Volume 48(1-4):61, 2004
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

DISSERTATION SUMMARY

Analysis of the mitochondrial genome of the human pathogenic yeast *Cryptococcus neoformans*

Judit Litter

Department of Microbiology, University of Szeged, Szeged, Hungary

Cryptococcus neoformans is an encapsulated basidiomycetous yeast. This opportunistic pathogen primarily infects immunosuppressed and immunodeficient patients. The disease is most frequently manifested as meningitis.

Well functioning respiratory system assured by well functioning mitochondria is one of the most important factors of the yeast's successful survival in the body. To date only a few data are available about the organization of the mitochondrial genome of basidiomycetous fungi. Varma and Kwon-Chung (1989) have demonstrated extensive polymorphism among the strains of *Cryptococcus neoformans* regardless of their serotype or varietal status by analysis of restriction fragment length polymorphisms (RFLP) in mitochondrial DNA.

The aim of the present work was the characterization of the structure and organization of the mitochondrial genome in *C. neoformans var. grubii* IFO 410 (serotype A) and *C. neoformans var. neoformans* α IFM 5844 (serotype D) strains. Physical and functional maps were constucted by *Eco*RI and *Eco*RV restriction enzymes using a reciprocal digestion technique (Hamari et al. 1999). Several genes could be localised to some fragments by Southern hybridizations using heterologous *Aspergillus nidulans* primers working on *Cryptococcus hungaricus* (Gácser et al. 2002). Additional genes were localised by cloning the majority of *Eco*RI and *Eco*RV fragments into pSK vector, and sequencing.

The correct order of several mitochondrial genes important in the respiration (*nad*1, *nad*2, *nad*3, *nad*4, *nad*4L, *nad*5, *nad*6, *atp*6, *atp*9, *cox*1, *cox*2, *cob*) and *rns* gene important in protein synthesis was determined. We did not find any differences in the order of the genes between the strains. However, they differed significantly in the sizes of their mt DNAs, measuring 32.6 kb and 24.1 kb for *C. n. var. neoformans* and *C. n. var. grubii*, respectively.

Comparing the maps it was found that two large regions, altogether of 8.5 kb, could give rise to this variance. The presence of introns and alteration of the length of intergenic regions was investigated in both regions.The greatest difference was observed in the size and number of introns of *cox*1 and *cob* genes. The size of *cox*1 gene in *C. n. var. grubii* was 1587 bp without any introns, while in *C. n. var. neoformans* it was 6004 bp and contained five introns. LAGLI-DADG motifs were found in several introns and there were also differences in the *rns*-*atp*6, *atp*6-*atp*9 and *atp*9-*cox*1 intergenic regions between the two strains.

Primer pairs for *cox*3, *rnl* and *nad*5 genes were designed using the *C. neoformans* mitochondrial genome database to execute detailed comparison. Using these primers in PCR reactions we found that there were some differences in the size of the *nad*5 and *rnl* genes, too. The PCR products of *rnl* genes were sequenced and concluded the presence of intron in *C. n. var. neoformans.* A 0.2 kb diffrence from the 8.5 kb size was revealed.

References

Gácser A, Hamari Zs, Pfeiffer I, Litter J, Kevei F, Kucsera J (2002) Organization of mitochondrial DNA in the basidiomycetous *Dioszegia hungarica* (*Cryptococcus hungaricus*) species. FEMS Microbiol Lett 212:1-6.

Hamari Zs, Pfeiffer I, Ferenczy L, Kevei F (1999) Interpretation of variability of mitochondrial genomes in the species *Aspergillus carbonarius*. Antonie van Leeuwenhoek 75:225-231.

Varma A, Kwon-Chung KJ (1989) Restriction fragment polymorphism in mitochondrial DNA of *Cryptococcus neoformans.* J Gen Microbiol 135:3353-3362.

Supervisor: Judit Kucsera
E-mail: liju@bio.u-szeged.hu