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DIVERSITY OF EXTRACHROMOSOMAL GENETIC ELEMENTS IN YEASTS

(A REWIEV)

ILONA PFEIFFER[1], JUDIT KUCSERA[1], A. GÁCSER[1], JUDIT LITTER[1] AND W. I. GOLUBEV[2]

[1]Department of Microbiology, Faculty of Sciences, University of Szeged,
P.O. Box 533, H-6701 Szeged, Hungary
[2]Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences,
Pushchino, Russia

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Mitochondrial DNA

Considering its function in the cell, the most important extrachromosomal genetic element is the mitochondrial DNA. A number of data accumulated about its structure and function since it was described in the sixties. Its size varies in the range from 17.3 kb (*Schizosaccharomyces pombe*) to 101.1 kb (*Brettanomyces custersii*) in yeast [1]. It seems that larger mtDNAs resulted mainly from changes in the length of the intergenic regions based on lower buoyant densities of the larger molecules (AT rich sequences), however, the presence of optional introns may undoubtedly contribute to it.

Despite its variable size, it possesses very similar information content in all of the examined species. Genes that generally occur in mitochondrial DNA of yeasts code the large (L)rRNA and the small (S)rRNA, 1–3 subunits of the cytochrome oxidase, cytochrome b and subunits 6, 8 and 9 of the ATPase complex and 23-25 tRNA. In addition, in *Saccharomyces cerevisiae* mtDNA encodes the var1 protein (also in *Candida glabrata*) and 9S RNA. Moreover several URFs (unassigned reading frames)

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and ORFs (open reading frames) were identified whose function is still not clear or their "products" take part in the intron-movement or intron-excision, therefore in the formation of mature mRNA [1, 2]. The complete nucleic acid sequence of mtDNA of *S. cerevisiae* and *Sch. pombe* is known. Neither of them contained genes for any subunits of the NADH dehydrogenase. This led to the generalisation that no NADH dehydrogenase genes can be found in yeasts mtDNA. This was a wrong assumption and both DNA-DNA hybridisation and DNA sequencing proved the presence of these genes in several other yeast species [3].

Early studies by electron microscope demonstrated that the common occurrence of mtDNA is circular [4]. A number of studies during the last decade revealed that several yeast species have linear mtDNA [5, 6]. Even the circular ones have linear forms arising from the rolling circle replication process, but these molecules do not possess thet specific terminal structures characterizing the end of linear mtDNA molecules [7, 8].

Most of the above-mentioned results came from studies carried on ascomycetous yeast. Basidiomycetous species, however, were not frequent subjects of mitochondrial DNA studies. Our results indicated significant intraspecific length polymorphism in the case of *Cryptococcus neoformans* [9] and *Cryptococcus hungaricus* [10] strains. Physical mapping revealed relatively small genomes (24.1–32.97 kb), with similar gene content characteristic for the ascomycetous species. Fragmented mitochondrial genome organisation was observed in the genus *Xanthophyllomyces* and *Cystofilobasidium* [10, 11].

MtDNA encodes several proteins and tRNAs that are essential for the function of the mitochondria therefore mutations concerning the coding regions can lead to the death of cells. This is not the case in some yeast species, those are called petite positives [12, 13]. Nearly all of the petite positive species belong to section Ascomycetes, the only one known among basidiomycetous yeast is *Phaffia rhodozyma* [14].

DNA plasmids

Many strains of different yeast species contain circular or linear DNA plasmids as well. The appearance of circular plasmids is restricted to the genus *Saccharomyces, Kluyveromyces* and *Zygosaccharomyces*. All of them have nuclear location, but their replication is independent of that of the chromosomal DNA. Despite previous suppositions, no obvious phenotypic changes can be attributed to them. Nevertheless 2 μm plasmid of *S. cerevisiae* is a very useful basis for designing cloning vectors in recombinant DNA technology [15].

Linear DNA plasmids have wider distribution among eukaryotic microbes than circular ones [16, 17]. Our study on yeasts, isolated from spring tree fluxes revealed the existence of plasmids in three genera [18]. All of the examined *Trichosporon pullulans* and *Xanthophyllomyces dendrorhous* isolates contained plasmids, while in the genus *Nadsonia* we could detect their presence only in two strains of *N. fulvescens var. elongata*. Majority of the linear DNA plasmids studied so far were localized in the cytoplasm but in *Pichia kluyveri* and *X. dendrorhous* they can be isolated from the mitochondria [19, 20]. Most of them are cryptic [21, 22, 23] and considered to be benign intracellular parasites with conserved autonomous replication ability. Nevertheless some of them have a well-defined function; thus, in *Kluyveromyces lactis* [24] and *Pichia acaciae* [25] they encode specific toxins causing killer phenomena of the host. In both cases they have pairwise occurrence, where the bigger plasmid encodes the specific DNA and RNA polymerases responsible for the replication and transcription of both plasmids, while the smaller one encodes the toxin and the immunity proteins.

DsRNA plasmids and viruses

DsRNA plasmids and viruses constitute the third group of extrachromosomal genetic elements of yeasts [26, 27, 28]. Electron microscopy and the non-mendelian inheritance demonstrate their cytoplasmic location [29]. In most cases their function is still unknown. However, in *S. cerevisiae* [30] and *Ustilago maydis* [31] they confer killer activity. Killer system of *S. cerevisiae* is very well characterized. Basically two types of isometric virus particle exist: L-A and M, with the same sizes (39 nm). The dsRNA genome of the L-A particle encodes the capsid protein and also the dsRNA-dependent RNA polymerase. The M virus is responsible for the killer activity as it harbours the killer toxin gene, and also an immunity region responsible for the resistance of the host cells against their own toxins. Similar system was observed in *Trichosporon pullulans* [32] where two types of dsRNA encapsidated into virus-like particles (VLPs) were isolated from a strain showing mycocinogenic activity. Elimination of the smaller dsRNA molecule was accompanied with the loss of mycocinogenic activity. DsRNA-associated VLPs were detected in killer strains of *Cryptococcus hungaricus* CBS 6569 [33], however in this case there is no direct evidence for the connection between the toxin production and the presence of VLPs.

The manifestation of killer phenomenon can be not always due to the presence of extrachromosomal genetic elements. As in *Saccharomyces dairensis* and *Filobasidium capsulogenum*, this phenotype can also be attributed to nuclear genes [34].

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