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Review

Molecular organization and heredity of the mitochondrial genome in Basidiomycetes

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Mitochondrial genomes, because of their rapid rate of sequence divergence, are appealing molecules to use in the study of eukaryotic population or evolutionary biology (Taylor, 1986). For example, in animals, nucleotide substitutions (point mutations) proceed ten to one hundred times faster in the highly compact mitochondrial genome than in the larger nuclear one (Brown et al., 1982). Hence, comparison of mitochondrial genomes allows an estimation of genetic variability, an establishment of genetic relations between species or higher taxa and a molecular characterization of strains, species or genus.

Until recently, studies of the molecular organization, evolution and genetics of the fungal mitochondrial genomes were largely limited to the yeasts *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe*, to the filamentous ascomycetes such as *Neurospora crassa* or *Podospora anserina*, or to the hyphomycete *Aspergillus nidulans* (for reviews see Böckelmann et al., 1986; Grossman and Hudspeth, 1985). Yet, in the last ten years, the molecular biology of basidiomycetes, especially of mushrooms and phytopathogenic fungi, has been developped and has furnished powerful tools to molecularly "fingerprint" individuals. Such tools are particularly important in most of the basidiomycetes, where the clonal "individual" is difficult to define because the thallus is a network of anastomosing hyphae (Smith et al., 1992).

Additionally, in some ascomycetes sub-cultured for a long time in laboratories, altered mtDNAs due to molecular rearrangements have been associated with modifications in mycelial growth in *N. crassa* (Taylor et al., 1986), in cellular growth in *S. cerevisiae*, (Clark-Walker et al., 1985) or with senescence in *P. anserina* (Cummings et al., 1985) and in *N. intermedia* (Griffiths and Bertrand, 1984). This suggests that the mitochondrial genetic material could be correlated with not yet suspected physiologically important functions. In the same way, most of the industrial varieties of the basidiomycete *Agaricus bisporus*, subcultured a long time, show an important phenotypic variability or vegetative decline with variations in growth which might be related to changes of the mitochondrial genome organization (Jin et al., 1992). Such reports give an important applied interest to investigations on basidiomycetous mitochondrial genomes.

Finally, an important question to analyse the mtDNA variability and evolution is to determine the mode of mitochondrial inheritance. If the inheritance is strictly uniparental, mtDNA molecules would propagate clonally (Fort et al., 1984); otherwise, if both parents contribute mtDNA to their offspring (biparental inheritance), and if recombination can occur, the mtDNA would have a non clonal mode of propagation (Wilson et al., 1985).

Moreover, in most of basidiomycetes, mating does not rely on organs or cells specialized for sexual reproduction, but on the fusion (plasmogamy) of two compatible vegetative hyphae, followed by nuclei exchange and migration. This particular mode of mating (somatogamy) allows the study of the molecular mechanisms implied in mitochondrial exchanges and transmission to the offspring.

This review will be focused on three points: (i) the molecular organization: size, location and role of the genic and intergenic sequences, especially of the repeated ones, (ii) the mitochondrial genome evolution, its contribution to the natural variability and to the obtention of molecular markers such as RFLP; additionally, in this second part, we will analyse mitochondrial molecular rearrangements, (iii) in the last point, we will analyse the complex results obtained on the basidiomycete mitochondrial heredity and its consequences on wild populations structure.

Molecular organization of the mitochondrial genome in basidiomycetes

The basidiomycetous mitochondrial genomes show a great variation in size (Table 1) from 36 kbp in *Suillus cavipes* (Bruns et al., 1988) to 176 Kbp in *Agaricus bitorquis* (Hintz et al., 1985). In other eukaryotes, larger mitochondrial genomes are found only in higher plants (Lonsdaale, 1985). In fungi, the largest size reported for an ascomycete mtDNA was 115 kbp in *Cochliobolus heterostrophus* (Garber and Yoder, 1985). In basidiomycetes, larger mtDNA sizes than this ascomycetous mitochondrial genome were reported in five species:

Table 1. Sizes of the basidiomycetous mitochondrial genomes.

Species	Molecular weight (Kbp)	References
Suillus cavipes	36	Bruns et al., 1988
Coprinus cinereus	43	Weber et al., 1986
Schizophyllum commune	50	Specht et al., 1992
Clavicorona pyxidata	56	Contolini et al., 1992
Ustilago maydis	61	Feng et al., 1991[b]
Pleurotus cornucopiae var. *citrinopileatus*	64	Fukumasa-Nakai et al., 1992
Pleurotus ostreatus	76	Fukumasa-Nakai et al., 1992
Ustilago cynodontis	76.5	Mery-Drugeon et al., 1981
Lentinula edodes	77	Fukumasa-Nakai et al., 1992
Puccinia graminis	80	Sock et al., 1994
Agrocybe cylindracea	80	Fukumasa-Nakai et al., 1992
Agrocybe aegerita	80	Moulinier et al., 1992
Coprinus stercorarius	91	Weber et al., 1986
Suillus grisellus	121	Bruns et al., 1988
Grifola frondosa	127	Fukumasa-Nakai et al., 1992
Agaricus brunnescens	136	Hintz et al., 1988
Armillaria ostoyae	147	Smith and Anderson, 1994
Agaricus bitorquis	176	Hintz et al., 1985

121 kbp in *Suillus grisellus* (Bruns et al., 1988), 127 Kbp in *Grifola frondosa* (Fukumasa-Nakai et al., 1992), 147 kbp in *Armillaria ostoyae* (Smith and Anderson, 1994), 136 kbp in *Agaricus bisporus* (Hintz et al., 1988b), and 176 kbp in *Agaricus bitorquis* (Hintz et al., 1985).

Interestingly, it should be noted that great size variations have been described in species belonging to the same genus. Within the *Suillus* genus, the analysis of 15 species has shown that mtDNA sizes varied over threefold (from 36 kbp in *S. cavipes* to 121 kbp in *S. grisellus*) and exhibited a nearly continuous distribution between these two values. Additionally, virtually every region of the mitochondrial genome appeared to be size variable in this genus (Bruns et al., 1989). In the same way, in the genus *Coprinus, Agaricus, Pleurotus* or *Ustilago*, the mitochondrial genomes of closely related species reveal important size variations: from 43.3 kbp in *Coprinus cinereus* to 91.1 kbp in *C. stercorarius* (Weber et al., 1986); from 136 kbp in *Agaricus bisporus* (Hintz et al., 1988b) to 176 kbp in *A. bitorquis* (Hintz et al., 1985); from 64.5 kbp in *Pleurotus cornucopiae* to 78.9 kbp in *P. ostreatus* (Fukumasa-Nakai, 1992); and from 61 kbp in the phytopathogenic species *Ustilago maydis* (Feng et al., 1991b) to 76.5 kbp in *U. cynodontis* (Mery-Drugeon et al., 1981). These large variations of size in the same genus have been analysed for the two *Coprinus* species by Weber et al. (1986). The authors are in favor of an evolution model in which the size variations would be imputed to mechanisms stochastically promoting deletions or insertions. Within *Suillus*, phylogenetic analysis are consistent with the hypothesis of an expansion (by gain of sequences such as, for example, intronic ones) of the mtDNAs during evolution from one species to another (Bruns et al., 1989).

The molecular mechanisms for such length mutations are largely unknown. In ascomycetes, much of the size variation was shown to be accounted for by variations in the amount of non-coding sequences (Lang et al., 1983); indeed, the coding capacity of mtDNA differed by the presence or absence, depending of the species, of only a little number of structural genes (Böckelmann et al., 1986).

Organization and location of genes

The basidiomycetous mitochondrial genomes encode the typical genes described in other mitochondrial genomes (Table 2): (i) structural genes for subunits of proteins involved in the respiratory chain and oxydative phosphorylation, i.e. the subunits I, II and III of cytochrome C oxydase, apocytochrome b from the cytochrome C reductase, two or three subunits (6, 8, and 9) from the ATPase complex; (ii) structural genes for components of the mitochondrial protein synthesis machinery, i.e. sequences encoding for the mitochondrial tRNAs and the L-rRNA and S-rRNA.

Additionally, some structural genes were shown to be located on the mitochondrial genome or on a nuclear chromosome, depending on the basidiomycetous species. For example, a gene coding for a ribosome associated protein was detected on the *C. stercorarius* mtDNA by heterologous hybridization with a DNA probe containing the *S. cerevisiae varl* gene. Interestingly, the same probe did not show any related sequence on the smallest mtDNA of *C. cinereus*, but hybridized with the

Table 2. Compilation of mitochondrial genes of different basidiomycetous species.

Species	Reference	Cytochrome C oxidase Subunit I	II	III	Cytochrome C reductase Apocytochrome b	ATP ase Subunit 6	8	9	rDNA L-rRNA	S-rRNA	Ribosomal associated protein
Armillaria ostoyae	Smith and Anderson (1994)	+	nd	+	+	+	+	nd	+	+	nd
Suillus sp.	Bruns and Palmer (1989)	+	+	+	+	+	nd	+	+	+	nd
Agaricus bisporus	Hintz et al. (1988)	nd	nd	+	+	+	+	nd	+	+	nd
Coprinus cinereus	Weber et al. (1986)	+	+	+	+	+	nd	+	+	+	−
Coprinus stercorarius	Weber et al. (1986)	+	+	+	+	+	nd	+	+	+	+
Schizophyllum commune	Specht et al. (1992)	+	+	+	+	+	+	+	−	+	nd
A. aegerita	Moulinier et al. (1992)	+	+	nd	nd	+	+	nd	+	+	nd
Clavicorona pyxidata	Contolini et al. (1992)	+	+	+	+	+	nd	+	+	+	nd
Puccinia graminis	Sock et al. (1993)	+	nd	+	+	nd	nd	nd	+	−	nd
Ustilago maydis	Feng et al. (1991[a])	+	+	+	+	+	nd	nd	+	+	nd

+: presence of the coding sequence, −: no evidence of gene, nd: not determined.

nuclear DNA of this species.

In the same way, transposition of genes were previously shown in *S. cerevisiae* (Farrelly et al., 1983), or in the filamentous ascomycete *N. crassa* (Boogaart et al., 1982) or in the hyphomycete *A. nidulans* (Brown et al., 1984). In these species, the functional gene for the subunit 9 of the ATPase was located on the mitochondrial genome in yeast and on the nuclear one in *N. crassa* and *A. nidulans*. In these two filamentous species, an intact copy of the gene was still present on the mtDNA but appeared to be a silent copy. The subunit 9 of the ATPase was detected on the mtDNA of *C. cinereus* and *C. stercorarius* (Weber et al., 1986), of *Suillus* species (Bruns and Palmer, 1989) and of *Clavicorona pyxidata* (Contolini et al., 1992). Functional study was performed in *Schizophyllum commune* where the subunit 9 of the ATPase was shown to be present as a pseudogene on the mtDNA whereas the functional copy was nuclear encoded (Specht et al., 1992).

In conclusion, by analogy with the ascomycetes, it appears that transposition of a copy of a gene from the mitochondrial genome to the nuclear one may occur during the evolution from one basidiomycetous species to another, even when these species are as closely related as belonging to the same genus.

Location of the mitochondrial genes on the restriction maps was determined by hybridizations with heterologous probes from other ascomycete or basidiomycete species. These probes were previously cloned mitochondrial genes or, in the case of *A. aegerita*, oligonucleotides specific to the 5′ coding part of the yeast genes (Moulinier et al., 1992). The structural gene order on the basidiomycetous mtDNA maps was shown to highly differ, even in closely related species. For example, the differences in gene order between the mitochondrial genome of *C. cinereus* and *C. stercorarius* require at least two molecular rearrangements (inversion and transposition) involving large blocks of genes. In contrast, in *Suillus* species, Bruns et al. (1989) have shown that mtDNA (from 36 kbp to 121 kbp) differed in gene order by only one major rearrangement (transposition). Three additional gene orders were detected in related genera (*Gyrodon, Paragyrodon* and *Xerocomus*) and imputed to either one or two rearrangements.

At that time, no sequence of basidiomycetous mitochondrial gene was published and, consequently, there was no data on the modalities of expression of these genes, i.e. their transcription, the RNA processing and translation and, especially the genetic code used in this subcellular compartment. Yet, the more recent studies of our laboratory on the sequence of the 5′ part of the *A. aegerita Cox I* gene have shown that the TGA codon was not a stop codon in this basidiomycete mitochondria and, by comparison with ascomycetous mitochondrial *Cox I* genic sequences, found to be a tryptophan codon. Additionally, the *A. aegerita Cox I* gene was shown to be spliced in exons and introns and to possess a high degree of homology in sequence and organization with the *Cox I* gene of the ascomycetous fission yeast *Schizosaccharomyces pombe* (Lang, 1984). The intron RNA secondary structures allowed to establish that the first two large introns of *A. aegerita Cox I* gene belonged to the group IB (Michel and Dujon, 1983) and contained coding sequences for potential RNA maturases involved in the splicing events of fungal introns.

Beside the number of structural genes, the large sizes variations between basidiomycetous mitochondrial genomes suggest the presence of numerous non-coding sequences such as: (i) repeated sequences of various sizes mainly involved in rearrangements of the mitochondrial genome, (ii) introns, (iii) Ori sequences.

In this context, the presence of large inverted repeated sequences has been reported in the cultivated species *Agaricus bisporus* (Hintz et al., 1988) and *Agrocybe aegerita* (Barroso et al., 1992). Such sequences were not found on *Armillaria ostoyae* mtDNA (Smith and Anderson, 1994). In *A. aegerita*, the large inverted repeated sequence was found to be involved in a intramolecular homologous recombinational event leading to an inversion of the orientation of the two unique copy regions

(the small single copy region SSC: 24 kbp and the large one LSC: 50 kbp), without any change in mtDNA complexity. However the two orientational isomeres were not equally present in the mitochondria; one form was largely more abundant that the other one (Barroso et al., 1992). In *A. bisporus*, Hintz et al. (1986b) failed to recover such orientational isomers.

The presence of two large intronic group 1B sequences has been reported above in the 5' part of the *A. aegerita Cox I* gene. Within *Suillus*, Bruns et al. (1989) gave indirect evidence for an intronic sequence in the *Cox I* gene of *Suillus luteus*, and for two large introns in the L-rDNA of several *Suillus* species.

Information on the Ori sequences in the basidiomycetous mtDNA is scarce. Yet, restriction fragments from basidiomycetous mtDNA were shown to act as ARS (autonomous replication sequences) in the ascomycete *Saccharomyces cerevisiae*. In this context, Katayose et al. (1986) have shown that a 3.2 kbp fragment from *Lentinula edodes* mtDNA confered to the plasmid YIp32 (a hybrid of pBR322 and of the yeast *Leu2*+gene) the same stability and copy number that an ARS sequence isolated from the *S. cerevisiae* mtDNA did, suggesting important sequences and functions homologies between the *L. edodes* and *S. cerevisiae* mtDNA fragments with ARS activity.

Evolution and molecular rearrangements of the basidiomycetous mitochondrial genomes

Evolution of the mtDNA was mainly studied by detection and analysis of restriction fragment length polymorphism (RFLP). Indeed, the restriction fragments can be considered as taxonomic characteristics and submitted to the analysis by the methods of the numerical taxonomy (Dunn and Everitt, 1982).

In animal mtDNA, the predominant mutations involved in molecular evolution and variability between genomes, are nucleotide substitutions (point mutations). On the contrary, in ascomycetes, mitochondrial genomes were shown to contain numerous length mutations, imputable to rearrangements, such as deletions, insertions or duplications (Taylor, 1986; Taylor et al., 1986). In evolutionary studies, these two kinds of mutations, point and length mutations must be recognized and compared independently. This characterization of the RFLP-generating mutations requires a restriction map of the mitochondrial genome.

In basidiomycetes, assessment of genetic variability was carried out with wild or industrial strains of different species, such as the cultivated mushrooms *Lentinula edodes* (Fukuda et al., 1994), *Pholiota nameko* (Babasaki and Ohomasa, 1993), *Agaricus brunnescens* (=*A. bisporus*), *A. bitorquis* (Hintz et al., 1985), and *Agrocybe aegerita* (Barroso et al., in press), or with strains collected in nature of the ectomycorrhizal *Laccaria* species (Gardes et al., 1991) or of the phytophatogenic *Armillaria* species (Smith and Anderson, 1989).

Initially, no RFLP was evidenced among four commercial canadian strains of *A. bisporus*, supporting the hypothesis that this cultivated mushroom represented essentially a monoculture with little variability (Hintz et al., 1985). Indeed, in contrast, abundant RFLPs were found in mtDNA from 10 wild isolates from the closely related species *A. bitorquis*. Yet, it should be noted that, more recently, four different mitochondrial genotypes were characterized in cultivated and wild strains of *A. bisporus* (Jin et al., 1992). Accordingly, mtDNA variability appears to be high in basidiomycetous fungi.

In this context, Fukuda et al. (1994), by combining the RFLP patterns obtained with *Eco*RI and *Bam*HI endonucleases, assigned 51 *Lentinula edodes* natural strains to 28 different mtDNA genotypes. A similarity matrix between pairs of mtDNA genotypes was submitted to UPGMA and Fitch-Margoliash analyses and showed that mitochondrial genotypes could be divided into five major clusters, closely related to the geographic origin of the strains.

In the same way, in wild strains of the ectomycorrhizal *Laccaria* species (25 *L. bicolor*, 8 *L. laccata*, 3 *L. proxima*, and 2 *L. amethystina*), RFLP analysis revealed a high level of variability. As most of the isolates had an unique overall mitochondrial pattern, the mitochondrial variability can be used for *Laccaria* isolate typing (Gardes et al., 1991a, b).

RFLP studies of mtDNA from *Armillaria* species was carried out with strains collected in a red pine forest plantation in Michigan (Smith et al., 1991, 1994). The analysis allowed to characterize the individual units of *Armillaria* population as genets, i.e. mitotic cell lineages established in a mating of two gametic nuclei. These vegetative clones ranged in size up to 635 m and were estimated to be the largest and oldest (the minimal age of the largest clone was estimated between 227 and 397 years) organisms. From the results, it appears clearly that mtDNA variations, evident at the population level in the study site, did not arise during the vegetative propagation of the clones. The implication of these studies on the mitochondrial heredity study will be developped in the last part of this review.

Finally, *Agrocybe aegerita* constitutes the unique report in which mitochondrial RFLPs were located on the restriction map of the genome (Barroso et al., in press). The analysis of 36 wild strains collected from widely distributed locations in Europe allowed identification of two types of mutations involved in the mitochondrial genome evolution: (i) point mutations which gave strain specific molecular markers and (ii) length mutations due to genome rearrangements, such as deletions, insertions or duplications. These rearrangements were located within two polymorphic regions which varied independently; one carrying the *Cox II* coding sequence, and the other carrying the *Cox I*, *ATP6* and *ATP8* coding sequences. In this context, it is interesting to note that, in *S. cerevisiae*, the three genes have a polycistronic expression (De Zamaroczy and Bernardi, 1986). Within each of the two *A. aegerita* polymorphic regions, the length differences defined only two mitochondrial types, suggesting that these mutations would not be randomly generated but resulted from rearrangemental mechanisms involv-

ing precise nucleotide sequences. For each of the two polymorphic regions, the two molecular types were distributed among the 36 strains, without obvious correlation with their geographic origin and the two mitochondrial types had different frequencies: a major and a minor type were recovered for each polymorphic region. Based on these two polymorphisms, it was possible to define four mitochondrial haplotypes which could be the result of intermolecular recombination between allelic forms present in the population long enough to reach linkage equilibrium. All of the 36 dikaryotic strains contained only a single mitochondrial type, suggesting mitochondrial sorting out after cytoplasmic mixing in *A. aegerita*.

Mitochondrial inheritance in basidiomycetes

Within the basidiomycetes, mitochondrial inheritance has been studied by using RFLP markers to follow mitochondria during matings of *Schizophyllum commune* (Specht et al., 1992), of the edible species *Agaricus bitorquis* (Hintz et al., 1988a), *Agaricus brunnescens* (Jin et al., 1992), *Coprinus cinereus* (May and Taylor, 1988), *Lentinula edodes* and *Pleurotus ostreatus* (Matsumoto and Fukumasa-Nakai, 1993) and of the phytopathogenic *Armillaria species* (Smith et al., 1991) and *Ustilago violacea* (Wilch and Castle, 1990, Wilch et al., 1992).

In the filamentous basidiomycetes, mating between two haploid homokaryotic mycelia possessing compatible incompatibility factors does not rely on organs or cells specialized for sexual reproduction but relies on a fusion (somatogamy) between the two mating vegetative hyphae. After cell fusion, the two hyphae exchange nuclei. A dikaryon with the two parental nuclei is thus formed in the existing thallus of the homokaryon. In most of the species (*Armillaria bulbosa, C. cinereus, L. edodes, P. ostreatus, A. aegerita*), reciprocal (bidirectional) nuclear migration occurs from the junction line of the two mating colonies since donor nuclei extensively migrate through the resident cells of each recipient homokaryon, resulting in two discrete dikaryons having identical pairs of nuclei but different cytoplams. Yet, in *C. cinereus*, mating asymmetry caused by nonreciprocal nuclear migration was described in one third of the matings and could constitute an important part of the reproductive biology of this species (May and Taylor, 1988). In the same way, although nuclear migration was rare in *A. bitorquis*, an unidirectional nuclear migration was described in this species (Hintz et al., 1988a). More precisely, a homokaryotic strain was identified, which, when paired with a compatible homokaryon, donates nuclei which migrate unilaterally through the resident mycelium of this later strain (recipient strain). In the closely related species *A. bisporus*, no nuclear migration was observed (Jin et al., 1992).

In all these cases, plasmogamy may result in the production of mycelial colonies composed of sectors differing in mtDNA (mitochondrial mosaics). This phenomenon is strengthened by the fact that numerous hyphal fusions are involved in the process of mating between the two parental homokaryons.

Mitochondrial inheritance was studied in dikaryons taken off from the following locations on the petri dish where the mating took place: on the junction line between the two homokaryotic strains and in regions distal from this line, on each parental side. In all cases, mitochondrai mixing was never observed in dikaryons from the regions distal from the junction line, suggesting the lack of mitochondrial migration with the nuclei. The mitochondrial type of these dikaryons was the type of the parental homokaryon located on the side from which the sample was taken.

On the junction line, the situation was more complex and showed important variations with the studied species. In *C. cinereus* (May and Taylor, 1988) and *L. edodes*, no evidence for mitochondria mixing was observed. Indeed, Matsumoto and Fukumasa-Nakai (1993), described the observance of only either type of parental mtDNAs in dikaryotic protoplasts regenerated from chimeric dikaryons of *L. edodes*, suggesting a uniparental inheritance of mtDNA without mtDNA mixing. Similarly, in *C. cinereus* where two types of mitochondrial markers were used to follow mitochondria during the fungal life cycle (RFLPs and the selectable biochemical marker chloramphenicol resistance), only one dikaryotic sample showing the two mitochondrial types was recovered from the junction line; but there was no evidence for the presence of the two parental mitochondrial types in the same hyphae and no recombinant mtDNA was observed, even by using the more sensitive test constituted by the selectable biochemical marker (chloramphenicol resistance). However, it should be noted that these results were in contrast with the previous reports of Baptista-Ferreira et al. (1983) and Economou et al. (1987) who frequently recovered mtDNA recombinants in *C. cinereus*. In this case, the recombination was detected using the mitochondrial gene mutation acu-10, which caused a cytochrome oxydase aa3 defect, and cap-1 mutation which confered chloramphenicol resistance.

In the second case, for *A. bitorquis, A. bisporus, A. bulbosa, P. ostreatus*, and *U. violacea*, analysis of the dikaryons collected in the junction zone gave evidence for a mitochondria mixing. This mixing is sometimes followed by recombinational events between the mitochondrial genomes.

In *A. bitorquis*, Hintz et al. (1988a) described chimeric dikaryons at the junction of the two monokaryotic cultures. A segregation of the two mitochondrial types was observed during vegetative growth of such chimeric dikaryons. More precisely, one mitochondrial type was lost after a few serial transfers. However, the two parental mitochondrial types were observed in fruiting bodies differentiated by such a chimeric dikaryon. When protoplasts were produced from a chimeric dikaryon, homokaryotic mycelia carrying a unique mitochondrial type were observed; and, in some cases, the resulting nucleus-mitochondria association differed from that of the two mated homokaryons. Thus, for this species, the results are in favor of the presence of the two mitochondrial types in the same hyphae.

In *P. ostreatus*, in the mtDNA restriction patterns of dikaryons from the junction zone, the appearance of bands that were not found in each of the two parental homokaryons and, at the same time, the disappearance of some parental bands were observed. Therefore, these results suggest a frequent occurence of mtDNA recombination in *P. ostreatus* dikaryons from the junction zone.

In the phytopathogenic *Ustilago violacea*, haploid yeast-like cells of opposite mating types (a1 and a2) that had different mitochondrial types, were selected from different geographic isolates. The haploid cells were mated, then treated with alpha-tocopherol (Vitamin E) to induce formation of dikaryotic hyphae. Upon depletion of the alpha-tocopherol, the hyphae budded off haploid cells. In these haploids expressing the a1 mating type, mitochondria from either parents were observed with equal frequencies. In haploids with the a2 mating type, mitochondria were almost exclusively (94%) from the a2 parent. Thus, it appears that, in *Ustilago violacea*, mitochondria mixing occurs during plasmogamy but that nuclear-mitochondria relations are critical in transmission of the mitochondrial type to the homokaryotic progeny and induce a strong bias in mtDNA inheritance.

The same type of bias was observed in *Agaricus bisporus*, during homokaryon pairing. Indeed, despite a heteroplasmon (heterokaryon containing nuclear and mitochondrial genomes from both parental homokaryons) can be formed at the junction zone, the uniparental inheritance observed in most of the studied crosses suggests a very strong bias toward the transmission of the mtDNA from one of the parental homokaryons (Jin et al., 1992).

Additionally, RFLP analysis of *Armillaria* strains collected in a North American (Michigan) red pine plantation, allowed a comparison of mitochondrial heredity rules deduced from laboratory matings with the observed transmission and propagation in a natural local population (Smith et al., 1991). On the basis of mating-type alleles, six different vegetative clones were identified, in sizes up to more than 600 m. A unique mtDNA restriction fragment pattern, corresponding to a unique mitochondrial type, was found in each vegetative clone. These results suggest that in *Armillaria*, mitochondria were uniparentally inherited or submitted to a rapid segregation and (or) elimination of mitochondria. A recent report on RFLP patterns of *A. ostoyae* genets in the same pine plantation, strengthens the hypothesis of a uniparental mtDNA transmission (Smith and Anderson, 1994). This uniparental (maternal) inheritance would be due to anisogamous sexual mating events (in which a newly established "female" monokaryon would be fertilized by an incoming compatible basidiospore or germling). The cytoplasmic input of this "male" gamete would be minimal and rapidly lost from the resulting fertile mycelium.

In conclusion, from the studies on mitochondrial heredity in basidiomycetous species, it appears that, in these somatogamic species, the nuclei migration is not followed by a mitochondrial one. In most of the species, the mating of homokaryotic mycelia leads to a mitochondria mixing, located at the junction line. However, in some species (*C. cinereus, L. edodes*), there is no evidence for such a mixing. When detected, the cytoplasmic mixing begins as a transient stage of heteroplasmy, in which recombination may occur; this stage appears to be generally followed by a rapid sorting out of mitochondria, i.e. the selection or elimination of one mitochondrial type in the recipient hyphae. Generally, the mitochondrial type associated with the donor nuclei was eliminated. At this time, the mechanisms implied in this mitochondrial selection are unknown.

Conclusion

The studies on basidiomycete mitochondrial genomes provide numerous amounts of information with important repercussions on fundamental as well as applied fields of mycology. This information is particularly interesting to furnish performant molecular markers (RFLPs) which allow a precise characterization of species and, within a species, constitute strain-specific markers.

Moreover, these markers will be useful to correlate sequences of the mitochondrial genome with physiologically important and, at this time, seemingly unrelated functions.

The analysis of the complex mitochondrial heredity of basidiomycetes will allow a better understanding of the transmission of mitochondria in eukaryotic life cycles and especially a characterization of the molecular mechanisms involved in mitochondria selection after plasmogamy in these somatogamic species and, certainly, in other eukaryotic cells.

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