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Department of Biochemistry and Molecular Biology *2001 Program*
University of Miami School of Medicine *Investigator Initiated (2-year project)*

Project Title: Regulation of genetic recombination and DNA repair by synaptic feedback

Project Summary: This research identified a large family of viral proteins involved in DNA repair, recombination and viral growth, and has yielded results that are guiding researchers world-wide in developing gene targeting strategies.

Red Beta protein was purified and its DNA binding properties characterized using a novel fluorescence-based assay. The exonuclease attenuation reaction and an in vitro recombination reaction were reconstituted, producing initial data showing regulation of the exonuclease by DNA pairing. This is an exciting result and has many implications for understanding the regulation of DNA repair, for gene therapy, and for biotechnology.

The team's prediction that Herpes would become dependent on DNA synthesis to produce packageable genomic DNA when recombination was compromised was confirmed, suggesting that the Herpes recombinase is an ideal target for antiviral drug development, especially in cocktails with recombination inhibiting drugs.

In the course of this work the research team developed a modification of their fluorescent nuclease assay that permits real time acquisition of DNA digestion data. A manuscript describing this method and its application to a number of DNA nucleases of medical and biotechnological interest was published. This assay will facilitate ongoing studies of the regulation of these nucleases by DNA pairing and has been adopted by at least two biotechnology companies for quality control measurements and has been written up in the Handbook of Fluorescence manual that Molecular Probes publishes for the research and biotechnology communities.

Project Successes: The results from this research shed light on the underlying mechanisms of recombination nucleases and their action on DNA strand breaks (such as are formed by tobacco-specific nitrosamines). Future studies planned by this research team promise to unfold the mechanisms regulating these highly processive enzymes so that faithful DNA repair occurs with minimal associated genotoxic events such as are associated with cancer.

Publications from BRP funded research in Peer Reviewed Journals:

Vellani TS, **Myers RS.** Bacteriophage SPP1 Chu is an alkaline exonuclease in the SynExo family of viral two-component recombinases. *J Bacteriol*. 2003;185(8):2465-2474.

Reuven NB, Staire AE, **Myers RS,** Weller SK. The Herpes Simplex Virus Type-1 alkaline nuclease and single-stranded DNA binding protein mediate strand exchange in vitro. *J Virol.* 2003;77:7425-7433.

Subramanian K, Rutvisuttinunt W, Scott W, **Myers RS.** The enzymatic basis of processivity in lambda exonuclease. *Nucleic Acids Res.* 2003;31(6):1585-96.

Jockovich ME, **Myers RS**. Disruption of cell division and nucleoid structure by nuclease-deficient helicase-proficient RecBCD mutants in Escherichia coli. *J Bacteriol***.** 2003 (under revision)

New grants based in part on BRP-funded work:
We are resubmitting a revised application for an RO1 grant from the NIH on July 1 (previous assignment number 1 RO1 GM068861-02). Results obtained from the FBRP have been instrumental in laying the foundation for this grant proposal, and the support by the State of Florida have been essential for our research. Thanks to your support of our research program, the results of these studies and their long-term applications in preserving and improving human health should continue to benefit Floridians.