Genetic differences between *Coccidioides* spp. and closely related nonpathogenic Onygenales

Theo N. Kirkland
University of California San Diego School of Medicine
Division of Infectious Disease
Departments of Pathology and Medicine
tkirkland@ucsd.edu

Abstract

Coccidioides spp. are dimorphic, pathogenic fungi that can cause severe human and animal disease. Like the other primary fungal pathogens, animal infection results in a morphologic transformation from the environmental mycelial phase to a tissue phase, known as a spherule. The sequencing and annotation of *Coccidioides* spp. and the genomes of several nonpathogenic Onygenales species allows orthology comparisons that may provide clues about the *Coccidioides* spp. genes involved in pathogenesis. There were a number of differences in the characterization of the genes that are *Coccidioides* spp.-specific and those that are found in both *Coccidioides* spp. and non-pathogenic Onygenales. Many more *Coccidioides* spp.-specific genes are up-regulated expression in spherules. *Coccidioides* spp.-specific genes more often lacked functional annotation, were more often classified as orphan genes and had SNPs with higher non-synonymous/ synonymous ratios. Review of individual genes in the *Coccidioides* spp.-specific group identified two genes in the Velvet family, a histidine kinase, two thioredoxin genes, a calmodulin gene and ureidoglycolate hydrolase. Velvet genes have been found to be important for mycelium differentiation to yeast in *Histoplasma capsulatum*. Hopefully, identification of these genes will be useful for pursuing potential *Coccidioides* spp. virulence genes in the future.

Keywords: Fungi, pathogenic fungi, *Coccidioides immitis*, *Coccidioides posadasii*, Onygenales, genomics, pathogenesis.

Introduction

Coccidioides immitis and *Coccidioides posadasii* are dimorphic fungi that are found in the soil in the desert regions of the American southwest, Mexico and South America [1-3]. Both species can cause disease known as Valley Fever in normal people. The two species are closely related, morphologically indistinguishable and the major phenotypic difference between the two that is currently recognized is their geographic distribution; *C. immitis* is found primarily in California (including Baja California) and *C. posadasii in* Arizona and other endemic areas [4,5]. The organism grows as mycelia in the soil and forms arthroconidia within the mycelium that are dispersed as the soil is disturbed by wind, construction or other events. If the arthroconidia are inhaled by susceptible hosts they undergo transformation into a spherical tissue form, known as a spherule. These structures divide internally to form as many as 100 endospores, which in turn can differentiate into spherules. Endospores can be released 96 hours after initial spherule formation, so the number of spherules can increase quickly. Infection with *Coccidioides* spp. causes symptomatic disease in 40-50% of immunocompetent hosts and in a substantial number of infections can be prolonged and/or severe. In some instances, dissemination of the infection beyond the lung occurs. Some infections require life-long treatment and severe infections can be fatal.

Coccidioides spp. are a member of the Ascomycete Onygenales order which contains a number of other human pathogenic fungi, including *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Paracoccidioides* spp., and the dermatophytes such as *Microsporum* spp. and *Trichopyton* spp.[6]. *Coccidioides* spp. are in the Coccidioides family, in contrast to *Histoplasma capsulatum*, *Blastomyces* spp., and *Paracoccidioides* spp. which are all Ajellomycetacae [7]. In addition to these pathogens, many fungal species that are not human pathogens are also Onygenales [6,8]. One of these, *Uncinocarpus reesii*, forms arthroconidia within mycelia that are morphologically

indistinguishable from *Coccidioides* spp. [9]. *U. reesii* is found in the soil and has a worldwide distribution. It is keratinophilic, thermotolerant and utilizes amino acids and peptides as well as carbohydrates for growth [10,11]. *U. reesii*. *U. reesii* does not cause human disease but a close relative, *Chrysosporium zonatum*, has been reported to cause an infection in a single patient with chronic granulomatous disease [12]. Despite the difference in biology, *U. reesii* is closely genetically related to *Coccidioides* spp. [8,9,13].

The genome sequencing of multiple isolates of *Coccidioides* spp. and one isolate of *U. reesii* has made it possible to identify genes that are present in *Coccidioides* spp. but not this close nonpathogenic relative. At least four other nonpathogenic species in the Onygenales are also closely related to *Coccidioides* spp. [6]. The genomes of these organisms, *Amauroascus mutatus*, *Amauroascus niger*, *Byssoonygena creatinophilia*, *Chrysosporium queenslandicum* and *Onygena corvina* have also been sequenced and the size of gene families and the gain or loss of genes compared to *Coccidioides* spp. and *U. reesii* and other fungi [6]. Whiston and Taylor found about 800 *Coccidioides* spp. genes that were not present in the nonpathogenic Onygenales. One distinctive feature of the *Coccidioides* spp.-specific genes was the increased frequency of preferential expression in spherules [6].

Comparison of the genes conserved within *Coccidioides* spp. but not found in closely related nonpathogenic Onygenales to those shared between *Coccidioides* spp. and nonpathogenic Onygenales might provide insights into genes that are required for pathogenesis of coccidioidomycosis. The purpose of the study is to identify these genes and analyze their characteristics.

Methods

Orthology

Initial orthololoogy searches were done using the orthology tool at FungiDB (http://fungidb.org/fungidb/showApplication.do). This tool compare two sets of proteins by BLASTP and computes the percent match length. The thresholds for blast results were an e

value < 10^{-5} and a percent match length of ≥ 50%. Paralogs, orthologs and ortholog groups were found using OrthoMCL Pairs. Orthologs shared by *C. immitis* RS, H538.4 and *C. posadasii* Silvera and RMSCC 3488 were identified. Sequences for all those strains are available at FungiDB. The sequence of *C. immitis* WA_211 is available at NCBI (https://www.ncbi.nlm.nih.gov/nuccore/RHJW00000000). Orthologs of *Coccidioides* spp. genes to *C. immitis* WA_211were identified using the online tool OrthoVenn2 (https://orthovenn2.bioinfotoolkits.net/home). The 7627 genes conserved within these *C. immitis* and *C. posadasii* isolates were then compared to *U. reesii* using the FungiDB orthology tool and reciprocal BLAST with a cut off e value of 10^{-6}. The reciprocal blast tool was Reciprocal-BB, which is available from https://github.com/marcusgj13/Reciprocal-BB. Comparison to the predicted proteins of non-pathogenic Onygenales, *A. mutatus*, *A. niger*, *B. creatinophilia*, *C. queenslandicum* and *O. corvina* was also done with Reciprocal-BB, with a cut off e value of 10^{-6}. The gene predictions and annotations for these species were provided by Jason Stajich, using DNA sequence data from Whiston and Taylor [14]. The *C. immitis* R.S. gene I.D. notation is used for all gene designations.

Blast

BLASTP searching was done using Stand-alone BLAST from NCBI, using the default settings. Blast hits were identified using an upper limit e value of 10^{-6}. Searches against fungal proteins were done using a representative sample (3 million predicted proteins) of the fungal protein database downloaded from NCBI. *Coccidioides* spp. genes without BLASTP matches in this search were defined as orphan genes.

Single Nucleotide Polymorphisms

The coding region SNPs of *C. posadasii* Silvera genes compared to a sample of 60 isolates were identified by Engelthaler et. al [15]. The SNP data for C. posadasii Silvera was downloaded from FungiDB. Because *C. immitis* RS SNPs were not identified in that study, *C. immitis* RS homologs of *C. posadasii* Silvera genes were assumed to have the same characteristics.

Gene characterization

Other characteristics, such as the genetic location, ortholog group, number of orthologs, predicted signal peptides, predicted transmembrane domains, PFAM and other functional domains. The functional domains included data from UniProt (www.uniprot.org), Superfamily 1.75 (http://supfam.org/), TIGR(https://www.jcvi.org/institute-genomic-research-tigr-j-craig-venter-institute-j-craig-venter-science-foundation) and NCBI (https://www.ncbi.nlm.nih.gov/cdd). The relative levels of expression in spherules and mycelia were downloaded from FungiDB. Up-regulation was defined by the criteria described by Whiston et. al., comparing mycelia to day 4 spherules [14]. The Chi-squared test was used to determine the statistical significance of differences between ratios; the Kruskal-Wallis test was used to compare determine the statistical significance of differences between continuous variables. All data analysis was done in R.

Supplementary data

Supplemental Table 1 is an attached Excel table containing all the data used for these analyses.

Results

7627 *Coccidioides* spp. genes are shared by *C. immitis* RS and H538.4, WA_211 and *C. posadasii* Silvera and RMSCC 3488. Since two strains of *C. immitis* from southern California and a *C. immitis* strain from Washington [16], as well two strains of *C. posadasii* were used to select this group, most of the core genome of *Coccidioides spp*. genome should be included. However, many more strains have been sequence but not annotated, so this may be an over-estimate of the total number of conserved genes. The total number of genes in these isolates varies between 9,905 and 117,815 (mean = 9,768) so, on average, about 20% of the genes appear to be species or strain specific. They will not be considered further here.

632 conserved *Coccidioides spp*. genes are not found in *U. reesii* or *A. mutatus*, *A. niger*, *B. creatinophilia*, *C. queenslandicum* and *O. corvina*. This value was similar to the estimate previously obtained by Whiston and Taylor [14]. For brevity, these genes are referred to as *Coccidioides* spp.-specific genes and those that are common to the *Coccidioides* spp. and nonpathogenic Onygenales are referred to as common genes. The term *Coccidioides* spp.-specific does not imply that these genes do not have homologs in other fungi.

Table I

Comparison of *Coccidioides* spp.- specific and common genes

	Number (%)				
Characteristics	*Coccidioides* spp.- specific		Common		P
Total	632	8[a]	6955	92[a]	$<10^{-10}$ [b]
Mean orthologous groups/gene	47		107		$<10^{-10}$
Up-regulated in spherules	184	29	911	13	$<10^{-10}$
Expression unchanged	363	57	4985	72	$<10^{-10}$
Down-regulated in spherules	85	13	1059	15	N.S.
Orphan genes	190	30[b]	0	0	$<10^{-10}$
Functional product description [c]	211	34	4624	66	$<10^{-10}$
Functional domains	331	52	5750	83	$<10^{-10}$
GO description	184	29	3628	52	$<10^{-10}$
Mean NS/S[d]	15.2		2		$<10^{-10}$
One or more transmembrane region	104	16	1313	19	N.S.
Signal peptide	78	12	930	13	N.S.

Table 1. Comparison of *Coccidioides* spp.- specific to common genes. a: Compared to total number of *Coccidioides* spp. genes conserved within the *C. immitis* and *C. posadasii* strains tested; b: Compared to total number of *Coccidioides* spp.- specific genes; c: Based on annotation of predicted proteins; d: Nonsynonymous/synonymous single nucleotide polymorphisms.

Table 1 shows a comparison of the two sets of genes. Ninety-two percent of all the *Coccidioides* spp. genes are common to nonpathogenic Onygenales and 8 % are *Coccidioides* spp.-specific genes. The predicted properties of proteins in the two groups are similar except for homology to other fungal proteins, the preferential expression in spherules, the amount of functional annotation and the degree of genetic variation. The mean number of orthologs per gene, the percentage of genes with blast matches, PFAM or other functional domains or functional product descriptions (not annotated as "hypothetical" or "predicted protein") are all higher in the common genes than the *Coccidioides* spp.-specific genes. As would be expected, common genes are homologous to genes in a wider diversity of fungi. The common genes had a higher proportion of genes with GO terms. The common group had a modest enrichment of GO:0016787, "hydrolase activity" and GO:0016817, "hydrolase activity, acting on acidic anhydrides" (Odds ratio 2.03, $p = 1.7 \times 10^{-4}$ and OR 3.19, $p = 2.35 \times 10^{-3}$ respectively) and the specific genes had no GO enrichment. Although many genes in both groups had a functional domain, very few terms were present in significant numbers. A larger proportion of the *Coccidioide*s spp.- specific genes are expressed at a higher level in spherules than mycelia, indicating that this group of genes is enriched for genes associated with differentiation to spherules. 30% of the *Coccidioides* spp.– specific genes have no BLASTP matches to other fungi with an e value less that 10^{-6}, which is our definition of orphan genes. 45% of the orphan genes are up-regulated in spherules. The origin of orphan genes is not clear. Although the function of these genes is difficult to determine, their conservation within *Coccidioides* spp. and up-regulation in spherules suggests that they may be important in differentiation into spherules. *Coccidioides* spp.-specific genes have a higher non-synonymous/synonymous SNP ratio than common genes suggesting that the *Coccidioides* spp.-specific genes are evolving rapidly. The *Coccidioides* spp.-specific genes did not cluster on the genetic map.

A previous study has identified 26 genes that are up-regulated in *Coccidioides* spp. and *Histoplasma capsulatum* as the spherule or yeast differentiation occurs [17]. Four of these are also found in the up-regulated *Coccidioides* spp.-unique group. One of the most interesting of these is CIMG_02628, an ARP2/3 complex subunit. This complex is composed of seven subunits that control nucleation of actin, which is important for cell wall remodeling and endocytosis [18]. The transformation of mycelia to spherules requires extensive cellular remodeling, so the up-regulation of this gene seems reasonable. Two GMC/SPRK kinases are up-regulated *Coccidioides* spp.-specific genes; one of these, CIMG_02373, is also up-regulated in *H. capsulatum* yeast.

Analysis of individual *Coccidioides* spp.-specific genes

Because the number of *Coccidioides* spp.-specific genes was relatively small, they could be examined individually. A small number of interesting genes are shown in Table 2

Table 2

Coccidioides spp.-specific genes of interest

Family	Product Description	Gene ID	Differentially regulated[a]	Previously Described[b]
Velvet	Ryp 2/Velvet	CIMG_01530	Y	Y
	Vel C	CIMG_02440	N	N
Histidine kinase	NIK1	CIMG_09624	N	N
Thioredoxin	TrxA	CIMG_09126	N	N
	Thioredoxin	CIMG_09274	N	N
Calmodulin	Calmodulin	CIMG_04786	N	N

Ureidoglycolate hydrolase	UGH	CIMG_02178	Y	Y

a) Up-regulated in spherules; b) previously described in references [6,7].

The velvet family of genes are transcription factors than respond to external stimulate, such as light or stress, and trigger adaptive responses [19]. Some of the responses include cell wall synthesis, trehalose synthesis, spore formation, secondary metabolism and a number of other functions in many fungi [20]. Webster and Sil have reported that two members of the Velvet family, Ryp2 (also known as VosA) and Ryp3, are required for yeast formation at 37° in *H. capsulatum* and the pathogenesis of invasive histoplasmosis [21,22]. The *C. immitis* gene that Sil annotated as Ryp2 (CIMG_01530) does not have a homolog in nonpathogenic Onygenales. In contrast, Ryp3 is common to *Coccidioides* spp. and nonpathogenic Onygenales. Ryp2 and Ryp3 are up-regulated in *H. capsulatum* yeast compared to mycelia and expression of CIMG_01530 is also up-regulated in spherules. Munoz has also reported that Ryp2 is present in *C. immitis* and *C. posadasii* but not in *U. reesii* [7].

Another velvet family gene that is conserved in *C. immitis* and *C. posadasii* but not found in nonpathogenic Onygenales is VelC. The *Coccidioides* spp. VelC orthologs are truncated at the N-terminal region of the protein, but highly conserved with *Aspergillus fumigatus* and *Aspergillus nidulans* at the C-terminal Velvet domain. This member of the velvet family has not been as extensively studied as the others, but a recent study found it played an important role in sexual development in *A. nidulans*, despite the fact that studies in *A. fumigatus* of VelC mutants did not show a distinct phenotype [23]. *Coccidioides* spp. contain genes required for mating, and recombination has been documented at the molecular level in some strains, but mating has not been observed. The function of VelC in *Coccidioides* spp. is unclear.

Histidine kinases are another group of proteins involved in responses to external stimuli [24]. Some of the best know functions of histidine kinases include osmosensing, adaption to oxidants, conidiation, cell wall integrity and virulence. In some other primary pathogenic fungi, the Drk1 has been found to be required for the temperature dependent conversion from

mycelia to yeast [25,26]. The Drk1 gene is found in both *Coccidioides* spp. and nonpathogenic Onygenales. Goldberg described three histidine kinases in *C. immitis*, CIMG_04512, CIMG_05052 and CIMG_06539, which are all present in *U. reesii* [27] . However, there are more than ten histidine kinases in most filamentous fungi, so it is likely that more genes exist [28]. In this analysis, we identified one other *Coccidioides* spp.-specific gene with a histidine kinase domain, which is not up-regulated in spherules. This gene, CIMG_09624, is almost completely identical in many isolates of *Coccidioides* spp. and very similar to histidine kinases in several other taxa despite its absence in non-pathogenic Onygenales. These data argue that this gene is very probably a conserved histidine kinase that may play an important role in the biology of *Coccidioides* spp.

Thioredoxin is an important regulatory enzyme that modifies target proteins by modifying their redox status [29]. Most organisms have multiple thioredoxin genes with overlapping functions. The biological role of thioredoxin includes antioxidant functions that are probably particularly important for *Coccidioides* spp. when ingested by phagocytic cells. *C. immitis* is relatively resistant to oxidative stress and mice lacking an oxidative burst are not more susceptible than control mice to coccidioidomycosis [30]. There are a total of three thioredoxin genes in *Coccidioides* spp., CIMG_09126, CIMG_09274 and CIMG_08211, none of which are up-regulated in spherules. Two of these, CIMG_09126 and CIMG_09274, are not found in nonpathogenic Onygenales but *U. reesii* does has have an ortholog of CIMG_08211. This difference in the number of thioredoxin genes may play a part in the ability of *Coccidioides* spp. to survive an oxidative burst and survive phagocytosis.

Calmodulin is a calcium binding protein in the calcineurin pathway which is also important in regulating growth, responses to stress and virulence in a variety of fungi [31,32]. When calmodulin binds to calcium it activates calcineurin, which is critical for thermotolerance and pathogenicity. Calcineurin inhibitors inhibit the growth of a number of different fungi, including *C. immitis,* at 37°C [32,33]. There are three genes coding for calmodulin in *Coccidioides* spp. and one of these, CIMG_04786 is a *Coccidioides* spp.- specific gene and the remaining two

calmodulin genes are common. This difference in the number of genes may play a role in determining thermotolerance.

Ureidoglycolate hydrolase (*UGH*), CIMG_02178, is a *Coccidioides spp.*-specific gene. Cole and his co-workers have studied the role of extracellular ammonia in spherule development [34]. There is evidence that first generation spherules release endospores into an alkaline environment [34]. One enzyme involved in ammonia production is *UGH* and the expression of this gene is up-regulated in spherules compared to mycelia [14]. The *C. posadasii* UGH deletion mutant is less virulent than the wildtype, even though the mutant grew normally as a mold in vitro [34]. The urease/UGH double mutant, which makes very little ammonia, was much less virulent than wildtype. These data suggest that the UGH gene plays an important role in the pathogenesis of infections caused by *Coccidioides* spp.

Summary

As part of a study of the genome of pathogenic Ajellomyces fungi compared to close non-pathogenic relatives Cuomo identified 46 *C. immitis* genes that were not found in *U. reesii* [7]. The current analysis identified many more genes that were found in multiple *Coccidioides* spp. isolates but not nonpathogenic Onygenales but 24 of the genes were found in both studies. Cuomo also proposed 32 genes that were important for fungal pathogenesis. This analysis found only two homologs of these genes in the Coccidioides spp. specific group: Ureidoglycolate hydrolase (*UGH*) and Ryp2. Whiston and Taylor have also compared the predicted proteins of *Coccidioides* spp. to nonpathogenic Onygenales [6]. The number of *Coccidioid*es spp.-specific genes and the tendency of those genes to be over-expressed in spherules is similar in their study and this one. A list of genes specific to *Coccidioides* spp. is not included in that work, so gene by gene comparisons cannot be done.

This attempt to identify *Coccidioid*es spp. genes that are not found in nonpathogenic Onygenales is done to try to identify candidate pathogenesis genes. The strengths of the study include the overlap of these results with previous studies and the internal consistency of the

data. This study used slightly different methods than others but a significant number of *Coccidioides* spp.-specific genes were found in more than one study. There are also a number of weaknesses. The identification of unique and common genes depends on the accuracy of the current annotation. Since *U. reesii* and the other nonpathogenic Onygenales may not be annotated as well as *C. immitis* and *C. posadasii*, some genes might be classified as unique in error. Furthermore, a weakness of all these types of studies is the possibility that genes have evolved to perform new functions, so the absence of a given homolog in an organism does not imply the absence of that biological process. In addition, in the instances where a number of genes are found (such as thioredoxin and calmodulin) it is not clear what to expect from the result of the absence of one or two of them.

A common theme of the genes that are *Coccidioides* spp.-specific is their role in responding to environmental changes. Genes in the Velvet family, histidine kinases, calmodulin and thioredoxin are all important for responses to stress. In *Histoplasma capsulatum*, velvet genes are required for the mycelium to yeast transformation. In addition, *Coccidioides* spp.-specific genes tended to be up-regulated in spherules more often than *Coccidioides* spp. shared with nonpathogenic Onygenales, suggesting that more *Coccidioides* spp.- specific genes are involved in, or a consequence of, spherule development. Because many of the up-regulated *Coccidioides* spp.-specific genes are orphan genes their role in mycelium to spherule transformation is intriguing even though their function is unknown. Hopefully this data will suggest genes that would be good candidates for further investigation of the molecular mechanisms of *Coccidioides* spp. pathogenicity.

Acknowledgement

The assistance of Jason Stajich in planning this study, providing the unpublished annotations and interpreting the results is deeply appreciated. I also appreciate the help of the FungiDB team and the help of Marcus Gallagher-Jones in using the Reciprocal-BB tool.

Bibliography

1. Kirkland, T.N.; Fierer, J. Coccidioidomycosis: a reemerging infectious disease. *Emerg Infect Dis* **1996**, *2*, doi:10.3201/eid0203.960305.

2. Kirkland, T.N.; Fierer, J. Coccidioides immitis and posadasii; A review of their biology, genomics, pathogenesis, and host immunity. *Virulence* **2018**, *9*, 1426-1435, doi:10.1080/21505594.2018.1509667.

3. Nguyen, C.; Barker, B.M.; Hoover, S.; Nix, D.E.; Ampel, N.M.; Frelinger, J.A.; Orbach, M.J.; Galgiani, J.N. Recent advances in our understanding of the environmental, epidemiological, immunological, and clinical dimensions of coccidioidomycosis. *Clinical Microbiology Reviews* **2013**, *26*, 505-525, doi:papers3://publication/doi/10.1128/CMR.00005-13.

4. Fisher, M.C.; Rannala, B.; Chaturvedi, V.; Taylor, J.W. Disease surveillance in recombining pathogens: Multilocus genotypes identify sources of human Coccidioides infections. *PNAS* **2002**, *99*, 9067-9071.

5. Teixeira, M.M.; Barker, B.M. Use of Population Genetics to Assess the Ecology, Evolution, and Population Structure of Coccidioides. *Emerg Infect Dis* **2016**, *22*, 1022-1030, doi:10.3201/eid2206.151565.

6. Whiston, E.; Taylor, J.W. Comparative Phylogenomics of Pathogenic and Nonpathogenic Species. *G3 (Bethesda)* **2015**, *6*, 235-244, doi:10.1534/g3.115.022806.

7. Munoz, J.F.; McEwen, J.G.; Clay, O.K.; Cuomo, C.A. Genome analysis reveals evolutionary mechanisms of adaptation in systemic dimorphic fungi. *Sci Rep* **2018**, *8*, 4473, doi:10.1038/s41598-018-22816-6.

8. Sharpton, T.J.; Stajich, J.E.; Rounsley, S.D.; Gardner, M.J.; Wortman, J.R.; Jordar, V.S.; Maiti, R.; Kodira, C.D.; Neafsey, D.E.; Zeng, Q., et al. Comparative genomic analyses of the human fungal pathogens Coccidioides and their relatives. *Genome Res* **2009**, *19*, 1722-1731, doi:10.1101/gr.087551.108.

9. Pan, S.; Sigler, L.; Cole, G.T. Evidence for a phylogenetic connection between Coccidioides immitis and uncinocarpus reesii (onygenaceae). *Microbiology* **1994**, *140*, 1481-1494.

10. Sigler, L.; Flis, A.L.; Carmicheal, J.W. The genus Uncinocarpus (Onygenaceae) and its synonym Brunneospora: new concepts, combinations and connections to anamorphs in Chrysosporium, and further evidence of relationship with Coccidioides immitis. *Canadian Journal of Botany-Revue Canadienne De Botanique* **1998**, *76*, 1624-1636, doi:DOI 10.1139/b98-110.

11. Desjardins, C.A.; Champion, M.D.; Holder, J.W.; Muszewska, A.; Goldberg, J.; Bailao, A.M.; Brigido, M.M.; Ferreira, M.E.; Garcia, A.M.; Grynberg, M., et al. Comparative genomic analysis of human fungal pathogens causing paracoccidioidomycosis. *PLoS Genet* **2011**, *7*, e1002345, doi:10.1371/journal.pgen.1002345.

12. Roilides, E.; Sigler, L.; Bibashi, E.; Katsifa, H.; Flaris, N.; Panteliadis, C. Disseminated infection due to Chrysosporium zonatum in a patient with chronic granulomatous disease and review of non-Aspergillus fungal infections in patients with this disease. *J Clin Microbiol* **1999**, *37*, 18-25.

13. Neafsey, D.E.; Barker, B.M.; Sharpton, T.J.; Stajich, J.E.; Park, D.J.; Whiston, E.; Hung, C.Y.; McMahan, C.; White, J.; Sykes, S., et al. Population genomic sequencing of Coccidioides fungi reveals recent hybridization and transposon control. *Genome Res* **2010**, *20*, 938-946, doi:10.1101/gr.103911.109.

14. Whiston, E.; Zhang Wise, H.; Sharpton, T.J.; Jui, G.; Cole, G.T.; Taylor, J.W. Comparative transcriptomics of the saprobic and parasitic growth phases in Coccidioides spp. *PLoS One* **2012**, *7*, e41034, doi:10.1371/journal.pone.0041034.

15. Engelthaler, D.M.; Roe, C.C.; Hepp, C.M.; Teixeira, M.; Driebe, E.M.; Schupp, J.M.; Gade, L.; Waddell, V.; Komatsu, K.; Arathoon, E., et al. Local Population Structure and Patterns of Western Hemisphere Dispersal for Coccidioides spp., the Fungal Cause of Valley Fever. *MBio* **2016**, *7*, e00550-00516, doi:10.1128/mBio.00550-16.

16. Litvintseva, A.P.; Marsden-Haug, N.; Hurst, S.; Hill, H.; Gade, L.; Driebe, E.M.; Ralston, C.; Roe, C.; Barker, B.M.; Goldoft, M., et al. Valley fever: finding new places for an old disease: Coccidioides immitis found in Washington State soil associated with recent human infection. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **2015**, *60*, e1-3, doi:papers3://publication/doi/10.1093/cid/ciu681.

17. Kirkland, T.N. A few shared up-regulated genes may influence conidia to yeast transformation in dimorphic fungal pathogens. *Med Mycol* **2016**, *54*, 648-653, doi:10.1093/mmy/myw019.

18. Higgs, H.N.; Pollard, T.D. Regulation of Actin Filament Network Formation Through ARP2/3 Complex: Activation by a Diverse Array of Proteins. *Annual Review of Biochemistry* **2001**, *70*, 649-676, doi:10.1146/annurev.biochem.70.1.649.

19. Calvo, A.M. The VeA regulatory system and its role in morphological and chemical development in fungi. *Fungal Genet Biol* **2008**, *45*, 1053-1061, doi:10.1016/j.fgb.2008.03.014.

20. Ni, M.; Yu, J.H. A novel regulator couples sporogenesis and trehalose biogenesis in Aspergillus nidulans. *PLoS One* **2007**, *2*, e970, doi:10.1371/journal.pone.0000970.

21. Webster, R.H.; Sil, A. Conserved factors Ryp2 and Ryp3 control cell morphology and infectious spore formation in the fungal pathogen Histoplasma capsulatum. *Proc Natl Acad Sci U S A* **2008**, *105*, 14573-14578, doi:0806221105 [pii] 10.1073/pnas.0806221105.

22. Laskowski-Peak, M.C.; Calvo, A.M.; Rohrssen, J.; Smulian, A.G. VEA1 is required for cleistothecial formation and virulence in Histoplasma capsulatum. *Fungal Genet Biol* **2012**, *49*, 838-846, doi:10.1016/j.fgb.2012.07.001.

23. Park, H.S.; Nam, T.Y.; Han, K.H.; Kim, S.C.; Yu, J.H. VelC positively controls sexual development in Aspergillus nidulans. *PLoS One* **2014**, *9*, e89883, doi:10.1371/journal.pone.0089883.

24. Herivaux, A.; So, Y.S.; Gastebois, A.; Latge, J.P.; Bouchara, J.P.; Bahn, Y.S.; Papon, N. Major Sensing Proteins in Pathogenic Fungi: The Hybrid Histidine Kinase Family. *PLoS Pathog* **2016**, *12*, e1005683, doi:10.1371/journal.ppat.1005683.

25. Nemecek, J.C.; Wuthrich, M.; Klein, B.S. Global control of dimorphism and virulence in fungi. *Science* **2006**, *312*, 583-588, doi:312/5773/583 [pii] 10.1126/science.1124105.

26. Boyce, K.J.; Andrianopoulos, A. Fungal dimorphism: the switch from hyphae to yeast is a specialized morphogenetic adaptation allowing colonization of a host. *FEMS microbiology reviews* **2015**, doi:fuv035 [pii].

27. Viriyakosol, S.; Singhania, A.; Fierer, J.; Goldberg, J.; Kirkland, T.N.; Woelk, C.H. Gene expression in human fungal pathogen Coccidioides immitis changes as arthroconidia

differentiate into spherules and mature. *BMC Microbiology* **2013**, *13*, 121, doi:10.1186/1471-2180-13-121.

28. Hagiwara, D.; Takahashi-Nakaguchi, A.; Toyotome, T.; Yoshimi, A.; Abe, K.; Kamei, K.; Gonoi, T.; Kawamoto, S. NikA/TcsC histidine kinase is involved in conidiation, hyphal morphology, and responses to osmotic stress and antifungal chemicals in Aspergillus fumigatus. *PLoS One* **2013**, *8*, e80881, doi:10.1371/journal.pone.0080881.

29. Meyer, Y.; Buchanan, B.B.; Vignols, F.; Reichheld, J.-P. Thioredoxins and Glutaredoxins: Unifying Elements in Redox Biology. *Annual Review of Genetics* **2009**, *43*, 335-367, doi:10.1146/annurev-genet-102108-134201.

30. Margolis, D.A.; Viriyakosol, S.; Fierer, J.; Kirkland, T.N. The role of reactive oxygen intermediates in experimental coccidioidomycois in mice. *BMC Microbiol* **2011**, *11*, 71, doi:10.1186/1471-2180-11-71.

31. Brown, S.M.; Campbell, L.T.; Lodge, J.K. Cryptococcus neoformans, a fungus under stress. *Curr Opin Microbiol* **2007**, *10*, 320-325, doi:S1369-5274(07)00081-1 [pii] 10.1016/j.mib.2007.05.014.

32. Steinbach, W.J.; Cramer, R.A., Jr.; Perfect, B.Z.; Asfaw, Y.G.; Sauer, T.C.; Najvar, L.K.; Kirkpatrick, W.R.; Patterson, T.F.; Benjamin, D.K., Jr.; Heitman, J., et al. Calcineurin controls growth, morphology, and pathogenicity in Aspergillus fumigatus. *Eukaryot Cell* **2006**, *5*, 1091-1103, doi:10.1128/EC.00139-06.

33. Kirkland, T.N.; Fierer, J. Cyclosporin A inhibits Coccidioides immitis in vitro and in vivo. *Antimicrob Agents Chemother* **1983**, *24*, 921-924.

34. Wise, H.Z.; Hung, C.Y.; Whiston, E.; Taylor, J.W.; Cole, G.T. Extracellular ammonia at sites of pulmonary infection with Coccidioides posadasii contributes to severity of the respiratory disease. *Microb Pathog* **2013**, *59-60*, 19-28, doi:10.1016/j.micpath.2013.04.003.