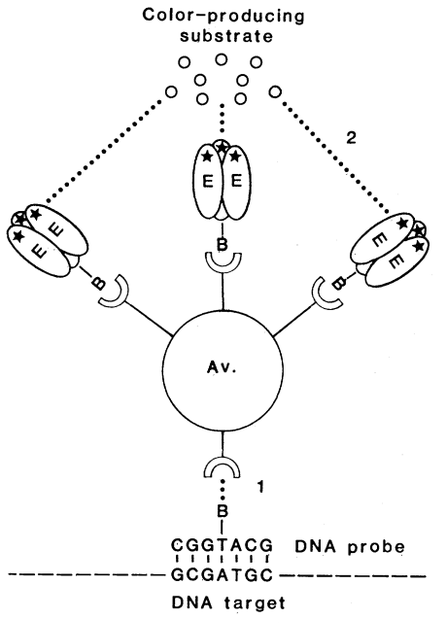
The relative ease with which specific stretches of DNA can be obtained through cloning means that, in principle, any genetic sequence can be located within a cell by "fishing" with the appropriate matching sequence. If the fishing probe is radioactive then the presence of the searched-for sequence can be revealed through autoradiography. Even though this is a one-step process it takes time and is often of poor spatial resolution.

The Yale approach, which has been directed by David Ward, employs a two-step process that produces a color reaction of some kind. Currently the method can reveal the presence of 10 to 20 copies of a genetic target in intact cells and the equivalent of one sequence copy per 100 cells in naked DNA. "We hope to be able to push up the sensitivity 100-fold, perhaps within a year," says Ward.

Ward and his colleagues began exploring the possibility of a nonisotopic method of pinpointing genetic sequences in the late 1970's. By 1981 they were able to publish the first key development, which was the synthesis of DNA probes that contained an effective "reporter molecule." The reporter, biotin, which is a small, water-soluble vitamin, has been used for some time in visualizing specific proteins, lipids, and carbohydrates on or within cells. Biotin binds tenaciously with avidin, a 68,000 dalton protein, the resulting complex being detectable in a number of biochemically simple ways. By incorporating biotin into DNA or RNA probes (usually linked to thymine or uracil bases) the standard detection methods already developed for other molecules could be applied to nucleic acids.

By late 1982 the Yale workers had shown that the technique could be used to locate specific sequences in *Drosophila* polytene chromosomes and in mammalian chromosomes. The detection of the reporter molecule involved conventional cytochemical techniques. "The system worked," says Ward, "but we needed to improve the sensitivity." This he and his colleagues have done during the past year, essentially by beefing up the detector molecule. The detection method is unexceptional: an enzyme molecule, which is attached to the biotin-labeled polynucleotide probe via an avidin residue, converts a soluble, colorless substrate into an insoluble pigment of some type. (The color grain can then be detected under a visible or ultraviolet microscope). "We are able to amplify this signal by polymerizing the enzyme, thus giving more active sites for substrate reaction."

Although this brute force approach gives a high sensitivity in locating sequences on naked chromosomes (as high as is achieved by certain isotopic techniques), the bulkiness of the avidin-polyenzyme complex impedes its passage into cells and thus reduces sensitivity in this context. "Tritium labeling is more sensitive in situ, but you have to remember that it might take 30 to 60 days to develop an autoradiogram as against an hour for our technique."



Nonisotopic genetic detection

When the biotin-containing DNA probe has hybridized with its target a detector complex (Av, avidin; E, enzyme) binds to it via the biotin (B) residue (step 1). The site of detection is revealed when a colorless, soluble substrate is metabolized into a colored, insoluble product (step 2).

The applications of the biotin-reporter technique are obvious. Wherever speed is required in detecting and locating specific, known genetic sequences, the technique is eminently applicable. This encompasses research, in mapping the chromosome location of a single copy gene for example, and biomedicine, especially diagnosis. "Even where monoclonal antibodies might be used, which also allow rapid detection, the biotin method might be sometimes superior," says Ward. "If you wish to locate a virus that is able to change its antigenic coat, for instance, a specific antibody will be unable to detect the virus in its new coat. By using the genome as the target, the biotin-labeling method will still pick up the virus."

Ward and his colleagues have already found that the probe does not have to match the target 100 percent. They were able to detect adenovirus type 34 in lung tissue using a type 2 virus, with which it has just 10 percent homology. "Immunological detection would have required using the correct one of a range of 37 serotypes."

The use of biotin as reporter is just one of several possibilities, says Ward. "You could envisage using multiple probes with differently modified nucleotides and different identification end points." With acute infections, such as bacterial meningitis, it would then be possible to detect the presence of several pathogens, as well as any major drug-resistance sequences, in a very short time.

"It took the isotope industry 20 years to reach the present sensitivity," notes Ward. "We have increased our sensitivity four orders of magnitude in 18 months, and we are already close to matching that level." The technique clearly has enormous promise, especially since the use of genetic probes in clinical medicine has just barely begun.—**Roger Lewin**

Additional Readings

1. *Virology*, **126**, 32 (1983).
2. *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4045 (1983).