Genome Plasticity in *Cryptococcus neoformans*: How It Contributes to Survival under Drug Stress

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Cryptococcus neoformans is an environmental fungus that causes brain infection most commonly in immunocompromised patients such as those with HIV infection or hematologic neoplasia (1, 6). The disease starts by inhalation of dehydrated asexual yeast cells or presumably basidiospores produced by sexual reproduction of *C. neoformans* (1, 6). The most commonly known ecological niche of *C. neoformans* is pigeon droppings and soil contaminated with avian guanos world-wide. The inhaled *C. neoformans* cells propagate in the lung and hematogenously disseminate to the brain by crossing the blood-brain barrier (2). Cryptococcal meningoencephalitis is fatal unless treated and the fatality rate is nearly 25% even with the most advanced antifungal therapy (6). Although *C. neoformans* produces no secondary metabolites such as toxins or antibiotics (7) to defend itself, the fungus survives in such extreme environments as pigeon guanos or soil. Considering the presence of 2,000 to 18,000 different genomes per gram of soil (4), *C. neoformans* must possess a mechanism that successfully defends and competes with coexisting organisms in its habitat. We recently discovered that the *C. neoformans* genome is extraordinarily malleable and such genome plasticity equips the fungus to overcome various stresses such as those exerted by fluconazole, a triazole drug. Fluconazole is the most commonly used antifungal drug for the treatment of cryptococcosis or other fungal diseases and resistant strains are increasingly being reported from recurrent infections during fluconazole maintenance therapy.

In 1999, we described a very unusual pattern of adaptive drug resistance termed “heteroresistance” to azoles in strains of *C. neoformans* isolated from therapy failure cases of cryptococcosis (8). Since azole drugs had been in use since the late 1980s (5), it was unclear as to whether the heteroresistance was azole induced or intrinsic. We have tested over 100 strains of *C. neoformans* which were isolated at least decade before the advent of azole antimycotics and found that “heteroresistance” was universal in *C. neoformans*. This indicated heteroresistance to be intrinsic and the drug level at which heteroresistance manifested was determined to be strain dependant.

We focused on the molecular mechanism of heteroresistance using H99, the genome sequenced serotype A strain of *C. neoformans*. The subpopulation that tolerated fluconazole concentrations higher than the MIC levels was observed to be disomic for several chromosomes. The number of chromosomal disomies correlated with exposure of the fungus to different levels of the drug. Higher drug concentrations resulted in more disomic chromosomes. The population that could tolerate 128ug/ml fluconazole revealed disomy in 4 out of 14

chromosomes. Chromosome 1 contains the azole target gene *ERG11* and the *AFR1* gene which encodes the only ABC transporter thus far known to be specific for azoles in *C. neoformans*. Chromosome 1 was always the first one to be duplicated in drug resistant subpopulations. While *AFR1* was not found to be associated with the disomy of chromosome 1, *ERG11* exhibited a direct association with the evolution of chromosome 1 disomy. The return of duplicated chromosomes to haploid status upon repeated transfers in drug free media indicated chromosomal duplications to be a mechanism of adapting to drug stress. (Chromosome 1 was also the first chromosome that returned from 2n to 1n during growth in drug free media.)

Our study reveals that plasticity of the *C. neoformans* genome is a major contributing factor that enables the fungus to cope with stress exerted by azole drugs. Massive tandem gene amplifications suggestive of genomic plasticity was also reported in a number of clinical isolates (2). We speculate that genome plasticity plays a major role in the survival of *C. neoformans* in extreme environmental sources and is a mechanism to overcome many different stresses including the host immune assault. This genomic plasticity may also explain the lack of identical karyotypes among *C. neoformans* isolated from various sources.

References

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