

Description

Long chain fatty acids(LCFAs) are required by cells for a number of functions including membrane synthesis, metabolism, intracellular signaling, and post-translational protein modification. Cells can get LCFAs through de novo fatty acid synthesis or through uptake from the environment. The mechanisms of uptake from the environment are not well understood and evidence has been presented that both passive diffusion and active transport may be involved. Some mammalian and bacterial proteins involved in active LCFA import have been identified but their functional significance and physiological roles have not been well studied.

This researcher proposes to use the genetically tractable yeast, *Saccharomyces cerevisiae*, to identify proteins involved in fatty acid import. 1. Wild-type yeast cells will be mutagenized with EMS and tested for decreased LCFA import using fluorescently labeled LCFA and a Fluorescence Activated Cell Sorter(FACS). This selection process may give unwanted mutants such as those that upregulate LCFA biosynthesis or upregulate LCFA metabolism such that they will contain less fluorescence. To select against these kinds of mutants, the cells will then be put through a second round of selection on LCFA containing media with an antibiotic to inhibit fatty acid synthesis. On this media, LCFA transport mutants will grow slowly or die while the others will be able to take up the LCFA from the media to survive. 2. These putative import mutants will be used to identify the specific genes involved in LCFA transport. Tetrad analysis will be used to select for single gene mutants which will then be used in noncomplementation tests to assess the number of genes isolated. A member of each complementation group will be used in a cDNA plasmid rescue scheme to identify the specific gene mutated. 3. These genes will then be used in multiple mutant tests to assess the role of active transport versus diffusional import.

Critique

This grant proposal is well organized and the experiments are thoroughly thought out. The investigator tackles the area of LCFA import in a way that should lead to new insights into the mechanisms of action and lay the foundation for future work in this field.

1. Mutagenesis and Selection- The double selection criteria used will greatly enrich the population of mutant cells for import mutants which will streamline the more labor intensive gene identification process. Use of the FACS at the first step is efficient and allows the initial screening of a large number of mutagenized cells.

2. Complementation and gene identification - The investigator exhibits a good knowledge of yeast genetics and uses it advantageously to sort through mutant cells isolated in the first step. In the process of complementation analysis, the investigator will be testing for recessive and dominant alleles as well which will aid in the final characterization of these genes. The detail given in this section shows that the researcher is very familiar with these techniques and can accomplish them.

3. Active transport vs. Diffusion - This portion of the grant proposal goes even further in using this mutational analysis to investigate an area of confusion in the literature. By using multiple mutants in the active import pathway, the true contribution of passive diffusion can be assessed.

Summary

This proposal is superior in quality, aim, and scope. The extensive background information provided and the detailed experimental protocol show that the investigator can accomplish the set goals. I rate it outstanding and give a priority score of 1.2.

Description

It is important to study the regulation of uptake of long-chain fatty acids (LCFA) because these molecules are important for membrane synthesis, intracellular signaling, transcriptional regulation, and post-translational modification of proteins. *S. cerevisiae* is a good model organism in which to study LCFA uptake because import of the fatty acids occurs only when yeast are grown under anaerobic conditions and when LCFAs are present in the media. The powerful genetics available with yeast, along with the fully sequenced genome, makes it practical to identify new genes involved in a pathway, such as that for fatty acid uptake.

This researcher proposes to determine the extent of involvement of LCFA import molecules in fatty acid uptake, while recognizing that diffusion is also likely part of the process. Mutagenized yeast will be selected for a decreased ability to import a fluorescent fatty acid analog (BODIPY) and for inability to grow in LCFA media with antibiotic inhibitor of LCFA biosynthesis. The mutated genes will then be cloned by complementation and functional assays will verify their role in LCFA uptake. Finally multiple LCFA transporter mutants will be created (with loss of several LCFA import genes) and the redundancy of these transport pathways will be assessed.

Critique

This is an excellent proposal which clearly and concisely describes the experiments important for elucidating fatty acid import in yeast. At every step the investigator considers several possible outcomes for each experiment and accounts for how each one will affect the subsequent experiments.

The two part mutagenesis screen is key in that it enriches the probability of isolating a gene that will be relevant to this researcher's study. Each step of the screen eliminates a different class of irrelevant mutants and, as such, will expediate the process of identifying an LCFA transporter. The methods and the yeast strains to be used are described in detail, reinforcing the idea that these experiments have been carefully thought out. One drawback is that we are not entirely convinced that an LCFA transporter will be isolated from this screen. One fatty acid transporter Fat1, whose disruption results in decreased BODIPY uptake, has already been isolated in yeast. Is it possible that different transporters are important for uptake of different types of LCFAs? If so, it might be possible that use of the BODIPY LCFA analog will not allow for identification of some important LCFA transporters which do not recognize BODIPY. Nonetheless, the screen is well designed and there is even an alternative plan to pursue if no death is seen after placing mutagenized yeast on cerulenin and LCFA supplemented media.

The tetrad analysis and complementation studies ensure that the researcher is studying one gene responsible for the phenotype and saves time in the cloning process. The investigator notes that mutants in the general cellular processes of protein post-translational modification or membrane localization will be deselected. The entire pool of plasma membrane proteins are to be labeled and examined for changes in localization/glycosylation. But this method will probably not pick up mutants which are important for processing select plasma membrane proteins nor will it deselect mutants in shuttles that may specifically move the LCFA transporters to the plasma membrane when they are needed.

The creation of a null mutant of an LCFA import gene along with multiple mutants where all LCFA transporters are knocked out will help determine how much the cells rely on LCFA import for viability and growth. This will also allow for placement of the transporters into different pathways.

Summary

This is a well-written, carefully planned proposal which will likely meet many of its goals within the expected time period. The strongest aspect of this grant is its thorough speculation of all possible results of experiments and the alternative proposals offered for each of these possible outcomes. This research will have implications for fatty acid uptake in yeast and in mammalian systems where cardiac myocytes, for example, rely heavily on LCFA import. I rate this proposal as outstanding (1.1 - 1.2).