



BACTERIA—Dr. George Fox, professor of biochemical and biophysical sciences, studies the effects of microgravity on bacteria. His research focuses on whether strains of bacteria might experience enhanced resistance to antibiotics during a long mission in space. Such changes would affect the health of astronauts and delay their recovery from illness during flight.

The Effect of Simulated Microgravity on Microbial Gene Expression

Investigative Team

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Abstract—

Contrary to popular belief, the virulence of *Salmonella* strains is not reduced in the microgravity of space. In fact, *Salmonella* strains might increase resistance to antibiotics if given the environment to multiply during missions of long duration. New technology allows researchers to study this serious problem with the development of a rotating bioreactor at the NASA-Johnson Space Center.

A team of researchers in biochemistry is devoting effort to bacterial growth in the bioreactor to determine whether changes in bacteria can, in fact, be attributed to microgravity or whether fluid effects cause changes. For the first time, scientists can examine gene expression in a comprehensive way, using arrays of surface-bound DNA probes that target large numbers of mRNAs in a single experiment.

ONE OF THE UNIQUE ASPECTS OF SPACE FLIGHT is extended exposure to microgravity. A large body of whole-organism-based work has demonstrated that prolonged microgravity exposure, has significant effects at a basic, cellular level. Far less work has been done on bacteria, and it is not obvious that they would be affected at all. However, recent evidence that exposure to microgravity modifies the virulence of *Salmonella* strains has dramatically demonstrated this is not the case. Another realistic possibility is that strains might evolve enhanced resistance to antibiotics over the course of a long-duration mission. Despite these concerns limited access to space flight has largely prevented detailed studies of the effect of microgravity on bacteria. Fortunately, an approximation of microgravity on Earth has been achieved through the development of a rotating bioreactor at the NASA-Johnson Space Center. This bioreactor (rotating wall vessel- RWV) simulates microgravity by randomizing the gravitational vectors such that the gravitational vector integrated over time approaches zero. Used in conjunction with genomics technology, the RWV makes it possible to study microbial gene expression under simulated microgravity conditions.

It is now possible to examine gene expression in a comprehensive way, using arrays of surface-bound DNA probes that target large numbers of mRNAs in a single experiment. This approach has taken a quantum leap in effectiveness, for the entire genomic sequences of well-characterized model organisms are now available. The whole-genome sequence information permits the design of hybridization probes for each and every gene, which may be expressed by the organism. Advances in detection and array construction now allow single experiments to simultaneously monitor the expression levels of every predicted mRNA. With the completion of numerous bacterial genomes, including *Escherichia coli* and *Bacillus subtilis*, it is especially timely to examine the effects of simulated microgravity on bacterial gene expression.

The specific aim of the project is to determine if bacterial gene expression is affected by simulated microgravity. In order to address this goal, we are initially using hybridization assays to monitor the expression of all genes in the Gram negative bacteri-

um, *E. coli*, for which the entire genome sequence is available and for which ordered arrays of probes for each open reading frame in the genome are commercially available from Genosys, Inc. (The Woodlands, TX). Because *E. coli* is by far the most extensively studied bacterium, its metabolism and gene regulation are extremely well understood. Initial studies indicated that over 100 open reading frames were expressed at levels that are either twice or less than half what is seen in a shaker flask control. We have developed software that allows us to automatically detect and quantify spots on the arrays and a display tool that allows one to display which genes are up or down, regulated within the context of genomic position.

Although growth in the bioreactor has significant effects on gene expression, it remains to be proven that microgravity rather than fluid effects causes the observed changes. Therefore, our focus in the coming months will be a series of experiments to distinguish responses to fluidic effects from microgravity effects. In order to do this we are analyzing a variety of physiological parameters including growth rate and yield, cell appearance, and the length of the lag and exponential phases. We will determine if these parameters are changed between controls and simulated microgravity when different growth conditions are imposed.

We will use two types of controls. The first is growth in the RWV held in the horizontal position and the second will be growth in an ordinary shaker flask. Results will be compared for growth in both minimal and rich media. Because *E. coli* is a facultative anaerobe it will also be possible to compare growth when oxygen is and is not the terminal electron acceptor.

Publications

- D'Souza, L. M., R. C. Willson, and G. E. Fox. "Expression of Marker RNAs in *Pseudomonas putida*," *Curr. Microbiol.* 40 (2000): 91-95.
- Hedenstierna, K. O. F., J. L. Siefert, G. E. Fox, and E. J. Murgola. "Co-conservation of rRNA Tetraloop Sequences and Helix Length Suggests Involvement of the Tetraloops in Higher-Order Interactions," *Biochimie* 82 (2000): 221-27.
- Kourentzi, K. D., G. E. Fox, and R. C. Willson. "Rapid Identification of Microorganisms Using 5S rRNA Specific Molecular Beacons," *Curr. Microbiol.* 43 (2001): 444-47.
- Larkin, D. C., S. A. Martinis, D. J. Roberts, G. E. Fox. "Do Small Dipeptides Mediate a Peptidyl Transferase Reaction with Aminoacylated RNA?" *Origins Life & Evol. Biosphere* 31 (2001): 511-26.
- Murphy, J. C., G. E. Fox, and R. C. Willson. "RNA Isolation and Fractionation with Compaction Agents," *Anal. Biochem* 295 (2001): 143-48.
- Nagaswamy, U., X. Gao, S. A. Martinis, and G. E. Fox. "Structure of a Conserved Penta-Loop Found in 16S rRNA," *Nucl. Acids Res.* 29 (2001): 5129-39.
- Nagaswamy, U., N. Voss, Z. Zhang, and G. E. Fox. "Database of Non-canonical Base Pairs Found in Known RNA Structures," *Nucl. Acids Res.* 28 (2000): 375-76.
- Siefert, J. L., M. Larios-Sanz, L. K. Nakamura, R. A. Slepecky, J. H. Paul, E. R. B. Moore, G. E. Fox, and P. Jurtshuk, Jr. "Phylogeny of Marine *Bacillus* Isolates from the Gulf of Mexico," *Curr. Microbiol.* 41 (2000): 84-88.

Presentations

- Fox, G. E. "Artificial Stable RNA," Invited Seminar Speaker, Jet Propulsion Laboratory, Pasadena, CA, Aug. 21, 2001.
- Fox, G. E. "Cyanobacteria: Then and Now," Invited Speaker, Earth System Processes at Edinburgh Int'l Conf. Centre, Edinburgh, Scotland, June 24-28, 2001.
- Fox, G. E. "Non-Canonical Interactions in RNA," *Nucleic Acids Research*, Database Issue 2001-Summary Papers, Abstract online at <http://www3.oup.co.uk/nar/database/summary/220>.
- Fox, G. E. "Progress Towards Monitoring Microbes in the Space Environment," Invited Speaker, Bioastronautics Investigator Workshop, Galveston, TX, Jan. 17-19, 2001.
- Kourentzi, K., G. E. Fox, and R. C. Willson. "Microbial Detection with Natural and Engineered RNAs," Gordon Research Conf. on Applied and Environmental Microbiology, Connecticut College, New London, CT, July 22-27, 2001.
- Kourentzi, K., G. E. Fox, and R. C. Willson. "Monitoring of Microorganisms Using Hybridization Assays Specific for RNA," National Meeting, American Chemical Society, San Diego, CA, April 2001.
- Moreno, P. A., J. D. Burgos, P. E. Velez, J. M. Gutierrez, A. K. Naik, A. Verdugo, and G. E. Fox. "Multifractal Analysis of Complete Genomes," 12th Int'l Genome Sequencing and Analysis Conf., Miami Beach, FL, Sept. 12-15, 2000.
- Murphy, J. C., G. E. Fox, R. C. Willson. "Immobilized Metal Affinity Chromatography of Nucleic Acids," National Meeting, American Chemical Society, San Diego, CA, April 2001.
- Murphy, J. C., G. E. Fox, and R. C. Willson. "Purification of Plasmid DNA Using Compaction Agents," National Meeting, American Inst. Chemical Engineers, Los Angeles, CA, Nov., 2000.
- Murphy, J. C., K. I. White, G. E. Fox, and R. C. Willson. "New Approaches to Nucleic Acid Separation," 10th Int'l Symp. on Analysis and Separation of Proteins, Peptides, and Polynucleotides, Ljubljana, Slovenia, Nov., 2000.

Funding and proposals

- "Artificial Stable RNA Sequestration of Heavy Metals." Texas ARP, Jan. 1, 2002-Dec. 31, 2003, \$176,076.
- "Comparative Genomics." UH Faculty Development Leave Application for 2001/2002.
- "Microbial Monitoring in Bioremediation." Texas Hazardous Substance Research Center, Sept. 1, 2000-Aug. 31, 2003, \$96,456.
- "Microorganisms in the Spacecraft Environment." Co-PI: R. Willson; National Space Biomedical Res. Inst. Oct. 1, 2000-Nov. 30, 2003, \$926,040.
- "Multicopy Gene Families in Eukaryotic Genomes." NIH Individual National Research Service Award (F33), July 1, 2002-June 30, 2003, \$50,000; *pending*.
- "Noncanonical Base-Base Interactions in Synthetic RNAs." Welch Foundation, June 1, 2000-May 31, 2003, \$141,000.
- "The Origins of Translation." Co-Investigator: S. Martinis; NASA-Exobiology Program, Feb. 1, 1999-June 30, 2002, \$240,000.
- "The Origins of Translation and Early Life." NASA Exobiology Program, April 1, 2002-March 30, 2005, \$343,177; *pending*.