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Short Communication

A few shared up-regulated genes may influence conidia to yeast transformation in dimorphic fungal pathogens

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Abstract

The small number of fungi that commonly cause disease in normal people share the capacity to grow as mycelia in the soil at 25°C and as yeast (or spherules) in mammals at 37°C. This remarkable conversion has long been a topic of interest in medical mycology. The conidia to yeast conversion has been studied by transcription profiling in several fungal species, including *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Coccidioides spp.*, *Blastomyces dermatitidis,* and *Talaromyces marneffei*. One limitation of transcriptional profiling is determining which genes are involved in the process of conversion to yeast as opposed to a result of conversion to yeast. If there are genes that are up-regulated in the yeast phase of more than one dimorphic, pathogenic fungus they might be required for conversion to yeast (or spherules). To address this issue, 24 up-regulated genes common to *Coccidioides spp*. spherules and *H. capsulatum* yeasts were identified. Four homologs of these genes were also found in *P. brasiliensis, B. dermatitidis* or *T. marneffei* genes that were up-regulated in yeast. 4-hydroxyphenylpurvate dioxygenase, a gene involved in tyrosine metabolism and melanin synthesis that has been shown to be required for yeast conversion, is conserved and up-regulated in yeast in all five species. Another up-regulated gene that is conserved in all five species is a MFS sugar porter. These results suggest that a minority of up-regulated yeast (or spherule) genes are conserved across species and raises the possibility that conserved up-regulated genes may be of special interest for differentiation of mycelium into yeast.

Key words: pathogenic fungus, fungal dimorphism, gene expression, transcription profile.

Introduction

There are 69,000 identified species of fungi (probably more than one million total) and most live on dead or dying organic matter.[1] A small number of these fungal species cause disease in human beings. Most pathogenic fungi are opportunistic pathogens that only cause disease in immunocompromised people. A very small number of fungi are able to frequently cause invasive disease in immunocompetent human beings.[2] A common feature of these organisms is the ability to transform to the yeast or spherule phase

at 37°C.[3,4] *H. capsulatum* yeast are found inside phagocytic cells but *Coccidioides spp.* spherules, *Paracoccidioides spp.*, and *B. dermatitidis* yeasts are facultative intracellular pathogens.[3] These dimorphic pathogens are a relatively diverse group of species in different orders: *Coccidioides spp.*, *Paracoccidioides spp.*, *Blastomyces spp.*, and *H. capsulatum* (order Onygenales), *T. (Penicillium) marneffei* (order Eurotiales) and *Sporothrix spp.* (order Ophiostomales). More than one species has been identified for *Coccidioides spp.*,[5] *Paracoccidioides spp.*,[6] *Sporothrix spp.*,[7] and *Blastomyces spp.*[8] The two *Coccidioides* species (immitis and posadasii) diverged about 1.5 million years ago but they can only be distinguished using molecular techniques and seem to cause the same clinical syndromes.[5] These fungi differ in geographic distribution and clinical syndrome. Most of the infections caused by dimorphic fungi are subclinical, but symptomatic infection commonly occurs and some of these infections are fatal.[2]

There are now a number of studies of gene transcription in yeast or spherules compared to mycelia or conidia in *H. capsulatum*,[9–12] *P. brasiliensis*,[14–17] *Coccidioides spp.*[18,19] *T. marneffei*,[20] and *B. dermatitidis*.[8] One difficulty with transcriptional profile data containing many up-regulated genes is distinguishing which genes are the most likely to be the mechanistically important for mycelial or conidial conversion to yeast or spherule. The goal of this work is to merge transcriptional data from conidia to yeast conversion studies done in several species and find the intersecting set of genes that are up-regulated in yeast (or spherules). The hypothesis is that the genes that are up-regulated in several species are more likely to be important for mycelium or conidial to yeast conversion.

Methods

To identify the intersection of up-regulated yeast (or spherule) genes the transcriptional profiling experiment done by Whiston was the starting point.[18] They studied *C. immitis* and *C. posadasii* mycelia compared to spherules after four days in culture in Converse media at 39°C. RNA-seq was used to determine the transcriptional profile. 1340 genes (about 13% of the genome) were up-regulated in spherules of both species. These data were merged with the spherule transcriptional profile obtained by Viriyakosol.[19] This study was done with an open reading frame microarray, and *C. immitis* mycelia expression was compared to spherules after two days in culture in Converse media. Four biological replicates of each condition were studied to maximize reproducibility. In sum, 795 genes were up-regulated in day two spherules, and 326 genes were common to both studies.

Five data sets from mycelium (or conidia) conversion to yeast in *H. capsulatum* were studied.[9–13] *H. capsulatum* orthologs of the up-regulated *C. immitis* genes were identified using InParanoid;[21] about half of the *C. immitis* genes had *H. capsulatum* orthologs so a maximum of 180 up-regulated genes could have been shared by the two species. In fact, homologs of only 24 of these genes were found in at least one *H. capsulatum* transcriptional study. This analysis was done by Mark Voorhies and Anita Sil (UC San Francisco). To look for genes that were up-regulated in *P. brasiliensis* yeast, data from the GEO database[22] (GPL2780) was analyzed by GEO2R and the top 50 up-regulated ESTs identified by BLASTN against all fungi. E values of $<10^{-20}$ were considered significant. In addition, published *P. brasiliensis* data was compared to the data in Table 1.[14–17] *B. dermatitidis* genes up-regulated in yeast were identified in a published study and compared to the data in Table 1.[8] *T. marneffei* genes were identified in a published study and compared to the data in Table 1.[20] The conservation of the 24 shared up-regulated genes (Table 1) within dimorphic, pathogenic fungi was determined by BLASTP.[23] E values of $<10^{-50}$ were considered significant. Shared up-regulated genes without meaningful annotation were searched against the Uniprot database and the NCBI database to find predicted conserved domains or Gene Ontology annotation. All the conserved hypothetical genes were evaluated using I-TASSER to identify functional domains.[24]

Results and discussion

The list of shared genes is in Table 1. In sum, 20 of 24 genes up-regulated in *H. capsulatum* yeast and *Coccidioides spp.* spherules were conserved in all of the dimorphic, pathogenic species tested. The four genes that were not conserved lacked a homolog in *Sporothrix spp.*, which are in the order Ophiostomales, distantly related to the order Onygenales. The two genes found in all five of the *H. capsulatum* studies and the two *Coccidioides* studies are conserved hypothetical proteins. CIMG_09539 is highly conserved in many fungi including all of the dimorphic fungi. This gene is predicted to contain a 2OG-Fe Oxy 2 domain (pfam 10014) that is found in 2-oxoglutarate, iron dependent dioxygenases.[24] One of these enzymes has been found to produce hydroxyisoleucine.[24] This unusual amino acid has effects on glucose metabolism in animals but its metabolic role in fungi is unknown. This gene seems to be a promising candidate for further study of conidium to yeast conversion.

Several genes were identified that have been found in previous studies: 4-hydroxyphenylpyruvate dioxygenase (4-HPPD or hdpA) is up regulated in *Coccidioides spp.* Spherules,[18,19] *H. capsulatum*,[13] *P. brasiliensis*,[14]

Table 1. Up-regulated genes in yeast spherule forms.

Locus ID	H. capsulatum[a]	P. brasiliensis	B. dermatitidis	T. marneffei	Annotation	Comment	Conserved[b]
CIMG_09539	5				Conserved hypothetical	GO:0006541, glutamine metabolic process	Yes
CIMG_09404	5				Conserved hypothetical		Yes
CIMG_04373	4				Glycerol dehydrogenase	Aldo-keto reductase	Yes
CIMG_06683	4				DUF1479 domain-containing protein		Yes
CIMG_01466	3	Yes	Yes	Yes	4-hydroxyphenylpyruvate dioxygenase		Yes
CIMG_08310	3				MFS monosaccharide transporter		Yes
CIMG_03142	3				alpha-(1,4)-amylase		No
CIMG_13019	3				Hypothetical protein	beta-beta-alpha zinc finger	No
CIMG_02373	2				CMGC/SRPK protein kinase		Yes
CIMG_00801	2				GDSL Lipase/Acylhydrolase		Yes
CIMG_03886	2				Conserved hypothetical	pfam05241:EBP, sterol binding membrane protein	Yes
CIMG_05899	2				Sulfite transporter Ssu1		Yes
CIMG_00945	2				Cyclin		Yes
CIMG_06445	2				trk potassium uptake		Yes
CIMG_09750	2				Polyketide synthase	Nonribsomal peptide synthase 1, J3K327 Uniprot	Yes
CIMG_02137	2				Ubiquitin hydrolase		Yes
CIMG_04558	1				Proline oxidase		Yes
CIMG_02628	1	Yes			Arp2/3 complex subunit Arc16		Yes
CIMG_07255	1				Ubiquitin domain		No
CIMG_03001	1	Yes		Yes	Sugar porter MFS		Yes
CIMG_04588	1				Aldo-keto reductase		Yes
CIMG_08724	1				Acetyl CoA hydrolase		Yes
CIMG_08168	1				Reversal of Tor2 lethality 1 (ROT-1)	chaperone for folding within ER	No
CIMG_04137	1				Tyrosine phosphatase		Yes

a) Five *H. capsulatum* datasets were searched for matched to the up-regulated genes common to spherule experiments. The values in this column indicate the number of data sets with positive results.
b) Genes conserved in all primary pathogenic, dimorphic fungi.

B. dermatitidis,[8] and *T. marneffei*[20] yeast. Studies in *P. brasiliensis* were the first to demonstrate the up-regulation of this gene in yeast.[14] In addition, nitisinone (also known as NTBC), a pharmacologic inhibitor of 4-HPPD, blocked conversion of *P. brasiliensis* conidia to yeast, suggesting that 4-HPPD activity is required for this conversion.[14] Nitisinone inhibits conidium to yeast conversion in *T. marneffei* and deletion of the hpdA gene results in an organism that can't form yeast.[26] Tyrosine metabolism is important for the production of 1,8-dihydroxynaphthalene (DHN) melanin, which is known to be a virulence factor in a variety of fungi.[26,27]

CIMG_03001, a MFS sugar transporter, is also up regulated in all four species. A second glucose transporter (CIMG_08310) is up-regulated in *H. capsulatum* yeast and *Coccidioides spp.* spherules. The process of transforming to yeast is probably energy intensive, requiring increased glucose uptake. Arp2/3 is one of a number of actin binding protein, and it is important for cell shape, mobility, and endocytosis.[28] The mycelial (or conidia) to yeast (or spherule) transformation involves dramatic cell shape changes, so up-regulation of this protein may play a role in the change in cell shape.

Amylase is known to be up regulated in transcriptional profiling studies and required for *H. capsulatum* mycelium to yeast conversion in gene deletion experiments.[29] This enzyme is required for synthesis of alpha-(1,3)-glucan. In many *H. capsulatum* isolates, alpha (1,3) glucan is required for pathogenicity.[30] Glycerol reductase and aldo-keto reductase are two reductase enzymes that are up regulated in *Coccidioides spp.* spherules and *H. capsulatum* yeast. There are a total of 13 aldo-keto reductases in the *C. immitis* genome and six of them are up-regulated in *Coccioides. spp.* spherules, so this gene family is clearly highly expressed in spherules.[18,19] Spherules are grown in 10–14% CO_2 compared to mycelia grown in air, so the spherule environment is relatively reduced. Since the major physiological role of NADP-dependent alcohol dehydrogenase is to dispose of excess reducing equivalents, up-regulation of these enzymes in spherules and yeast seems reasonable.

CMGC/SRPK protein kinase was up-regulated in *Coccidioides spp.* spherules and *H. capsulatum* yeast. This up-regulated protein kinase is especially remarkable because 23 other protein kinases are down-regulated in day 2 spherules.[19] The CMGC/SRPK protein kinases have been identified in *Trichophyton spp.*; their function is not understood at present.[31] One Type I polyketide synthase (PKS) was up regulated in *Coccidioides spp.* spherules and *H. capsulatum* yeast. *Coccidioides spp.* have at least nine PKS genes and six of these are closely related to the up-regulated gene.[32] Type I PKS genes are large, highly modular genes that produce complex organic molecules.[33] These

secondary metabolites include anaphlotoxins, antibiotics and pigments such DHN-melanin, which is an important virulence factor in many fungi.[27] In *T. marneffei*, deletion of a single PKS gene, alb1, reduces DHN-melanin production and virulence,[32] which is further evidence that DHN-melanin production is important for virulence. Another conserved protein is CIMG_00945 which is predicted to have a cyclin domain (pfam 08613). This domain is found in many different proteins including G1/S-specific cyclins and phosphate system cyclins. The Ssu1 sulfite efflux pump makes yeast resistant to sulphite. *Candida albicans* Ssu1 deletion mutants are unable to switch to the filamentous form.[34] A gene coding for sulfite oxidase, another enzyme that protects against sulfite toxicity, is also up-regulated in *C. immitis* spherules.[19]

In summary, this study demonstrates the importance of DHN-melanin synthesis, since both 4-HPPD and a Type 1 polyketide synthase genes are unregulated in yeast (or spherules). Other functions that this study suggests may play a role in the transformation to yeast include cell wall biosynthesis, transporters and oxidation/reduction.

The results of this kind of analysis depends heavily on which fungal species are used as the starting point. If other dimorphic fungi were used as the starting point for this analysis, the shared genes would be different. I chose *Coccidioides spp.* and *H. capsulatum* because the data from multiple studies was easily available at the time this study was begun. Of the 24 genes found to be up-regulated in *Coccidioides spp.* spherules and *H. capsulatum* yeast, only four also up-regulated in *P. brasiliensis*, *B. dermatitidis* or *T. marneffei* yeast. This suggests that the transcription profile of mycelium to yeast conversion is very different among the thermal dimorphic fungi. Sil has previously suggested that the genomic pathways of conidia to yeast (or spherule) conversion diverge.[3] Understanding the process of transformation into yeast is much more difficult than documenting the transcriptional profile of yeast and mycelia. The best way to prove that a gene is required for yeast formation is to delete it, or decrease the level of expression by a knock down technique. In addition, some genes that are required for yeast transformation are not up-regulated in the yeast phase. Drk1, for example, is a histidine kinase that is required for the transformation of *B. dermatitidis* conidia into yeast, is not overexpressed in the yeast phase.[35] This may be true for a number of genes that are required for transformation of conidia into yeast.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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