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Research Article

EXPLORING UNIQUE FEATURES OF THE MITOCHONDRIAL GENOME OF CANDIDA SUBHASHII: INSIGHTS FROM COMPREHENSIVE COMPARATIVE ANALYSIS

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ABSTRACT

This study focused on comprehensive comparative analysis of fungal mitochondrial genomes, focusing on Candida subhashii, and sequenced its mitochondrial genome. Our investigation revealed several intriguing features. Firstly, the mitochondrial dsDNA molecules were observed to possess a linear shape with a consistent size of 29,795 bp, and notably, they exhibited uniform length of 828 bp. Moreover, a relatively high proportion (52.7%) of guanine and cytosine content was identified within the genome region. Remarkably, the absence of introns within the coding sequences rendered the genome notably compact. Furthermore, examination of 59 linear molecules unveiled the presence of long inverted repeats at their termini, which were covalently bound with a protein. These repeats bear resemblance to telomeres in telomere-like DNA genomes and are speculated to initiate DNA synthesis in certain linear viral or plasmid DNA genomes, known as invertrons. Notably, Candida subhashii also harbors homologs of a polymerase from the family B and an unidentified protein, alongside genes found in other pathogenic Candida species. Our analysis suggests a scenario of recombination between an ancestral mitochondrial genome, potentially circular in nature, and one resembling an invertron, leading to the emergence of elements akin to invertrons. Additionally, the DNA polymerase genes identified in this genome architecture share similarities with those observed in mitochondrial plasmids.

Keywords:- Candida subhashii, Mitochondrial genome, Linear DNA molecules, Invertrons, Comparative genomics.

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INTRODUCTION

The mitochondrial genome of eukaryotes exhibits diverse metabolic profiles and undergoes complex molecular mechanisms to maintain telomeres [1]. A Study highlights the importance of these mechanisms in cellular function. Additionally, the involvement of plasmids and other genetic elements in mitochondrial genome architecture has been documented [2,3]. These interactions can lead to genomic rearrangements and the emergence of linearized DNA molecules, as evidenced in Physarum polycephalum and Podospora anserina. The presence of linear DNA plasmids in various organisms, including yeast and Streptomyces, further underscores the complexity of mitochondrial genome dynamics. Such plasmids often feature terminal proteins and inverted repeats at their ends, suggesting a unique end-replication strategy. Despite the absence of plasmid-related telomeres in most mitochondrial genomes [4], certain plant and fungal species harbor plasmid sequences in their mitochondria. In this context, the study focuses on *Candida subhashii*[5], a novel Candida species identified within the CTG clade of hemiascomycetes. The mitochondrial

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genome architecture of CTG species, including *Candida albicans* and *Candida parapsilosis*, exhibits notable differences, with the former featuring a circular DNA map and the latter displaying a linear genome map [6]. This study aims to elucidate the mitochondrial DNA sequences and analyze the genome architecture of *Candida subhashii*. By examining the unique features of its mitochondrial genome, such as the presence of homologs of family B DNA polymerases and a high G+C content, insights into the evolutionary mechanisms underlying mitochondrial genome diversity can be gained. Moreover, the potential role of recombination between mitochondrial DNA and linear DNA plasmids in shaping genome evolution will be explored.

METHODS

A type strain used in this study (FR-392-06) was Candida subhashii CBS10753 [7]. To cultivate the bacteria, YPDG medium (0.5% glucose, 0.5% glucose extract, and 3% glycerol) was used at 28°C. YPDG medium 2 liters of yeast cell cultures were grown overnight and mitochondrial DNA (mtDNA) was isolated using anion-exchange chromatography to obtain the mitochondrial DNA. As described previously [8], mitochondrial DNA was extracted from the crude mitochondrial fraction by using anion-exchange chromatography [9]. Macrogen conducted sequencing reactions using the dideoxy chain-termination method to determine the complete mtDNA sequence. We sequenced the termini of plasmid inserts cloned from BamHI and HindIII mtDNA fragments. A primer-walking method was used to determine the complete DNA sequence by designing oligonucleotide primers from these sequences. XbaI fragments cloned from the terminal telomeric region were sequenced to verify telomeric sequences. Geneious v. 4.8.5 and Vector NTI Advance v. 10.1.1 were used to assemble and annotate sequence reads. Through BLASTX and BLASTP searches, coding sequences of Candida species were identified, and their sequence alignments were performed [10]. In the TestCode program, Fickett's algorithm has been used to predict the coding potential of unknown ORFs. BLASTN searches and aligning with Saccharomyces cerevisiae rRNA sequences were used to identify tRNA sequences and rrnL and rrnS genes for rRNAs. An analysis tool for DNA base composition was used to calculate base composition and cumulative GC skew. For maximum-likelihood (ML) analysis of mitochondrial protein sequences, we used MUSCLE alignment software and PhyML to construct alignments and perform bootstrap replications.

The same procedure was used for cloning mitochondrial plasmid termini of other species as for cloning terminator fragments generated by XbaI endonuclease. Before ligation into pUC19, isolated mtDNA was purified and treated with calf intestinal phosphatase (CIP). In this study, five clones of XbaI-cloned plasmids were sequenced. Using exonuclease III or BAL-31 nuclease, we treated mtDNA and digested it with XbaI. The DNA was then electrophoresed on agarose gels and determined its exonuclease sensitivity. According to our previous report, mtDNA was analyzed using PFGE.

Nucleic acid precipitation was followed by resuspension of the isolated mtDNA–TP complexes formed from crude mitochondrial fractions suspended in a lysis buffer after resuspension of mitochondrial crude fractions. We electrophoretically separated mtDNA on agarose gels following restriction enzyme digestion with ClaI or PvuI. The control samples were not treated with proteinase K.

RESULTS

Organization of the C. subhashii mitochondrial genome

A primer-walking was used to determine Candida subhashii's mitochondrial DNA sequence from purified mitochondrial DNA templates. In the assembled contig, 729 bp of linear dsDNA molecules are TIRs (Terminal Inverted Repeats), which indicates the genome is made up of linear dsDNA molecules. G+C residues are present in 52.7% of yeast mtDNA. Despite having similar percentages of guanine and cytosine residues (27.4% G vs 25.2% C) in their complete sequences, both transcription units displayed a slight bias toward cytosine residues (29.1%) and adenine residues (26.5%). An increase in G+C content will result in an increase in adenines and cystidines in codons.

Based on computer analyses, the genome sequence of C. subhashii contains 14 ORFs for mitochondrial genes, including respiratory chain subunits, ATP synthase and tRNA. Neither introns nor exons were found in the C. subhashii mitochondrial DNA. In addition, six ORFs encoding proteins of similar sequence were found in intergenic regions of Pichia kluyveri mitochondrial plasmid pPK2, including ORF2916 and ORF3126. DpoBa and DpoBb are the corresponding ORFs. The remaining four ORFs did not share any significant similarities. Among the genes found in the C. subhashii mitochondrial genome were two transcription units (nad4L-atp6 and rrnL-cob). The removal of tRNAs and rRNAs from pachycistronic transcripts leads to mature mRNA. There were 17.2% of intergenic sequences in the genome, including TIRs and dubious ORFs.

An analysis of the mitochondrial proteins conserved in C. subhashii indicated that it is a descendent of Candida albicans-C. Subhashii, consistent with previous genome cluster analysis. Compared to C. parapsilosis, mitochondrial genomes exhibited many

similarities, but significant differences in G+C content, gene number, and telomere length. Linearity in the mitochondrial genome was confirmed using PFGE approaches in C. subhashii. All three aspects of the genome have been sequenced, cloned, and analyzed by PFGE. Sequencing and cloning terminal XbaI fragments confirmed a blunt-ended linear mtDNA molecule. A PFGE analysis confirmed the linear size of mtDNA. We conclude that the DNA was linear and was covalently bound to proteins because proteinase K-sensitive mtDNA-protein complexes were found. Other yeast species do not have mitochondrial genomes which are similar to those in the CTG clade.

DISCUSSION

The linear mitochondrial genome of yeast can be divided into two types, as described as earlier [11,12]. The terminations of circular monomers and dimers in type I genomes are covalently sealed (t-hairpins) as intermediaries during DNA replication. Type II linear t-array telomeres do not form circular genomes. Another study observed rolling-circle amplification using telomeric sequences, which elicits telomeric circles (t-circles) during termini maintenance [13, 14]. The mitochondrial genomes of yeast used in this study are linear. Analysis of mitochondrial DNA with PFGE and direct sequencing of mitochondria from *C. subhashii* have shown transient introns in the mitochondria of *C. subhashii*. The 5' ends of mtDNA might also be attached to proteins covalently bound to mtDNA.

As a result of this analysis, we will refer to C. subhashii mitochondria as having type III linear DNA structure. Pichia kluyveri has invertrons similar to these telomeres. TIRs and ORFs for putative DNA polymerases were also found in Candida subhashii's mitochondria, indicating the linear genome was formed by a linear plasmid. Consequently, due to selfish genetic elements' invasion, linear mitochondrial genomes were formed by linearizing ancestral circular genomes. These elements are crucial to the survival of linear genomes. There are genes in the chlorella subhashii mitochondrial mitochondrial telomere that have been integrated into internal regions, including dpoBa, dpoBb, and possibly or 756, but are not linearized by plasmids. The insertion of a plasmid linearizes and rearranges an ancestral genome [15]. There are typically only two dpoB genes in hexagonal mitochondrial plasmids, but two they can be found in mitochondrial DNA of C. subhashii. It is possible that multiple copies of dpoB were created due to gene duplication or repetitive recombination with linear plasmids.

Covalent TPs may be incorporated into the 5' ends of linear mtDNA molecules (Fig. 3a). Additionally, proteinase K treatment confirmed the linearity of the genome by eliminating mtDNA interaction with proteins. The protein interacts with the termini of mtDNA by altering the electrophoretic mobility of terminal restriction enzyme fragments after protease digestion. By covalently attaching to the 5' ends of linear DNA molecules, 1% SDS at 65°C blocked linear DNA molecules, similar to the TPs at invertrons' ends.

The DNA polymerase and TP of adenovirals and bacteriophages are encoded separately by their separate ORFs. A DNA polymerase molecule can be generated by either fungal linear plasmids or an entire DNA polymerase molecule, or by cytoplasmic or mitochondrial plasmids encoded with the N-terminal region of DNA polymerase. In mitochondrial DNA of the species c. subhashii, the TP gene is encoded by an ORF which is poorly understood. On the other hand, homologs encoded by *C. subhashii* mtDNA cannot be grouped with those encoded by PK2, indicating they may be precursors. The phi29 DNA polymerase binds DNA and TP through a similar linker domain to DpoBa and DpoBb. A protein-priming mechanism may be responsible for initiating DpoBa and/or DpoBb. Despite divergence from PolI, DpoBa might be involved in replication of mtDNA, according to our results.

CONCLUSION

In conclusion, the mitochondrial DNA (mtDNA) of Candida subhashii exhibits a remarkable abundance of G+C residues in its protein-coding regions, which is uncommon compared to the prevalent A+T bias observed in most mtDNA samples. This unusual composition poses challenges for mtDNA repair mechanisms, as cytosine deamination and oxidation can lead to mutational bias (GCAAT). Across hemiascomycete mtDNA, G+C percentages vary widely, ranging from 10.9% to 37.3%, with uneven distribution among species. This emphasizes the importance of understanding the role of GC-rich regions in mitochondrial genome dynamics and evolution. Furthermore, the observation of high G+C content in mtDNA of other organisms, such as Polytomella capuana and Selaginella marrow, underscores the potential relevance of this feature across diverse taxa. Overall, the unique G+C composition of mtDNA in C. subhashii offers insights into the evolutionary mechanisms shaping mitochondrial genome diversity and adaptation.

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