**Specific Aims**

Nijmegen breakage syndrome (NBS) is an autosomal recessive disorder characterized by small head size, distinctive facial features, intellectual disability, short stature, immunodeficiency, and cancer predisposition1. Not as obvious, however, are the unstable chromosomes, accelerated shortening of telomeres, sensitivity to DNA-damaging agents (such as ionizing radiation), and abnormal cell cycle checkpoints experienced by these patients2. Mutations in nibrin (NBN), a gene known for its role in eukaryotic DNA-damage repair, are responsible for these molecular phenotypes. NBN mediates protein-protein interactions at sites of DNA breakage, but lacks any enzymatic activity of its own. However, working with two other proteins, MRE11 and RAD50, NBN forms a complex called MRN, which senses DNA double-strand breaks (DSB) and mediates DSB-related cell cycle checkpoint signaling and telomere maintenance3. Nbs1\_C, an NBN domain located at the C-terminus, is responsible for interaction with MRE11 and ultimately the formation of MRN4. Because of this NBN can be considered a tumor suppressor protein for, without this process, cells can accumulate errors that may lead to cell death or uncontrolled growth, offering an explanation for NBS patients predisposition to cancer.

Mutations in NBN generally lead to a truncated protein. Today, eleven mutations, if not more, are directly linked to NBS. 90 percent of these mutations are caused by a 5-nucleotide deletion in the sixth exon, creating a premature stop codon. This mutation, 657del5, results in fragmented version of NBN, p26, lacking the Nbs1\_C domain. However, the remainder of the gene, post p26, is also translated and results in a complementary fragment, p70, whose expression is found to be essential for organismal viability. The levels of expression of this protein fragment appear to be negatively correlated with cancer predisposition and other NBS phenotypes.

Additionally, recent research has shown that NBS also affects heterozygotes. Experiencing higher spontaneous and induced chromosome instability and predisposition to malignancies, it’s concluded that expression of one unaffected gene is not sufficent7. *However*, *the molecular mechanisms that regulate NBN expression in wild type, heterozygotes and mutants, such as 657del5, remain unclear.*

**We will test the hypothesis that the expression levels of nibrin are critical indicators of cancer susceptibility in wild type and heterozygous organisms.** Our ***primary goal*** is to understand the molecular mechanism behind the regulation of NBN expression in these patients.

***Aim 1.*** Determine the degree of variability of NBN expression levels in wild type and heterozygous mice. **Hypothesis.** There will exist more variability among heterozygotes than wild type mice. **Approach.** Perform quantitative mass spectrometry and compare levels of NBN.

***Aim 2.*** Identify NBN expression levels in organism with naturally low cancer susceptibility, such as naked mole rats. **Hypothesis.** Average expression levels will be higher compared to that in mice and humans. **Approach**. Perform quantitative mass spectrometry and compare levels of NBN.

***Aim 3.*** Identify potential sites of translation initiation conserved among various organisms. **Hypothesis.** Translation and expression of NBN will decrease as translation is initiated further down the protein. **Approach.** Obtain a multiple sequence alignment using Basic Local Alignment Search Tool (BLAST) and Clustal Omega.

We attempt to understand the regulation of NBN expression at a genomic and proteomic level. With knowledge gained from this research, we peruse a ***long-term goal*** to help those affected by NBS by providing information that may lead to new treatment options, such as gene therapy, or drug developments.

Naked mole rats -- [http://biomedgerontology.oxfordjournals.org/content/60/11/1369.full.pdf+html](http://biomedgerontology.oxfordjournals.org/content/60/11/1369.full.pdf%2Bhtml)