Evidence-based Annotation Revision to Genes Involved in the Virulence Factor Melanin Production in Fungal Pathogen, *Cryptococcus neoformans*

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Introduction

C. neoformans is a common opportunistic fungal pathogen that infects immunosuppressed individuals.

- Infection most commonly occurs as meningitis and infects the brain by crossing the blood-brain barrier (14, 17).
- Approximately 1 million cases of cryptococcal meningitis are reported annually (6, 10, 17). Of the annual 220,000 cases in people with HIV/AIDS globally, 181,000 result in death (9).
- Contraction through inhalation of spores from the environment can lead to no infection due to immune response, fungal latency, primary pulmonary infection, and dissemination (10, 12).
- *C. neoformans* has evolved many virulence factors including melanin production.
- Melanin protects the yeast cell from phagocytosis (10, 13, 16) and confers resistance to antifungal drugs (7, 10).

Purpose

The goal of this project is to use experimental RNA sequencing data and an improved gene prediction algorithm, Program to Assemble Spliced Alignment (PASA) (4), within the genome annotation platform GenSAS (4) to improve gene and transcript accuracy in a community-based curation space (Apollo) for genes implicated in melanin production in fungal pathogen, *C. neoformans* (4).

Methods

- Nine melanin coding genes in *C. neoformans* were identified from the literature (Table) (2, 5, 8, 15).
  - Transcriptome data from multiple strains (clinical, estrogen, scytovirin) have been loaded into GenSAS (4) and used to generate PASA model.
- The coordinates and gene structures found (Table) were compared to the 3 sets of transcriptomic data within GenSAS (4).
- Using transcript data, version 1.0 gene model, and the new PASA Refinement gene model (version 2.0), the gene structure was analyzed.
- Areas of gene structure with possible inaccuracies: exon structure, gene structure, alternative splicing were identified based on data and noted.
- After using GenSAS and RNAseq data, new revised gene transcript models were curated in the Apollo user space (4).

Results

- Transcriptome data showed inaccuracies in all nine melanin genes (Table). Most of the genes required multiple transcripts to be curated based on data.
- For each gene, the transcripts were combined into one super-transcript model. The super-transcripts represent the possible combinations of translation occurring for the gene.

Table: *C. neoformans* Genes Associated with Melanin-Production. The gene names, CNAG ID and chromosomal coordinates were found using EnsemblFungi (3) and FungiDB (1).

Gene Name	CNAG ID	Coordinates
LAC1	02087	6: 1,232,467 - 1,234,881
LAC2	02086	6: 1,235,018 - 1,237,477
PKA	04162	9:166,848 - 169,568
AGC/PKA	00396	1: 1,039,957 - 1,042,941
RAD53	05216	4: 755,013 - 758,097
CHK1	03167	8: 230,927 - 233,605
VPH1	02702	3: 964,483 – 968,105
CLC1	07647	6: 1,051,970 – 1,056,522
VAD1	01537	11: 225,540 - 228,664

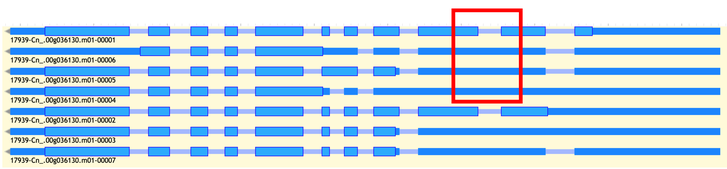

Figure 1. Transcript Reannotations of *LAC1* Gene. The top model shows the original PASA algorithm (4) generated model for the *LAC1* gene. All the models underneath are reannotations based on the transcriptome data. The red box indicates one region the PASA algorithm (4) proposes is intron 2 and the data suggested could be retained as shown in the following three models.

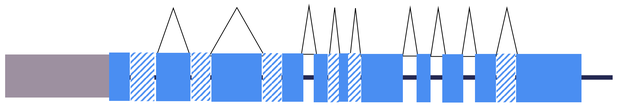

Figure 2. Super-Transcript Model of *LAC1* Gene. The model above shows the super-transcript for the *LAC1* gene. The grey rectangle is the untranslated exon sequence before the start codon. The blue rectangles are the exon regions in the gene that are translated. The blue striped regions represent that in some RNAs are introns and other RNAs are exon (retained intron).

Conclusions

- The transcriptome data supported the nine genes required reannotation to their transcript models due to inaccuracies like intron retention and spliced regions.
- Super-transcript models of possible translational paths were curated for each gene to improve gene model accuracy in GenSAS (4). Once all the models are curated, a new annotated genome will be published to the community.

Future Directions

Further research is needed:

- New curated models will be translated and compared to the original version to analyze potential protein changes using Clustal Omega (11).
- Hypothetical protein domains and isoforms will be analyzed for changes in function.

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