Variance of Ploidy in *Candida albicans*

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Determination of ploidy was performed on isolates of *Candida albicans* from clinical sources by measuring nuclear DNA content with fluorescent microscope photometry. By this criterion and UV irradiation survival experiments, haploid, diploid, and tetraploid strains were identified in this organism. The dimensions of nucleus-associated organelles (equivalent to spindle pole bodies) in these strains increased as a function of ploidy number.

Recently, the ploidy of a dimorphic yeast, *Candida albicans*, was concluded to be diploid from studies on its nuclear DNA content measured with flow cytometry (10) or on its genetic heterozygosity and segregation (18). As we noticed our clinical isolates of this organism showing a variety in cell dimension, we decided that variation of ploidy may exist in the strains from natural sources. In this study, a technique of fluorescent microscope photometry was used to measure nuclear DNA contents of the isolates to determine their ploidy, since the advantage of the technique was the simultaneous detection of the DNA content and the morphology of an individual cell under a microscope (7).

The *C. albicans* strains were isolated from patients at the University Hospital, Nagoya University School of Medicine, or at Toyohashi City Hospital, Toyohashi, Aichi Prefecture, Japan, except for one strain, IAM4966, which we obtained from the Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan. Each strain was ascertained by its antigenicity, checked by slide agglutination tests with mono-specific antibodies against *C. albicans* antigens (3, 14; products of Iatron Laboratories, Inc., Tokyo, Japan), its ability to form chlamydospores and hyphal filaments on corn meal agar (17), and its carbohydrate fermentation profile (17). *C. albicans* cells were grown at 25°C in a semidefined medium, a modification of the synthetic medium of Lee et al. (8), containing 0.5% (NH₄)₂SO₄, 0.02% MgSO₄ · 7H₂O, 0.25% NaCl, 0.3% Casamino Acids (Difco Laboratories), 2% (wt/vol) glucose, and biotin (1 µg/ml). Logarithmically growing cells in liquid semidefined medium (0.1-ml portion) were plated on an agar plate containing the same medium, and the incubation was continued for 2 h. Then a square block (10 by 10 mm) of the agar plate was cut with a knife

and was pressed to the surface of a glass slide so that the monolayer of growing cells was transferred to the glass. The cells were then stained for 1 to 2 h at 25°C with propidium iodide (10 µg/ml) dissolved in NS buffer (10 µl), which consisted of 10 mM Tris (pH 7.4), 0.25 M sucrose, 7 mM β-mercaptoethanol, 0.4 mM phenylmethylsulfonyl fluoride, 1 mM disodium EDTA, 1 mM MgCl₂, 0.1 mM CaCl₂, and 0.1 mM ZnCl₂. An equal volume of RNase A (Sigma Chemical Co., from bovine pancreas, DNase activity inactivated by heating) dissolved in NS buffer at a concentration of 1 mg/ml was added to the staining solution. The fluorescent dye was found to stain nuclei of this organism specifically. As described by Hutter and Eipel (6) on yeast species and by Olaiya and Sogin (10) on *C. albicans* by flow cytometry, the intensity of fluorescence emitted from each nucleus was expected to be proportional to the DNA content. Since the data obtained from the measurement of this type were of a relative nature, it was necessary to determine them quantitatively through the use of *Saccharomyces cerevisiae* cells of known ploidy as the reference. *S. cerevisiae* strains T26 α (haploid), T556 α α (diploid), GM5 α α α (triploid), and 30T α α α α (tetraploid) were used, which were kindly given to us by N. Yanagishima (Biological Institute, Faculty of Science, Nagoya University, Nagoya, Japan). These *S. cerevisiae* cells were grown at 25°C in modified Burkholder medium as described by Hayashibe and Sando (4) and were prepared in the manner described above. The fluorescent intensity was measured in a Zeiss MPM 01K-FL/03FL epifluorescent microscope-photometer. Excitation of fluorescence was performed with an instantaneous excitation apparatus which applied a 1-s pulse of exciting beam (546-nm wavelength) to the sample through a pinhole.

TABLE 1. Ploidy determination and germ tube formation

Strain	Ploidy		Source	Serotype ^b	Germ tube formation ^c
	Expected	Determined by FMP ^a			
<i>S. cerevisiae</i>					
T26	1	1.00			
T556	2	2.09 ± 0.21			
GM5	3	2.87 ± 0.31			
30T	4	3.92 ± 0.43			
<i>C. albicans</i>					
NUM2		0.98 ± 0.15	Vaginal secretion	B	—
NUM43		0.95 ± 0.26	Sputum	A	—
NUM63		0.97 ± 0.15	Kidney	A	—
NUM1		1.93 ± 0.21	Urine	A	+
NUM24		2.02 ± 0.17	Vaginal secretion	A	+
NUM25		2.03 ± 0.33	Vaginal secretion	A	+
NUM29		1.95 ± 0.42	Urine	A	+
NUM33		1.98 ± 0.21	Vaginal secretion	A	+
NUM37		1.90 ± 0.20	Sputum	A	±
NUM42		1.94 ± 0.20	Sputum	A	+
NUM46		2.03 ± 0.27	Sputum	A	+
NUM58		1.92 ± 0.12	Pus	A	+
NUM110		1.96 ± 0.42	Feces	A	+
NUM113		1.99 ± 0.26	Swab	A	+
NUM114		2.01 ± 0.26	Swab	A	+
NUM249		1.94 ± 0.31	Urine	B	—
NUM961		2.10 ± 0.17	Swab	A	+
IAM4966		1.92 ± 0.20		A	+
NUM678		4.23 ± 0.38	Swab	A	+

^a Relative ploidy was determined after normalization to the value of the fluorescence intensity obtained from G1-phase nuclei of the haploid *S. cerevisiae* T26. The values are means ± standard deviations of multiple determinations. FMP, Fluorescent microscope photometry.

^b Serotypes A and B were designated according to Hasenclever and Mitchell (3) and Tsuchiya et al. (14).

^c Yeast cells grown in a liquid medium containing yeast extract (1%, wt/vol), peptone (2%, wt/vol), glucose (2%, wt/vol), and KH₂PO₄ (0.4%) were washed with sterile water and starved for 4 h at 37°C. A 0.1-ml amount of the starved cells (10⁷) was transferred onto a plastic dish and mixed with 2 ml of 25 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffer (pH 7.0) containing 5% (wt/vol) calf serum (Commonwealth Serum Laboratories, Melbourne, Australia). The mixture was incubated at 37°C for 3 h, and the cells were observed by phase-contrast microscopy. The percentage of cells producing germ tubes was counted, and the results were expressed as follows: —, less than 0.1%; ±, from 0.1 to less than 2%; +, from 2 to 100%.

Pinholes of 2 and 4 µm in diameter were used to cover the area of an individual nucleus. For determination of ploidy, G1-phase nuclei of both organisms were picked up to measure the intensity. The G1-phase cell was defined here as a yeast cell having no bud, since the bud emergence occurs before nuclear DNA synthesis (2). More than 100 G1-phase nuclei were measured for a given strain. The data of the fluorescence intensity were computerized, using a program of Zeiss CYFLAN. The average intensity in the haploid *S. cerevisiae* strain was standardized as one unit, and those of the other strains of both organisms were expressed as the ratio to the standard intensity. The ratios in *S. cerevisiae* strains were in close agreement with the values predicted from their ploidy (Table 1). In the isolates of *C. albicans*, diploid strains were most frequently observed, and haploid and tetra-

ploid ones were also detected. The table also includes the serotype and source of each isolate, which shows no correlation between the serotype and the ploidy number or between the source and the number.

Survival to UV light has been shown to be a function of ploidy number (10, 11, 18). Figure 1 shows representative data on inactivation kinetics after exposure to UV light examined in the *C. albicans* strains, in comparison with *S. cerevisiae* strains of known ploidy. Compared with the latter organism, *C. albicans* strains showed slightly sigmoidal curves. This may be a reflection of the property of *C. albicans* cells to form clumps or chains of yeasts. Inactivation kinetics of *C. albicans* strains were nearly identical to those of *S. cerevisiae* strains of the corresponding ploidy (Fig. 1).

The ploidy of a cell can be directly determined

FIG. 1. Survival of *C. albicans* strains as compared with *S. cerevisiae* strains of different ploidies when exposed to UV light. The method was according to Olaiya and Sogin (10). Samples of agar plates which contained 1% (wt/vol) peptone (Difco), 1% (wt/vol) yeast extract (Daigo Eiyo Kagaku Inc., Osaka, Japan) and 2% (wt/vol) glucose were plated with yeast cells (500 to 800 cells per plate) and exposed to UV light (254 nm, 15 ergs/mm² per s) for increasing periods of time. Symbols: *C. albicans* NUM43 (●), IAM4966 (▲), and NUM678 (■); *S. cerevisiae* haploid (○), diploid (△), triploid (◇), and tetraploid (□).

by counting the number of chromosomes. However, countable chromosomes could not be seen in *C. albicans*, and they were thought to be associated with noncontinuous microtubules (MTs) in the spindle (5). The determination of the noncontinuous MTs was suggested but was not possible due to poor preservation of MTs in electron microscopy. Thus, we compared the size of nucleus-associated organelles (NAOs) which were equivalent to spindle pole bodies in cells of different ploidy in *C. albicans* (Fig. 2). The size is a function of number of MTs associated with NAO (5). Measurement of NAO diameter was carried out by serial thin-sectioning electron microscopy (K. Tanaka and A. Hirata, J. Cell Sci., in press) on the spindle at metaphase or at the stage when the spindle formation was just completed, NAOs facing each other. We discovered that the NAO of a haploid strain, NUM43, was about 80 nm in diameter; that of a diploid strain, IAM4966, was about 150 nm in diameter, and that of a tetraploid strain, NUM678, was about 220 nm in diameter. These results indicate that, as in *S. cerevisiae* (12), the size of the NAO becomes larger with the increase in ploidy number in *C. albicans*.

The ability to produce germ tubes has been

considered a useful criterion for identifying the species of *C. albicans*, as has been the ability to form chlamydospores (1, 9). Olaiya et al. (11) found that germ tube-negative laboratory variants of *C. albicans* were haploid. Also in this study, the efficiency of germ tube formation was examined in serum on each strain (Table 1). All of the haploid isolates and two of the diploid ones showed very rare formation of germ tubes, whereas the other diploid and tetraploid ones produced the tubes at high frequency. From these results, efficient formation of germ tubes in serum seems to depend upon the ploidy number in this organism, and the ability to form chlamydospores appears to be independent of it (unpublished data).

This study is the first report of the finding of haploid and tetraploid strains of *C. albicans* in clinical sources, and it suggests the presence of a parasexual cycle of this organism in nature. The organism has been generally considered to exist naturally only in the imperfect state, although a parasexual cycle employing polyethylene glycol fusion of spheroplasts was reported for this species (13). A life cycle of this organism was postulated by van der Walt and Pitout on sexually active strains (15, 16) in which presumed

FIG. 2. Electron micrographs showing nucleus-associated organelles in *C. albicans* strains of different ploidies. (A) NUM43 haploid; (B) IAM4966 diploid; (C) NUM678 tetraploid. Bars indicate 100 nm.

haplophase yeast cells developed into diplophase ones after karyogamy and mycelial formation occurred in the diplophase. Studies on the line to isolate such a sexually active strain as

described by them should be reexamined on this organism from natural sources.

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