Linear versus circular mitochondrial genomes: intraspecies variability of mitochondrial genome architecture in *Candida parapsilosis*

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**INTRODUCTION**

Although the yeast *Candida parapsilosis* is considered to be a benign commensal micro-organism in healthy individuals, it is frequently associated with cases of severe infection in patients with diminished immune function (Weems, 1992; Hazen, 1995; Garber, 2001; Arendrup et al., 2002). Several recent surveys have described genetic and/or genomic heterogeneity among *C. parapsilosis* isolates (Lott et al., 1993; Branchini et al., 1994; Cassone et al., 1995; Pfaffer et al., 1995; De Bernardis et al., 1999). Data from DNA–DNA reassociation, restriction length polymorphism (RFLP) and isoenzyme profiling suggest that the form species *C. parapsilosis* consists of the three variant groups that may even represent three distinct species (Lin et al., 1995; Roy & Meyer, 1998). This conclusion was further supported by comparison of the DNA sequence within the internal transcribed spacer (ITS) of the rDNA and D1/D2 domain of the gene encoding 26S rRNA (Kurtzman & Robnett, 1998). Although strains from each group have been found in samples from human patients (Lin et al., 1995; Roy & Meyer, 1998; Enger et al., 2001), strains from group I, including the type strain of the species (CBS 604T/ATCC 22019T), seem to predominate among clinical isolates. One of the problems that hampers epidemiological studies is the incorrect identification of group, or even species, which may result from using conventional diagnostic methods (Fenn et al., 1994; Ramani et al., 1998). To solve this problem several approaches employing molecular techniques, such as electrophoretic karyotyping, PCR, randomly amplified polymorphic DNA (RAPD) and DNA fingerprinting have been developed for species- and group-specific identification of *C. parapsilosis* (Carruba et al., 1991; Branchini et al., 1994; Cassone et al., 1995; Pfaffer et al., 1995; Pontieri et al., 1996, 2001; Enger et al., 2001). In addition, several studies have reported that molecular markers derived from mitochondrial DNA (mtDNA) are applicable in molecular diagnostics of *C. parapsilosis* isolates (Camougrand et al., 1988; Su & Meyer, 1989, 1991; Yokoyama et al., 2000; Nosek et al., 2002). Importantly, studies of genetic organization of mtDNA in *C. parapsilosis* have uncovered that this yeast, in contrast to the related species *C. albicans* and *C. tropicalis*, possesses a linear mitochondrial genome terminating with specific structures termed mitochondrial telomereres (Kovac et al., 1984; Nosek et al., 1984; Yokoyama et al., 2000; Nosek et al., 2002).
et al., 1995). Due to the uniqueness of mitochondrial telomeres in C. parapsilosis compared to related species, these structures may represent a promising diagnostic or therapeutic target (Nosek et al., 1998, 2002).

The three groups of C. parapsilosis have been defined without any knowledge regarding structural differences in their mtDNA. In this report, we investigated the variability of mitochondrial genomes in the strains from different C. parapsilosis groups and compared their molecular profiles with the closely related species, Lodderomyces elongisporus. The analysis revealed a group-specific pattern at the level of restriction fragments of C. parapsilosis mtDNA. More importantly, the results indicate the striking differences between groups regarding the molecular architecture of the mitochondrial genophore. The presence of variant mitochondrial genomes of mtDNA in strains belonging to the same species opens up the unique possibility to analyse the structural differences between linear- and circular-mapping mtDNA, specifically questions concerning the evolutionary origin and replication strategy of linear mitochondrial genomes. Therefore, we developed a strategy for identification of strains with rearrangements in the telomeric region of the mtDNA. This effort uncovered several strains that might be considered as mutants in the mitochondrial telomeres.

METHODS

Yeast strains. Strains (Table 1) designated CBS were from the Centraalbureau voor Schimmelcultures (Delft, The Netherlands); MCO and PL strains were kindly provided by P. F. Lehmann (Medical College of Ohio, Toledo, USA) and S. A. Meyer (Georgia State University, Atlanta, USA), respectively. Yeast cultures were grown in YPD medium [1% (w/v) yeast extract (Difco), 2% (w/v) Bacto Peptone (Difco), 2% (w/v) glucose] at 28 °C. Biotyping profiles of yeast strains were confirmed by using an API20C kit (Biomerieux) according to the manufacturer’s instructions.

Electrophoretic karyotyping. Samples of chromosomal DNA prepared in agarose blocks were separated in a 0–8% (w/v) agarose gel in 45 mM Tris/borate–1 mM EDTA buffer. Electrophoresis was carried out in a Pulsaphor apparatus (LKB) in a contour-clamped homogeneous electric field (CHEF) configuration with the pulse switching from 60 to 600 s (interpolation) for 72 h at 100 V and 9 °C throughout.

Comparison of ITS rDNA sequences. Total cellular DNA was isolated from 5 ml yeast cultures as described by Philippson et al. (1991). Subsequently, the ITS region of the rDNA was amplified using ITS oligonucleotide primers (Table 2) in a reaction with a 10:1 mixture of Taq DNA polymerase (Gibco-BRL) and Vent DNA polymerase (New England Biolabs). The resulting PCR products were cloned into the pDrive cloning vector (Qiagen) and their sequences were determined as indicated below.

Analysis of mtDNA. Screening for linear mtDNA by PFGE was performed as described by Fukuhara et al. (1993). Briefly, whole-cell DNA samples were prepared in agarose blocks and separated in a 0–8–1% (w/v) agarose gel in 45 mM Tris/borate–1 mM EDTA buffer using a Pulsaphor apparatus (LKB) in CHEF configuration with pulse switching from 5 to 50 s (interpolation) for 24 h at 150 V and 9 °C throughout. Restriction fragment patterns of mtDNAs were

examined on small-scale preparations (Defontaine et al., 1991) using the restriction endonucleases HindIII, EcoRV, PvuI or KpnI (New England Biolabs) and separated by agarose gel electrophoresis. For physical mapping and cloning purposes mtDNA was prepared and purified by CsCl gradient centrifugation as described previously (Nosek et al., 1995). The HindIII and EcoRV restriction fragments of mtDNA were cloned into the corresponding sites of the pCR-Script-Amp®-SK(+) plasmid vector (Stratagene) and the sequences at the boundaries of inserts were determined using universal (M13) forward and reverse primers.

PCR analysis. The analysis was performed as described previously (Nosek et al., 2002) with modifications in the primer pairs (Table 2). Briefly, amplification reactions (20–50 µl) were performed using Taq DNA polymerase (Gibco-BRL) on cell lysates or isolated total cellular DNA (see above). Reactions were done in a thermal cycler (Biometra) using a standard three-step programme: 3 min at 95 °C, followed by 25–30 cycles of 45 s at 94 °C, 1 min at 43–49 °C and 1 min at 72 °C, with a final step of 3 min at 72 °C. Samples were then separated by agarose gel electrophoresis (1–0.5–1.5%, w/v agarose containing 0.5 µg ethidium bromide ml–1) at 5–10 V cm–1 for 45–60 min in 45 mM Tris/borate–1 mM EDTA buffer.

Miscellaneous. Enzymic DNA manipulations, Southern blot analysis and cloning procedures were performed as described by Sambrook & Russell (2001). The DNA sequence was determined using the BigDye terminator sequencing kit and an ABI 310 automatic analyser (Applied Biosystems). Sequence assembly and analysis was performed using the Vector NTI package (Informax) and BLAST (http://www.ncbi.nlm.nih.gov/blast/). The sequence alignments and tree calculation were done using the AlignX program (Informax).

Reproducibility of results. All PCR and PFGE analyses were repeated at least twice with the same results.

RESULTS

Strains of C. parapsilosis display group-specific molecular profiles of mtDNA

The variability of strains (Table 1) belonging to genetically distinct groups of C. parapsilosis was examined by PFGE and by restriction enzyme analysis of mtDNA. The results of both approaches indicate that electrophoretic karyotypes as well as patterns of mtDNA restriction fragments are group-specific features (Figs 1 and 2). Only a minor polymorphism in chromosome length was found among strains of the same group (data not shown). However, mtDNA-derived profiles appear to be highly conserved within groups I and III, while some heterogeneity was observed among strains in group II (see below). Strain CBS 5301, whose original classification as C. parapsilosis was questioned recently (Nosek et al., 2002), displays a profile unrelated to any of the three groups (lane 4 in Figs 1 and 2). To examine its relationship to distinct groups of C. parapsilosis and the closest related species, L. elongisporus, the sequences of the ITS region of the rDNAs were determined and compared. As described by Lin et al. (1995) and Enger et al. (2001), the strains from distinct C. parapsilosis groups form three adjacent branches on the phylogenetic tree. However, the ITS sequence of CBS 5301 clearly clusters with the sequences of two strains of L. elongisporus (CBS 2603T and CBS 2606; Fig. 3). Together
with the electrophoretic karyotype and restriction enzyme analysis of mtDNA, the results strongly suggest that CBS 5301 represents an anamorphic strain of *L. elongisporus*.

**Physical mapping of mtDNA in different groups of C. parapsilosis**

Previous investigations demonstrated that the mitochondrial genome in *C. parapsilosis* CBS 7157 (SR23) consists of a population of linear DNA molecules that terminate with a variable number of the 738 bp unit repeated in tandem (Nosek et al., 1995). Southern hybridization and subsequent DNA sequence analysis uncovered a standard set of mitochondrial genes starting with *nad3* near the left telomere and terminating with *atp6* close to the right telomere (Fig. 4) (Kovac et al., 1984; Nosek & Fukuhara, 1994a, b; Nosek et al., 1995; J. Nosek, M. Novotna, Z. Hlavatovicova, D. W. Ussery, J. Fajkus & L. Tomaska, unpublished). Results of the restriction enzyme analysis of mtDNA by *Hin*dIII, *Eco*RV, *Pvu*II and *Kpn*I indicated that the restriction fragment patterns seem to be constant among group I strains. This is in agreement with results

### Table 1. List of yeast strains and summary of molecular profiling results

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Group*</th>
<th>Linear mtDNA†</th>
<th>TMC‡</th>
<th>PCR§</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>rDNA TEL-A</td>
<td>TEL-B</td>
<td>TEL-C</td>
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<tr>
<td><em>C. parapsilosis</em></td>
<td>CBS 604&lt;sup&gt;T&lt;/sup&gt;</td>
<td>I</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>CBS 1954</td>
<td>I</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>CBS 2152</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>CBS 5301</td>
<td><em>L.e.</em></td>
<td>-</td>
<td>-</td>
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<tr>
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<td>I</td>
<td>+</td>
<td>+</td>
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<tr>
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<td>I</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>MCO 456&lt;sup&gt;II&lt;/sup&gt;</td>
<td>I</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>MCO 457&lt;sup&gt;II&lt;/sup&gt;</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>MCO 462&lt;sup&gt;II&lt;/sup&gt;</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>MCO 471&lt;sup&gt;II&lt;/sup&gt;</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>PL 429&lt;sup&gt;III&lt;/sup&gt;</td>
<td>I</td>
<td>+</td>
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<td>PL 448</td>
<td>III</td>
<td>-</td>
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<tr>
<td>PL 452&lt;sup&gt;II&lt;/sup&gt;</td>
<td>I</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>L. elongisporus</em></td>
<td>CBS 2605&lt;sup&gt;T&lt;/sup&gt;</td>
<td>-</td>
<td>NA</td>
<td>+</td>
<td>-</td>
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<tr>
<td>CBS 6181</td>
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<td>NA</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>CBS 6182</td>
<td>-</td>
<td>NA</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*C. parapsilosis* group is as defined by Lin et al. (1995) and Roy & Meyer (1998). *L.e.*, Strain originally classified as *C. parapsilosis*; however, molecular analyses described in this work indicate that this strain belongs to *L. elongisporus*.

†Presence/absence of a distinct band corresponding to linear mtDNA after separation by PFGE (Fig. 1b).

‡Presence/absence of mitochondrial telomeric minicircles (TMC; Fig. 5). NA, Not analysed.

§Summary of PCR analysis (for details see Methods, Table 2 and Fig. 6).

||Identical to the type strain (CBS 604<sup>T</sup>/ATCC 22019<sup>T</sup>).

Since strains MCO 456 and MCO 448 from groups II and III, respectively, exhibit restriction fragment patterns different from the type strain CBS 604 T and other group I strains, it was of interest to find whether these alterations represent an intraspecies polymorphism or reflect profound differences in genetic organization of the mtDNA. Restriction enzyme mapping of mtDNA by a combination of single- and double-digestions with restriction endonucleases indicate that strains CBS 2916 and MCO 448 (group III) possess linear-mapping mitochondrial genomes, while MCO 456 (group II) displays a circular-mapping mtDNA (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4). These results are in agreement with PFGE analysis (Fig. 4).

Table 2. Sequences of oligonucleotide primer pairs

<table>
<thead>
<tr>
<th>Primer specificity</th>
<th>Sequence (5’–3’)</th>
</tr>
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<tbody>
<tr>
<td>ITS</td>
<td>GTCGTAACAGGTTTCCGTAAGGTA</td>
</tr>
<tr>
<td>rDNA</td>
<td>CAAAGATACCTCTCAGAATTACAAC</td>
</tr>
<tr>
<td>TEL-A</td>
<td>TAAAATAGAGAGAAATGATATATATTTTAC</td>
</tr>
<tr>
<td>TEL-B</td>
<td>TCTTTTTACTATTATTAGTTTAGATAATTT</td>
</tr>
<tr>
<td>TEL-C</td>
<td>AGGATAATAGAGAGGTAAGGA</td>
</tr>
<tr>
<td>TEL-D</td>
<td>TAAATTATGATATGTTTGCTATATCTTA</td>
</tr>
</tbody>
</table>

Fig. 1. PFGE analysis. DNA samples were prepared and separated by PFGE using a programme for chromosomal (a) and mtDNA separation (b) as described in Methods. Note that circular-mapping mitochondrial genomes are represented mainly by polydisperse linear mtDNA molecules (Williamson, 2002) and, in contrast to linear-mapping mtDNAs, do not exhibit a distinct band in PFGE (Fukuhara et al., 1993). Lanes: 1, C. parapsilosis CBS 604 T (group I); 2, MCO 456 (II); 3, MCO 448 (III); 4, CBS 5301; 5, L. elongisporus CBS 2605 T; 6, CBS 2606; 7, CBS 6120.

Fig. 2. Comparison of C. parapsilosis and L. elongisporus strains. Samples of mtDNA were digested with EcoRV (a) and PvuII (b) and separated by agarose gel electrophoresis (see Methods). Lanes: 1, C. parapsilosis CBS 604 T (group I); 2, MCO 456 (II); 3, MCO 448 (III); 4, CBS 5301; 5, L. elongisporus CBS 2605 T; 6, CBS 2606; 7, CBS 6120; M, molecular mass marker (λ DNA digested with PstI).
linear mitochondrial genome in the group I and III strains, but not in the group II strain (Fig. 1b).

To study the genetic organization of mtDNA in strains from groups II and III, the plasmid libraries of EcoRV and HindIII fragments of mtDNA from strains MCO 456 and MCO 448 were constructed. Next, the gene contents in both mtDNAs were inferred by sequencing the termini of the restriction fragments inserted within the plasmid vector. The data were then compared with the GenBank database as well as with the complete sequence of mtDNA of the group I strain CBS 7157 (Nosek & Fukuhara, 1994a, b; Nosek et al., 1995; J. Nosek, M. Novotna, Z. Hlavatovicova, D. W. Ussery, J. Fajkus & L. Tomaska, unpublished). This approach led to the identification of nad1-5, cytb, atp6 and rrnL genes in MCO 456 and nad1-5, cytb, cox1, cox3, atp6 and rrnL in MCO 448. Subsequently, these sequences were localized on the restriction enzyme maps of both mtDNAs. The results indicate that mitochondrial genomes in group II and III strains have the same gene order as found in group I strains (Fig. 4). In several cases the coding regions were fully sequenced and the data show a significant degree of homology when compared with their counterparts from group I (Table 3).

The overall length of the mitochondrial genome was estimated to be approximately 23 kbp in both MCO 456 (group II) and MCO 448 (group III) strains. Since mtDNA of CBS 7157 (30-9 kbp) contains two and three introns within the cytb and cox1 genes, respectively (J. Nosek, M. Novotna, Z. Hlavatovicova, D. W. Ussery, J. Fajkus & L. Tomaska, unpublished), shorter mtDNAs in group II and III strains may reflect the absence of some of these sequences. Our preliminary results support this idea, since a partial DNA sequence analysis of the cytb gene indicated that strain MCO 448 lacks the bi2 intron (data not shown).

Mitochondrial telomeres differ between strains belonging to the variant groups

DNA sequence analysis of terminal regions uncovered that linear mitochondrial genomes from strains in groups I (CBS 7157) and III (MCO 448) differ in the sequence of their telomeric regions. While the mitochondrial telomeres in the strain belonging to group I consist of arrays of tandem repeats of a 738 bp unit (Nosek et al., 1995), the telomeric repeat in the group III strain is remarkably shorter (620 bp). However, the two sequences possess highly conserved regions and overall identity is 72% (for details see the GenBank/EMBL database entries AY391851, AY391852, X76196 and X76197).
Survey of \textit{C. parapsilosis} strains

To analyse the frequency of linear- and circular-mapping mtDNA in this species, we employed the PFGE method for screening of linear mitochondrial genomes (Fukuhara et al., 1993). This approach revealed a distinct band corresponding to linear mtDNA molecules in almost all strains from groups I and III, but only in one group II strain (MCO 471; Table 1).

Mitochondria of strain CBS 7157 harbour extragenomic minicircular DNA molecules derived solely from the mitochondrial telomere repeat motif that seem to play a key role in the dynamics of the linear mtDNA terminal structures (Tomaska et al., 2000; Nosek & Tomaska, 2002). Therefore, we examined their occurrence in strains from the three groups by probing Southern blots of electrophoretically separated undigested total cellular DNA with an \( [\alpha^32\text{P}]d\text{CTP} \)-labelled telomeric probe. The results show that the presence of the minicircles (Fig. 5) correlate with the linear form of mtDNA (Table 1), supporting the hypothesis that minicircles are involved in mitochondrial telomere maintenance.

Recently, we have demonstrated that mitochondrial telomeres represent specific molecular markers with potential in molecular diagnostics of \textit{C. parapsilosis} (Nosek et al., 2002). Based on the differences in the mitochondrial telomeres of CBS 7157 and MCO 448 and in the corresponding region (\textit{nad3–atp6}) of the circular-mapping mtDNA of MCO 456, we designed a panel of oligonucleotide primers derived from telomeric and subtelomeric regions (Table 2). Subsequent PCR analysis (Fig. 6, Table 1) showed that these primers allow the identification of strains with alterations within the telomeric regions of their mitochondrial

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**Table 3.** Percentage identity of DNA sequences and deduced protein products of mitochondrial genes among strains from the three different \textit{C. parapsilosis} groups and \textit{C. albicans} (GenBank accession no. AF285261)

Numbers in parentheses indicate the homology when conservative amino acid substitutions are included. NA, Complete sequence is not available.

<table>
<thead>
<tr>
<th>Group I (CBS 7157)</th>
<th>Group II (MCO 456)</th>
<th>Group III (MCO 448)</th>
<th>\textit{C. albicans}</th>
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</thead>
<tbody>
<tr>
<td>\textit{nad2}/\textit{Nad2p}</td>
<td>NA</td>
<td>95·1/96·6 (99·8)</td>
<td>69·9/60·8 (86·4)</td>
</tr>
<tr>
<td>\textit{nad3}/\textit{Nad3p}</td>
<td>95·9/96·2 (100)</td>
<td>94·1/95·4 (100)</td>
<td>69·5/66·4 (90·8)</td>
</tr>
<tr>
<td>\textit{atp6}/\textit{Atp6p}</td>
<td>94·2/98·0 (100)</td>
<td>NA</td>
<td>77·6/81·3 (96·3)</td>
</tr>
<tr>
<td>\textit{trnR}</td>
<td>NA</td>
<td>100</td>
<td>83·1</td>
</tr>
<tr>
<td>\textit{trnS}</td>
<td>NA</td>
<td>100</td>
<td>96·4</td>
</tr>
</tbody>
</table>

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**Fig. 5.** Screening for the presence of mitochondrial telomeric minicircles by Southern blot analysis. Samples of undigested total cellular DNA were electrophoretically separated in a 1% agarose gel and transferred onto a nylon membrane. Blots were then hybridized with a radioactively labelled probe derived from the 620 bp \textit{EcoRV} telomeric fragment from mtDNA of strain MCO 448 (group III). The scale pattern indicates the presence of multimers of the telomeric minicircular DNAs. Lanes: 1, CBS 604\textsuperscript{T} (group I); 2, CBS 1954 (I); 3, CBS 2152 (I); 4, CBS 2193 (I); 5, CBS 2194 (I); 6, CBS 2195 (I); 7, CBS 2197 (I); 8, CBS 2211 (I); 9, CBS 2215 (I); 10, CBS 2916 (III); 11, CBS 6318 (I); 12, CBS 7157 (I); 13, CBS 8050 (I); 14, CBS 8181 (I); 15, CBS 5301; 16, MCO 433 (I); 17, MCO 441 (I); 18, MCO 448 (III); 19, MCO 456 (II); 20, MCO 457 (II); 21, MCO 482 (II); 22, MCO 471 (II); 23, MCO 478 (I); 24, PL 429 (III); 25, PL 448 (III); 26, PL 452 (II). All strains except CBS 5301 (lane 15) are \textit{C. parapsilosis}.

**Fig. 6.** Screening for strains with an altered molecular architecture. PCR reactions were performed as described in Methods with the indicated primer pairs (Table 2) and products were electrophoretically separated on a 1·5% agarose gel. Lanes: 1, CBS 604\textsuperscript{T} (group I); 2, MCO 456 (II); 3, MCO 448 (III); 4, CBS 2194 (I); 5, MCO 457 (II); 6, MCO 462 (II); 7, MCO 471 (II); 8, PL 448 (III); 9, PL 452 (II).
Polymorphisms in mtDNA

In contrast to the conserved restriction fragment profiles of mtDNA in strains from the groups I and III, we observed a polymorphism within group II (Fig. 7a). The variability is within the sequence between the genes *rrnL* and *nad4* and is present on the 5·0 kbp *EcoRV* fragment in strain MCO 456. In strains MCO 457, MCO 462, MCO 471 and PL 452, the length of the corresponding restriction fragment is approximately 6·5 kbp. The mtDNA of strain PL 452 also contains an additional 0·6 kbp *EcoRV* fragment, the position of which was not precisely determined. Since mtDNAs of strains from groups I and III contain the *cox1* gene within this region, it is possible that the differences are attributed to the presence of intronic sequence(s) that might be absent in strain MCO 456. The polymorphism also affects the largest *EcoRV* fragment. In strains MCO 456, MCO 457, MCO 462 and PL 452 the length of this fragment varies from 13·0 to 13·5 kbp while in strain MCO 471 this same region is divided into two separate fragments of approximately 6·8 kbp split exactly between the *atp6* and *nad3* genes. This corresponds with the results of PFGE analysis that indicate the presence of a linear mitochondrial genome in MCO 471 (Table 1) and suggests that this strain has the molecular architecture of mtDNA typical of group I and III strains.

Restriction enzyme mapping using *EcoRV* and *HindIII* localized the polymorphic region between genes *atp6* and *nad3* (Fig. 7b). The 1·1 kbp *HindIII* fragment of MCO 456 varies in size from 0·45 to 1·3 kbp in strains MCO 457, MCO 462 and PL 452. Interestingly, four non-stoichiometric bands produced by *HindIII* (0·9, 1·0, 1·2 and 1·3 kbp) that map in this region were observed in strain MCO 462, indicating either instability of this region or a heteroplasmic state of the cells (Fig. 7a, lane 3). Furthermore, the circular-mapping mtDNA of strain PL 448 lacks telomeres and possesses a restriction fragment corresponding to the fusion of the left and right termini (Fig. 7c). This indicates that, similar to group II strains, mitochondrial genomes identified in group III strains have identical genetic organization and differ only in their linear/circular state.

DISCUSSION

Taxonomic remarks

Differences among *C. parapsilosis* isolates led to the suggestion that distinct groups may represent separate species (Lin et al., 1995). A relatively low degree of DNA relatedness among the groups argues for this possibility (Roy & Meyer, 1998). Kurtzman & Robnett (1997, 1998) found that strain NRRL Y-17456 belonging to group II differs from the type strain (CBS 604^T^/ATCC 22019^T^) by 6 nt in the D1/D2 genomes. To confirm the results, restriction fragment maps of mtDNA were constructed and compared (Fig. 7).
domain of rRNA and concluded that this strain represents a new species of *Candida*. Although comparisons of rRNA sequences are successfully employed in the molecular taxonomy of yeasts, in an evolutionary perspective, a species does not necessarily need to be homogeneous in molecular terms and therefore biological criteria for the species need to be examined (Naumov, 1987). However, crossing strains from different groups followed by an analysis of fertility in the progeny is not possible in yeasts where the sexual state is unknown. Regardless of this taxonomical problem, the data presented in this work clearly show that genetic organization as well as coding sequences in mtDNA are highly conserved among the three groups of *C. parapsilosis*, pointing to their close phylogenetic relationship. Moreover, the results indicate that mtDNA profiles enable discrimination between the groups, exemplifying that investigations of mtDNA polymorphism represent a powerful tool to distinguish subspecies variants or close, recently separated species.

**Evolutionary implications**

The existence of linear genophores in mitochondria of diverse phylogenetic taxa evokes questions concerning their evolutionary origin and relatedness to circular-mapping mtDNAs. In several cases it has been observed that the form of mitochondrial genome may differ among closely related species (Bridge et al., 1992; Fukuhara et al., 1993; Martin, 1995; Laflamme & Lee, 2003). Investigations of mtDNA-binding proteins indicate that similar machineries are responsible for the maintenance of both forms (Miyakawa et al., 1995, 1996; Nosek et al., 1999; Kaufman et al., 2000; Tomaska et al., 2001). The occurrence of linear- and circular-mapping genomes in different strains of the same species indicates that both types of mitochondrial genome do not differ radically in their life styles (Fukuhara et al., 1993). One such example has been reported in two strains of the saturn-spored yeast, *Williopsis suaveolens*. While mitochondria of strain CBS 1670 with a linear-mapping genome harbour mtDNA molecules terminating with inverted repeats closed by a single-stranded hairpin loop, strain CBS 255 possesses a mitochondrial genome with similar genetic organization except that its map is circular (Fukuhara et al., 1993; Drissi et al., 1994). Here we report another example of intraspecies variability in mtDNA architecture in several strains of *C. parapsilosis*. The structure of the linear mitochondrial genome found in this species substantially differs in the type of mitochondrial telomere from that of *W. suaveolens* (Nosek et al., 1995). While nearly all strains in *C. parapsilosis* groups I and III possess a linear mtDNA that terminates with arrays of tandem repeats, only one group II isolate (MCO 471) has a linear genome in its mitochondria. However, most of the strains in group II and PL 448 from group III display a circular map of their mtDNAs. Moreover, the linear mtDNAs in groups I and III differ in the sequences of their telomeric motifs.

The genetic organization and homology of the coding regions appear to be highly conserved among linear- and circular-mapping mitochondrial genomes found in *C. parapsilosis* strains, indicating that both forms originated from a common ancestor via a relatively simple mechanism. At present it is not possible to make any definitive conclusion as to whether a circular-mapping mtDNA represents either an ancestral form or a rearranged derivative (mutant) that lost the mitochondrial telomeres and solved the ‘end-replication problem’ (Olovnikov, 1971,1973; Watson, 1972) by circularization of the genophore, similar to bacteriophage λ (reviewed by Taylor & Wegrzyn, 1995) or nuclear chromosomes in the telomerase-deficient mutant of the fission yeast *Schizosaccharomyces pombe* (Nakamura et al., 1998).

Mitochondrial telomeres play essentially the same biological role(s) as their nuclear counterparts, i.e. they have to (i) ensure the complete replication of the linear genophore, (ii) mask the ends from DNA repair machinery and (iii) protect them from exonucleolytic degradation and/or end-to-end fusions. Identification of a specific mitochondrial telomere-binding protein (mtTBP) (Tomaska et al., 1997; Nosek et al., 1999) and mitochondrial telomeric loop (t-loop) structures (Tomaska et al., 2002) similar to the t-loops present at the ends of mammalian chromosomes (Griffith et al., 1999) substantiates the analogy. Observation of extragenomic telomeric minicircles in mitochondria of several yeast species (Tomaska et al., 2000) uncovered yet another general theme in telomere biology. Recently, we have demonstrated that the minicircles replicate via a rolling-circle mechanism which generates tandem arrays of the telomeric sequence that may recombine with linear molecules of mtDNA to lengthen the telomeres (L. Tomaska, A. M. Makhov, J. D. Griffith & J. Nosek, unpublished). This may parallel alternative mechanisms of nuclear telomere maintenance involving telomeric small polydisperse circular DNAs detected in several telomerase-negative tumour cell lines (Regev et al., 1998) and *Xenopus* oocytes (Cohen & Mechali, 2002), or an elongation of telomeres by recombination with DNA circles as demonstrated in the yeast *Kluyveromyces lactis* (Natarajan & McEachern, 2002).

Recently, we proposed a hypothesis that mitochondrial telomeres might have evolved from mobile genetic elements (e.g. transposons, plasmids, telomeric minicircles) that invaded mitochondria, integrated into a circular-mapping mtDNA and eventually resulted in the formation of linear mtDNA molecules of defined length, terminating with specific telomeric structures (Nosek & Tomaska, 2002, 2003). Insertions and excisions of such elements may represent a simple mechanism for switching between the two forms of the mitochondrial genophore. Some support for this idea comes from the correlation between the occurrence of the linear-/circular-mapping genome and the presence/absence of extragenomic telomeric minicircles (Fig. 5, Table 1). One possibility, although speculative, is that some drugs might interfere with the replication of
mitochondrial telomeres and/or telomeric minicircles thus selecting for the formation of a circular-mapping genome without any apparent changes in mitochondrial physiology and/or maintenance of the mtDNA. Hence, the variability in the telomeric region might be generated by antifungal treatment of patients infected with C. parapsilosis. The identification of a collection of strains with altered mtDNA architecture among C. parapsilosis isolates opens the unique possibility of uncovering molecular mechanism(s) that trigger the alteration of the mtDNA form and may be instrumental in experimental testing of the above hypotheses.

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