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Mechanisms of Uniparental Mitochondrial DNA Inheritance in *Cryptococcus neoformans*

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In contrast to the nuclear genome, the mitochondrial genome does not follow Mendelian laws of inheritance. The nuclear genome of meiotic progeny comes from the recombination of both parental genomes, whereas the meiotic progeny could inherit mitochondria from one, the other, or both parents. In fact, one fascinating phenomenon is that mitochondrial DNA in the majority of eukaryotes is inherited from only one particular parent. Typically, such unidirectional and uniparental inheritance of mitochondrial DNA can be explained by the size of the gametes involved in mating, with the larger gamete contributing towards mitochondrial DNA inheritance. However, in the human fungal pathogen *Cryptococcus neoformans*, bisexual mating involves the fusion of two isogamous cells of mating type (*MAT*) a and *MAT*α, yet the mitochondrial DNA is inherited predominantly from the *MAT*a parent. Although the exact mechanism underlying such uniparental mitochondrial inheritance in this fungus is still unclear, various hypotheses have been proposed. Elucidating the mechanism of mitochondrial inheritance in this clinically important and genetically amenable eukaryotic microbe will yield insights into general mechanisms that are likely conserved in higher eukaryotes. In this review, we highlight studies on *Cryptococcus* mitochondrial inheritance and point out some important questions that need to be addressed in the future.

KEYWORDS : Isogamy, Mating, *MAT* locus, Mitochondrial inheritance, Morphogenesis

Mitochondria are important organelles of eukaryotic cells. They serve as the major source of energy [1] and are vital even for organisms that do not depend on respiration for energy [2]. In addition, mitochondria are involved in other important processes, including aging, calcium homeostasis, and apoptosis [2, 3]. The number of mitochondria per cell varies widely among different cell types. In general, cells that need more energy, such as skeletal muscle cells, neurons, or cardiac cells, have a lot more mitochondria than other cell types [4].

One unique feature of eukaryotic organisms is the ability or potential to undergo sexual reproduction. Because organelles such as mitochondria cannot be synthesized *de novo*, it is essential to have accurate and faithful transmission of important organelles from parents to their progeny during sexual reproduction. Although the mitochondrial genome in the meiotic progeny can occasionally be a novel recombination of parental mitochondrial genomes, usually, it comes from one, the other, or both parents [5].

The different inheritance pattern of organelle genomes from that of nuclear genomes was first recognized in plant chloroplasts in 1909 [6]. Later, cytoplasmic inheritance of mitochondria was observed in the budding yeast *Saccharomyces cerevisiae* through the inheritance of the petite character, a small colony phenotype resulting from respiration deficiency due to inability to synthesize mitochondrial cytochrome oxidase [7]. Similarly, cytoplasmic inheritance of mitochondria was observed in the filamentous fungus *Neurospora crassa* through the inheritance of the "poky" character [8], a slow growing phenotype resulting from respiration deficiency due to mutations in mitochondrial cytochromes aa3 and b that are involved in the electron transport [8, 9]. The "poky" character was transmitted only if it was carried by the parent with protoperithecia, which is considered the maternal parent [8]. Since then much progress has been made with regard to its function, genetic makeup and pattern of inheritance.

In the majority of eukaryotes, mitochondrial DNA in the meiotic progeny is derived from only one particular mating partner [3, 5, 10]. Detailed description about mitochondrial inheritance in plants, fungi, and animals has been published for plants [10, 11], fungi [3, 10], and animals [10]. In this article, we will consider paradigmatic

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mitochondrial DNA inheritance studies in other organisms, but will primarily focus on studies carried out in the fungal pathogen *Cryptococcus neoformans*. Mating in this fungus involves isogamous partners of mating type (*MAT*) **a** and *MAT*α, yet mitochondrial DNA of meiotic progeny originates predominantly from the *MAT***a** parent. This is similar to what has been observed in higher eukaryotes. Because *Cryptococcus* is a genetically amenable fungal pathogen, its natural population structure and mating system have been intensively investigated, and robust molecular and genomic tools have become available to study various aspects of its biology and pathogenesis. Thus, this eukaryotic microbe could serve as an ideal system to study the underlying mechanisms of uniparental mitochondrial DNA inheritance.

Cryptococcus Life Cycle

Cryptococcus is a human fungal pathogen that is responsible for the majority of fungal meningitis cases in immunocompromised and immunosuppressed people [12, 13]. This fungus is found worldwide and is acquired by the host through inhalation. *Cryptococcus* can remain dormant without producing any clinical symptoms for years and can be activated once the immune system of the host gets weakened [12, 14].

Cryptococcus usually exists as capsulated haploid yeast. However, this fungus can undergo transition between the yeast and various other morphological forms (Fig. 1) [15]. *Cryptococcus* has a bipolar mating system. The single genetic locus (the *MAT* locus) that encodes one of the two idiomorphic alleles determines whether the cell is of the *MAT***a** or the *MAT*α sexual types. This is equivalent to two sexualities in higher eukaryotes [15, 16]. *Cryptococcus* is capable of undergoing unisexual mating [17] as well as bisexual mating [18]. Unisexual mating involves the fusion between two haploid cells of the same mating type. Nuclear fusion occurs prior to or at the basidium, which is a swollen structure at hyphal tip (Fig. 1) [15, 18]. Meiosis and sporulation subsequently occur at the basidium [19]. In case of bisexual mating, cell fusion takes place between haploid cells of opposite mating types, *MAT***a** and *MAT*α, as shown in Fig. 1. Unlike unisexual mating, cell fusion is followed by the formation of a dikaryotic heterokaryon (zygote), which then generates dikaryotic hyphae with fused clamp cells. The two parental nuclei remain congressed

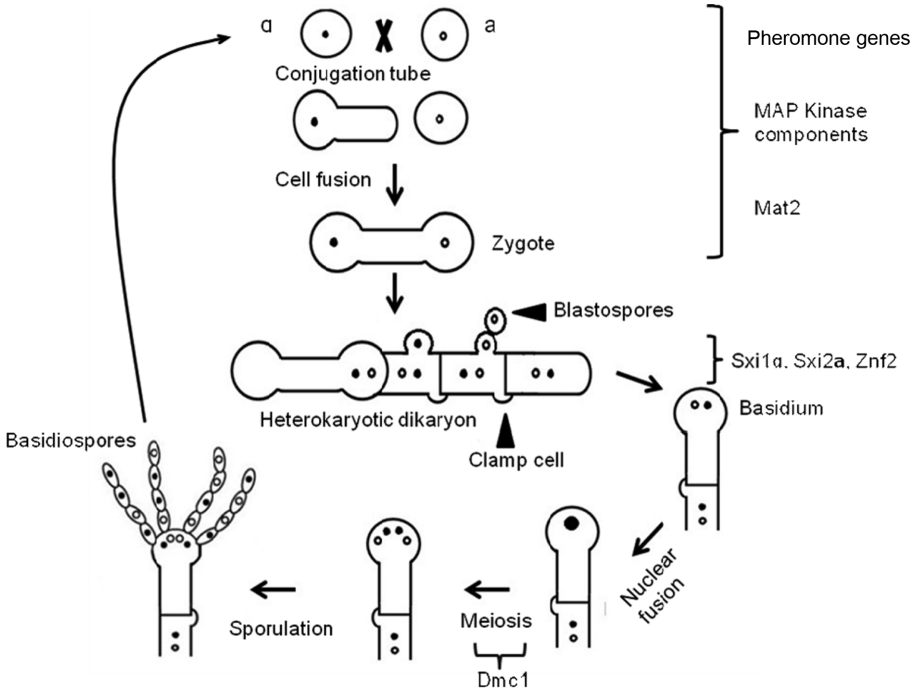

Fig. 1. Schematic diagram of Cryptococcus neoformans bisexual mating. **a** and α represent cells of the opposite mating type controlled by the mating type locus (MAT). In response to mating induction, the α cell usually produces a conjugation tube, after which cell fusion takes place. Important events that take place during sexual development are shown in the figure. The genes on the right show the key known components involved in mating. For example, pheromone genes, components of the Cpk1 mitogen-activated protein (MAP) kinase pathway [23], and the HMG domain transcription factor Mat2 are important for cell fusion [20], whereas the homeodomain transcription factors Sxi1α and Sxi2**a** are necessary for the dikaryotic hyphae formation [21, 22]. The zinc finger transcription factor Znf2 is crucial for hyphal formation in both unisexual and bisexual mating [20]. Dmc1 is the meiotic recombinase which plays a role in homologous recombination during meiosis [17].

Table 1. Summary of mitochondrial DNA inheritance patterns in bisexual crosses of *Cryptococcus neoformans*

	Crosses Strain type (mt genome)	Cell type tested	Mitochondrial DNA inheritance pattern	References
a	Serotype A (mtA) × Serotype D (mtD)	Basidiospores, hyphae	Uniparental from *MAT***a**	Xu *et al.* [25]
b	Serotype D (mtD) × Serotype D (mtA)	Basidiospores, hyphae, blastospores	Uniparental from *MAT***a**	Yan and Xu [26]
c	Serotype D (mtD) × Serotype D (mtA)	Diploid zygote	Uniparental from *MAT***a**	Yan *et al.* [29]
d	Serotype D (mtD); *sxi1*αΔ × Serotype D (mtA)	Diploid zygote	Bipatental	Yan *et al.* [29]
e	SeroD (mtA) × SeroD (mtA); *sxi2***a**Δ	Diploid zygote	Biparental	Yan *et al.* [24]
f	SeroD (mtA); *sxi1*αΔ × SeroD (mtD); *sxi2***a**Δ	Diploid zygote	Biparental	Yan *et al.* [24]
g	SeroD (mtA) × SeroD (mtD) UV irradiation	Diploid zygote	Biparental	Yan *et al.* [27]
h	SeroD (mtA) × SeroD (mtD) Temperature (33°C)	Diploid zygote	Biparental	Yan *et al.* [27]
i	SeroD (mtA) × SeroD (mtD) Unisexual mating	Diploid zygote	Biparental	Yan *et al.* [24]
j	Haploid (mtA) × Non-haploid (mtD)	Basidiospores, hyphae	Majority from *MAT***a**	Skosireva *et al.* [28]

Serotype A and serotype D are two varieties of *C. neoformans*. Here, the mtA and mtD represent Serotype A and serotype D specific mitochondrial genome respectively. Biparental refers to the inheritance pattern in which some of the progenies of a cross inherited mitochondrial DNA from the mating type (*MAT*) **a** parent while others inherited from the *MAT*α parent. All crosses in this table involved bisexual mating between haploid *MAT***a** and *MAT*α parental strains except crosses (i) and (j). The cross (i) involved unisexual mating between two *MAT*α strains and the cross (j) involved mating between a haploid strain and either a diploid or an aneuploid strain referred to as non-haploid.

but unfused until the formation of the basidium [15, 18]. Again, meiosis and sporulation occur at the basidia. For the discussion of mitochondrial DNA inheritance, we will mostly focus on the bisexual mating of *Cryptococcus*, as progeny from unisexual mating between two *MAT*α cells are shown to inherit mitochondrial DNA from either parent (Table 1) [24].

Molecular and cell biological studies on *Cryptococcus* bisexual mating have led to the discovery of the essential roles of the pheromone sensing mitogen-activated protein kinase (MAPK) pathway in sexual reproduction. Some of these components involved in this pathway are encoded in the *MAT* locus [30, 31]. For more detailed information on the molecular events of mating, please refer to the following reviews [19, 20, 32, 33].

In the majority of higher eukaryotes, mitochondrial DNA is inherited from only one of the two parents involved in the mating. Likewise, mitochondrial DNA is inherited uniparentally during bisexual crosses in *Cryptococcus* [25]. Given that bisexual mating involves the fusion between two isogamous cells of the **a** and α mating types, the observation that mitochondrial DNA inheritance is uniparental from the *MAT***a** parent is mesmerizing [25, 26]. Possible mechanisms that contribute to such inheritance pattern will be discussed here.

Mitochondrial DNA Inheritance in *Cryptococcus*

The first mitochondrial DNA inheritance study in *Cryptococcus* was done in 2000 [25]. The authors expected a biparental mitochondrial inheritance as bisexual mating involves the fusion of two isogamous cells. Surprisingly, their results showed that the mitochondrial DNA of the progeny tested was inherited uniparentally from the *MAT***a** parent in a cross between the *MAT***a** and the *MAT*α cells [25]. To differentiate the mitochondrial genotype of the *MAT***a** or the *MAT*α parent, the authors used two different varieties of *Cryptococcus*, known as serotype A and serotype D, as the mating partners. These two serotypes have different mitochondrial genotypes, which can be easily distinguished by restriction fragment length polymorphism of the mitochondrial ribosomal RNA subunit region [25]. The results for mitochondrial inheritance in *C. neoformans* involving various crosses are summarized in Table 1.

To examine when the mitochondrial DNA inheritance pattern is determined during mating process, the authors obtained various cell types generated at different stages of mating (cell fusion products, vegetative blastospores, hyphae, and meiotic basidiospores, as shown in Fig. 1) by collecting cells at locations with varied distances from the original site of parental yeast cells. The authors expected that cells generated nearer to the original mating site would be heteroplasmic; that is, mitochondria of these cells were a mix of two types of mitochondria originated from both the parents. Surprisingly, the authors found that the sampling location, which reflects different cell type or different stages of mating, did not have any measurable effect on the uniparental mitochondrial DNA inheritance pattern observed [25]. This suggests that mitochondrial DNA inheritance is determined at an early stage during mating.

To avoid any complication in mitochondrial inheritance due to potential differences in mating behavior of different serotypes, follow-up experiments using strains of the same

serotype but carrying different mitochondrial DNA was conducted [26]. Strains were generated by dissecting vegetative haploid uninuclear blastospores budded off from dikaryotic hyphae (Fig. 1). The nucleus of a blastospore could come from either one of the two parental nuclei from the dikaryotic hyphae. However, the mitochrondrial genome of the blastospores would primarily be from the *MAT***a** parent, based on the previous study [25]. Thus, the authors could potentially switch the mitochondrial genome of a *MAT*α strain to a different one by crossing this *MAT*α strain with a *MAT***a** strain with the desired mitochondrial genome, and then dissect blastospores containing the *MAT*α nuclei. Using such strategies, a serotype D *MAT*α strain with a serotype A mitochondrial DNA (from a serotype A *MAT***a** strain) could be generated, or *vice versa*. This is because mitochondrial DNA inherited in blastospores is from the *MAT***a** parent. Subsequently, such strains derived from blastospores could be crossed with other strains having the same nuclear genetic background (same serotype) with their native mitochondria. Crosses between these strains with the same nuclear genetic background would avoid any potential biases introduced by using strains of different serotypes (Table 1) [26]. The results from analyzing the mitochondrial DNA in the progeny from crosses of these strains again showed the uniparental mitochondrial DNA inheritance from the *MAT***a** parent [26]. Consistent with the previous study by the same authors, the use of hyphae, blastospores, or basidiospores all gave the same result of uniparental mitochondrial DNA inheritance from the *MAT***a** parent.

To understand the factors affecting uniparental mitochondrial inheritance in *Cryptococcus*, various environmental factors and chemicals have been tested, including temperature, ultraviolet (UV) irradiation, 5-adc (a methylation inhibitor), and ammonium chloride (an inhibitor of ubiquitination) [27]. The use of 5-adc or ammonium chloride did not have any effect on the inheritance pattern whereas strong UV irradiation and high temperatures did seem to have an effect (Table 1). In these crosses, the frequency of mitochondrial inheritance from the *MAT*α parent increased compared to that of control, which was done at room temperature [27]. Ploidy also seems to affect mitochondrial inheritance [28]. In crosses between haploid and diploid parental strains (*MAT***a** × *MAT*α/α or *MAT*α × *MAT***a**/**a**), the number of progenies that were not homoplasmic for the mitochondrial DNA from the *MAT***a** or the *MAT***a**/**a** parent were significantly higher than in a typical bisexual cross involving two haploid parents, although the majority of the progeny still inherited mitochondrial DNA from the *MAT***a** or the *MAT***a**/**a** parent [28]. This is surprising as one would expect more extreme uniparental mitochondrial DNA inheritance from the diploid *MAT***a/a** parent given that *MAT***a/a** diploid cells are larger than *MAT*α haploid cells [34]. Their result supports that cell size is not an important factor in determining mitochondrial inheritance pattern in *Cryptococcus*.

Mechanisms of mitochondrial inheritance in higher eukaryotes. In the majority of eukaryotes, mitochondrial DNA is inherited uniparentally [5, 10]. The simplest explanation for this phenomenon is that the mating partner with the greater cytoplasmic content contributes towards mitochondrial inheritance. The quantitative difference of mitochondria eventually gives the gamete with larger cytoplasm a replicative advantage. This morphological/ size difference between mating partners is conceptually simple for the understanding of mitochondrial inheritance in organisms where matings involve anisogamous partners. However, the molecular mechanisms underlying mitochondrial inheritance even in these organisms appear to be much more complicated, as will be discussed below. The uniparental mitochondrial inheritance pattern observed in organisms where mating involves isogamous partners cannot be simply explained by the quantitative difference in cytoplasm (or the mitochondria) of the mating partners. Many hypotheses have sought to explain the predominantly uniparental mitochondrial inheritance trend across various kingdoms of eukaryotes. No single hypothesis or mechanism has been able to satisfactorily explain this wide-spread phenomenon.

Mitochondrial inheritance in mammals is uniparental and maternal [35, 36]. Because an egg is much bigger than a sperm, it was assumed that the uniparental mitochondrial inheritance in mammals was simply due to the failure of mitochondria from the sperm to enter the egg. However, studies utilizing electron microscopy and molecular techniques have shown this not to be the case. Mitochondria in the sperm midpiece (middle segment of spermatozoa that consists of mitochondria) are present in a newly fertilized egg [37] and are likely eliminated later. Elimination of the mitochondria from sperm is proposed to be mediated by ubiquitination, a process of degradation of protein tagged with ubiquitin. Sperm mitochondria can already be tagged with ubiquitin during spermatogenesis prior to fertilization; studies on rhesus, bovine, and human have shown that ubiquitination of sperm mitochondria might be responsible for the uniparental mitochondrial inheritance in mammals [38]. Another proposition about uniparental mitochondrial inheritance is the dilution effect of sperm mitochondria in the oocyte [38]. The fertilized egg contains about 50~100 mitochondria from sperm midpiece, whereas the oocyte contains 10^5 to 10^8 mitochondria [39]. During each replication cycle, the sperm mitochondria become further diluted due to the replicative advantage offered by the high copy number of mitochondria from the oocyte. An alternative hypothesis to explain the targeted elimination of sperm mitochondrial was proposed based

on the mitochondrial theory of aging [40]. According to this theory, production of ATP by oxidative phosphorylation, the major function of mitochondria, is inimical for its own maintenance due to the generation of free radicals during electron transport. Fertilization requires the sperm to be highly mobile, which demands a large amount of ATP, whereas the egg cell remains immobile and, thus, minimizes oxidative damage by repressing oxidative phosphorylation. Hence, the sperm sacrifices its mitochondrial genome due to oxidative damage, whereas the female mitochondrial genome is protected so it can be transmitted faithfully to offspring [40].

Similarly, elimination of mitochondrial DNA from one parent through exclusion of its cytoplasm during fertilization has also been proposed for plants [41].

Mechanisms of mitochondrial inheritance in lower eukaryotes. In *S. cerevisiae*, mitochondrial DNA inheritance is biparental. Mating in *S. cerevisiae* involves fusion of two isogamous cells of mating types **a** and α, and the mitochondrial inheritance pattern in cells derived from the zygote is biparental sometimes and uniparental at other times [42-44]. Mitochondrial inheritance in *S. cerevisiae* depends on the position where the first bud arises from the zygote. If the first bud arises from the center of the zygote, then it contains mitochondrial DNA from both parents; if the first bud arises from the end position of the zygote, then it contains mitochondrial DNA from only one of the parents [43]. The choice of parents depends on which parental end of the zygote the bud arises from. For instance, about 80% of the first end buds are pure for uniparental mitochondrial genotype. In case of first center buds, only 30~45% are pure for one parental genotype [43]. Indeed, such inheritance pattern requires that the parental mitochondrial DNA not be mixed completely in the zygote. Consistently, the mixing of the mitochondrial DNA in the zygote has been found to be a slow proces [43].

Limited mixing and non-random sorting of mitochondrial DNA in *S. cerevisiae* were also documented in another study [42]. Through labeling of mitochondrial proteins by vital dye and GFP, the authors found that there was complete mixing of the mitochondrial proteins from both the parents in the zygote. In contrast, the mitochondrial DNA remained localized to one place in the zygote [42]. For zygotes that contained mitochondria from both parents, most of their mitotic progeny were pure for mitochondrial DNA from one parent or the other by around 20 generations [42]. The authors suggested that sorting of the mitochondrial DNA in the progeny is a non-random process; otherwise it would take more than 20 generations to reach homoplasmic state with just one mitochondrial DNA type.

Given the findings in *Saccharomyces*, uniparental mitochondrial inheritance in isogamous species like the fungus *C. neoformans* and the alga *Chlamydomonas reinhardtii* is surprising. In the unicellular alga *C. reinhardtii*, mating involves the fusion of two isogamous cells, mt^+ and mt^-. However, mitochondrial DNA inheritance is uniparental from the mt^- parent [45, 46]. Mitochondrial DNA from mt^+ parent is eliminated after zygote formation [45]. The latter study examined the identity of mitochondrial DNA in single zygote cells by nested PCR followed by restriction digestion. To determine the timing of the elimination of mt^+ mitochondrial DNA, single zygotes from matings were isolated at various time points and the identity of their mitochondrial DNA was examined. Zygotes retained mitochondrial DNA from both parents at 6 hr after mating. However, only the mt^- parent's mitochondrial DNA was detected in zygotes by 12 hr [45]. Thus, elimination of mitochondrial DNA from one parent after the formation of zygote takes time and is not an immediate process.

Mitochondrial inheritance mechanism in *Cryptococcus*. Although mating in *C. neoformans* involves fusion of two isogamous cells, the mitochondrial inheritance is uniparental from the *MAT***a** parent, similar to what is observed in *C. reinhardtii*. It is not known whether mitochondrial DNA from the *MAT*α parent gets transferred to the zygote, and, if it does, when it is destroyed or eliminated during sexual development. Although mechanisms underlying the uniparental mitochondrial DNA inheritance in *Cryptococcus* are still unclear, it is conceivable that it must be intimately associated with the mating process. Indeed, disruption of the *MAT*α cell identity gene, *SXI1*α, changes the mitochondrial DNA inheritance pattern from uniparental to biparental [29]. The cross between a WT *MAT***a** strain and a *MAT*α *sxi1*α mutant resulted in mitochondrial inheritance from either the *MAT***a** or the *MAT*α parent [29]. The *SXI1*α gene encodes for a sex-specific homeodomain protein in α strains [21]. The corresponding sex specific homeodomain gene *SXI2***a** in *MAT***a** strains is also crucial for the uniparental mitochondrial DNA inheritance (Table 1) [24]. This is consistent with previous findings that Sxi1α and Sxi2**a** form a heterocomplex to direct sexual development after the cell fusion event during bisexual mating (Fig. 1) [22]. The fact that Sxi1α or Sxi2**a** do not impair unisexual mating may explain the biparental mitochondrial inheritance during unisexual mating (unpublished results) [19, 24]. Despite the apparent importance of these two sex specific transcription factors, how their hetero-complex controls the uniparental mitochondrial inheritance in bisexual mating remains unknown.

Several possible mechanisms may operate to control mitochondrial DNA inheritance in *Cryptococcus*. Some may function at pre-zygotic stages, while some may

function at post-zygotic stages. One hypothesis for the uniparental mitochondrial inheritance in *C. neoformans* was that the nucleus from the *MAT*α cell migrates unidirectionally to the *MAT***a** cell, leaving behind its mitochondria as a result. McClelland and his colleagues in 2004 demonstrated that there was indeed unidirectional migration of the α nucleus into **a** cell based on cytological evidence [16]. In contrast, nuclear migration after hyphal fusion (anastomosis) between compatible mating types in another basidiomycetous fungus *Coprinus cinereus*, is bidirectional. The resulting *Coprinus* dikaryon contains both parental nuclear genomes, but its cytoplasmic content could be from either parent [47]. This is because cytoplasm, including mitochondria, does not migrate along with nuclei. Consequently, the mitochondria inherited in the *Coprinus* dikaryon rely on the mitochondria of the recipient mycelia [47]. Hence, mitochondrial inheritance in this black ink mushroom is biparental.

One possible post-zygotic mechanism that controls mitochondrial DNA inheritance in *C. neoformans* is based on the observation that after nuclear migration and conjugation between **a** and α cells, preferential dikaryotic hyphal formation takes place only from the end of the original *MAT***a** parent (Fig. 1) [16]. This phenomenon is analogous to the budding from *Saccharomyces* zygotes where the mitochondrial DNA of the progeny depends on the position of the bud from the zygote. Thus, uniparental mitochondrial inheritance in *Cryptococcus* from the *MAT***a** parent could be the result of the position of the emerging dikaryotic hyphae from the *MAT***a** side of the zygote.

Consistent with these observations, it has been proposed that incomplete mixing of the cytoplasmic content and the preference of hyphal formation from the *MAT***a** parent side determine the uniparental mitochondrial inheritance in *Cryptococcus* [26]. To test this hypothesis, the incubation temperature after the early stages of mating was increased, presumably right after **a** and α cells fuse to form a zygote. High temperatures inhibited growth of dikaryotic hyphae, and induced the fusion between the two parental nuclei in the dikaryon to become a diploid [48]. Yet again, uniparental mitochondrial inheritance from the *MAT***a** cell was observed, refuting the original hypothesis [26]. However, several factors may complicate such study. It was assumed that the zygote formed a diploid after cell and nuclear fusion of the two parental **a** and α cells, and that the diploid did not go through the filamentous stage and cytoplasmic mixing occurred. However, it is possible that there might be incomplete cytoplasmic mixing in the diploid cells, or that subsequent cytokinesis occurred prior to sufficient cytoplasmic mixing. Furthermore, diploids could be derived from the original zygote, or dikaryotic hyphal compartments subsequently formed from the zygote. Thus, diploids obtained might not reflect the state of the original zygote prior to filamentation. The temperature increase needs to be controlled at the right moment after cell fusion to prevent the emergence of filament. It is difficult, if not impossible, to control a heterogeneous population undergoing mating and to stop the process of every mating pair at the same stage where cell fusion has just occurred. Thus, some zygotes might well be on their way of sending emerging hyphae, or had already done so, when the temperature increased. By this time, the mitochondrial DNA inheritance pattern of the collected cells might have been already determined as expected from normal matings. So, to test the contribution of incomplete mixing of the cytoplasmic content, or the preference of hyphal formation from the *MAT***a** parent side of the initial zygote to mitochondrial inheritance, techniques allowing exact control of developmental stages of zygotes are going to be critical.

Selective degradation of the *MAT*α mitochondrial DNA in the zygote through the action of Sxi1α and Sxi2**a** complex is also proposed to explain the unidirectional and uniparental mitochondrial inheritance in *Cryptococcus* [24, 29]. According to this hypothesis, deletion of the *SXI1*α or the *SXI2***a** gene may prevent such degradation, resulting in the biparental mitochondrial inheritance as shown in Table 1. However, this hypothesis still needs to be vigorously tested as other events of mating controlled by this heterocomplex might also yield the same results.

Conclusion

It is possible that no one single mechanism can fully explain the uniparental mitochondrial inheritance pattern seen in majority of eukaryotes. For example, the oxidative theory for mammalian mitochondrial inheritance is unable to explain why there is uniparental mitochondrial inheritance even during *in vitro* fertilization where the oxidative damage to sperm cell is nominal [38]. In case of mammals, both size differences during gametogenesis and ubiquitin tagging of sperm mitochondria during spermatogenesis prior to the cell fusion event [38] contribute to the uniparental mitochondrial inheritance. The fate of mitochondria is possibly already determined when the cell identity is established. It is puzzling why one or the other events predominate in various species. In *C. neoformans* cell size is not an important factor in determining mitochondrial inheritance and there is still a lack of convincing evidence to support the hypothesis regarding selective degradation of the *MAT*α mitochondria. First, the use of methylation inhibitor (5-adc) or ubiquitination inhibitor (ammonium chloride) did not influence the mitochondrial inheritance pattern in bisexual mating [27]. Second, the ectopic integration of Sxi1α or Sxi12**a** into *MAT***a** or *MAT*α cells, respectively, prior to mating produced a minimal impact on the mitochondrial inheritance during mating [24]. However, one common theme that can be extracted from

all these hypotheses is that mating events and critical mating components like Sxi1α and Sxi2**a** are going to be the determining factors of the mitochondrial inheritance. Transmission of mitochondrial DNA can be controlled at various checkpoints during sexual development including the prezygotic stages and postzygotic stages. Techniques allowing controlled manipulation of these developmental events will help shed light on the mechanisms of mitochondrial inheritance.

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