Creation of an *in silico* metabolic model for the fungal pathogen *Cryptococcus neoformans*

Samantha Gonyea

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 organisms such as *Escherichia coli* (Reed and Palsson, 2003) and *Saccharomyces cerevisiae* (Duarte *et al*., 2004) Using the *S. cerevisiae* model, 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 the pre-existing model of *S. cerevisiae* and building a new model through genome comparisons. The second aim of this study is to possibly connect the capsular pathways to the central metabolism. By computationally making predictions of capsule mechanisms, it will give a familiarity to the process of simulations as well as give designs that can be validated experimentally in *C. neoformans* in the future. The understanding of how the capsular pathways interact with the central metabolism may lead to further clues of virulence and may show new metabolic pathways for *C. neoformans* that are currently unknown.

Summer Progress

When beginning the model for *C. neoformans*, it was a bit troublesome to find that there was a lack of information since *C. neoformans* had not been well studied in terms of metabolism and there had been no previous systematic analysis performed. However, we were able to locate *C. neoformans* on the The Institute for Genomic Research (TIGR) database and this provided us with a partially annotated genome. This TIGR database showed the TC annotation numbers in relation to the metabolic pathways of *C. neoformans*. I used these relationships and organized them further into an Excel worksheet by enzyme classification (EC) number, subsystems, and the enzymes related to the pathway. Therefore, the known genes of *C. neoformans* could be directly related to the enzyme and pathway it affects.

Now that I had organized the already known *C. neoformans* data, I compared these pathways to a pre-existing metabolic model of the non-pathogenic yeast *Saccharomyces cerevisiae.* Since *S. cerevisiae* already had a working model, it made sense to use it as a starting point rather than starting a brand new model. The data was compared by having a large printout of the metabolic pathways of *S. cerevisiae* and highlighting the corresponding known pathways in *C. neoformans*.

After the known pathways for *C. neoformans* was compared to the pre-existing model, it was found that the central metabolism for the pathogen was fairly complete with few gaps in between. However, looking at the other metabolism groups such as nucleotide and amino acid metabolism, there were many gaps making the model for *C. neoformans* incredibly incomplete. It was surprising to see the complete central metabolism due to the lack of metabolic data on the TIGR website for *C. neoformans.* Also, due to the fact that there are so many differences between *C. neoformans* and *S. cerevisiae,* we were expecting to see a few genes from the pathogenic *C. neoformans* that were not present in *S. cerevisiae*. However, this was not the case with the genomic data from TIGR.

Future Directions for Academic Year

Due to the numerous gaps in pathways for the metabolism of *C. neoformans,* the most logical next step is to attempt to fill in those gaps. The way I plan to do this is using the BLAST program for comparison of known *S. cerevisiae* genes to the entire genome of *C. neoformans.* Therefore, if strong nucleotide sequence matches are found within the *C. neoformans* genome, it is highly likely that *C. neoformans* possesses the same gene as S. cerevisiae. Also, by attempting to grow *C. neoformans* on various substrates such as glycerol, maltose, etc., we will be able to

determine if *C. neoformans* possesses the enzymes and pathways to break down these particular substances, leading to more clues about its metabolism.

If enough of the metabolic pathway gaps from *C. neoformans* can be filled within the academic year, a very ambitious goal would be to begin constructing a metabolic model over the school year and eventually running gene deletion simulations.

References

Bose, I., A.J. Reese, J.J. Ory, G. Janbon, & T.L. Doering. 2003. A yeast under cover: the capsule of *Cryptococcus neoformans*. *Eukaryotic Cell* 2: 655-663.

Casadevall, A., A.L. Rosas, & J.D. Nosanchuk. Melanin and virulence in *Cryptococcus neoformans*. *Current Opinion in Microbiology* 3: 354-358.

Duarte, N.C., M.J. Herrgard, & B.O. Palsson. 2004. Reconstruction and validation of *Saccharomyces cerevisiae* iND750, a fully compartmentalized genome-scale metabolic model. *Genome Research* 14: 1298-1309.

Edwards, J.S. & B.O. Palsson. 2000. The *Escherichia coli* MG1655 *in silico* metabolic genotype: Its definition, characteristics, and capabilities. *PNAS* 97: 5528-5533.

Edwards, J. S., M. Covert, & B. Palsson. 2002. Metabolic modeling of microbes: the flux-balance approach. *Environmental Microbiology* 4(3): 133-140.

Kauffman, K.J., P. Prakash, & J.S. Edwards. 2003. Advances in flux balance analysis. *Current Opinion in Biotechnology* 14: 491-496.

Reed, J.L. & B.O. Palsson. 2003. Thirteen years of building constraint-based *in silico* models of *Escherichia coli*. *Journal of Bacteriology* 185: 2692-2699.