Malhotra, Arun

Department of Biochemistry and Molecular Biology *2001 Program*
University of Miami School of Medicine *New Investigator (3-year project)*

Project Title: Crystallographic studies of exoribonucleases

Project Summary: The DNA in genes is copied into RNA and the RNA then carries out some particular cellular function. Before it can operate, however, the RNA is further processed. One common processing step involves enzymes called exoribonucleases trimming off the ends of the RNA strands. This research uses X-ray crystallography to study the structure of exoribonucleases and figure our how they are able to identify specific sites near the ends of the RNA strands, attach to them, and break the strands at that point. Many tobacco-related cancers depend on RNA function. Results from this study can help in the design of drugs to control RNA function.

Project Successes: The project has mapped the three-dimensional structures of two exoribonucleases (oligoribonuclease and RNase D) in the bacterium, Escherichia coli, using protein crystallography. Crystals of the third enzyme in this family (RNase T) have also been obtained, and work is underway to map its structure. The structure of the first of these enzymes, oligoribonuclease, shows that the protein works as a dimer, with a pair of identical units. The project results suggests that one of the molecules in the dimer participates in "holding" the RNA while its partner chops the RNA. On the other hand, RNase D works as a mononer, and different domains of this protein carry out the RNA binding and catalytic functions. The project results have enabled us to obtain a NIH research grant ($1.46 million over 5 years) to carry out detailed structural and mechanistic studies on these and other related exoribonucleases. Equipment and resources from this Florida Biomedical research grant also enabled us to obtain the structures of two enzymes (RluD and TruD) involved in RNA modification in E. coli. Results from this project have improved the understanding of the molecular basis of RNA degradation and modification, which are important processes in cancer biology.

Selected publications from BRP funded research in Peer Reviewed Journals:

Kaya Y, Del Campo M, Ofengand J, **Malhotra A.** Crystal structure of TruD, a novel pseudouridine synthase with a new protein fold. *J Biol Chem*. 2004;279:18107-18110.

Del Campo M, Ofengand J, **Malhotra A**. Crystal structure of the catalytic domain of RluD, the only rRNA pseudouridine synthase required for normal growth of Escherichia coli. *RNA.* 2004;10:231-239.

Fiedler TJ, Vincent HA, Zuo Y, Gavrialov O, **Malhotra A**. Purification and crystallization of Escherichia coli oligoribonuclease. *Acta Crystallogr.* 2004;D60:736-739.

Del Campo M, Ofengand J, **Malhotra A**. Purification and crystallization of Escherichia coli pseudouridine synthase RluD. *Acta Crystallogr.* 2003;D59:1871-1873.

Presentations from BRP funded research:

Fiedler TJ., Zuo Y, **Malhotra A.** Crystallographic Studies of DEDD Family Exoribonucleases Involved in tRNA/rRNA 3' End Maturation and mRNA Degradation.

FASEB Summer Research Meeting – Posttranscriptional Control of Gene Expression: Mechanisms of mRNA Decay, Tucson, AZ. June 26- July 1, 2004.

Fiedler TJ., Zuo Y, **Malhotra A.** Crystallographic Studies of DEDD Family Exoribonucleases Involved in tRNA/rRNA 3' End Maturation and mRNA Degradation. Annual Symposium of the Protein Society, Boston, MA. July 26th-30th, 2003.

Malhotra A., Fiedler TJ., Zuo Y. Crystallographic Studies of DEDD Family Exoribonucleases Involved in tRNA/rRNA 3' End Maturation and mRNA Degradation. 8th Annual Meeting of the RNA Society, Vienna, Austria. July 2003.

New grants based in part on BRP-funded work:
NIH/NIGMS
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Project period: 9/30/2003 - 8/31/2008
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