N91-18993

NASA/ASEE SUMMER FACULTY FELLOWSHIP PROGRAM

MARSHALL SPACE FLIGHT CENTER
THE UNIVERSITY OF ALABAMA

VISUALIZATION OF YEAST CHROMOSOMAL DNA

Prepared by:	Seth Lubega
Academic Rank:	Professor
University and Department:	Oakwood College Department of Biological Sciences
NASA/MSFC:	
Laboratory:	Space Science
Division:	Microgravity Science & Application
Branch:	Biophysics
MSFC Colleague:	Dr. Robert S. Snyder

Contractor No.:	NGT-01-002-099 The University of Alabama

VISUALIZATION OF YEAST CHROMOSOMAL DNA

Introduction

The DNA molecule is without doubt the most significant life molecule since it codes the blue print for other structural and functional molecules of all living organisms. It is also by far the largest. Some chromosomal DNA molecules are as long as several centimeters. With a diameter of only 20 Å, the aspect ratio may be to the magnitude of 10^6 - 10^7. This makes it extremely fragile. Agarose gel electrophoresis is now being used widely to separate DNA of virus, bacteria, and lower eukaryotes (fungi and protozoa). Individual chromosomal DNA molecules have been observed using the fluorescent microscope but the physical basis of their behavior is still poorly understood. Dr. Robert Snyder and his team have undertaken the task of reviewing the existing methods of DNA fractionation and microscopic visualization of individual chromosomal DNA molecules by gel electrophoresis as a basis for a proposed study to investigate the feasibility of separating DNA molecules in free-fluids as an alternative to gel electrophoresis. It has been an exciting experience to learn the various techniques as I worked with the team this summer. Since the study is in its initial stages, negotiations are under way for some arrangements to make it possible for me to continue to work with the team through the academic year. This will be necessary for me to keep up with the progress of the study and be ready to work with the team next summer.

The chromosomes of lower eukaryotes such as fungi and protozoa have poor mitotic and meiotic visualization and present a challenge for both genetic and cytogenetic studies. On the molecular level, agarose gel electrophoresis is being widely used to separate chromosomal DNA according to molecular weight. Carl and Olson (1985) separated and characterized the entire

karyotype of a laboratory strain of Saccharomyces cerevisiae. Smith et al. (1989) and Schwartz and Koval (1989) independently reported the visualization of individual DNA molecules migrating through agarose gel matrix during electrophoresis. The techniques used by these researchers are being reviewed in our laboratory as a basis for the proposed studies.

Preparation of Intact Chromosomal DNA

'The separation of chromosomal DNA based on molecular weight requires that that DNA remain intact without breakage. For DNA of 50 kb or less from most biological sources, standard methods of purification such as phenol extraction, alcohol precipitation, and micropipetting are quite effective. Larger molecules, especially those above 300 kb in size cannot be effectively purified by these methods without breakage. Schwartz and Cantor (1984) developed a method of preparing DNA samples by in situ lysis of cells or spheroplasts in a semi-solid matrix. Several modifications of this method have been developed but they all employ an agarose matrix. The intact cells or spheroplasts are suspended in low gelling agarose. The liquid-cell mixture is poured in molds known as inserts and allowed to solidify before treating with reagents to lyse the cells and remove the proteins leaving the naked DNA in the stable gel. The insert is then treated with 0.5 M EDTA to reduce nuclease activity to levels where no double stranded breaks will occur. In this form the insert can be stored at 4 °C for long periods of time although best results are obtained when electrophoresis is carried out within 2 weeks.

Separation of DNA by Pulse Field Gradient Gel Electrophoresis (PFGE)

Most chromosomal DNA molecules are extremely large. Small molecules 50 Kb or less are easily separated on the basis of their molecular weight using polyacrelamide or agarose gel electrophoresis. DNA molecules larger than 50 kb tend to migrate at the same rate making separation by these methods impossible. 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 more than 30 kb 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. 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.

Visualization of Individual DNA Molecules During Agarose Gel Electrophoresis

The visualization of individual DNA molecules migrating through agarose gel matrix during electrophoresis using a fluorescent microscope was a giant step toward the understanding of the dynamics involved in the orientation of these macromolecules. Smith et al. (1989) and Schwartz and Koval (1989) both observed that DNA molecules advanced lengthwise through the

gel in an extended configuration. They alternately contracted and lengthened as they moved. Often they became hooked around obstacles in a U-shape for extended periods and displayed elasticity as they extended from both ends at once. The obstacles may be due to the fact that commercial agarose is contaminated with other polysaccharides, salts, and proteins.

Smith et al. also observed an aqueous layer under the cover slip on the slide on which the gel was. In the absence of an electric field the DNA molecules in the aqueous layer could be seen "tumbling and wriggling" in response to Brownian motion while the DNA in the gel was virtually motionless. In an electric field the DNA in the quueous layer "tumbled and streamed" quickly toward the positive electrode. The DNA in the gel underwent a slower and more constrained motion as described above.

The studies of chromosome-sized DNA fractionation and visualization reported to date involve viral, prokaryotic (bacteria), and lower eukaryotic (fungi and protozoa) DNA. Higher plants and animals have much larger and more complex chromosomal DNA's that will require other methods of preparation.

Our team is in the initial stages of reviewing the established techniques as a basis of improving on them. Our initial challenge is the investigation of the feasibility of developing a method of visualizing and separating DNA-sized molecules in free-fluids. We hope that this method will have the capability of visualizing and separating chromosomal DNA of higher eukaryotes (plants and animals) and provide an invaluable tool in genetic studies of these organisms.

References

Carle, George F., and Maynard V. Olson (1985): "An Electrophoretic Karyotype for Yeast (DNA/Chromosomes/Orthogonal-Field-Alteration Gel Electrophoresis)," Proc. Natl. Acad. Sci. USA, Vol 82: 3756-3760.

Carle, George F., and Maynard V. Olson (1987): "Orthogonal-Field-Alternation Gel Electrophoresis" Methods in Enzymology, 155:468-475.

Schwartz, David C., and Charles R. Cantor (1984): "Separation of Yeast Chromosome-Sized DNAs by Pulsed Field Gradient Gel Electrophoresis, Cell. 37:67-75.

_____ and Michael Koval (1989): "Conformational Dynamics of Individual DNA Molecules During Gel Electrophoresis," Nature, Vol. 338, No. G215, pp. 520-522.

Smith, Steven B., Paul K. Aldridge, and James B. Callis (1989): "Observation of Individual DNA Molecules Undergoing Gel Electrophoresis," Science, 243:203-206.