Chapter 6

Conclusions and future perspectives
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In the current study, we have delineated the interaction of a human opportunistic fungal pathogen, *C. glabrata*, with human macrophages. Employing a signature tagged-mutagenesis approach, we screened a Tn7 insertion mutant library, representing ~ 50% of *C. glabrata* genome, for altered survival profiles in THP-1 macrophages and identified a set of 56 genes which are required for intracellular proliferation of *C. glabrata* cells in THP-1 cells. Of these, 53 genes are novel and orthologs of *CgENA1*, *CgRTT109* and *CgSEF1* have been implicated in the virulence in *Cryptococcus neoformans* and *C. albicans*, respectively. Our findings established chromatin remodeling as a key regulator of reprogramming of cellular energy metabolism for survival and/or replication of macrophage-internalized *C. glabrata* cells.

Utilizing an *in vitro* THP-1 macrophage model system, we showed that *C. glabrata* cells employed multiple strategies to survive/counteract antimicrobial response of macrophages and replicate intracellularly. These strategies include prevention of phagolysosomal maturation, suppression of IL-6 secretion in THP-1 cells as well as large-scale reprogramming of their own chromatin architecture and carbon and energy metabolism. To identify factors which govern these changes upon macrophage internalization, we screened a *C. glabrata* mutant library, comprised of 18,350 mutants, generated by homologous recombination of *in vitro* created Tn7 insertions in *C. glabrata* genomic clones, for altered survival profiles in THP-1 macrophages via signature-tagged mutagenesis approach and identified a total of 168 mutants with 35 and 133 mutants displaying increased and reduced survival, respectively. Mapping of Tn7 insertion in mutants showing diminished intracellular proliferation identified a set of 56 genes which are required to survive and/or replicate in THP-1 macrophages. Functional annotation of identified genes to biological processes using GO Slim Mapper revealed 5, 5, 7, 9 and 12% of total identified genes to be involved in signaling, cell wall organization, endocytosis, Golgi vesicle transport and chromatin organization, respectively.

Further, using a combined approach of genome-wide transcriptional profiling, cell biological, microscopy and biochemical analyses, we demonstrated that *C. glabrata* wild-type cells respond to the THP-1 macrophage internal milieu by down-
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regulating translational machinery and modifying their chromatin structure to a closed, compact form. Since chromatin resistance to micrococcal nuclease digestion, altered epigenetic signature (diminished active (H3K9Ac, H3K14Ac) and elevated repressive (H3K9Me3, H3K27Me3) chromatin marks) and increased cellular lysine deacetylase activity appear to be characteristics of macrophage-internalized C. glabrata cells, it will be intriguing to investigate whether C. glabrata cells utilize similar mechanisms to evade antifungal response of other immune cells viz., neutrophils and dendritic cells.

Reorganization of chromatin in response to host immune cells was found to be pivotal for pathogenesis of C. glabrata as mutants defective in chromatin organization (Cgrσc3-aΔ, Cgrσc3-bΔ, Cgrσc3-aΔbΔ and Cgrtt109Δ) and DNA damage repair (Cgrtt107Δ and Cgsσ51Δ) displayed attenuated virulence in a murine model of disseminated candidiasis. Although it remains to be determined if chromatin remodeling is a common fungal virulence mechanism, an important role for Rtt109 in virulence of C. albicans lends support to this notion. Additionally, since global heterochromatinization is a key response of C. glabrata cells to the intracellular environment of THP-1 macrophages, identification of genomic loci whose expression is down-regulated upon macrophage ingestion will help elucidate the molecular basis underlying the metabolic reconfiguration of C. glabrata cells for survival in mammalian host.

In addition to chromatin reorganization, we uncovered an essential role for phosphatidylinositol-3-kinase (PI-3 kinase) in virulence of C. glabrata as disruption of either regulatory (CgVps15) or catalytic (CgVps34) subunit of PI-3 kinase completely abolished the ability of C. glabrata cells to survive in primary murine and THP-1 macrophages and mice model of systemic candidiasis. Additionally, Cgvs15Δ and Cgvs34Δ cells exhibited increased adherence to epithelial cells, missorted adhesin, Epa1, and vacuolar protease, carboxypeptidase Y, and colocalized with acidified lysosomes in THP-1 cells. An intriguing finding of the current study is an important function of C. glabrata PI3-kinase in modulation of phagosome acidification in THP-1 macrophages and future studies will be focused on identification of factors that actively inhibit phagolysosomal maturation and enable C. glabrata cells survive in host immune cells.
In conclusion, we have developed an *in vitro* cell culture model system using THP-1 macrophages which is simple, fast and amenable to high-throughput mutant screens, and uncovered two key mechanisms, viz., chromatin remodeling and PI-3 kinase-mediated signaling, of *C. glabrata* virulence.