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VISUALIZATION OF DNA MOLECULES IN TIME DURING ELECTROPHORESIS

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Visualization of Individual DNA Molecules

For several years individual DNA molecules have been observed and photographed during agarose gel electrophoresis. The DNA molecule is clearly the largest molecule known. Nevertheless, the largest molecule is still too small to be seen using a microscope. A technique developed by Morikawa and Yanagida has made it possible to visualize individual DNA molecules. When these long molecules are labeled with appropriate fluorescence dyes and observed under a fluorescence microscope, although it is not possible to directly visualize the local ultrastructure of the molecules, yet because they are long light emitting chains, their microsocpic dynamical behavior can be observed. This visualization works in the same principle that enables one to observe a star through a telescope because it emits light against a dark background. The dynamics of individual DNA molecules migrating through agarose matrix during electrophoresis have been described by Smith et al. (1989), Schwartz and Koval (1989), and Bustamante et al. (1990). DNA molecules during agarose gel electrophoresis advance lengthwise thorough the gel in an extended configuration. They display an extension-contraction motion and tend to bunch up in their leading ends as the "heads" find new pores through the gel. From time to time they get hooked on obstacles in the gel to form U-shaped configurations before they resume their linear configuration.

Fractionation of DNA by Electrophoresis

The visualization of the conformation dynamics of DNA molecules during electrophoresis has contributed much to the design of improved methods of DNA size fractionation. In particular, efforts to obtain complete physical maps of human chromosomes require techniques capable of separating large DNA fragments whose dimensions differ only in a small percentage of their total length. DNA molecules smaller than 50Kb are easily separated on the basis of their size. Large DNA molecules tend to migrate at the same rate. Schwartz and Cantor (1984) described a method that effectively separates large DNA molecules by agarose gel electrophoresis. The Pulse Field Gel Electrophoresis (PFGE) method is based on the fact that DNA molecules in solution behave like a worm-like coil. The pores in the agarose are smaller than the dimension of the coils formed by molecules larger than 30Kb in size. When a large DNA molecule enters such a gel in response to an electric field, the coils must elongate parallel to the field. When the field is shut off and a new field is applied perpendicular to the long axis of the DNA molecule, it finds itself lying across the openings of several pores. It will have to reorient itself and enter one of them. Bustamante et al. (1990) described and

photographed the beads and kinks that form along the molecule during the orientation process. The key to separation is the fact that larger molecules take longer to reorient than smaller ones. As the cycle is repeated, each molecule will have a characteristic net mobility along the diagonal of the gel.

Considerable progress has been made in our lab in preparation of materials and techniques for fractionation and visualization of chromosomal DNA. Intact yeast chromosomal DNA was prepared, fluorescently labelled with ethidium bromide, and microscopically visualized and photographed (1990). A new Zeiss research microscope has been installed in Dr. Percy Rhodes' lab equipped with a 35mm photographic system and video image system. The design of the electrophoretic chamber and power systems to be used with the microscope is almost complete. Phage lambda DNA has been stained with ethdium bromide and visualized by brownian motion in distilled filtered water using a small black and white video camera and video monitor.

As soon as the above preparations are completed the following will be investigated: 1) fractionation and visualization of T2 chromosomal DNA molecules during agarose gel electrophoresis using constant and pulse field direct currents. 2) Intact yeast chromosomal DNA molecules will be prepared and investigated using the same methods. 3) The feasibility of modifying the techniques used to prepare intact yeast chromosomal DNA and using them to obtain intact chromosomal DNA from animal cells grown in tissue culture, spermatozoa, and protozoa is being investigated.

The experience of working with the MSFC team for two summers has been enlightening and exciting. Special appreciation goes to MSFC colleagues Robert Synder, Percy Rhodes, and Teresa Miller, as well as numerous others who have been so helpful and friendly. It is hoped that colloboration will continue through the Summer Faculty Fellow Research Continuation Grant.

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