

## **NNPDF-Funded Research Grant # 29**

**TITLE: A Yeast System for Analysis of Sterol Trafficking Defects in NPC**  
**PROJECT INVESTIGATOR: Melanie Jean Dobson, Ph.D.**

**PERIOD: 2/15/2002 - 2/14/2003**

### **PROJECT DESCRIPTION**

The first step in understanding any inherited human disorder is to understand the fundamental cellular process that has been disrupted due to the loss of the functional gene. Most cases of Niemann-Pick type C (NPC) disease, a fatal inherited neurodegenerative disorder, result from mutations in the NPC1 gene. NPC disease is characterized by a defect in intracellular cholesterol transport. We do not understand the role that the NPC1 plays in regulating the production and movement of sterols within cells. Pathways of sterol synthesis and regulation have been conserved from yeast to man, and yeast contains a single gene with remarkable similarity to the human NPC1 gene. Our approach to determining the function of human NPC1 is to analyze the yeast version of this gene. The yeast model system offers several advantages over mammalian cell systems including the ease with which genes can be manipulated, simple genetics allowing yeast with different mutant genes to be crossed to each other so that the combined effects of the mutant genes can be assessed in their progeny, and the presence of only one copy of each chromosome that allows rapid identification of other genes that may interact with the NPC1 gene. We have constructed yeast strains in which the yeast's version of the NPC1 gene has been deleted or mutated. Biochemical and molecular genetic analysis of this yeast will be used to determine what effect mutations in the gene have on sterol metabolism and cell physiology and to identify where and how the normal protein functions within the cell. We will use several standard yeast techniques to identify other genes that interact with the yeast version of NPC1. The availability of the complete sequence of both the yeast and human genome allows for a rapid transition from yeast to direct tests of the functions of the mammalian versions of any genes identified in our study. This research will help determine the basis for NPC disease and is therefore of direct relevance to hundreds of people with the disease and their families. The findings, by providing a better understanding of cholesterol and lipid regulation, will also have broader relevance for many common human disorders, such as hypercholesterolemia, atherosclerosis and coronary heart disease.

### **FINAL STATUS REPORT**

**Dated 2/14/2003**

Niemann-Pick type C (NPC) disease is a fatal inherited neurodegenerative disorder characterized by a defect in intracellular trafficking of lipids and other metabolites. Most cases of NPC disease result from mutations in the NPC1 gene. The function of NPC1 has not been determined but its conservation from yeast to man and the pathology associated with its mutation indicate it plays an important role in cell biology. Intracellular trafficking pathways have been conserved from yeast to man. Our approach to determining the function of human NPC1 is to analyze the yeast Niemann-Pick C related gene, NCR1.

Yeast genes are easily manipulated, and crossing yeast with different mutations allows the combined effects of the mutant genes to be assessed in their progeny. The presence of only one copy of each chromosome allows the consequences of mutant genes to be directly observed in yeast. Through our NNPDF-supported research we discovered that yeast lacking the NCR1 gene are less efficient than wild-type yeast at delivering material from the extracellular environment to their intracellular digestive compartment, the vacuole, and that the NCR1-encoded protein is localized in the vacuolar membrane. Biochemical and molecular genetic analysis are now being used to determine which aspect of intracellular trafficking is impaired in the mutant yeast. We have used several standard yeast techniques to identify other genes that interact with NCR1 or participate in the same cellular process. The availability of the complete sequence of both the yeast and human genome allows for a rapid transition from yeast to direct tests of the functions of the mammalian versions of genes identified in our study. This research will help determine the basis for NPC disease and is therefore of direct relevance to individuals with the disease and their families. The findings, by providing a better understanding of intracellular trafficking of lipids, will also have broader relevance for other human disorders, such as hypercholesterolemia, atherosclerosis and coronary heart disease.

**PUBLICATIONS:**

No Publications on this Work To Date