Building an *in silico* metabolic model of *Cryptococcus neoformans* to study membrane protein production

Introduction

As technology in biological research continues to advance, large amounts of new data have become available in such fields as genomics and proteomics. In order to comprehend this flood of information, the data must be interconnected so the organism can be understood as a whole, not just many different parts (Edwards *et al*., 2002). To accomplish this seemingly difficult task, an *in silico* model of metabolism is required. This computer simulation is based on physicochemical laws and principles to recreate the complex interconnected pathways of a cell's metabolism (Kauffman *et al*., 2003).

A method known as flux balance analysis (FBA) is often used for *in silico* modeling. Based on the stoichometric coefficients of all the metabolic reactions, FBA uses mass balance constraints to predict growth performance (Edwards and Palsson, 2000). *In silico* metabolic models have already been constructed for both *Escherichia coli* (Reed and Palsson, 2003) and *Saccharomyces cerevisiae* (Duarte *et al*., 2004) Using these pre-existing models, as well as in depth literature searches, a model for the fungal pathogen *Cryptococcus neoformans* will be attempted.

C. neoformans is a yeast-like fungus that is pathogenic to immunocompromised individuals, especially those with AIDS (Bose *et al*., 2003). The yeast has several important virulence factors that aid in its pathogenicity such as the ability to grow at 37ºC, the ability to produce melanin on specific substrates, and a complex polysaccharide capsule (Casadevall *et al.*, 2000). Two families of genes, *CAP* and *CAS*, have already been identified as playing a role in capsule synthesis (Bose *et al*., 2003). Using *in silico* models and experimentation with live cells,

the deletion of *CAP* and *CAS* genes may lead to further knowledge of the metabolism associated with this particular process.

There is still much to discover about the metabolic pathways of *C. neoformans* and the creation of an *in silico* model may aid in the complete understanding of this organism. An aim of this study is to create a working *in silico* model for *C. neoformans* which will initially be done starting with existing models of *E. coli* and *S. cerevisiae* and building a new model through genome comparisons. The second aim of this study is to use these models to study membrane protein production. Initially, a target membrane protein in *E. coli* or *S. cerevisiae* will be selected based upon genome comparisons. By computationally making predictions of protein product, it will give a familiarity to the process of simulations as well as give designs that can be validated experimentally in those organisms if a working *C. neoformans* model is unattainable. If a working *C. neoformans* model can be achieved, computational designs could be applied to *C. neoformans.*

Methods

Flux balance analysis will be used in order to determine membrane protein products in either the *E. coli* or *S. cerevisiae* models. Using particular constraints, a computer simulation will be performed of possible growth outcomes of the organism. These mass balance constraints are mathematically represented by the matrix equation: $S \cdot v = 0$. The S is the $m \cdot n$ stoichometric matrix, where *m* is the number of metabolites and *n* is the number of reactions in the metabolic network. The v is the vector that represents all the fluxes in the network. We will be using the software developed in the Edwards and Palsson study of *E. coli* for our analysis of either *E. coli* or *S. cerevisiae* (Edwards and Palsson, 2003).

The gene databases MetaCyc (www.metacyc.org) and KEGG (www.genome.jp/kegg) will be used for comparisons of *C. neoformans* and the other two organisms. A BLAST search will also be performed for sequence alignments between the organisms to determine sequence homology.

Possible Results and Future Directions

Considering how little is actually known about *C. neoformans* and its metabolism, it is quite possible that an *in silico* model for *C. neoformans* will not reflect its true metabolism. The gaps in the metabolic pathways could lead to other tests to determine what reactions are actually present. If a working model is able to be created, however, much could be learned about this organism and clues to its virulence may eventually be discovered. The more that is known about its pathogenic qualities, the easier it may be to create specific drug targets.

The experimental testing on the *E. coli* or *S. cerevisiae* models may lead to a more complete metabolic network of the organisms depending on the protein product studied and the genes associated with that protein. To this point, this modeling approach has not been used to predict protein productions in any organism.

References

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