

Cystinosis Research Foundation

Progress Report Sept 1, 2014- March 1, 2015

Principal Investigator: Anand K Bachhawat, IISER Mohali, India

Project Title: Genetic and biochemical investigations on the cystinosin transporter using a novel genetic screen

Specific Aims (As proposed):

1. Development of a yeast screen for genetic investigations of the human lysosomal transporter, cystinosin
2. Isolation of gain-of-function and loss-of-function cystinosin mutants using a yeast screen
3. Genetic isolation of suppressor mutants to delineate functional domains
4. Biochemical characterization cystinosin mutants in yeast cells
5. Biochemical characterization cystinosin mutants in mammalian cell lines

Executive overview of Progress: Suitable for public disclosure

The human cystinosin protein is localized to an internal organelle in the cell, the lysosome. The localization of the protein to this organelle makes it difficult to assay functionally as it requires the tedious method of lysosome purification. To get around this difficulty researchers mistarget the protein to the plasma membrane of humans and investigate it functionally on whole cells. However, mammalian cells are not easy to work with and they are not easily manipulable from a genetics points of view. To get around this difficulty we have initiated studies where we have begun to investigate the protein function in yeast. In yeast also, human cystinosin is localized to an internal organelle, the vacuole, which is the yeast equivalent of the human lysosome. To be able to investigate this transporter effectively one needs to be able to express the protein on the yeast cell surface (plasma membrane) and then carry out functional studies. Using a mutagenic strategy we have been able to isolate mutants of the human cystinosin protein that are mistargeted to the cell surface. Evaluation of these mutants has revealed that the targeting of the human cystinosin protein is very similar in yeasts and mammals. This observation was further confirmed when we were able to show that the human variants that are mislocalized to the plasma membrane in humans are also mislocalized to the plasma membrane in yeasts. In addition to this we have also carried out the converse study where we have investigated

how mutants in yeast can mislocalize the protein to the plasma membrane. This has led to information into the genes involved in targeting of the human cystinosin in yeast. This information, will be exceedingly useful both from understanding how the protein trafficks in yeast and humans, and also in refining the yeast model for investigating the cystinosin protein. Finally using the yeast model we have been able to isolate several mutants of the cystinosin protein that show increased activity in yeast. The mutations were sequenced and we are currently in the process of analyzing the reasons for the increased activity of these mutants.