MINIREVIEW

Mitochondrial chromosome structure: an insight from analysis of complete yeast genomes

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Abstract

Recent progress in the analysis of protein components of the mitochondrial nucleoid and replisome of baker’s yeast, Saccharomyces cerevisiae, opens a unique opportunity for understanding the molecular principles of mitochondrial inheritance. In this work we identified homologs of proteins involved in the mitochondrial DNA packaging and replication in the complete genome sequence of the petite-negative yeast Kluyveromyces lactis. Comparative analysis of their counterparts from phylogenetically diverse yeast species revealed conserved as well as diverged features of the organellar chromosome structure and its replication strategy. Moreover, it provides a basis for subsequent functional studies of the structure and dynamics of the mitochondrial nucleoids.

Introduction

The concept of organellar nuclei, termed also nucleoids or chromosomes, was introduced into cell biology by Kuroiwa (1982; for a recent review see Sakai et al., 2004). These specific cytological structures are involved in the stabilization, maintenance and inheritance of chloroplast and mitochondrial genomes. Data from many laboratories indicate that nucleoids are fundamental segregating units of mitochondrial DNA (mtDNA) (Okamoto et al., 1998; Zelenaya-Troitskaya et al., 1998; MacAlpine et al., 2000; Zuo et al., 2002; Meeusen & Nunnari, 2003). Investigations of their structure and dynamics provide an opportunity to uncover the molecular principles that govern the mitochondrial inheritance as well as the organellar genome evolution.

Studies of the slime mold, Physarum polycephalum, revealed that the mitochondrial nucleoids contain mtDNA molecules compacted with RNA and proteins into chromatin-like fibers organized into three-dimensional rod-shaped structures bound to the mitochondrial inner membrane (Kawano et al., 1982; Suzuki et al., 1982; Kuroiwa et al., 1994). In baker’s yeast, Saccharomyces cerevisiae, the nucleoids were originally visualized by Miyakawa et al. (1984, 1987), who estimated that a diploid cell contains about 50–70 of these mtDNA-protein complexes exhibiting spherical configuration (0.3–0.6 μm in diameter). The physiological state of the cells, life cycle stages, and treatment with various drugs alter the nucleoid morphology and result in forms such as strings, giant nucleoids or aggregates (Miyakawa et al., 1984, 1988, 1994; Shiiba et al., 1997). The quantitative analysis revealed that the number of nucleoids per cell and the DNA content in individual nucleoids vary depending on the culture conditions. Stationary phase cells grown aerobically contain on average 1.5 genome equivalent (calculated as 75 kbp per genome) per nucleoid, whereas the cells grown anaerobically in the same medium possess giant nucleoids with about 20 genome copies (Miyakawa et al., 2004).
Deeper insight into the structure of mitochondrial chromosomes resulted from biochemical studies of a specific set of proteins associated with the mtDNA. In S. cerevisiae, the major nucleoid component is a high mobility group (HMG) nonhistone protein Abf2p (Caron et al., 1979; Miyakawa et al., 1987; Diffley & Stillman, 1991, 1992). Analyses of its DNA-binding properties revealed that Abf2p sharply bends the DNA backbone, resulting in compaction of double-stranded DNA molecules into 190-nm structures resembling the nucleoids (Brewer et al., 2003). The protein belongs to the HMG-box superfamily, which contains two tandem HMG boxes, mediating their ability to bind the minor groove of DNA in a sequence nonspecific fashion. Importantly, the HMG-box-containing proteins exhibit an affinity to bent or distorted DNA (e.g., four-way DNA junctions, synthetic DNA cruciforms, DNA bulges and cross-overs in supercoiled DNA), and they can cause looping of linear DNA molecules. These biochemical properties, some of which were demonstrated experimentally (Diffley & Stillman, 1991, 1992; Brewer et al., 2003; Fridle et al., 2004), made Abf2p a candidate for a guardian of the yeast mitochondrial genome. Indeed, several reports pointed out that this might be the case. For example, deletion of the ABF2 gene resulted in a rapid loss of mtDNA from cells grown on a fermentable carbon source (Diffley & Stillman, 1991). Furthermore, it was shown that Abf2p influences the level of yeast mtDNA recombination intermediates in vivo (MacAlpine et al., 1998; Zelenaya-Troitskaya et al., 1998). Finally, the ability of bacterial protein HU, human mitochondrial transcriptional activator mtTF1/TFAM and mitochondrially targeted nonhistone protein NHP6A (other members of the HMG-box superfamily) to replace Abf2p functionally both in vivo and in vitro (Kao et al., 1993; Megraw & Chae, 1993; Parisi et al., 1993) indicated that the presence of HMG boxes in otherwise nonhomologous proteins is sufficient for the stability of the organellar genome. In Podospora anserina, mitochondrial HMG-like protein mtHMG1 was shown to protect mtDNA from deletions (Dequard-Chablat & Allandt, 2002). With the exception of two HMG boxes, mtHMG1 does not display structural homology with any mitochondrial HMG-box-containing protein. The repertoire of currently known mtDNA packaging proteins was recently expanded by a protein, Glom, purified from the mitochondrial nucleoids of the slime mold, Physarum polycephalum. Glom has a lysine-rich region with a proline-rich domain in the N-terminal half and two HMG boxes in the C-terminal half (Sasaki et al., 2003).

Two high-throughput studies from Butow’s laboratory resulted in the identification of more than 20 polypeptides in mitochondrial nucleoids of S. cerevisiae (Kaufman et al., 2000; Chen et al., 2005). Some of these proteins have previously been shown to interact with mtDNA or function in mtDNA maintenance. In addition to Abf2p, the nucleoids include Mgm101p, a protein possibly involved in mtDNA repair as well as mtDNA stability (Chen et al., 1993; Meuseen et al., 1999), Rim1p, mitochondrial single-stranded DNA-binding (mtSSB) protein (Dyck et al., 1992), and Ilv5p, a mitochondrial matrix protein catalyzing a step in branched-chain amino acid biosynthesis that serves as a high copy suppressor of mtDNA instability in a Δabf2 strain (Zelenaya-Troitskaya et al., 1995). However, others, such as the mitochondrial chaperones Hsp60p and Hsp10p, the tricarboxylic acid (TCA) cycle proteins Kgd1p, Kgd2p, Lpd1p [three regulatory subunits of α-ketoglutarate dehydrogenase; see also Sato et al. (2002)], Pda1p (catalytic subunit of pyruvate dehydrogenase complex), Aco1p (aconitase), Ald4p (aldehyde dehydrogenase), and Atp1p (α-subunit of F1-ATPase) were not previously known to interact with mtDNA. Genetic analysis confirmed a role for these proteins in the stabilization of mtDNA. Interestingly, Aco1p has been shown to act as a suppressor of Δabf2 mutation, and the mtDNA stabilization mediated by Aco1p is independent of its aconitase activity (Chen et al., 2005). A surprising finding was that the chaperone Hsp60p is an ssDNA binding protein that binds with strand specificity to a putative origin of mtDNA replication (Kaufman et al., 2000). Another unexpected member of the nucleoid complex is Mnp1p, a mitochondrial ribosomal protein (Sato & Miyakawa, 2004). Bi-functionality, apparently a common feature of nucleoid-associated proteins, implies that during the evolution of the eukaryotic cell, the persistence of a remnant of the endosymbiont genome together with its reduction may have necessitated the evolution of mechanisms to retain and express mtDNA. One such mechanism would be the evolution of genes encoding both nuclear and mitochondrial isoforms of the same enzyme, such as Pif1p (Schulz & Zakian, 1994) and Cdc9p (Willer et al., 1999). A second mechanism might require maintaining proteins that have acquired multiple functions, such as Rpo41p, a bacteriophage type RNA polymerase, which functions both as a transcriptase and a stabilizer of the mtDNA (Wang & Shadel, 1999), or Aco1p (see above). The list of nucleoid components may contain additional members as the analysis of animal mitochondria revealed that ADP/ATP carrier (ANT1) and prohibitin (PHB2) associate with the nucleoids (Bogenhagen et al., 2003). Besides the nucleoid components, the mtDNA interacts with several other proteins involved in mtDNA replication, recombination, repair and gene expression (reviewed in Contamine & Picard, 2000). The repertoire of proteins that bind mtDNA was recently extended by Arg5.6p (Hall et al., 2004) and, in higher eukaryotes, Lon protease (Liu et al., 2004).

Although most of the nucleoid components and interacting proteins were already identified, little is known about their biological role(s) in the formation of higher-order
architecture of the mitochondrial chromosome, and its dynamics under various physiological states of the eukaryotic cell. Data on the structure of nucleoids, mtDNA replication and segregation originate mainly from studies of S. cerevisiae. Investigations of species that differ from baker's yeast in physiology, mitochondrial genome organization and stability may uncover novel aspects of the nucleoid architecture and organellar inheritance.

Kluyveromyces lactis is a petite-negative, aerobic, yeast species that does not tolerate the loss of the mtDNA, although it is not dependent on the intact respiratory chain (Clark-Walker & Chen, 1996; Murray et al., 2000). Its mitochondria contain a circular genome with a length of 40 291 bps (Zivanovic et al., 2005). Although almost half the size of the mtDNA of baker's yeast, S. cerevisiae (Foury et al., 1998), it encodes essentially the same set of gene products. These include RNAs of the small (rrnS) and large (rrnL) subunit of the mitochondrial ribosome, a set of 22 transfer RNAs (trn genes), RNA subunit of RNase P (rpm1), apocytochrome b (cob), subunits I, II and III of the cytochrome oxidase (cox1,2,3), three subunits of the ATP synthase (atp6,8,9) and the ribosomal protein (var1). With a few exceptions, such as the presence/absence of the genes encoding subunits of the NADH:ubiquinone oxidoreductase (nad1-6), rpm1 and var1, the mitochondrial genomes of other yeast species have similar genetic makeup (Sekito et al., 1995; Foury et al., 1998; Kerscher et al., 2001; Bullerwell et al., 2003; Koszul et al., 2003; Langkjaer et al., 2003; Nosek et al., 2004; Talla et al., 2005; Zivanovic et al., 2005). As mitochondria of several yeast species (e.g. S. cerevisiae, Candida glabrata) were shown to contain mainly linear concatemeric DNA molecules longer than the genome unit and a relatively small fraction of monomeric circular molecules (Maleszka et al., 1991), it is likely that the molecular architecture of the circular-mapping mtDNA of K. lactis is similar. In spite of the conserved gene contents and possibly also the molecular architecture of the mtDNA, this species displays several differences (Zivanovic et al., 2005). In contrast to S. cerevisiae, the mtDNA seems to play a vital function in the wild-type cells of K. lactis that can be bypassed by mutations (mgi) in the nuclear genes ATP1, 2 and 3 coding for the subunits of F1 ATP synthase (Chen & Clark-Walker, 1993, 1996). Moreover, K. lactis mtDNA lacks canonical ori/rep elements found in the mtDNA of baker's yeast, contains different types of guanine and cytosine (G+C) rich clusters and uses a different variant of the mitochondrial genetic code.

To gain an insight on the evolution of the organellar chromosomes in yeasts and to provide a basis for functional studies of their dynamics and inheritance, we analyzed the presence of homologs of the components of mitochondrial nucleoids as well as proteins implicated in the mtDNA transactions in the complete genome sequence of K. lactis. The protein sequences were also compared among phylogenetically diverse yeasts. The analysis revealed conserved as well as diverged features of the mitochondrial nucleoid and replisome structure, respectively.

Materials and methods

Genome sequence analysis

The sequences of mitochondrial nucleoid proteins were identified and downloaded from the Saccharomyces genome database (http://www.yeastgenome.org; S. cerevisiae), the Genolevures database (http://cbi.labri.fr/Genolevures; Kluyveromyces lactis, Candida glabrata, Debaryomyces hansenii and Yarrowia lipolytica), the Candida genome database (http://www.candidagenome.org/; C. albicans), the Schizosaccharomyces pombe Gene Database (http://www.genedb.org/genedb/pombe/index.jsp) and the NCBI genomes database (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi?organism=euk). Analyses of genes encoding proteins involved in mitochondrial genome replication, stabilization and inheritance were performed by BLAST searches using S. cerevisiae sequences as queries, and hits were usually found within annotated genes. Depending on the site used and the type of sequences, BlastP, BlastN, tBlastN, or tBlastX were used, usually with default parameters (http://Blast.wustl.edu). Sequence alignments were performed using the Clustal X program (Thompson et al., 1997) and shaded using the GeneDoc utility (Nicholas & Deerfield, 1997).

Miscellaneous analyses

Phylogenetic relationships among yeast species were deduced from alignments of concatenated sequences of proteins encoded by their mtDNA (i.e. atp6-8-9-cob-cox1-2-3) using the MEGA 2.1 package (Kumar et al., 2001) (Fig. 1). Two computational methods were used to predict mitochondrially imported proteins, PSORTII (http://psort.nibb.ac.jp/; Nakai & Kanehisa, 1992) and MITOPROT (http://ihg.gsf.de/ihg/mitoprot.html; Claros & Vincens, 1996). The HMG box domains were detected by SMART (http://dylan.embl-heidelberg.de/; Letunic et al., 2004).

Results and discussion

Structural components of mitochondrial nucleoids

Preliminary analyses detected more than 20 protein components of the Kluyveromyces lactis nucleoids whose profile on the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels was different from that of Saccharomyces cerevisiae (Miyakawa et al., 2003). The study led to
identification of the 17-kDa DNA-binding protein homologous to the Abf2p implicated in the mtDNA packaging. Our survey of the complete genome sequence of *K. lactis* revealed the presence of homologs of essentially all nucleoid components known from baker’s yeast (Table 1). A similar picture resulted from an analysis of the complete genome sequences of three unrelated species, *Candida albicans*, *Schizosaccharomyces pombe* and *Yarrowia lipolytica*, indicating that the fundamental features of the mitochondrial nucleoid structure are highly conserved among phylogenetically distant organisms.

On the other hand, the degree of sequence conservation of individual proteins differs significantly. Although most of the *K. lactis* proteins are highly conserved and the similarity with their *S. cerevisiae* counterparts exceeds 90%, the homology of a number of components (i.e. Abf2p, Cha1p, Ilv6p, Kgd2p, Mgm101p, Mnp1p, Rim1p, Rpo41p, Sls1p) ranges between 65–90%. A similar tendency was observed in other species. For some of these proteins the conservation is very weak, which confront us with the question of the presence of true orthologs. Such is the case of Abf2p, Mnp1p and Sls1p, which are the least conserved components of the *K. lactis* nucleoid. The Abf2p homolog is not even detected in the genomes of *C. albicans*, *Y. lipolytica* and *Sch. pombe*.

Proteins participating in the formation and function of a multicomponent complex are presumed to co-evolve; thus, they would accumulate mutations at similar rates (Fraser et al., 2002). However, our analysis revealed different degrees of sequence conservation of several components of the mitochondrial nucleoids. With a few exceptions, bi-functional nucleoid components are highly conserved (Table 1). One possible explanation is that these proteins may be more sensitive to mutations due to simultaneous selection for their metabolic functions. However, different mutational rates in certain nucleoid components may also reflect evolutionary changes of functional cis-elements in the mtDNA (e.g. ori/rep elements, promoters, transcription initiation and termination signals), replication strategy and/or mode of transmission. The lower degree of sequence similarity that is observed predominantly in the sequences of proteins that are known to interact directly with the mtDNA (Abf2p, Mgm101p, Rim1p, Rpo41p, Mtf1p) supports the hypothesis.

The packaging of mtDNA is presumed to be crucial for its maintenance. However, experiments with baker’s yeast revealed that mutants lacking the Abf2p are able to maintain their mtDNA when propagated on a nonfermentable carbon source, such as glycerol. It has been shown that mitochondrial carrier proteins Yhm1p (= Ggc1p, YDL198C) and Yhm2p (YMR241W), the latter containing a DNA-binding domain (Kao et al., 1996; Cho et al., 1998), and the nucleoid component implicated in branched-chain amino acid synthesis Ilv5p (Zelenaya-Troitskaya et al., 1995) are able to suppress the abf2 mutation. Moreover, Chen et al. (2005)
recently demonstrated that mitochondrial aconitase (Aco1p) substitutes the Abf2p in mtDNA packaging in abf2 mutants under de-repressed conditions. This indicates that baker’s yeasts have a back-up system for the stabilization of mtDNA in the absence of Abf2p. Thus, Abf2p is a dispensable member of the nucleoid and one would expect that its gene is a subject of relatively frequent evolutionary changes. The analysis of yeast genomes supports this idea.

Table 1. Proteins implicated in the mitochondrial nucleoid structure and mtDNA transactions identified in the nuclear genome of Kluyveromyces lactis and their similarity with Saccharomyces cerevisiae counterparts

<table>
<thead>
<tr>
<th>Gene</th>
<th>Saccharomyces cerevisiae systematic name</th>
<th>Kluvveromyces lactis systematic name</th>
<th>Identity/ Homology (%)</th>
<th>Probability of import to mitochondria (MITOPROT) (%)</th>
<th>PSORTII (PSORTII)</th>
</tr>
</thead>
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<tr>
<td>ABF2</td>
<td>YMR072W</td>
<td>KLLA0F01485g</td>
<td>29.8/72.0</td>
<td>0.4012</td>
<td>43.5</td>
</tr>
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<td>ACO1</td>
<td>YLR304C</td>
<td>KLLA0C17314g</td>
<td>86.5/95.9</td>
<td>0.9933</td>
<td>43.5</td>
</tr>
<tr>
<td>ALD4</td>
<td>YOR374W</td>
<td>KLLA0F00440g</td>
<td>55.1/195.4</td>
<td>0.9828</td>
<td>65.2</td>
</tr>
<tr>
<td>ATP1</td>
<td>YBL099W</td>
<td>KLLA0E06644g</td>
<td>89.2/97.8</td>
<td>0.9999</td>
<td>82.6</td>
</tr>
<tr>
<td>CHA1</td>
<td>YCL064C</td>
<td>KLLA0C00440g</td>
<td>51.4/84.2</td>
<td>0.8750</td>
<td>56.5</td>
</tr>
<tr>
<td>HSP10</td>
<td>YOR020C</td>
<td>KLLA0E09284g</td>
<td>83.0/97.2</td>
<td>0.4126</td>
<td>8.7</td>
</tr>
<tr>
<td>HSP60</td>
<td>YLR259C</td>
<td>KLLA0F09449g</td>
<td>87.4/97.7</td>
<td>0.7101</td>
<td>82.6</td>
</tr>
<tr>
<td>IDH1</td>
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<td>78.6/94.2</td>
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<td>0.9559</td>
<td>73.9</td>
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<td>KGD1</td>
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<td>KLLA0F04477g</td>
<td>78.3/94.1</td>
<td>0.9872</td>
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<td>KGD2</td>
<td>YDR148C</td>
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<tr>
<td>RIM1</td>
<td>YCR028C-A</td>
<td>KLLA0A06149g</td>
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<td>0.9403</td>
<td>82.6</td>
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<td>RPO41</td>
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<td>KLLA0D05247g</td>
<td>54.8/81.3</td>
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<td>SLS1</td>
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<td>89.3/97.1</td>
<td>0.9814</td>
<td>34.8</td>
</tr>
</tbody>
</table>

gene-encoding this protein is a subject of relatively fast evolution.

Moreover, analysis of available genome sequences did not reveal a clear sequence homolog of Abf2p in species below the Ashbya gossypii branch on the phylogenetic tree (Fig. 1). Therefore, species such as C. albicans, D. hansenii, Y. lipolytica and Sch. pombe seem to employ alternative solution(s) of mtDNA packaging. There are several possible scenarios that would explain this divergence. These species may tolerate dramatic alterations in the Abf2p sequence without affecting its function, provided that the DNA-binding domain remains functional. Alternatively, another HMG-box-containing protein might have been recruited to ensure the mtDNA packaging in species lacking ‘true’ Abf2p homolog. Indeed, inspection of the genomic sequences of C. albicans and D. hansenii revealed the presence of proteins [EAK92709 in C. albicans (CamtHMG1); CAG90643 in D. hansenii (DhmtHMG1)] containing at least one HMG box, one coiled-coil domain and a putative mitochondrial N-terminal leader sequences predicted by both PSORT II (69.6% for CamtHMG1; 43.5% for DhmtHMG1) and MITOPROT (0.9939 for CamtHMG1; 0.8536 for DhmtHMG1) software (Fig. 2b). There are two interesting features exhibited by both proteins: (i) their lengths (245 amino acids in CamtHMG1; 275 amino acids in DhmtHMG1) are similar to that of the human mtTFI (246 amino acids), and (ii) they are relatively lysine-rich, analogous to the N-terminal domain of mitochondrial packaging protein of the P. polypephalum Glom (Sasaki et al., 2003). In contrast to Abf2p, mtTFI, mtHMG1, Glom and DhmtHMG1, the SMART analysis revealed a presence of only one HMG box in CamtHMG1, indicating that in C. albicans a single HMG box is sufficient for mtDNA packaging. Alternatively, CamtHMG1 may still possess a second HMG box, whose sequence is too vague to be uncovered by in silico analysis. In any case, CamtHMG1 and DhmtHMG1 seem to represent a novel class of mtDNA packaging proteins, and their biochemical properties and physiological functions are worthy of experimental analysis. Finally, the absence of Abf2p may be compensated by proteins with dual functions, such as Aco1p, I1v5p, Yhm1p or Yhm2p (Zelenaya-Troitskaya et al., 1995; Kao et al., 1996; Cho et al., 1998; Chen et al., 2005).

Importantly, all these proteins are highly conserved (e.g. similarity between K. lactis and S. cerevisiae Aco1p homologs is above 95%) and may functionally replace and/or compete with Abf2p under specific physiological circumstances. In mitochondria of phylogenetically distant species such as Sch. pombe or Y. lipolytica, where neither Abf2-like nor putative mitochondrial HMG-box-containing proteins have been identified by BLAST searches, these proteins may even play the major role in packaging of the mtDNA.

In the case of the ribosomal protein subunit Mnp1p, lower overall similarity (about 77%) between K. lactis and S. cerevisiae homologs results from the absence of short stretches of amino acid sequence in the N-terminal and central parts of the K. lactis protein, rather than from accumulation of random substitutions (Fig. 2c). This may be due to compensations reflecting differences observed in the sequences of the mitochondrial ribosomal RNAs that allows the ribosome function(s).

On the other hand, the reason for the lower similarity between Sls1p homologs is less clear (Table 1). In S. cerevisiae, the SLS1 gene encodes a 73-kDa protein with a presumed role in delivering mRNAs to membrane-bound translational activation complexes. Interestingly, its deletion is viable on glucose-containing media and generates a high frequency of petites in a strain containing introns in mitochondrial genes, but not in the intron-less strain (Rouillard et al., 1996).

Another important component of the nucleoid is a putative replication initiator protein Mgm101p. The thermostressive mgm101-1 mutant gradually loses mtDNA in subsequent cell divisions when grown at a restrictive temperature (Zuo et al., 2002). It has been suggested that the Mgm101p may participate in the repair of oxidatively damaged mtDNA molecules (Meeusen et al., 1999). Importantly, Meeusen and Nunnari (Meeusen & Nunnari, 2003) have shown that the Mgm101p has subnucleoid localization and is present only in a subpopulation of nucleoids. The nucleoids containing Mgm101p associate with the mitochondrial replisome which spans both mitochondrial membranes, whereas those lacking this protein localize in the mitochondrial matrix. The Mgm101p thus possibly provides a functional connection between the nucleoids and the replication machinery. The same study also demonstrated that the replisome co-localizes with the outer membrane protein Mmm1p, linking the mitochondria to the cytoskeleton (Boldogh et al., 2003), and that mmm1-1 mgm101-2 mutant displays a synthetic growth defect on glycerol.

In contrast to baker’s yeast, K. lactis Mgm101p was shown to be essential for the viability of the wild-type cells (Clark-Walker & Chen, 1996). The sequence comparison of Mgm101p homologs from different yeast species confirmed the original observation (Clark-Walker & Chen, 1996) that the C-terminal part of the protein is highly conserved. The homology of K. lactis Mgm101p with its counterpart from S. cerevisiae exceeds 97% in the region 92–262. However, the N-terminus of the protein does not display any significant sequence similarity among diverse yeast species (Fig. 2d). This region contains a signal sequence for import to mitochondria and MITOPROT predicts a putative cleavage site at the position 62. In addition, the presumed role of Mgm101p in the initiation of mtDNA replication and the
Fig. 2. Amino acid sequence alignments of selected proteins implicated in the maintenance of mtDNA. (a) Abf2p, (b) mthMG1, (c) Mnp1p, (d) Mgm101p, (e) Rim1p, (f) Cce1p, and (g) Mhr1p. ASHGO, Ashbya gossypii; KLULA, Kluyveromyces lactis; CANAL, Candida albicans; CANGL, Candida glabrata; DEBHA, Debaryomyces hansenii; SACCE, Saccharomyces cerevisiae; SCHPO, Schizosaccharomyces pombe; YARLI, Yarrowia lipolytica. Dashed lines indicate the positions of high mobility group boxes and coiled-coil domain in Abf2p and mthMG1 proteins.
absence of canonical ori/rep elements in most of the species imply that a nonconserved region may play a species-specific role and/or that a highly conserved domain interacts with an as yet unknown, conserved motif/structure within the mtDNA. The latter possibility is supported by functional complementation of the thermosensitive mgm101 mutation by the K. lactis homolog (Clark-Walker & Chen, 1996) as well as by a conserved lysine-arginine rich region near the C-terminus that contains a putative DNA-interacting domain. However, these and related questions need to be addressed experimentally.

The next nucleoid component that plays an essential role in the mtDNA replication is Rim1p. Its role contrasts with a relatively low sequence conservation (Fig. 2e). The similarity of the K. lactis homolog with S. cerevisiae Rim1p is only about 83%. It is therefore of interest to find whether the sequence variation among yeast mtSSB proteins reflects specific changes in their function (e.g. sequence preference).

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One such example has been demonstrated in our laboratory. The mtSSB protein (mtTBP) from Candida parapsilosis, a species harboring a linear mitochondrial genome, gained an additional role in the stabilization of termini of mitochondrial telomeres, illustrating the evolutionary emergence of a telomeric DNA-binding factor from a sequence nonspecific protein (Tomaska et al., 1997, 2001; Nosek et al., 1999). In a similar way to animal mtSSB (Schultz et al., 1998; Ruiz de Mena et al., 2000), Rim1p may control the number of mtDNA copies. Interestingly, it has been demonstrated that glucose affects the ratio between precursor and mature mRNA of S. cerevisiae RIM1, suggesting its regulation at the level of intron splicing (Li et al., 1998). In contrast, yeasts which lack intron in the RIM1 gene have to rely on different

Fig. 2. Continued.
control mechanisms. The study of C. parapsilosis mtTBP points out that its DNA-binding activity but not oligomerization is inhibited upon phosphorylation with protein kinase A (Tomaska, 1998). The BLAST search using the S. cerevisiae Rim1p amino acid sequence as a query did not result in the identification of a homolog in C. glabrata. It seemed improbable that C. glabrata, as a close S. cerevisiae relative, would not contain Rim1p homolog, we performed the search using the ScRIM1 nucleotide sequence as a query and identified a 168-nt region at the chromosome L of C. glabrata highly homologous to 50–218 nt of RIM1. Importantly, this region lies between the loci annotated as similar to YOR028C and YOR030C of S. cerevisiae, respectively, and this position in S. cerevisiae is occupied by RIM1. We therefore analyzed this region in more detail and identified an ORF containing a 159-nt-long intron. Translation of this ORF resulted in a protein highly homologous to S. cerevisiae Rim1p (Fig. 2e).

The GroEL family chaperonin Hsp60p was shown to be associated with the template strand of the active, transcriptionally competent, replication origin (ori5) of S. cerevisiae mtDNA (Kaufman et al., 2003). It may participate in nucleoid division rather than mtDNA replication. Despite the high conservation among yeast species (e.g., K. lactis homolog displays 97.7% similarity with its counterpart from S. cerevisiae), its role in the nucleoid remains unclear because the ori/rep elements were not identified in the mtDNA of K. lactis and other yeast species (Sekito et al., 1995; Kerscher et al., 2001; Bullerwell et al., 2003; Langkjaer et al., 2003; Nosek et al., 2004; Zivanovic et al., 2005).

Mitochondrial replisome and the strategies of mtDNA replication and transmission

In vitro experiments indicate that the minimal core of the mitochondrial replisome in mammalian cells consists of three components; mitochondrial DNA polymerase (POLγ), single-stranded DNA-binding protein (mtSSB) and DNA helicase (TWINKLE) (Korhonen et al., 2004). The S. cerevisiae genome encodes Mip1p (a single subunit DNA polymerase γ) and Rim1p. Although yeasts lack a homolog of TWINKLE, two helicases, Hmi1p and Pif1p, were identified in their mitochondria (Lahaye et al., 1991; Sedman et al., 2000). Moreover, replication of yeast mtDNA likely involves activities of topoisomerase I and II. Although corresponding genes were not yet unequivocally identified (Ezekiel et al., 1994; Murthy & Pasupathy, 1994; Tua et al., 1997), an analysis of S. cerevisiae suggests the presence of topoisomerase II (Top2p) in the mitochondrial proteome (Sickmann et al., 2003).

The absence of Mip1p and/or Rim1p activities results in rapid elimination of DNA from yeast mitochondria. In contrast, the pif1 null mutants are able to maintain wild-type mtDNA unless cultivated at an elevated (36 °C) temperature (Lahaye et al., 1991, 1993) and this phenotype is partially rescued by the Rim1p (Dyck et al., 1992). The Hmi1p is essential for replication of the wild-type mtDNA (Sedman et al., 2000) and seems to be implicated in the formation of mtDNA concatemers (Sedman et al., 2005).

In addition, maintenance of the wild-type mtDNA requires several proteins such as mitochondrial RNA
polymerase (Rpo41p), a putative initiator protein Mgm101p, HMG-like protein Abf2p (on glucose media), DNA ligase (Cdc9p), as well as functional protein synthesis within the organelle. Some of these proteins (e.g., Rpo41p, Mgm101p, Hmi1p, Pir1p) are not essential for the maintenance of hypersuppressive ρ− genomes, thus indicating that different modes of mtDNA replication operate in S. cerevisiae mitochondria. These include rolling-circle mechanism, recombination-dependent mode and possibly a replication via Cairns-type theta intermediates (Maleszka et al., 1991; Williamson, 2002).

Genes coding for homologs of all key proteins implicated in mtDNA replication were identified in the K. lactis genome as well as in other analyzed yeast genomes. In general, these proteins display a relatively high degree of sequence conservation (Table 1), although Sch. pombe lacks a clear homolog of Hmi1p.

In addition, the faithful transmission of mtDNA into daughter cells requires a Cce1p- and/or Mhr1p-dependent recombination pathway. Cce1p is a cruciform-cutting endonuclease (X-solvase) and the absence of its activity results in the aggregation of nucleoids into large complexes and the accumulation of Holliday junctions in mtDNA, indicating its participation in mtDNA recombination transactions (Lockshon et al., 1995). Moreover, Cce1p participates in the recombination-dependent initiation of mtDNA replication and/or in the resolution of linear monogenic molecules from concatemeric mtDNA forms. Although cce1 mutants have impaired preferential transmission of hypersuppressive ρ− derivatives of the mtDNA and generate increased proportion of petites, they do not display any significant change in the maintenance of the wild-type mtDNA (Zweifel & Fangman, 1991; Lockshon et al., 1995). Mhr1p is implicated in the homologous recombination of mtDNA, the repair of oxidatively damaged mtDNA molecules, the formation of linear concatemeric molecules of the mtDNA and the partitioning of nascent mtDNA in the form of monomeric molecules into buds. The mhr1-1 mutation increases the induction of cytoplasmic petites and delays the transmission of mtDNA into daughter cells. Importantly, the cce1 mhr1 double mutants are unable to maintain the mtDNA (Ling & Shibata, 2002, 2004).

The K. lactis homologs of Cce1p and Mhr1p display 65.6% and 81.4% similarity, respectively, to their S. cerevisiae counterparts. In contrast, BLAST searches of complete genome sequences of other yeast species revealed that they lack clear sequence homologs (E value < 10−10) of Cce1p (C. albicans, Y. lipolytica, Sch. pombe) and Mhr1p (Sch. pombe). However, it has been demonstrated that fission yeast mitochondria contain Holliday junction resolvase Ydc2p. In spite of low overall sequence conservation with Cce1p, the Ydc2p possesses a conserved core typical for bacterial resolvase RuvC (White & Lilley, 1997; Ceschini et al., 2001). This prompted us to search for homologs of Cce1p and Mhr1p more carefully, based on this specific signature. We identified three candidates, EAK99006, DEHA0E17996g and YALI0E08910g in the C. albicans, D. hansenii and Y. lipolytica genomes, respectively, that possess conserved resolvase domains. Interestingly, the deduced protein product of the A. gossypii syntenic homolog (ADR157W) shares only a little similarity with other Cce1 proteins (Fig. 2f).

The viability of the Δydc2 mutant indicates that the Sch. pombe genome should contain a homolog of the Mhr1 protein as well, and our analysis suggests that ORF SPBC18H10.17c may encode this homolog (Fig. 2g).

Based on these results it is possible to conclude that both Cce1p- and Mhr1p-dependent mtDNA transmission pathways are evolutionarily conserved among distant yeast species, although it remains unclear why the sequences of key players, Cce1p and Mhr1p, vary significantly and if or how this affects the mode of mtDNA replication and/or transmission.

Concluding remarks and unresolved questions

Comparative analysis of yeast genomes provides a unique insight to the structure, function and evolution of the mitochondrial chromosomes. In this study we demonstrated that homologs of genes which are required in S. cerevisiae for the nucleoid structure and dynamics of the mitochondrial genome can be identified in different yeast genomes, even though their protein sequence has in some cases strongly diverged during evolution. However, mtDNAs are very diverse in terms of the gene arrangement and molecular architecture (e.g. circular vs. linear with different types of telomeric structures Nosek et al., 1998; Nosek & Tomaska, 2003), are more or less dispensable and can be maintained or not as ρ− derivatives. We might in turn expect more diversity in the systems that drive the mitochondrial maintenance, as in S. cerevisiae some genes act differently upon ρ+ and ρ− genomes, respectively. Although no apparent difference is detected, fine nucleoid structure and/or replication strategy between the petite-positive and petite-negative yeasts might appear in dynamic studies.

The presence of ori/rep elements is also puzzling. The S. cerevisiae mtDNA includes up to eight ori/rep elements which contain a typical GC cluster, and four of them also contain an active promoter. However, the mtDNA of K. lactis, as well as of most of the other yeast species, lacks these motifs (Sekito et al., 1995; Kerscher et al., 2001; Bullerwell et al., 2003; Langkjær et al., 2003; Nosek et al., 2004; Zivanovic et al., 2005). Although mtDNAs of K. lactis contain multiple GC clusters, their pattern differs from that found in S. cerevisiae. Moreover, GC-rich sequence stretches...
are scarce in some species (e.g. *C. parapsilosis*), indicating that yeast mtDNA may contain another, evolutionarily conserved, yet unidentified, element that plays a role of the mtDNA replication origin.

In contrast to chromosomes present in eukaryotic nuclei, virtually nothing is known about the higher-order (3D) structure of the mitochondrial nucleoid and its dynamics under different circumstances. A key problem of the nucleoid architecture is the packaging of an individual mtDNA molecule and the regulation of the number of mtDNA copies. Therefore, it is crucial to uncover what roles are played by individual nucleoid components and how their functions are orchestrated to ensure the stability and segregation of the mitochondrial genome. One of the major challenges of mitochondrial research is to understand the asymmetrical segregation of organelles into the daughter cells (Aguilaniu et al., 2003). It is possible that nucleoids are subject to a checkpoint control that would ensure that the daughter cells will receive undamaged mtDNA. In that case it would be highly interesting to uncover the nature of such a checkpoint and its possible connection to the cell cycle. Another important problem is the role of different mtDNA forms found in yeast mitochondria (i.e. monogenicomic linear and circular mtDNAs, concatemeric molecules and branched structures) and their participation in gene expression and organellar inheritance.

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**References**


Mitochondrial chromosome structure


Tomaska L (1998) Phosphorylation of mitochondrial telomere
binding protein of Candida parapsilosis by cAMP-dependent
Tomaska L, Makhov AM, Nosek J, Kucejova B & Griffith JD
(2001) Electron microscopic analysis supports a dual role for
the mitochondrial telomere-binding protein of Candida
putative mitochondrial telomere-binding protein of the yeast
topoisomerase I of Saccharomyces cerevisiae. Biochimie
Umezaki T & Miyakawa I (2002) Use of SDS-DNA PAGE for
detection of mitochondrial Abf2p-like proteins and
mitochondrial nuclease in Saccharomyces yeasts and
Van Dyck E, Foury F, Stillman B & Brill SJ (1992) A single-
stranded DNA binding protein required for mitochondrial
dNA replication in S. cerevisiae is homologous to E. coli SSB.
EMBO J 11: 3421–3430.
geno requires an amino-terminal domain of yeast
mitochondrial RNA polymerase. Proc Natl Acad Sci USA 96:
8046–8051.
junction-resolving enzyme from Schizosaccharomyces pombe.
DNA. Nat Rev Genet 3: 475–481.
Zelenaya-Troitskaya O, Newman SM, Okamoto K, Perlman PS &
Butow RA (1998) Functions of the high mobility group
protein, Abf2p, in mitochondrial DNA segregation,
recombination and copy number in Saccharomyces cerevisiae.
Zelenaya-Troitskaya O, Perlman PS & Butow RA (1995)
An enzyme in yeast mitochondria that catalyzes a
step in branched-chain amino acid biosynthesis also
functions in mitochondrial DNA stability. EMBO J 14:
3268–3276.
Zivanovic Y, Wincker P, Vacherie B, Bolotin-Fukuhara M &
Fukuhara H (2005) Complete nucleotide sequence of the
mitochondrial DNA from Kluyveromyces lactis. FEMS Yeast Res
5: 315–322.
Zuo XM, Clark-Walker GD & Chen XJ (2002) The mitochon-
drial nucleoid protein, Mgm101p, of Saccharomyces
cerevisiae is involved in the maintenance of rho(+) and
ori/rep-devoid petite genomes but is not required
for hypersuppressive rho(−) mtDNA. Genetics 160:
1389–1400.
a biased transmission of yeast mitochondrial DNA. Genetics