

May 31, 2005

Final Report – Research Enhancement Proposal

PI: L. Kevin Lewis
Department: Chemistry and Biochemistry

Title: Role of Oxidative DNA Damage in Telomere Shortening and Cellular Aging

Abstract:

The major aims of this research proposal were to develop assays for quantitating cellular aging and telomere shortening rates using yeast as a model system and to investigate factors that affect the kinetics of these processes. During the past year we have performed most of the experiments described in the Specific Aims and added additional experiments to clarify some of the results. For example, (a) we have created special yeast strains that have telomerase under the control of a new, regulatable *GALI* promoter and used these to develop assays for monitoring cell senescence, including measurement of changes in cell growth rate, killing and cell cycle checkpoint arrest, (b) we have tested effects of the pro-oxidant chemicals hydrogen peroxide, iron, and bleomycin, as well as the antioxidant chemicals N-acetylcysteine and resveratrol, on cell aging kinetics, and (c) we have constructed yeast strains with antioxidant genes *GPX3* (glutathione peroxidase) and *CTTI* (catalase) inactivated and evaluated the impact on cell aging. These early experiments have demonstrated that two of the pro-oxidants, bleomycin and iron, can act to accelerate cell senescence. The *GPX3* and *CTTI* mutations did not affect cell aging, but new mutant strains with multiple antioxidant genes inactivated (e.g. *GPX3* + *CTTI* as well as three other mutants) are currently being tested. In addition, during the past year we have worked to develop a new, simpler method for quantitating rates of telomeric DNA shortening that is based on the use of polymerase chain reaction (PCR) techniques.

Publications during past year:

L. Kevin Lewis, Francesca Storici, Shanna Calero and Michael A. Resnick. **2004**. Role of the nuclease activity of *Saccharomyces cerevisiae* Mre11 in repair of DNA double-strand breaks in mitotic cells. *Genetics* 166:1701-1713.

Ling Chen, Kelly Trujillo, Lumir Krejci, L. Kevin Lewis, Michael A. Resnick, Patrick Sung and Alan E. Tomkinson. **2005**. Effect of amino acid substitutions in the Rad50 ATP binding site on the function of the Rad50/Mre11/Xrs2 complex in the repair of DNA double strand breaks. *J. Biol. Chem.* 280:2620-2627.

Papers submitted in past year:

L. Kevin Lewis, G. Karthikeyan, Jared Cassiano and Michael A. Resnick. **2005**. Reduction of nucleosome assembly during new DNA synthesis impairs both major pathways of double-strand break repair. Submitted to journal *Nucleic Acids Research*.

L. Kevin Lewis, Kirill Lobachev, Jim Westmoreland, G. Karthikeyan, Kelly Williamson and Michael A. Resnick. **2005**. Use of a dominant negative nuclease assay to identify inducible *GALI* promoter variants with reduced basal activity. Submitted to journal *Gene*.

Grant proposals submitted in past year:

The Welch Foundation –

“Chemical Oxidation of DNA Regulates Telomere Stability and the Rate of Cellular Aging” - (3 yrs, \$150,000). - Not Funded.

NIH AREA grant –

“Role of Telomere Fusions in Replicative Cell Senescence”– (3 yrs, \$150,000 direct). - Not Funded.

NSF RUI grant –

“Chromosome Fusions and Replicative Senescence: Testing the Model”– (3 yrs, \$236,143 direct). In review.

Presentations:

Invited Seminar – Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, NIH, June 30, 2004.