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Mitochondrial DNA inheritance in the human fungal pathogen *Cryptococcus gattii*

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ABSTRACT

The inheritance of mitochondrial DNA (mtDNA) is predominantly uniparental in most sexual eukaryotes. In this study, we examined the mitochondrial inheritance pattern of *Cryptococcus gattii*, a basidiomycetous yeast responsible for the recent and ongoing outbreak of cryptococcal infections in the US Pacific Northwest and British Columbia (especially Vancouver Island) in Canada. Using molecular markers, we analyzed the inheritance of mtDNA in 14 crosses between strains within and between divergent lineages in *C. gattii*. Consistent with results from recent studies, our analyses identified significant variations in mtDNA inheritance patterns among strains and crosses, ranging from strictly uniparental to biparental. For two of the crosses that showed uniparental mitochondrial inheritance in standard laboratory conditions, we further investigated the effects of the following environmental variables on mtDNA inheritance: UV exposure, temperature, and treatments with the methylation inhibitor 5-aza-2′-deoxycytidine and with the ubiquitination inhibitor ammonium chloride. Interestingly, one of these crosses showed no response to these environmental variables while the other exhibited diverse patterns ranging from complete uniparental inheritance of the MAT**a** parent mtDNA, to biparental inheritance, and to a significant bias toward inheritance of the MAT**α** parental mtDNA. Our results indicate that mtDNA inheritance in *C. gattii* differs from that in its closely related species *Cryptococcus neoformans*.

1. Introduction

The mitochondrion is the principal energy-producing organelle within most eukaryotes, and is commonly referred to as the "powerhouse" of the cell (Gillham, 1994). However, mitochondria play important roles in other cellular processes including apoptosis (Green and Reed, 1998), drug/host resistance (Brun et al., 2005; Cheng et al., 2007), virulence (Olson and Stenlid, 2001), nuclear genome stability (Dirick et al., 2013), metabolite homeostasis (Gillham, 1994), aging (Basse, 2010), and male sterility (Saumitou-Laprade et al., 1994). In humans, mitochondrial DNA (mtDNA) mutation contributes to the pathogenesis of many diseases (Taylor and Turnbull, 2005), including diabetes (Lowell and Shulman, 2005), neurodegenerative disorders (Lin and Beal, 2006), and cancers (Chatterjee et al., 2006; Fliss et al., 2000).

In the majority of sexual eukaryotes, a zygote will normally inherit equal contributions of paternal and maternal genetic makeup for nuclear genes. However, mitochondrial inheritance is predominantly uniparental, with the zygote receiving mtDNA from the maternal parent (Gillham, 1994; Birky, 2001; Wilson and Xu, 2012). Although uniparental mitochondrial inheritance is the predominant pattern, paternal and biparental patterns of inheritance have also been observed in some organisms (Zouros et al., 1992; Jannotti-Passos et al., 2001; Schwartz and Vissing, 2002; Yan and Xu, 2005; Basse, 2010; Wilson and Xu, 2012). In addition, environmental factors such as temperature and UV irradiation can also influence mtDNA inheritance (Yan et al., 2007b). At present, the detailed molecular mechanisms governing mtDNA inheritance remain poorly understood (Basse, 2010; Wilson and Xu, 2012).

There are 37 species in the fungal genus *Cryptococcus*, of which only two (*C. gattii* and *C. neoformans*) are relevant to the majority of clinical infections in humans (Casadevall and Perfect, 1998; Heitman et al., 2011). These basidiomycete yeasts infect hosts by first colonizing the lungs of susceptible individuals and then spreading to multiple organs, with dissemination to the central nervous system causing the most problematic symptoms, especially in immune-compromised patients. The majority of cryptococcosis cases are caused by *C. neoformans*, and this species is distributed worldwide, often found in association with bird guano (Casadevall and Perfect, 1998). There are three serotypes (A, D and AD) and two varieties (var. *grubii* and var. *neoformans*) in *C. neoformans* and they have become model organisms for fungal molecular

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genetics and pathogenesis research (Heitman et al., 2011). In contrast, its closely related sister species, *C. gattii* (serotypes B and C), is clinically rare, causing <1% of global cryptococcosis cases and is largely limited to tropical and subtropical regions (Litvintseva et al., 2011). Compared to *C. neoformans*, relatively little was known about *C. gattii*, until recently when it was found to cause a significant number of infections in humans as well as both wild and domesticated animals in the temperate region of the west coast of North America (Kidd et al., 2004; Datta et al., 2009).

Historically, *C. gattii* was considered as a varietal form of its closest relative, *C. neoformans* (Casadevall and Perfect, 1998). However based on differences in ecology, epidemiology, biochemical and molecular characteristics, *C. gattii* was recently defined as a separate species from *C. neoformans* (Kwon-Chung et al., 2002; Kwon-Chung and Varma, 2006). Because of historical connections and that hybrids between *C. neoformans* and *C. gattii* have been reported (Litvintseva et al., 2011), *C. neoformans* and *C. gattii* are often collectively referred to as the *Cryptococcus neoformans* species complex (CNSC). One of the defining differences between *C. neoformans* and *C. gattii* is their susceptible host range. *C. gattii* is capable of infecting healthy humans, whereas *C. neoformans* is mostly found to infect immune-compromised individuals, and as such *C. neoformans* is commonly referred to as an opportunistic pathogen while *C. gattii* is a primary pathogen (Litvintseva et al., 2011; Kwon-Chung and Varma, 2006).

Within *C. gattii* there are four well-established lineages or molecular types (VGI, VGII, VGIII and VGIV), which are genetically distinct from each other, as shown by multi-locus sequence typing (Kidd et al., 2005; Bovers et al., 2008; Litvintseva et al., 2011). Among the four lineages, VGI and VGIII are the most closely related with VGIV being the next closely related to them and VGII being the most distantly related to all other lineages (Bovers et al., 2008). The VGII lineage is prevalent in the environments of South America, the Pacific Northwestern United States and Vancouver Island, Canada (Litvintseva et al., 2011). VGII is also the second most prevalent lineage in Australia, but mostly in the northern and western regions (Litvintseva et al., 2011). The VGI lineage is the most prevalent in Australia and India accounting for the majority of environmental and clinical isolates (Litvintseva et al., 2011; Chowdhary et al., 2011). VGI has also been found in northwestern United States and Canada (Kidd et al., 2007). VGIII is found commonly in Columbia and India as well as southwest United States, while VGIV is rare and found mainly in Africa and Central America (Campbell et al., 2005; Litvintseva et al., 2011; Xu et al., 2011).

Mitochondrial inheritance in *C. neoformans* is uniparental under normal mating conditions (Xu et al., 2000a; Yan and Xu, 2003). In *C. neoformans*, there are two mating types: *MATa* and *MATα*. Progeny from mating between wild type strains within and between var. *neoformans* and var. *grubii* predominantly or exclusively inherit mtDNA from the *MATa* parent (Xu et al., 2000a; Yan and Xu, 2003), with the sex-determining genes *sxi1α* and *sxi2a* playing determining roles (Yan et al., 2007a). Aside from the sex-determining genes, other independent genetic factors have also been shown to influence mtDNA inheritance in *C. neoformans*. For example, a key pre-zygotic transcription factor Mat2 that governs the pheromone sensing and response pathway plays a critical role in mtDNA inheritance in *C. neoformans* and that role is independent of the Sxi1α/Sxi2a complex (Gyawali and Lin, 2013). In addition, in matings between haploid and diploid (MATa/a or MATα/α) strains, mtDNA from the MATα parent was commonly detected in the meiotic progeny, suggesting an influence of the ploidy level on mtDNA inheritance (Skosireva et al., 2010).

Similar to *C. neoformans*, mating in *C. gattii* is also controlled by a single locus with two alleles, *MATa* and *MATα*. Two recent studies examined mtDNA inheritance in *C. gattii* (Zhu et al., 2013; Voelz et al., 2013). Zhu et al. (2013) examined an isogenic cross between two strains of VGII that differed only in mating type and mtDNA genotype and they identified that all 12 of their analyzed progeny inherited mtDNA from the MATa parent. However, only one marker, a SNP within the *cob2* gene, was examined (Zhu et al., 2013) and thus whether mtDNA recombination occurred was unknown. In the study by Voelz et al. (2013), seven crosses were examined, including both intra-lineage and inter-lineage crosses. Three to 24 progeny were analyzed for each cross, with an average of 11 progeny per cross. Five of the crosses were analyzed using a single genetic marker, the same one used in the Zhu et al. (2013) study. Voelz et al. (2013) found that mtDNA was inherited uniparentally in their inter-lineage crosses. However, in the two intra-VGII crosses where additional mtDNA markers were used, recombinant mtDNA genotypes were found and suggested that genetic factor(s) other than mating type locus also played a role in mtDNA inheritance in *C. gattii*. Due the relatively limited number of crosses conducted, the relatively small progeny sizes analyzed for most crosses, and the use of a single mtDNA marker for analyzing most of these crosses, it remains to be determined whether the results obtained above by Zhu et al. (2013) and by Voelz et al. (2013) could be generalized for *C. gattii*. In addition, experiments conducted by Yan et al. (2007b) showed that environmental factors such as high temperature and UV irradiation could impact mitochondrial inheritance in *C. neoformans*. Specifically, at certain elevated temperatures and UV dosages, increased leakage from the MATα parent was found, which in some cases resulted in the formation of recombinant mitochondrial genomes (Yan et al., 2007b). These environmental factors can be considered as stressors to the organisms and the increased leakage and mtDNA recombination were hypothesized to be a response by the organisms to increase mitochondrial genetic variation among the progeny (Yan et al., 2007b).

In this study, we examined the mitochondrial inheritance in *C. gattii* using genetically diverse strains that belonged to three different evolutionary lineages. Crosses between these lineages are analogous to hybrid crosses since the strains from different lineage of *C. gattii* are genetically distinct from each other (Bovers et al., 2008; Xu et al., 2009; Litvintseva et al., 2011). In addition, we examined the effects of four environmental factors [temperature, UV irradiation, the methylation inhibitor 5-aza-2′-deoxycytidine (5-adc), and the ubiquitination inhibitor ammonium chloride] on mtDNA inheritance in two representative crosses in *C. gattii*. Two of these four factors, UV irradiation and temperature, were previously found to influence mtDNA inheritance in *C. neoformans* (Yan et al., 2007b). However, 5-adc and ammonium chloride showed no effect at a wide range of concentrations (Yan et al., 2007b) that have previously shown to influence mtDNA inheritance in animals (Sutovsky et al., 2000). We hypothesize that the mtDNA inheritance pattern among natural strains in *C. gattii* would be more variable than in *C. neoformans*, similar to that found in Voelz et al. (2013). However, due to their close evolutionary relationships, we hypothesize that its response to environmental factors should be similar to those in *C. neoformans*.

2. Materials and methods

2.1. Strains

In this study, twelve *C. gattii* strains were used in mitochondrial inheritance analyses (Table 1). Strains B4545, B4492, and B4495 belong to the VGI lineage; strains LA55n, LA61n, and R265 belong to the VGII lineage; and the remaining six strains (B4544, B4546, B4499, ATCC32608, JF101, and JF109) belong to the VGIII lineage (Fraser et al., 2003; Kidd et al., 2005; Xu et al., 2009). Strains B4544, B4492, B4499, LA61n, JF101, and R265 are of the MATα mating type while the remaining six strains have the MATa allele